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The Neuropharmacology of Capsaicin: Review of Some Recent Observations*

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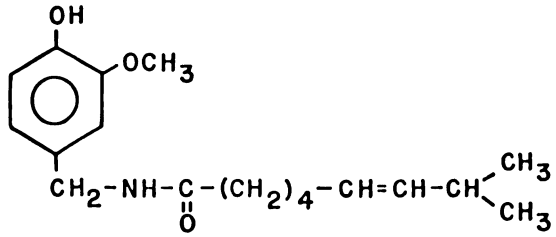
I. Introduction

CAPSAICIN (8-methyl-N-vanillyl-6-nonenamide) (fig. 1) is the major pungent ingredient of hot peppers of the plant genus *Capsicum*. Owing to the diverse and peculiar biological effects of these fruits in humans, hot peppers have been used since antiquity as food additives and preservatives, as ingredients in certain social rituals and practices, and as herbal medicines for maladies ranging from itch and pain to constipation. Apparently intrigued initially by some of the acute physiological consequences of the customary feeding of paprika-containing (Hungarian red pepper) foods to children, the Hungarian pharmacologist Nicholas Jancso and his laboratory began in the late 1940s an extensive characterization of the pharmacological effects of capsaicin and its congeners on certain sensory processes in mammals. These studies, continued after Jancso's death in 1966 by Janos Szolcsanyi, Gabor Jancso, and Aurelia Jancso-Gabor, revealed that most of the biological effects of capsaicin result from an initial intense excitation of certain sensory neurons that is followed by a prolonged period of insensitivity to physicochemical stimuli, presumably including the natural environmental stimuli for the sensory nerve endings. Discussions of the early work on capsaicin can be found in the review articles by Jancso (120), Virus and Gebhart (249), and Szolcsanyi (237).

Two of the early reports on capsaicin from eastern Europe suggested that, in addition to altering the neurophysiology of sensory neurons, capsaicin produced changes in some biochemical processes in these cells. Gasparovic and coworkers (78) observed that the amount of bioassayable substance P (SP) was reduced in the spinal cord, but not in the brain, of rats treated systemically with capsaicin. SP is an undecapeptide that is thought to be a neurotransmitter or neuromodulator in sensory neurons giving rise to type C fibers (unmyelinated cutaneous fibers 0.2 to 1.5 μm in diameter) (138). Similarly, Jancso and Knyihar (117) determined that capsaicin depleted fluoride-resistant acid phosphatase activity, known to be associated with central terminals of some primary afferent neurons, from the dorsal horn of the spinal cord. The neurophysiological and neurochemical effects of capsaicin were remarkably specific for primary afferent neurons, and in animals treated neonatally, but not in those treated while adults, the compound produced degeneration and loss of the majority of the type C sensory fibers (115).



CAPSAICIN

FIG. 1. Chemical structure of capsaicin.

A surge of new interest in capsaicin among neuroscientists began in 1978 with the report by Jessell et al. (123) that radioimmunoassay (RIA)- and immunohistochemically detectable SP was reduced in the dorsal horn of the spinal cord of adult rats treated systemically with capsaicin. The simultaneous reduction in dorsal horn fluoride-resistant acid phosphatase activity was also confirmed. These investigators extended their findings by observing that the levels of spinal glutamic acid decarboxylase activity and of opioid binding sites were not altered by capsaicin, thus ruling out a general neurotoxicity of the compound and the destruction of primary afferent central terminals. Numerous studies have now confirmed the peptide and sensory neurotoxic effects of capsaicin (table 1), and intriguing aspects of these actions have been uncovered. Some of the newer investigations dealing with capsaicin are included in reviews by Nagy (179), Jessell (122), and Fitzgerald (63).

Unlike the other treatises on the subject, it was our intention to review in detail all the findings of studies on the neuropharmacology of capsaicin that appeared in the recent peak publication period, i.e., studies published between 1978 and 1983 inclusive. This review is limited to full papers (abstracts and proceedings are excluded) published during this time in English language journals indexed in *Index Medicus*. Notwithstanding articles published in journals too new to be indexed and omissions by the indexing service, we have compiled approximately 250 such papers on the neuropharmacology of capsaicin. Readers interested in earlier work and in other aspects of the compound are referred to the review articles cited above and to the review by Monsereenusorn et al. (174) on the biochemical and metabolic actions of capsaicin. Highlights of major findings on the neuropharmacology of capsaicin that have appeared since 1983 are covered at the end of the text in "Supplemental Information."

II. Systemic Treatment of Adult Animals

A. Neurochemical Investigations

Jessell and coworkers (123) observed that systemic treatment (s.c.) of adult rats with a cumulative dose of capsaicin (950 mg/kg) depleted spinal cord dorsal horn SP by 48% and produced a marked, but incomplete, depletion of fluoride-resistant acid phosphatase. The greatest portion of this effect appeared histochemically to occur in the substantia gelatinosa of the dorsal horn. In adult rats treated with substantially smaller doses of capsaicin, there was marked depletion of radioimmunoassayable SP in saphenous nerve, dorsal root ganglia, and dorsal roots, as well as a 50% decrease in levels in dorsal cord (74). A s.c. dose of as little as 50 mg of capsaicin per kg produced significant depletion in all sensitive tissues, but an i.p. dose of 125 mg/kg produced a 70 to 80% decrease in SP in dorsal root ganglia and

TABLE 1
Reports of SP depletion by capsaicin

Tissue	Species	Refs.
CNS		
Amygdaloid nucleus	Rat	251
Medulla oblongata	Rat	74
	Guinea pig	77
Dorsal spinal cord	Rat	7, 27, 30, 46, 70, 74, 78, 79, 90, 91, 93, 97, 111, 123, 134, 146, 168, 180, 183, 186, 208, 222, 227, 252, 261
	Guinea pig	26, 77
	Mouse	70
Spinal cord lamina X	Rat	111
Dorsal roots/ganglia	Rat	1, 7, 27, 72, 74, 76, 85, 86, 95, 111, 134, 140, 171, 183, 184
	Guinea pig	26, 77
Ventral roots	Rat	74
Ganglia/nerves		
Trigeminal ganglia/nuclei	Rat	46, 74, 91, 93, 97, 111, 146, 155, 184, 208, 213, 222
	Guinea pig	155
Nucleus tractus solitarius	Rat	46
Vagus nerve	Rat	74
	Guinea pig	77
Superior cervical ganglia	Rat	251
	Guinea pig	77
Middle/inferior cervical ganglia	Guinea pig	77
Sphenopalatine ganglia	Rat	155
	Guinea pig	155
Thoracic ganglia	Guinea pig	77
Stellata ganglion	Rat	96
Celiac ganglia	Rat	251
	Guinea pig	77
Mesenteric ganglia	Rat	96
Prevertebral sympathetic ganglia	Guinea pig	163
Cuneate fasciculus	Rat	146
Splanchnic nerve	Guinea pig	77
Saphenous nerve	Rat	74, 140
Sciatic nerve	Rat	39, 70, 76, 184
	Mouse	70
Sural nerve	Rat	146
Cardiovascular system		
Gut/mesenteric vasculature	Guinea pig	57, 258
Pial arteries	Guinea pig	57
General vasculature	Rat	9
	Guinea pig	68
Nasal vasculature/glands	Rat	155
	Guinea pig	155
	Mouse	204
Heart/pericardium	Rat	96
	Guinea pig	178, 202
Peripheral tissues		
Cornea	Rat	74, 140
	Mouse	132
Mucosal surfaces	Rat	96, 155
Skin	Rat	7, 72, 76, 79, 90, 96, 140
Tongue	Rat	181
Submandibular gland duct	Rat	80
Trachea	Rat	96
Lungs	Rat	96
Hepatic duct	Rat	96
Ureter	Rat	96
	Guinea pig	178, 216
Urinary bladder	Rat	96, 224
Adrenal gland	Guinea pig	30

dorsal roots, the maximal depletion seen in these particular tissue at any capsaicin dose. Administration of 125 mg of capsaicin per kg s.c. also depleted SP in cornea, trigeminal ganglion, vagus nerve, and medulla oblongata; in some of the tissues (cornea, vagus, and medulla), this depletion was still present 9 mo later. There was also some indication of SP depletion by these doses of capsaicin in ventral roots, but in view of the lack of a simultaneous depletion in ventral cord and of the lack of effect on ventral root SP levels in animals treated while neonates (see section III, A and C), this finding is puzzling (74). A combination of s.c. and i.p. dosing of adult rats by these same investigators resulted in no significant SP depletion by capsaicin in the proximal or distal gastrointestinal tract (97) or in the adrenal gland (30).

Priestley and coworkers (208) treated adult rats with a systemic cumulative dose of 266 mg of capsaicin per kg and 1 wk later observed that SP immunofluorescence in the substantia gelatinosa of the spinal trigeminal nucleus and of the cord was markedly depleted, although not to the extent seen in animals treated while neonates (see section III, A and C). Virus and coworkers (251) examined the effects of capsaicin in adult WKY (strain controls) and SHR (spontaneously hypertensive strain) rats and found SP depletion in both strains in superior cervical and celiac ganglia, but not in adrenal gland in either strain. There was no depletion in either strain in the nuclei tractus solitarius, raphe magnus, and locus coeruleus, or in the periaqueductal grey or periventricular preoptic area of the brain. In the SHR strain only, there was a suggestion of SP depletion by capsaicin in the amygdaloid nucleus. Interestingly, SHR controls had lower SP levels than WKY controls in both sympathetic ganglia and higher levels in raphe, preoptic, and amygdaloid nuclei.

Several laboratories have investigated the effects of capsaicin in adult guinea pigs, a species with higher SP levels than rats in some areas of the spinal cord and in some peripheral tissues (25). Buck et al. (26) treated animals s.c. with 1250 mg of capsaicin per kg and observed a comparable proportional decrease in SP levels as in adult rats (vide supra) in dorsal cord and dorsal roots plus ganglia. Gamse and coworkers (77) reported a similar effect of one-tenth of this dose as well as marked SP depletion in superior cervical, middle plus inferior cervical, thoracic, and celiac-superior mesenteric ganglia, splanchnic and vagus nerves, and medulla oblongata. Systemic treatment of adult guinea pigs with capsaicin produced almost total depletion of immunofluorescent SP fibers in prevertebral sympathetic ganglia and in para- and perivascular areas of the ileum and mesentery (163, 258). SP levels measured by RIA were totally abolished in pial and mesenteric arteries (57), and SP-positive immunofluorescence was nearly obliterated in pericardium, epicardium, myocardium, endocardium, cardiac valves, and chorda tendinae of capsaicin-treated guinea pigs (202). This depletion in the heart after a total dose of capsaicin of 775 mg/kg lasted for at least 45 days. Furness and colleagues (68) conducted an extensive immunohistochemical study of SP innervation of the entire guinea pig cardiovascular system and found that this same dose of capsaicin markedly depleted the peptide from nearly every vascular bed in the animal. The only exception was an intriguing capsaicin-resistant group of SP-containing fibers located on arteries to the distal colon and rectum. These same investigators used combined high-pressure liquid chromatography (HPLC) and RIA to confirm depletion in the ureter, atrium, and superior mesenteric artery of capsaicin-treated animals (178). In contrast to adult rats, capsaicin depleted SP in the adrenal gland of the guinea pig (30).

The results of these investigations indicate that the neurochemical effects of capsaicin are remarkably specific for primary afferent neurons. In adult rats, systemic capsaicin treatment failed to deplete SP in ventral cord, hypothalamus and other brain areas, gut, or adrenal gland (30, 74, 97, 251). In guinea pigs, a similar lack of effect was seen in brain (other than brainstem), ventral cord, and enteric neurons (26, 68, 77, 163, 178).

Systemic treatment of adult animals did not alter spinal cord levels of neurotensin or Leu-enkephalin immunoreactivity (208) or levels of vasoactive intestinal polypeptide (VIP) in the cardiovascular system or sensory afferents at times when SP was markedly depleted (28, 49, 162, 202). The compound did produce a small, relatively short-lived reduction in sensory neuron somatostatin levels (74), a decrease in dorsal horn cholecystokinin (CCK) immunofluorescence (208) in rats, and a partial reduction of acetylcholinesterase staining in guinea pig heart (202). The effects on somatostatin and CCK were not seen in adult guinea pigs (28).

Virus et al. (252) found small to moderate changes in spinal cord levels of norepinephrine and 5-hydroxytryptamine 16 days after a cumulative dose of capsaicin (150 mg/kg). At this time point, spinal cord SP was depleted by 40 to 60% in WKY and in SHR rats. Spinal levels of 5-hydroxytryptamine were elevated by 15% in SHR, but not in WKY, animals. Norepinephrine content was increased by 10% in spinal cord of WKY rats and by 25 to 35% in spinal cord of SHR rats treated with capsaicin. It is difficult to imagine that these small changes reflect any major nonspecific actions of capsaicin in the spinal cord, since many other neurochemical parameters are not affected by the compound. Furthermore, other investigators have not observed alterations in levels of these biogenic amines after treatment of adult or neonatal animals with capsaicin (vide supra; see sections II C and III, A and C). The changes in norepinephrine and 5-hydroxytryptamine levels observed by Virus and coworkers (252) may be secondary to the actions of capsaicin on primary afferent neurons and may or may not be involved in the physiological consequences of these ac-

tions. Thus, capsaicin depletes SP from susceptible tissues for days to weeks after treatment with minimal, or no, changes in levels of other putative neurochemicals.

B. Analgesic Investigations

Hayes and coworkers (89) administered capsaicin s.c. in rats in doses up to 10 mg/kg and observed a marked, long-lasting reduction of the response to plantar injections of formalin. The same doses of capsaicin also produced a reduction in responses to nociceptive paw pressure. This latter effect was rapid in onset and lasted for 3 h. The treated animals responded normally to pinch and startle and did not show any signs of motor depression. Doses of capsaicin up to 90 mg/kg had no effect on nociceptive responses in the hot-plate and tail-immersion tests in adult rats 30 min after dosing. In the same study, capsaicin (20 mg/kg) had no effect on hot-plate latencies, but it reduced noxious chemical-induced writhing with a 50% effective dose (ED_{50}) of 1.4 mg/kg in adult mice 30 min after dosing.

Gamse (70) treated adult rats with much higher doses of capsaicin and obtained a slight and short increase in the threshold in the tail-withdrawal test and an increase in response time in the hot-plate test that persisted approximately 2 wk. Even with a total systemic dose of 950 mg/kg, the tail-withdrawal latency in capsaicin-treated rats was increased by only 64% compared to a 224% increase in the hot-plate threshold in the same animals. Open field rearing behavior was depressed, whereas grooming and locomotor behavior were not affected in male rats. Female rats did not show depression of rearing, but still exhibited similar changes as males in the thermal thresholds. The changes in nociceptive thermal sensitivity in females, however, were of much shorter duration than those in males. This is the only report of a possible sex difference in the neuropharmacological actions of capsaicin. Capsaicin in a dose of 10 mg/kg rendered adult rats substantially less sensitive than controls to chemical irritation of the cornea. At doses of 50 and 125 mg/kg, but not at 10 mg/kg, this chemical insensitivity lasted for at least 6 wk.

In the same investigation, Gamse (70) studied the effects of capsaicin in adult mice. A dose of 50 mg/kg, but not 10 mg/kg, increased the response time in the hot-plate test. Repeated administration of 10 mg/kg/day for 5 days, as well as the single 50-mg/kg dose, prolonged the hot-plate latency for up to 8 wk. A 300% increase in tail-withdrawal latency was seen in animals treated for 5 days with capsaicin (10 mg/kg/day), but not with a single 10-mg/kg dose, and the latency declined to a 100% increase at 1.5 to 7 wk after treatment. There was no difference in sensitivity to capsaicin between male and female mice or between different strains of mice with differential baseline thermal sensitivity. A 50-mg/kg dose of capsaicin in adult mice produced a marked, long-lasting reduction in sensitivity to noxious chemical-induced writhing and to corneal chemical irritation.

Jancso and Jancso-Gabor (112) reported that adult rats treated systemically with a single 50-mg/kg dose of capsaicin had a marked attenuation in the analgesic effect of a single i.p. dose of 10 mg of morphine per kg in the tail-withdrawal test. Unfortunately, no age-matched, non-capsaicin-treated controls were tested nor were other doses of morphine. These authors speculated that the hypothalamus was the site of this capsaicin action, but it is just as likely that the site was primary afferent neurons or even the liver (hepatic drug metabolism) (170). In rats and mice, capsaicin is less potent, but equieffective and longer lasting in its analgesic action than morphine (137).

C. Combined Neurochemical and Analgesic Studies

Several laboratories have been unable to find altered thermal sensitivity in adult rats treated with capsaicin at times when primary afferent SP was markedly depleted. Hayes and Tyers (90) administered high systemic doses of capsaicin and found no change in thresholds in the tail-withdrawal and hot-plate tests, even though the treated animals had a long-lasting decrease in sensitivity to nociceptive paw pressure and a marked decrease in dorsal horn and paw skin SP levels. Buck et al. (27) obtained similar results when they studied the effects of capsaicin and of dihydrocapsaicin on tail-flick latencies in rats. Gamse (70) saw no difference in tail-withdrawal or hot-plate latencies in capsaicin-treated rats 2 mo after 50 or 125 mg/kg even though both dose levels depleted SP in the dorsal cord and in the sciatic nerve.

Lembeck and Donnerer (140) compared the time course of altered plasma extravasation and of corneal insensitivity to the SP depletion after a 50-mg/kg dose of capsaicin in adult rats. Although the SP reductions seen after this dose were small in most tissues, they were evident in some tissues (dorsal cord, dorsal root ganglia, saphenous nerve, and skin) as early as 5 h after dosing. The authors also reported one of only two instances where capsaicin caused an increase in sensory neuron SP levels, a 40% increase in dorsal root ganglia levels beginning 1 day after capsaicin administration. Gamse and coworkers (74) reported a similar increase in the trigeminal ganglion 4 mo after a dose of 125 mg/kg and following an initial decrease in SP levels in this tissue. Since a comprehensive analysis of the dose-response effects of capsaicin on SP levels in tissues of the adult rat has not been carried out, however, it is dangerous to ascribe conclusions to effects of capsaicin administered at doses which may be close to the threshold for sensory neuron peptide neurotoxicity in these animals (50 mg/kg). The fact that adult rats appear to be more resistant than adult guinea pigs (vide infra) and adult mice (70) to the SP depletion induced by capsaicin in sensory neurons reinforces this caveat. When Lembeck and Donnerer (140) measured plasma extravasation and corneal sensitivity in animals treated with the 50-mg/kg dose of capsaicin, these functions were markedly reduced as

early as 10 min after systemic dosing of the compound. Plasma extravasation in the skin in response to saphenous nerve stimulation was also inhibited by 70% at 30 min after capsaicin, but extravasation induced by topical application of mustard oil on the skin was not reduced until many hours after paw surgical denervation. Although corneal SP depletion was not assessed by these investigators, it was clear that the functional changes induced by capsaicin in skin preceded substantially any effects of the compound on SP levels in peripheral, somatic, or central aspects of the affected sensory neurons.

Buck and coworkers (26) observed that treatment of adult guinea pigs with high doses of capsaicin depleted SP in dorsal roots plus ganglia by 80 to 90% and in dorsal cord by 35% and also produced a marked reduction in sensitivity to nociceptive heat as determined in a hot-plate test and a high-intensity light-induced skin-flinch test. The capsaicin-treated animals showed no response to intense heat even at times when the stimulus was clearly initiating tissue damage in the animals. These investigators have characterized the dose-response relationship of SP depletion in guinea pigs (28). Capsaicin in a s.c. dose as low as 2.5 mg/kg produced a significant depletion in dorsal roots plus ganglia, while 10 mg/kg produced maximal depletion in roots plus ganglia (85% decrease) and in dorsal cord (40% decrease). Capsaicin at 5 mg/kg abolished corneal sensitivity to chemical irritation while sensitivity to noxious heat in the hot-plate test was somewhat reduced at 10 mg/kg. The selectivity of capsaicin for altering only certain sensory functions was apparent when high doses of the compound reduced sensitivity to noxious and nonnoxious heat and to chemical irritation without affecting responses to touch, vibration, nociceptive pressure, and nociceptive and nonnociceptive cold. Capsaicin also produced corneal opacities/ulceration and skin lesions in the treated animals. The thermal and chemical insensitivity and the SP depletion produced by a single 50-mg/kg dose of capsaicin lasted for at least 10 wk. Sensory neuron levels of VIP, CCK, and somatostatin were not altered 4 or 10 days or 10 wk after capsaicin, even though SP levels were markedly reduced. There was a transient 50% reduction in ventral cord and dorsal roots plus ganglia levels of CCK 4 days after capsaicin administration.

Additional evidence for a dissociation between the functional and SP-depleting actions of capsaicin was provided by Miller et al. (171) who observed that thermal insensitivity in adult guinea pigs preceded SP depletion in dorsal root ganglia. In addition, localized injection of 8 μg of dihydrocapsaicin into a foot pad produced localized thermal insensitivity within 2 h, but did not affect SP levels in innervating dorsal root ganglia for up to 10 days after the injection.

D. Neuroanatomical Investigations

Numerous studies have demonstrated the value of capsaicin as a pharmacological tool with which to map the innervation of peripheral tissues by sensory neurons. Sikri and coworkers (225) took the novel approach of incubating guinea pig ureters in vitro with capsaicin and then assessing by microscopy the effects on axons and terminals in the tissue. After incubation with the compound, nerve plexuses contained fibers exhibiting a loss of microtubules, a reduction in axoplasmic electron density, and axonal dilation. Even though the capsaicin concentration used (20 mg/100 ml) was quite high, the neuronal damage seemed to be limited to axons containing large dense-cored vesicles which are characteristic of peptide-containing neurons. Axons and terminals containing small vesicles were not visibly harmed by the capsaicin treatment. Similar experiments with the trachea of the rat indicated an identical pattern of damage in intraepithelial axons and terminals (107). When adult rats were treated s.c. with a 50-mg/kg dose of capsaicin, there was a reduction in the number of axon profiles containing a high proportion of large dense-cored vesicles in the tracheal epithelium. In the gut of these capsaicin-treated rats, evidence of axonal degeneration was seen in arteriolar perivascular plexuses and occasionally in the myenteric, muscularis externa, and mucosal plexuses. Again, axonal terminals containing small closely packed vesicles did not appear susceptible to damage by capsaicin (106). Systemic treatment of adult guinea pigs also affects SP-containing fibers in the ureter (216; see sections IX and X I).

Lundblad et al. (155) used capsaicin to investigate innervation of the nasal mucosa by SP-containing neurons. Treatment of adult rats and adult guinea pigs with a single s.c. dose of 50 mg/kg 7 days prior to sacrifice resulted in a near-total loss of SP immunofluorescent fibers from around blood vessels and in the nasal epithelium. At the same time, VIP and tyrosine hydroxylase immunostaining were not altered. Capsaicin-sensitive SP fibers were also seen around principal cells of the sphenopalatine ganglion and the spinal trigeminal nucleus. SP-containing, small cell bodies were observed in the trigeminal ganglia of rats and guinea pigs, but unfortunately no mention was made of the appearance of these SP-positive cell bodies after capsaicin treatment. Sectioning of the maxillary branch of the trigeminal nerve depleted most SP immunofluorescence in the nasal mucosa without affecting VIP or tyrosine hydroxylase-containing fibers in the tissue. Papka and Matulionis (204) also observed capsaicin-sensitive SP immunoreactivity in murine olfactory mucosa. Most of this was present in fibers at the border of the olfactory epithelium and lamina propria or in close proximity to glands and blood vessels in the lamina propria. No SP immunoreactivity was seen in the epithelium or in the fila olfactoria.

The SP-containing fibers that innervate large peripheral blood vessels in the rat are also capsaicin sensitive. Most of these fibers were observed in the adventitia and adventitia-media border. Arteries contained more SP

immunoreactivity than veins with the superior mesenteric artery being heavily innervated. There was generally a lack of correlation in rat vasculature of sensitivity to exogenous SP and degree of SP innervation which supports the hypothesis that the SP-containing neurons are sensory (9).

III. Systemic Treatment of Neonatal and Prenatal Animals

A. Neurochemical Investigations

Treatment of neonatal rats with capsaicin has revealed a neurotoxicity that shows the same specificity as in adults for primary afferent neurons. Within this class of neurons, however, the toxicity in neonates appears greater and less limited to SP-containing cells. Treatment with capsaicin (50 mg/kg) s.c. on day 2 of life markedly reduced SP immunofluorescence and immunoreactivity in adulthood in the dorsal horn of the cord, in the trigeminal nucleus, and in the substantia gelatinosa, but not in ventral horn, interpeduncular nucleus, substantia nigra, posteromedial amygdala, posterior or dorsomedial hypothalamus, paraventricular nucleus, medial preoptic nucleus, globus pallidus, salivary gland, other medullary nuclei, or any part of the gastrointestinal tract (46, 91, 93, 97, 208, 222). There was a permanent loss of some immunohistochemically detectable SP in the nucleus tractus solitarius (46) that was not detectable by RIA (91, 93). Treatment of neonatal rats with the same dose of capsaicin on day 10 or day 20 resulted in a lesser decrease in SP immunofluorescence in adulthood than did treatment on day 2 (46). Outside the central nervous system (CNS), treatment of neonates on day 2 with 50 mg of capsaicin per kg resulted in depletion of SP in skin from all body regions, in oral, nasal, and lingual mucosae, and in stellate ganglion, mesenteric ganglia, urinary bladder, ureter, hepatic duct, myocardium, lungs, and trachea. The degree of depletion ranged from 22% in lingual mucosa to 84% in urinary bladder (96).

The effects of capsaicin in newborn rats, which apparently result from a massive, permanent loss of unmyelinated sensory neurons (see section III D), are not specific for SP-containing cells, since several of the other sensory neuron peptides are also affected. In a comprehensive study of spinal and medullary neuropeptides after 50 mg of capsaicin per kg on day 2 of life, Jancso and coworkers (111) observed a substantial decrease in dorsal horn SP, CCK, VIP, and somatostatin immunofluorescence when the animals were 2 mo old. SP and somatostatin-positive fluorescence was also obliterated in cell bodies in spinal ganglia. SP staining was reduced in the trigeminal nucleus as well. In none of these regions was any reduction in enkephalin, neurotensin, or 5-hydroxytryptamine immunofluorescence evident. Spinal enkephalin, neurotensin, and 5-hydroxytryptamine are known not to be of sensory neuron origin. SP immunofluorescence in laminae deeper than I and II of the dorsal horn and in the ventral horn was not affected by capsaicin. Priestley and coworkers (208) confirmed these results for SP, CCK, somatostatin, enkephalin, and neurotensin in the spinal cord and trigeminal nucleus in rats treated while neonates. Capsaicin also depleted SP and CCK immunoreactivity in a small area just ventral to the central canal (111). Whereas CCK determined by immunofluorescence techniques is markedly decreased after neonatal capsaicin in dorsal cord (111, 208, 222), CCK immunoreactivity quantitated by RIA was not changed in capsaicin-treated animals in dorsal cord, dorsal root ganglia, or celiac ganglia (159, 222). After denervating the dorsal cord from sensory cell input, Marley and coworkers (159) were also unable to detect a change in CCK determined by RIA in the cord. This inconsistency between immunohistochemical and RIA results with CCK indicates that the much greater amount of antiserum used in the former technique is detecting some material not recognized by the more dilute antiserum concentrations used in RIAs. The identity of this material is unknown, but it is presumably CCK related and occurs in capsaicin-sensitive primary afferents.

Nagy et al. (183) conducted an intensive investigation of the biochemical and neuroanatomical effects of a 50-mg/kg dose of capsaicin administered to 2-day-old rats. Morphometric analysis in adulthood of the numbers of unmyelinated and myelinated fibers in cross-sections of lumbar dorsal roots revealed that capsaicin treatment had reduced the number of unmyelinated fibers by 90% with no change in myelinated fiber numbers. SP levels in the dorsal horn were reduced by 55%, and somatostatin was depleted by 20% in this region. Ventral horn SP was unchanged, as expected (vide supra). Dorsal root SP levels were reduced by 85%, while somatostatin was depleted by 90% in this tissue. Fluoride-resistant acid phosphatase activity was markedly reduced in dorsal horn and in cell bodies of dorsal root ganglia in the animals treated while neonates. In one of few studies of the dose-response character of capsaicin neurotoxicity in neonates, this same group (184) determined that the ED_{50} for reduction of the number of unmyelinated sensory fibers was approximately 10 mg/kg with an additional loss of myelinated fibers beginning at 25 mg of capsaicin per kg. This more rigorous analysis of myelinated fibers than the previous study (183) suggested that as many as 40% of the myelinated fibers in lumbar dorsal roots could be destroyed by 75 to 100 mg of capsaicin per kg. Determination of the ED_{50} for SP and somatostatin depletion indicated values of 10 mg/kg for both peptides in lumbar dorsal roots, 15 mg/kg in sciatic nerve, 10 to 15 mg/kg in lumbar dorsal root ganglia, and 10 to 15 mg/kg in trigeminal ganglia. From this informative investigation, it was concluded that there are doses (5 mg/kg) of capsaicin which can destroy unmyelinated neurons containing neither SP nor somatostatin in animals

treated while neonates and that there are doses (10 to 25 mg/kg) of the compound which can destroy 90% of the unmyelinated fibers without affecting myelinated fibers. It was also noted that only Aδ myelinated sensory fibers (myelinated cutaneous fibers 1–5 μm in diameter) were destroyed at the high doses which affected myelinated and unmyelinated neurons.

Kessler and Black (134) administered a cumulative dose of 950 mg of capsaicin per kg to newborn rats on days 1 to 5 of life and observed SP decreases in dorsal root ganglia that were smaller than those reported by others using much lower doses (vide supra). Somatostatin was depleted by only 34% in spinal ganglia, but by 53% in dorsal spinal cord (compare to ref. 183). In view of the near complete loss of unmyelinated cells and the additional loss of myelinated A cells that would be expected with this capsaicin dose (184), the discordant amounts of depletion observed by these investigators probably reflect methodological differences. Kantner and Kirby (127) found that 15-day-old offspring of mothers treated with capsaicin while pregnant had less fluoride-resistant acid phosphatase activity in the spinal substantia gelatinosa than newborn rats from vehicle-treated mothers. Surprisingly, when formalin was injected as a nociceptive stimulus into the forepaw of these neonates, acid phosphatase activity increased in the gelatinosa only in the capsaicin-exposed animals. If the increased phosphatase activity is a reflection of increased neuronal activity in pain-sensing neurons, then it is peculiar that the increase occurred in animals exposed to a neurotoxin thought to destroy most of the phosphatase-containing sensory cells. Since the effects of prenatal exposure to capsaicin have not been carefully studied, the observations of Kantner and Kirby (127) must be interpreted with caution. Offspring mice of mothers treated with capsaicin during pregnancy and mice that had been injected in utero with capsaicin had extensive depletion of SP in the dorsal horn, particularly in the substantia gelatinosa. There was also depletion in skin and in cells in dorsal root ganglia. No depletion could be detected in cells or axons in the gut wall of the prenatally treated animals. No determination was made of whether this treatment actually produced degeneration and loss of the affected neurons (7).

In an admirably thorough investigation, Singer and coworkers (227) assessed the effects of a 50-mg/kg dose of capsaicin on day 2 of life on 14 neurochemical parameters in the dorsal spinal cord in adulthood. The animals exhibited an unusually large 75% decrease in dorsal cord SP levels, while at the same time there was no change in neurotensin, glutamic acid, aspartic acid, glycine, γ-aminobutyric acid, taurine, norepinephrine, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, or histamine levels and no alteration of glutamic acid uptake, glutamic acid decarboxylase activity, or choline acetyltransferase activity. Holzer et al. (100) observed increased 5-hydroxytryptamine levels in dorsal hindpaw skin and dorsal cord, and elevated histamine concentrations in dorsal hindpaw skin, dorsal and ventral spinal cord, and lungs after a similar treatment of neonatal rats. The authors speculated that these changes occurred in mast cells and neurons as a secondary response to the loss of unmyelinated sensory neurons. The reasons for the conflicting findings with dorsal cord 5-hydroxytryptamine and histamine in these two studies are not apparent, but given the massive number of determinations made, it may be that some of the differences reported by Holzer and coworkers (100) were due to chance.

In a novel SP antibody immunoprecipitation study of incorporation of [^{35}S]methionine into immunoreactive material by dorsal root ganglia in tissue culture, Harmar and coworkers (85, 86) reported an 80 to 90% decrease in incorporation in ganglia taken from adult rats treated with capsaicin on the second day of life. The reduction occurred in immunoreactivity thought to be authentic SP as well as in an unknown accompanying HPLC peak and presumably reflected the capsaicin-induced loss of the majority of the SP-containing primary afferent neurons.

After ligation of the sciatic nerve, McDougal et al. (166) found that lysosomal and nonlysosomal acid phosphatase accumulated at the tie to a much lesser degree in animals treated neonatally with capsaicin. Treatment of neonates with guanethidine, a sympathetic neuron toxin, had a much lesser effect on the appearance of these enzyme activities at the time. Acetylcholinesterase accumulation was reduced by guanethidine treatment, but not by capsaicin, a finding contradictory to that of Papka and coworkers (202) who observed a reduction in cholinesterase staining in the heart of adult guinea pigs treated with capsaicin. Each neurotoxin reduced the accumulation of glutaminase, hexokinase, and glutamic dehydrogenase by one-third. A similar pattern of effects for each of these agents was found in dorsal root ganglia. The retrograde transport of ^{125}I-nerve growth factor was reduced 20% after forepaw injection in animals treated with capsaicin as neonates. Guanethidine had marked inhibitory action on the enzyme activities in the superior cervical ganglia as well as on the activity of tyrosine hydroxylase. Capsaicin had no effect on the enzymes assayed in this tissue, suggesting a lack of innervation of the ganglion by unmyelinated primary afferents (but see refs. 74 and 251).

Avian pancreatic polypeptide exists in the dorsal horn and trigeminal nucleus of the rat in a distribution similar to that of SP. The pancreatic peptide, however, is not of sensory neuron origin, since dorsal rhizotomy or neonatal capsaicin treatment do not deplete the peptide in these regions (94). SP-containing nerves that innervate the duct of the rat submandibular gland are apparently sensory, since lesions of the mandibular division of the trigeminal nerve or neonatal treatment with capsaicin

markedly reduced SP immunoreactivity in the duct. In contrast, each of these manipulations produced only a small decrease in SP levels in the submandibular gland itself (80).

Panerai and coworkers (201) reported that systemic treatment of neonatal rats and intraventricular treatment of adult rats with capsaicin (see section VI) produced a specific depletion of β-endorphin in the hypothalamus. Newborn rats were treated on days 1 to 5 of life with a total dose of capsaicin of 750 mg/kg and killed for peptide determinations 6 mo later. Hypothalamic levels of β-endorphin were reduced by 75%, but levels in the hindbrain or midbrain were not altered. Hypothalamic levels of SP, somatostatin, or Met-enkephalin were also not altered. In view of the high dose of capsaicin used in neonatal rats by these investigators (vide supra; see section III C), and since a microscopic histological examination of the hypothalamus was not conducted in treated neonates (but see section VI), it is possible that the compound produced a nonspecific depletion of the endorphin from the arcuate nucleus. This region contains very high levels of the peptide and is located in close proximity to the third ventricle. Since the β-endorphin depletion was evident 6 mo after capsaicin treatment of neonatal rats, it is unlikely that this was a reversible effect as implied by Panerai et al. (201) (but see section VI).

B. Analgesic Investigations

The initial report by Nagy and coworkers (186) of permanent SP depletion and altered thermal sensitivity after treatment of neonatal rats with systemic capsaicin ushered in considerable interest in the analgesic effects of this treatment and a substantial amount of controversy about these effects. Their report indicated that 12-wk-old rats, injected with a single 50-mg/kg dose of capsaicin on day 2 of life, exhibited a 66% increase in tail/flick latency and a 44% increase in latency to paw lick on a hot-plate (186). Another major capsaicin research laboratory also reported that the identical treatment increased tail-flick time by 90% and hot-plate response by 50% (98). The interesting observation was also made that this treatment with capsaicin on day 10 of life, but not on days 15 or 20, produced the analgesic effect in the hot-plate test when the animals had reached 3 to 4 mo of age. Jancso and Jancso-Gabor (112) observed a marginally significant increase in the tail-withdrawal latency from 55°C water in adult rats treated as neonates with 50 mg of capsaicin per kg. Hayes and coworkers (88) administered the same dose of capsaicin to neonatal rats and were unable to detect adulthood changes in the hot-plate or tail-withdrawal thresholds even though significant analgesia in a nociceptive paw pressure test was observed. Adult mice treated with the compound as neonates were substantially analgesic to chemical-induced abdominal writhing, but also showed no insensitivity to heat in the hot-plate test. Faulkner and Growcott (60) also observed a reduced sensitivity to nociceptive pressure in paws (normal and yeast-inflamed) of rats treated on day 2 of life with capsaicin. The treated animals had a substantial reduction in their response to the nociceptive effect of tail-vein injection of dilute hydrochloric acid.

Cervero and McRitchie (37) could find no indication of an elevated threshold in the hot-plate test in 4-mo-old rats treated on day 2 of life with capsaicin (50 mg/kg). There was, however, a 30% increase in the threshold to nociceptive paw pressure. These investigators then used the same rats to make the interesting observation that, whereas abdominal nociceptive heating and pinching induced a reflex, short-lived drop in blood pressure and in gastric motility in anesthetized vehicle-treated animals, only the pinch stimulus produced these effects in the capsaicin-treated animals. This gastric response to noxious abdominal stimuli was believed to be mediated by cutaneous afferents and sympathetic splanchnic efferent neurons.

Saumet and Duclaux (219) administered a cumulative capsaicin dose of 475 mg/kg on days 2 to 7 of life to rats and found that, among the 40% of the treated animals that survived this dose regimen, there was a reduced sensitivity to all forms of nociceptive stimuli tested. These stimuli included radiant heat in the tail-flick test, nociceptive tail pressure, nociceptive pinprick, and a nociceptive i.p. chemical injection. In view of the magnitude of the dose used and possible widespread effects on primary afferents it may have had (see section III A), it is difficult to interpret these observations.

Nagy and vander Kooy (185) tested the effects of capsaicin doses of 5 to 100 mg/kg administered neonatally on thermal, pressure, and chemical nociceptive responses. Despite considerable variability within treatment groups, they were able to detect significant reductions in sensitivity to all three types of stimuli. Mechanical sensitivity appeared to be most susceptible to capsaicin with an effect of the compound at 5 mg/kg being observed. The tail-flick test required higher doses of capsaicin than other tests for a significant effect. These investigators concluded that extreme variability in animal responsiveness to capsaicin may have contributed to previous conflicting results with the antinociceptive effects of capsaicin (see sections II and III, A to D). They also concluded that SP and somatostatin may not be involved in nociceptive mechanical sensation in rats, since the 5-mg/kg dose of capsaicin had no effect on levels of these peptides in dorsal roots (184). It is possible, however, that afferents containing SP or somatostatin could be physiologically altered by this dose of capsaicin without actual peptide depletion (see section II C).

C. Combined Neurochemical and Analgesic Studies

Nagy et al. (186) supplemented their analgesic findings cited in the preceding section by observing through electron microscopy that the dorsal horn of the cord con-

tained many degenerating terminals and unmyelinated axons 2 h after administration of capsaicin to neonatal rats. By 6 h, most of the degenerating elements were engulfed by glial cells. Twelve wk after this treatment, at the time of the reported thermal analgesia, SP was depleted by 48% in the dorsal horn, but unchanged in the striatum, substantia nigra, and hypothalamus. Dorsal horn levels of glutamic acid decarboxylase and choline acetyltransferase activities were also unaltered, but the capsaicin-treated animals had a 37% decrease in the number of dorsal horn [^{3}H]naloxone binding sites. This latter finding presumably indicated the existence of opioid receptors on central terminals of the destroyed unmyelinated fibers. SP was near-maximally depleted in dorsal spinal cord and in dorsal roots plus ganglia in adult rats treated on day 2 of life with capsaicin, even though the tail-flick latencies in these animals were not elevated (27).

Gamse (70) conducted an extensive reinvestigation of the actions of neonatally administered capsaicin on thermal sensitivity. Using large numbers of treated animals, he was able to detect a 55 to 120% increase in hot-plate latency that was significant at ages 6, 13, and 20 mo. A 39 to 52% increase in the tail-flick latency was also present at ages 2 wk and 5, 13, and 20 mo. SP depletion was confirmed in the treated rats by the finding that, at 4 mo of age, the neuropeptide was depleted by 76% in the sciatic nerve. At 20 mo, the depletion was 69% in the nerve and 48% in the dorsal spinal cord. Similar experiments in mice led to more complex results. Treatment with capsaicin (50 mg/kg) s.c. at age 2 days had no effect on hot-plate or tail-flick latencies and little effect on nociceptive chemical sensitivity when the animals were 3 mo old. Treatment at age 4 days had little effect on the hot-plate latencies, but significantly increased tail-withdrawal times. Treatment at age 7 or 10 days resulted in moderate increases in the hot-plate latencies and substantial increases in the tail-withdrawal latencies at 3 mo of age. Sensitivities to capsaicin applied topically to the cornea or a hindpaw and to acetylcholine-induced writhing followed the same pattern in that they were reduced more by treatment at age 4 or 7 days than by treatment at age 2 days. Even more perplexing was the finding that SP content was depleted to the same extent at age 8 wk in sciatic nerve (55% decrease) and in dorsal cord (35% decrease) no matter what neonatal age the mice were when treated with capsaicin (70).

D. Neuroanatomical Investigations

The value of capsaicin as an anatomical tool with which to study the primary afferent system has also been demonstrated by the widespread use of the compound to characterize innervation by sensory neurons after neonatal treatment. Jancso (109) used capsaicin-induced sensory neuronal process degeneration in the dorsal horn of the spinal cord as a means to investigate the characteristics of glial cells. These glial cells appeared within 4 h of neonatal capsaicin treatment in laminae I and II of the dorsal horn and in white matter adjacent to lamina I. The glial cells were frequently seen to send long processes into the neuropile. Their cytoplasm contained numerous darkly staining granules. The cells were most numerous at 24 h after capsaicin and had largely disappeared by 72 h. Although cells exhibiting this appearance were not seen in control animals, occasional apparent glial cells were observed. The glial cells in the capsaicin-treated rats were apparently reacting to and participating in the destruction of the moribund unmyelinated fibers. Jancso et al. (118) observed that histochemically detectable calcium occurred in 30% of the cells of the semilunar ganglion of rats treated on day 2 of life with 50 mg of capsaicin per kg. These cells appeared to be sensory ganglion type B cells, consistent with the cell type thought to be destroyed by capsaicin. Animals treated later than postnatal day 2 had fewer calcium-positive cell bodies in the ganglia. No such stained cells could be seen in controls. Although this occurrence of intracellular calcium is characteristic of injured cells in general, it is possible that it is related to the mechanism of the neurotoxicity of capsaicin (see section XII F).

Scadding (220) quantitatively characterized the loss of neuronal fibers in the sural nerve of mice treated while neonates with capsaicin and in the saphenous nerve of rats similarly treated. In the former, capsaicin resulted in a 50% reduction, and in the latter, a 64% reduction in the number of unmyelinated fibers. In mice, there was no effect on myelinated fiber counts, a finding contrary to that reported by Nagy and coworkers (184) in a study in rat dorsal roots. Scadding was, unfortunately, not able to get an accurate picture of any possible effect on myelinated fiber numbers in rats because of fibers already being given off the nerve at the point of biopsy. Jancso and Kiraly (113) used the Fink-Heimer technique to stain and map the distribution of degenerating sensory neuron terminals in the spinal cord and brainstem after neonatal capsaicin treatment. The time course of degeneration was similar to that reported by Jancso (109) (vide supra). Degeneration was most intense in the spinal substantia gelatinosa, although there was some present elsewhere in lamina I as well. The particularly pertinent observation was made that, within the gelatinosa, no distinct pattern of distribution of degenerating terminals was observed, in contrast to what is sometimes seen after dorsal root section in the rat. In the brainstem caudal trigeminal nucleus, degeneration was confined to superficial zones and to small islands of terminals in the deeper layers of the nucleus. A small area of degenerating structures was also found in the most dorsal part of the nucleus oralis, but not in the sensory nucleus. Marked terminal degeneration was seen throughout the solitary nucleus with the exception of the rostral part. An unexpected small area of dying processes was also seen in the dorsolateral part of the area postrema. The marked de-

generation of terminals reported in the solitary nucleus is opposed to the loss of little or no SP immunoreactivity in this region after neonatal capsaicin (46, 91) and probably indicates a loss from the nucleus of more than just SP-containing primary afferents. Despite the assumptions of Jancso and Kiraly (113) that all of the degenerating terminals were from chemosensitive sensory neurons, it is also likely that more than just this functional type of neuron was being detected. Jancso and Kiraly (114) examined a series of capsaicin congeners for their ability to produce axon terminal degeneration in the spinal dorsal horn and brainstem sensory nuclei. In addition to capsaicin, N-vanillylnonanamide, N-octylhomovanillamide, N-dodecylhomovanillamide, N-cyclohexylhomovanillamide, octylhomovanillate, and nonylhomovanillate produced terminal degeneration. According to these investigators, the distribution of damaged elements differed for N-cyclohexylhomovanillamide and the homovanillate esters compared to the other neurotoxins. Degeneration produced by these three compounds was confined only to the lateral portions of laminae I and II in the dorsal horn and to the ventral nucleus gelatinosus of the caudal trigeminal nucleus with little or no effect on terminals in the solitary nucleus. A dissociation between pain-producing ability of the capsaicin congeners and their ability to produce chemogenic desensitization was suggested by the observation that the production of sensory neuron terminal degeneration was correlated with the former, but not with the latter, action of these compounds. However, a confounding aspect of these functional comparisons is that the neurotoxic actions were determined in neonatal animals, while the pain-inducing and desensitizing effects were assessed in adult animals.

Two laboratories have used capsaicin to determine the effects of removal of unmyelinated sensory neurons on the neuronal plasticity of the CNS. Wall and coworkers (255) observed that receptive fields of cells in the rat spinal cord and mouse cortex were increased after neonatal capsaicin treatment. This increase was apparently due to abnormal innervation of the cells by myelinated fibers brought about by the loss of unmyelinated neurons. Similar findings were reported for the nucleus gracilis of the rat (182). In an elegant microscopic study, Nagy and Hunt (182) also observed that larger Aδ fibers in the dorsal horn expanded their arborization pattern from lamina III into lamina II after neonatal capsaicin-induced destruction of type C fibers in laminae I and II. Most of the destroyed unmyelinated axons in lamina II were confined to the inner layer with some sparing of these axons in the outer layer. The investigators also obtained further evidence (see ref. 184) in this study of an effect of high neonatal doses of capsaicin on small-diameter myelinated fibers. These fibers were located in lamina I and the outer layer of lamina II and were presumably fine Aδ axons (182).

Nagy et al. (181) investigated the effects of capsaicin treatment of neonatal rats on SP-containing neuronal innervation of taste papillae of the tongue. This treatment reduced SP levels by 71% in the tongue. Taste buds were present and appeared structurally normal in the circumvallate and fungiform areas. Immunofluorescence staining for SP revealed a rich innervation by SP-positive fibers in both vallate and fungiform papillae, and this fluorescence was dramatically reduced by neonatal capsaicin treatment. Holje and coworkers (95) found that the number of unmyelinated axons in the inferior alveolar nerve of adult rats treated with capsaicin (50 mg/kg) on day 2 of life was reduced by 58%. A similar loss was seen in the mental nerve. Surprisingly, these investigators could find no reduction in unmyelinated fibers in the pulp of molar teeth in the treated animals. Tooth pulp contains many unmyelinated sensory fibers containing SP which are thought to be almost exclusively nociceptive in functional character. The effectiveness of the capsaicin treatment was verified by the observation that 85% of the unmyelinated axons were depleted from the fourth lumbar dorsal root. Lundblad and coworkers (155) used capsaicin treatment of neonatal and adult rats to determine that most of the SP-containing fibers of the nasal mucosa were sensory neurons (see sections II D and X I).

After treating neonatal rats with capsaicin (50 mg/kg), Chad et al. (39) concluded that the sural and sciatic nerves contain large numbers of capsaicin-sensitive sensory afferents that contain SP or somatostatin. The muscular nerves (i.e., nerve to the soleus muscle), however, contain few capsaicin-sensitive, dorsal root ganglia-derived afferents, although capsaicin-insensitive afferents might still contain SP or somatostatin. All three nerves contained similar proportions of sympathetic ganglia-derived fibers. Clearly, skeletal muscle in at least some species does contain capsaicin-sensitive afferent fibers (see section X C).

In one of few reports in cats, Feher and Vajda (61) treated newborn animals with systemic capsaicin and conducted an electron microscopic examination of damage to neuronal processes in the small intestine. These investigators observed numerous degenerating fibers in the gut including some in the myenteric plexus, submucosal plexus, and mucosa. The puzzling finding that some cell bodies in the two intestinal plexuses were severely damaged with swollen mitochondria, dilated rough endoplasmic reticulum, and aggregated ribosomes makes this the only report of possible effects of capsaicin on intrinsic neurons of the gut. Degeneration of axons that appeared to be postsynaptic was also claimed in this study. Since Feher and Vajda (61) treated their neonatal animals with the high dose of 200 mg of capsaicin per kg, it is possible that some nonspecific damage to intestinal cells occurred. Furthermore, since SP depletion was not assessed by this group, and since no characterization

of the neurotoxicity of capsaicin in cats has been conducted, the results of this investigation on the actions of capsaicin on enteric neural elements must be viewed with caution.

IV. Effects of Systemic Treatment on Ligand-Receptor Binding

In addition to the reported decrease in number of binding sites for [^{3}H]naloxone after neonatal capsaicin treatment (186), changes in binding have been observed for other labeled molecules as well. Gamse et al. (71) confirmed the loss of opioid binding sites in the dorsal horn of the spinal cord when they observed a 37% decrease in B_{max} for [^{3}H]diprenorphine binding in adult rats treated with capsaicin while neonates. There was no change in the affinity of these binding sites. Singer and Placheta (226) similarly found that this treatment reduced the number of [^{3}H]muscimol binding sites in the dorsal cord by 27% without altering the affinity of these γ-aminobutyric acid receptors. At the same time, a presynaptic localization of glycine receptors was ruled out on capsaicin-destroyed sensory terminals by the lack of a change in [^{3}H]strychnine binding.

Mayer and coworkers (164) examined the ether-chloroform extractable association of ^{125}I-Tyr^8-SP with synaptic vesicles after neonatal or adult treatment of rats with capsaicin (50 mg/kg). After both treatments, there was a 40% decrease in number of binding sites with no change in affinity in spinal cord and dorsal roots, but not in ventral roots, midbrain, or hypothalamus. Since Tyr^8-SP does not bind to neuronal or synaptic SP receptors, these investigators interpreted their results to indicate the loss of an intracellular vesicular site with affinity for Tyr^8-SP, possibly a form of SP storage and/or transport mechanism. The reduction in the number of these sites after capsaicin treatment of adult animals presumably indicates that these binding sites are destroyed, even though there may not be an actual destruction of the sensory neurons (see sections I and II, A and C).

Kirby et al. (135) found that prenatal rates of mothers injected twice with capsaicin (50 mg/kg) during pregnancy had a 25% decrease in the number of spinal [^{3}H]naloxone binding sites with no change in affinity. Acid phosphatase staining in the same animals on postnatal day 10 was reduced in the dorsal horn as well. Surprisingly, these investigators observed that, on postnatal day 24, there was no difference in the number of [^{3}H]naloxone binding sites after in utero capsaicin exposure. As pointed out before (see section III A), the significance of these results is tempered by the fact that the effects on sensory neurons of capsaicin exposure during gestation have not been well characterized.

V. Effects of Systemic Treatment on Neural Electrophysiology

When s.c. doses of capsaicin from 5 to 20 mg/kg were given to adult rats, electrical spiking activity was recorded in the medial habenula, anterior hypothalamus, dorsal raphe, and substantia nigra. This activity was seen first in the habenula, and it became more intense and occurred in all four areas at the highest capsaicin dose. Evoked acoustic responses in the same animals were increased in amplitude in a dose-related manner in the habenula and anterior hypothalamus. Portions of the electroencephalogram evoked by visual stimuli were increased by 5 mg of capsaicin per kg and subsequently depressed by the doses of 10 and 20 mg/kg. This visual effect was most pronounced in the medial habenula (209).

Salt and coworkers (213) administered capsaicin systemically to neonatal and to adult rats and determined the proportion of cells in the caudal trigeminal nucleus that responded to nociceptive and nonnociceptive mechanical stimuli and to nociceptive thermal stimuli applied to the face. In animals treated while neonates, there was no change in the proportion of nucleus caudalis neurones responding to either type of mechanical stimulus. Among the 55 cells that were observed to respond to noxious mechanical stimuli, however, there was a dramatic 80% reduction in the proportion that also responded to nociceptive heat in the capsaicin-treated animals. In rats treated with the compound as adults, there were a 50% decrease in the number of cells responding to noxious mechanical stimuli and a 28% decrease in the proportion of cells that responded to noxious heat. When SP levels were determined in the nucleus by RIA, they were reduced by 56% in the animals treated while neonates and by 58% in those treated with capsaicin as adults. The similarity in extent of SP depletion after the two treatments was confirmed by immunohistochemistry and, in view of the markedly different changes in thermal sensitivity observed, led these investigators to conclude that SP could not be the mediator of thermal nociceptive stimuli in the rat.

Wall (253) injected 50 mg of capsaicin per kg i.p. in 2-day-old rats and found that, in adulthood, these animals had a marked decrease in the primary afferent depolarization induced in neighboring myelinated afferent central terminals by orthodromic stimulation of other myelinated afferents. The expected abolishment of the C-wave in the antidromic action potential recorded from the sural nerve was confirmed after capsaicin treatment. Wall et al. (256) extended these findings by observing that a preceding A-fiber orthodromic volley in the sciatic nerve of neonatally capsaicin-treated rats was only 15% as effective as in controls in increasing the height of a subsequent sciatic antidromic A-fiber action potential. As reported by Wall and coworkers (255) in another study, there was also a marked increase in the distribution of medial lumbar dorsal horn cells responding to mechanical stimulation of the leg above the knee in the treated rats.

Administration of capsaicin i.v. in doses of 125 to 500 μg/kg in cats increased spontaneous discharge, a C-fiber

reflex response, and heat-evoked discharge recorded in ventral roots. Aδ-fibers were claimed to be more sensitive than C-fibers to the acute effects of capsaicin in this species (161). The rabbit aortic nerve contains capsaicin-sensitive C-fibers which are capable of inducing a sympathoexcitatory reflex recorded from the renal nerve (190). These fibers may be chemogenic nociceptive afferents, but since the neurotoxic effects of capsaicin have not been carefully studied in rabbits, it is impossible to say if these are SP-containing afferents.

VI. Effects of Nonsystemic administration

Some of the controversy about SP depletion being involved in capsaicin-induced thermal analgesia arose from studies in which capsaicin was administered intrathecally. Yaksh and coworkers (261) first reported the effects of such administration in 1979. They found that an intrathecal injection of 30 μg of capsaicin in adult rats depleted SP in the spinal cord and blocked responsiveness in the hot-plate and tail-flick tests 7 days later. The thermal insensitivity in the hot-plate test seemed to be correlated with the peptide depletion, since a 3-μg dose of capsaicin was much less effective in both actions. The increased latencies in both analgesia tests were apparent as early as 24 h after the injection and persisted for 5 mo after 30 μg of capsaicin. The same animals were also less sensitive to formalin injected into a hindpaw and to i.p. injection of a noxious chemical. However, they are normal in their response to tail pinch. Administration i.v. of 30 μg of capsaicin into the tail vein had no effect on thermal sensitivity or on spinal cord SP levels. Thus, these data suggested that SP depletion after intrathecal capsaicin was responsible for the altered heat sensitivity. Palermo et al. (200) confirmed that intrathecal capsaicin elevated tail-flick latencies and also reported that there was an altered morphology of C-type terminals in the substantia gelatinosa (also see ref. 110). The alteration was described as a degeneration that was "highly selective" for lamina II synaptic glomeruli (see section III D). It is not clear if this meant that there was no such effect on lamina I terminals of the gelatinosa. Also pertinent are the observations in this investigation that the damaged terminals contained substantial electron-dense material without evidence of glial cell engulfment 6 days after capsaicin injection (see refs. 113 and 186).

Aside from the lack of consistent correlation between capsaicin-induced thermal analgesia and SP depletion after systemic administration of the neurotoxin (see sections II, B and C, and III, B and C), some additional studies of intrathecal actions questioned the role of SP in thermal sensitivity. Hayes et al. (89) could find no evidence of altered reactions in the hot-plate or tail-flick tests after intrathecal doses of up to 100 μg of capsaicin. This was in spite of an increased rear paw pressure threshold at doses as low as 25 μg and that persisted for 24 h in rats. The elevation in nociceptive pressure latencies was no longer present 4 days after intrathecal capsaicin. Nagy and coworkers (180) also studied the effects of intrathecal capsaicin. One wk after injection of 60 μg of capsaicin, there was no effect on hot-plate or tail-flick latencies. Four wk after the treatment, some of the treated animals had markedly increased latencies in the analgesia tests, and some exhibited no difference from controls. When dorsal horn levels of SP, somatostatin, and glutamic acid decarboxylase activity were assessed in the group with no thermal analgesia, only the SP was reduced. In the group exhibiting thermal analgesia, SP was depleted by 77%, somatostatin by 36%, and enzyme activity by 34%. These observers speculated that only after nonspecific spinal damage by intrathecal capsaicin was thermal sensitivity reduced.

Piercey and coworkers (207) also provided evidence against a role of SP in thermal sensitivity in mice when they found that the SP antagonist, D-Pro^2,D-Phe^7,D-Trp^9-SP, did not increase hot-plate or tail-flick latencies when administered intrathecally except at doses which also produced motor deficits. The antagonist did block the biting and scratching behavior elicited by intrathecal SP and also blocked the irritation response to capsaicin solutions swabbed on the skin.

Topical application of capsaicin to the cornea also produces a response in animals suggesting irritation. This response is abolished in animals pretreated with capsaicin as adults or as neonates (see sections II, B and C, and III, B and C). Gamse et al. (74) compared this desensitization after topical capsaicin to the SP depletion produced by the same treatment. Application of several drops of a 10-mg/ml capsaicin solution to the cornea induced a 100% reduction in eye-wiping movements and a 90% decrease in corneal SP content by 4 h after the treatment. There was an interesting dissimilarity in the recovery of these changes at 5 days after treatment, when the wiping behavior had nearly returned to control values, but the corneal SP content was still reduced by 80%.

An assessment of the effects of intrathecal capsaicin on dorsal horn peptide immunofluorescence was undertaken by Micevych and coworkers (168). They observed that, after an intrathecal injection of 70 μg of capsaicin or 1-nonenoyl-vanillylamide (N-vanillyl-nonenamide), there was a disappearance of SP- and CCK-positive immunofluorescence in the substantia gelatinosa as well as in lamina III of the dorsal horn. Neither compound had any effect on dorsal horn Met-enkephalin or 5-hydroxytryptamine immunostaining. Both compounds produced an incomplete reduction in somatostatin immunofluorescence as did piperine, a chemical irritant that is the pungent ingredient in black pepper, but unrelated in structure to capsaicin. Immunoreactive visualization of all the neurochemicals was unchanged after intrathecal treatment with homovanilloyl-dodecylamide (N-dodecylhomovanillamide), -hexadecylamide, and -cyclohexylamide (N-cyclohexylhomovanillamide). Piperine at a dose of 70 μg also had the effect of depleting SP

from the gelatinosa without depleting CCK at the same time. Met-enkephalin immunofluorescence and 5-hydroxytryptamine immunofluorescence were not affected by piperine. When these investigators measured tail-flick latencies 7 days after the intrathecal treatments, these latencies were higher in the capsaicin-treated and in the 1-nonenoyl-vanillylamide-treated animals. Thus, among the irritants that depleted dorsal horn neuropeptides, only the two which depleted CCK also produced thermal insensitivity. The fact that homovanilloyl-dodecylamide produced a doubling of tail-flick latencies but had no effect on any neuropeptide in the study was not addressed by the authors. Neither was the point that homovanilloyl-cyclohexylamide, also lacking an intrathecal action on the primary afferent peptides, is a potent irritant that produces sensory neuron degeneration (114).

Three laboratories have investigated the neuropharmacological actions of capsaicin administered intraventricularly. Gamse et al. (74) injected 200 μg of capsaicin by this route and measured brain and dorsal cord levels of SP, somatostatin, and neurotensin 1 and 10 days later. SP was significantly depleted in the medulla at both time points, but only by 25 to 31%. There were no changes for either of the other two peptides or in any other region examined. Bodnar and coworkers (22) administered intraventricular capsaicin doses of 25, 50, and 100 μg and determined the effects on flinch-jump thresholds of animals placed on an electrified grid. All doses of capsaicin produced a lowering of the jump threshold only when it was measured 3 days after treatment. There was no effect at 1 or 2 days after the intraventricular injections. When morphine was assessed for analgesic effectiveness in the capsaicin-treated animals, the opiate was significantly less analgesic as the intraventricular capsaicin dose was increased in both the jump and flinch testing. When these investigators assessed SP immunoreactivity in the capsaicin-treated rats, they could find no evidence of depletion in brain or in the spinal cord. Since the neurotoxic effects of capsaicin have not been well characterized after intraventricular administration, it would have been much more informative for these experiments to have been conducted in rats treated systemically with capsaicin as adults or as neonates. The same can be said for a subsequent study in which Bodnar et al. (23) found that the same intraventricular administration of capsaicin reduced the analgesia (not in a simple dose-related manner) produced by 2-deoxy-D-glucose and by forced swimming, but not that produced by low-frequency (nonopioid analgesia) and high-frequency (opioid analgesia) inescapable foot shocks.

One of only two reports to appear in recent years showing an effect of capsaicin on neurons in the brain came from Dawbarn and coworkers (48). These investigators injected 30 μg of capsaicin bilaterally into the substantia nigra pars reticulata and found that the treated animals had increased spontaneous motor activity for up to 6 days after the injections. In addition, the capsaicin-treated rats exhibited reduced hyperactivity in response to systemic amphetamine and an attenuated cataleptic response after systemic fluphenazine compared to intranigral vehicle-treated animals. When nigral levels of 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, SP, dopamine, norepinephrine, γ-aminobutyric acid, glutamate, aspartate, and glycine were measured, the concentrations of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid were significantly reduced at times when the altered behavioral activity was observed. None of the other neurotransmitters was affected. At the same times, levels of dihydroxyphenylacetic acid and homovanillic acid were elevated in the corpus striatum and nucleus accumbens in the capsaicin-treated animals. These results suggested that the enhanced motor activity resulted from increased activity of the nigrostriatal dopaminergic pathway, although a direct action of capsaicin on a descending striatonigral system could not be ruled out. Panerai et al. (201) administered 10 μg of capsaicin twice intraventricularly in adult rats and determined levels of β-endorphin, SP, somatostatin, and Met-enkephalin by RIA in the hypothalamus and two other brain regions. Hypothalamic β-endorphin levels were reduced approximately 50% at 3, 5, and 7 days after the capsaicin injections, but not at 15 days after the injection. Levels of the endorphin were not altered at any of these times in the hindbrain or midbrain. SP, somatostatin, and Met-enkephalin levels were not altered in the hypothalamus at any of the time points. Whether these intriguing findings were due to an increased turnover or a decreased synthesis of the opioid peptide was not determined. The authors reported that no microscopic anatomical lesions of the arcuate nucleus were found in the capsaicin-treated rats, possibly ruling out nonspecific damage. Additional studies will be necessary to clarify these potentially important findings, but it is tempting to speculate that some of the central thermoregulatory effects of capsaicin are mediated by β-endorphin-containing hypothalamic neurons (section X A, 1 and 2).

Kenins (133) applied capsaicin solutions topically to the skin of rats and assessed the effects on various types of sensory fibers in the saphenous nerve. Based on conduction velocities and responses to stimuli, this investigator identified Aβ-hair mechanoreceptors, Aβ-slowly adapting type I, Aβ-slowly adapting type II, Aδ-high threshold mechanical nociceptors, C-low threshold mechanoreceptors, C-warm thermoreceptors, C-cold thermoreceptors, C-freezing thermoreceptors, and C-polymodal nociceptors. When a 1% solution of capsaicin was applied to the field of innervation, only the polymodal nociceptors responded with a discharge. All 23 of the polymodal nociceptors tested showed this response, whereas none of the other Aβ, Aδ, or C-receptors responded with discharge. Repeated topical application of

capsaicin resulted in a desensitization to the applications. Desensitized fibers were also insensitive to application of turpentine oil or hydrochloric acid. At the same time, the threshold was increased, and the evoked discharge was reduced to nociceptive mechanical and to nociceptive heat stimulation of the capsaicin-desensitized polymodal nociceptors. Kenins (133) concluded that capsaicin affects only polymodal nociceptors and most likely exerts its effects on the receptors rather than on the axons of these sensory neurons.

Local intraarterial infusion of capsaicin produced a biphasic vasodilatory response in cat nasal mucosa. The initial phase of this response was suggested to be due to SP release from sensory fibers, while the second phase could have resulted from activation by the released SP of sphenopalatine ganglion cells which released another vasodilatory substance (154).

VII. Effects of Direct Application to Neurons and Nerves

Capsaicin has also aroused considerable excitement and excitation within neurophysiology laboratories. Jancso et al. (116) provided evidence that local application of capsaicin onto a major innervating nerve could affect sensory function in peripheral tissues. They wrapped either the sciatic or the saphenous nerve for 15 min with a sponge soaked in a 1% solution of capsaicin and measured the hindpaw response latency in a hot-plate test and the extravasation of Evans blue dye in response to nerve stimulation or to a topical skin irritant. Capsaicin treatment of the sciatic nerve markedly increased the hot-plate latency for up to 33 days. Treatment of the saphenous nerve markedly reduced the dye accumulation in the paw after antidromic saphenous nerve stimulation and after topical application of mustard oil. These actions were also of long duration after the nerve treatment. At the same time, treatment of either nerve did not produce motor effects or any signs of atrophy in the innervated limb. In a later series of experiments, Jancso and Such (119) characterized the cardiovascular and respiratory effects of capsaicin applied in the same manner to the vagus nerve of the cat. They divided these actions into three phases. The first was a marked decrease in mean arterial blood pressure and heart rate, and apnea, all of which were attributed to a direct excitatory action of the compound on chemosensitive afferents in the vagus. The second phase was a generalized block of vagal afferents, since norepinephrine-induced cardiovascular effects were altered as well as those of capsaicin. This local axonal block was reversible within 1 to 2 h. The third phase was characterized by a specific blockade of the effects of capsaicin and its congeners that developed several days after treatment of the nerves. The cardiovascular and respiratory effects of systemic phenyldiguanidine, veratrine, and lobeline were still evident during this period of specific insensitivity. In addition to capsaicin, the reflex triad seen in phase I was also induced by i.v. injection of N-vanillylnonanamide, N-dodecylhomovanillamide, N-cyclohexylhomovanillamide, and zingerone. It was suggested that the specific blockade of phase III was due to an action of capsaicin on SP-containing vagal afferents, while the general blockade of phase II was due also to a short-lived effect on other afferents, including myelinated afferent fibers.

Gamse and coworkers (76) made the interesting observation that capsaicin applied to a nerve inhibits the axoplasmic transport of SP and somatostatin. When they applied a sponge soaked in capsaicin solutions of 0.1 to 10 mg/ml, there was a concentration-dependent increase in SP in the sciatic nerve proximal to the capsaicin application site. There was also an increase in the immunoreactive somatostatin level, but this was not clearly concentration dependent. The effect of SP transport was seen in sciatic nerve from rats, guinea pigs, rabbits, and cats. Equimolar concentrations of capsaicin and of colchicine produced similar SP build-up above the site of application. Capsaicin had no effect on proximal levels of norepinephrine or acetylcholinesterase, whereas nerve ligation of the sciatic produced increases in both. Several days after capsaicin, SP was also depleted in innervated skin, distal nerve, proximal nerve, and dorsal root ganglia, dorsal roots, and dorsal cord containing afferents to the sciatic nerve. The depletion lasted for at least 2 wk. Ligation of the nerve produced the same pattern of SP depletion. Application of mustard oil to the innervated skin evoked normal plasma extravasation until 24 h after capsaicin application to the sciatic nerve when the response was markedly reduced. Extravasation induced by antidromic stimulation of the nerve was reduced within a few hours after the capsaicin treatment, as was the reaction of the innervated foot to nociceptive heat. When ligation studies were carried out in rats treated systemically with capsaicin, there was a slight reduction in the accumulation of SP proximal to the ligation site in the treated animals and slight retrograde SP transport that was surprisingly increased in the capsaicin-treated animals.

Petsche et al. (206) found that the standard capsaicin solvent consisting of 10% ethanol, 10% Tween 80, and 80% saline, when applied to the rat coccygeal nerve, blocked compound action potentials from both A- and C-fibers. They used a solvent of 10% Tween 80 in paraffin oil, which was without effect on action potentials itself, to assess the actions of local capsaicin administration to the nerve. A 1% solution applied to the nerve for 30 min diminished C-fiber action potentials across the site by up to 75% within 4 min, and the block lasted for up to 100 min after removal of the capsaicin applicator. A-fiber action potentials were not affected nor were C-fiber potentials that did not have a pass through the capsaicin application site. The latencies and durations of the C-fiber action potentials across the site were

reportedly not consistently altered by capsaicin. Individual A- or C-fibers were isolated from the nerve, and the same pattern of capsaicin blockade of action potentials was observed. All of the A-fibers that were not affected by capsaicin responded to mechanical stimulation of the skin. It was mentioned that occasionally a C-fiber would be depolarized by capsaicin before being blocked by the compound. Petsche and coworkers (206) made the interesting observation that C-fibers that responded to both nociceptive heat and mechanical stimulation (MH-units or polymodal nociceptors) were invariably blocked by capsaicin when electrical stimulation was applied to the fiber or when radiant heat was applied to the skin. Unfortunately, these authors omitted the important point of whether or not mechanical stimulation was also blocked, but presumably it was. Another type of C-fiber in the nerve, unmyelinated fibers responding only to cold stimulation of the skin, was not blocked by capsaicin application to the nerve. These results are remarkably similar to what has been observed in sensory testing of the conscious adult guinea pig after systemic administration of capsaicin (28). In another report from the same neurophysiology laboratory, Welk et al. (257) observed the same loss of responsive MH units after capsaicin application to the saphenous nerve, but an increase in the number of units responding only to noxious heat (H-units). It was suggested that many of the MH-units may have been changed to exclusively H-units. This observation is in harmony with the findings that capsaicin treatment of adult rats reduces their sensitivity to nociceptive pressure (see section II, B and C), but it is difficult to reconcile with reduced sensitivity to noxious heat that these investigators (206) and others (vide supra and infra) have reported after application of capsaicin solutions to nerve trunks. In this study, Welk and coworkers (257) used the standard capsaicin solvent apparently without complications.

Wall's laboratory and their coworkers have carried out numerous investigations of the effects of neural application of capsaicin. In a study complicated somewhat by the effects of the ethanol-Tween 80-saline solvent on neural conduction, Wall and Fitzgerald (254) observed a slightly increased latency (20 ms) of some C-waves after capsaicin in the sural nerve with stimulation of lumbar dorsal roots. The mean conduction velocity was significantly reduced from 0.87 ms to 0.7 ms for C-fibers in the nerve. When C-fiber evoked responses of dorsal horn cells were measured in rats in which capsaicin had been applied to the sciatic nerve, only late responses that were much weaker than in control animals could be elicited. The latency of response to the C-fiber input was also significantly increased. No effect on A-fiber electrophysiology was seen in these experiments. In a follow-up biochemical analysis of the effects of topical capsaicin applied to the sciatic nerve, Ainsworth et al. (1) found that local application reduced fluoride-resistant acid phosphatase, SP, and CCK levels in the region of the central termination of the nerve. No evidence of intraaxonal damage or of degeneration and loss of either unmyelinated or myelinated fibers was seen in the nerve at the site of capsaicin application. However, the electron microscopy was conducted 14 days after the capsaicin application and may not have detected reversible structural effects in unmyelinated axons of the rat. Fitzgerald (62) extended the studies on dorsal horn cell responsiveness when she found that, after capsaicin application to the sciatic nerve, the number of cells in the dorsal horn excited by C-fibers and by noxious heat was reduced by 70% and 50%, respectively. A-fiber input and the proportion of cells responding to low-threshold and to high-threshold mechanical stimuli were unchanged by capsaicin. Receptive field size was increased for many cells on the ipsilateral side of the cord (see section V). The interesting observation was also made that, after capsaicin treatment of the sciatic nerve, excitatory receptive fields were seen in the contralateral dorsal horn, whereas in control preparations, these inputs were invariably inhibitory. Wall et al. (256) applied capsaicin topically to the sciatic nerve and confirmed the decrease in number of cells responding to C-fiber afferent volleys. Primary afferent depolarizations and A-fiber inhibition of A-volleys were not affected by topical capsaicin, in contrast to what was seen after neonatal treatment (see section V). Topical application increased the receptive fields of cells in the spinal dorsal horn more than in the nucleus gracilis of the adult rat (167).

Fitzgerald and Woolf (64) applied a sponge soaked in a 1.5% capsaicin solution to the sciatic nerve of adult rats for 15 min and assessed the time course of sensory response changes and dorsal horn cell sensitivity changes. One day after the capsaicin application, there were a 3-fold increase in the escape latency of the treated leg to noxious heat and no change in the response to nociceptive pressure. The thermal insensitivity was less pronounced at 4 days after treatment and plateaued at this time for an additional 12 days. Pressure sensitivity was unchanged over the entire 16-day period. The number of dorsal horn cells responding to noxious heating of the skin was reduced by 66% beginning 24 h after capsaicin treatment, whereas the number responding to C-fiber evoked peripheral volleys was not reduced maximally (66%) until day 7 after capsaicin. These reductions in dorsal horn cell responses lasted for at least 16 days. There was no difference between capsaicin-treated and vehicle-treated sides of the dorsal cord in the number of cells responding to action potentials evoked by noxious mechanical stimuli. Gibson and coworkers (79) conducted an intensive investigation of the biochemical consequences in the dorsal horn of topical capsaicin application to the ipsilateral sciatic nerve. Application of as little as 0.1 mM capsaicin (30 μg/ml) to the nerve produced a slight decrease in SP and CCK immunostaining

in the dorsal horn. A maximal concentration of 49 mM (15 mg/ml) produced a slight decrease in SP and CCK immunostaining by 1 wk after application, and these reductions were maximal by 2 wk. A reduction in somatostatin staining was also evident by 2 wk after capsaicin treatment. These changes were reported to persist for 4 to 5 mo. No change in neurotensin, Met-enkephalin, neurophysin, or bombesin immunostaining was seen at any time after the higher dose of capsaicin. Fourteen days after capsaicin, the few SP-positive fibers that could normally be seen in the innervated skin were no longer visible. No immunostaining for any of the other neuropeptides could be seen in the skin of control or capsaicin-treated legs. RIA determination of peptide levels indicated that 49 mM capsaicin topically applied to the sciatic nerve depleted dorsal horn SP by 27% and somatostatin by 48% at 2 wk after treatment. Radioimmunoassayable neurotensin and bombesin levels were unchanged, as was the level of CCK measured by RIA (see section III A). SP measured by RIA in the innervated skin was depleted by 70% at 2 wk. Levels of the other neuropeptides were too low for determination by RIA of capsaicin-induced depletion. Fourteen days after application of 49 mM capsaicin to the sciatic nerve, the response of the ipsilateral leg to noxious heat was increased 76%; at 1.5 mM, this increase was 33% at the same time point. It is unfortunate that thermal sensitivity was not assessed at earlier time points as the present data of Gibson et al. (79) provide little information as to which neuropeptide changes, if any, parallel in time the development of thermal analgesia (see ref. 64; vide supra).

Williams and Zieglgansberger (259) applied capsaicin to the bathing fluid of acute rat dorsal root ganglia preparations and found a fall in input resistance and depolarization in 70% of the C-cells and 58% of the A-cells examined. The effects in A-cells were rapidly reversible. There was also marked desensitization to the capsaicin effects in both cell types. Capsaicin incompletely blocked the passage of antidromic spikes into the soma from the dorsal roots, and γ-aminobutyric acid-induced depolarization of the soma was blocked during capsaicin-induced depolarizations. Application of capsaicin to the dorsal root entry area of the spinal cord increased the firing rate of 11 of 13 cells monitored in the dorsal horn. The increase was reversible, and desensitization occurred to repeated capsaicin applications. Glutamate still evoked responses in the capsaicin-desensitized cells. Cells that responded to noxious heating and light touch and pinch of the skin were all excited by capsaicin application to the dorsal root entry zone. During periods of capsaicin desensitization, these cells were no longer responsive to heat but continued to respond to mechanical stimulation and to glutamate. In an elegant study of sensory neurons in cell culture, Baccaglini and Hogan (8) found some cells (69% of those tested) in trigeminal ganglion cultures that responded to capsaicin pressure applications with a combination of action potentials, fast depolarizing potentials, and/or slow depolarization. Interestingly, the remainder of the cells in culture were less sensitive to capsaicin, exhibiting only small, slow depolarizing responses and weak depolarizations at the highest capsaicin concentration (10 μM) applied. In the sensitive cells, the excitation produced by capsaicin was not calcium dependent. When SP immunostaining of the cultured trigeminal ganglion cells was conducted, 40 to 45% of the cells were SP positive. Cells from rat dorsal root ganglia were also cultured, and 80% of these were responsive to as little as 0.1 μM capsaicin pressure applied to their surface. The pattern of responses was the same as in cells from the trigeminal ganglion. These investigators added that, when superior cervical ganglion cells of the rat were grown in culture, none of 20 cells surveyed responded in any way to application of capsaicin (10 μM). Salt and Hill (214) microiontophoretically applied capsaicin to neurons in the trigeminal nucleus caudalis of the rat and the cat and found that, even though few cells (13%) were excited by the compound alone, 74% exhibited potentiation by capsaicin of their responses to excitatory amino acids (glutamate and aspartate). SP excited or potentiated the amino acid-induced excitation of 89% of the neurons tested, and many of these cells were also excited by capsaicin. Application of capsaicin and SP simultaneously led to excitation of most cells, and this effect was observed equally on cells which responded to nonnoxious stimulation, to noxious and nonnoxious stimulation, or exclusively to noxious stimulation. Although SP excited some cells recorded in the cerebellum, capsaicin alone depressed or had no effect alone or in combination with SP on cerebellar neurons.

In the only recent paper to examine the ionic basis of the neuronal membrane effects of capsaicin, Dubois (56) made the interesting observation that capsaicin reversibly blocked one of the two fast components of the potassium current in the node of Ranvier of the frog sciatic nerve. This component was apparently the f_2K^+ channel with no effect on the f_1K^+ channel or on slow potassium conductance. This investigator also postulated that the affinity of capsaicin for the channel receptor is greater in the open condition than in the closed condition, since part of the f_2K^+ channels was blocked in the resting state and the remainder during depolarization. Aside from supporting the existence of two types of fast potassium channels in myelinated axons, these results provide additional evidence that capsaicin can have effects on myelinated primary afferent neuronal fibers (see refs. 182, 184, 253, 255, and 259).

Andoh et al. (4, 5) recorded from single units of the cat thalamus and observed that 93% of the cells that were excited by pinching of the skin were also activated by capsaicin and dihydrocapsaicin injected intraarterially. This activation was blocked in a naloxone-sensitive

manner by i.v. morphine. The capsaicinoids had no effect on activity of thalamic neurons that were excited by nonnoxious peripheral stimuli. Prostaglandin E_2 potentiated the excitation in 55% of the thalamic neurons that were activated by capsaicin or by bradykinin. Aspirin suppressed intraarterial bradykinin excitation of thalamic neurons, suggesting a role of prostaglandins in the bradykinin-induced activation. However, aspirin treatment had no effect on activation by intraarterial capsaicin of thalamic cells, suggesting different nociceptive mechanisms of the two algesic agents.

VIII. Effects in in Vitro Tissue Preparations

Extensive investigations have been reported of the neuropharmacological effects of capsaicin in the isolated guinea pig ileum preparation. Szolcsanyi and Bartho (239) found that capsaicin at a bath concentration of 10 ng/ml to 1 μg/ml blocked ileal contraction induced by periarterial nerve stimulation in the presence of guanethidine plus hexamethonium. This blockade followed an initial capsaicin-induced tonic contraction of the ileum and could not be overcome even by repeated washing of the tissue for several hours. Stimulation frequencies over the entire range of 2 to 50 Hz were without effect after capsaicin. Field stimulation of intestinal cholinergic postganglionic fibers in the tissue preparation produced twitch responses that were inhibited by capsaicin with a 50% inhibitory concentration (IC_{50}) of 50 μg/ml. The inhibition of twitch contractions was fully reversible with repeated washing of the tissue for 30 min. Capsaicin in bath concentrations up to 1 μg/ml had no effect on periarterial nerve stimulation-induced sympathetic (guanethidine-sensitive) inhibition of field stimulation-induced ileal twitch responses. In a vagus nerve-stomach-duodenum preparation, stimulation of the vagal preganglionic parasympathetic fibers induced duodenal contraction that was blocked by hexamethonium or mecamylamine, but not by capsaicin in a concentration of 1 μg/ml. Addition of capsaicin to the tissue bath produced a contraction of the duodenum to which a complete tachyphylaxis rapidly developed. These investigators concluded from their findings that capsaicin first excites and then blocks neurons (sensory) that traverse the mesenteric nerve and subsequently innervate enteric ganglion cells and smooth muscle cells.

Bartho and Szolcsanyi (12) investigated further the contraction of the isolated guinea pig ileum produced by capsaicin. Contractions were elicited by capsaicin concentrations ranging from 5 ng/ml to 10 μg/ml, and a dose-related tachyphylaxis was induced to further capsaicin challenges, but not to the contractile effects of nicotine. The tachyphylaxis to capsaicin did not dissipate with repeated washing over 3 to 4 h. Cold storage of the tissue to damage neurons or preaddition of tetrodotoxin to the bath markedly attenuated the contractile effects of capsaicin. The interesting observation was made that tachyphylaxis to subsequent doses of capsaicin still occurred when the initial contraction was blocked by tetrodotoxin. Perivascular mesenteric denervation blocked the contractile effects of capsaicin while bilateral vagotomy had no effect. The muscarinic antagonist, hyoscine, blocked 60 to 70% of a capsaicin-induced contraction, whereas ganglionic blockers did not antagonize contraction. Morphine, which inhibits acetylcholine release in the ileum, also reduced the magnitude of capsaicin-induced contractions. Desensitization of the ileum produced by 5-hydroxytryptamine, bradykinin, or SP was without effect on contractions produced by capsaicin. Other than those mentioned above, a series of pharmacological receptor antagonists was screened, but none was found to block the actions of capsaicin in the guinea pig ileum. Similar to the previous study (vide supra), these results suggested an action of capsaicin on extrinsic neuronal fibers to the gut which were capable of exciting cholinergic myenteric ganglion cells through nonnicotinic receptors.

Although these early experiments with capsaicin in the isolated guinea pig ileum did not provide evidence for a role of SP in the contractions induced by capsaicin, Bartho et al. (10) subsequently used a different recording scheme to show that SP desensitization and D-Pro^2,D-Trp^{7,9}-SP antagonized these contractions. Desensitization to SP reduced contractions induced both by capsaicin and by mesenteric nerve stimulation. Atropine inhibited capsaicin-induced contractions only partially, and the atropine-resistant contractions were almost totally blocked by SP desensitization or by the SP antagonist, D-Pro^2,D-Trp^{7,9}-SP. After tachyphylaxis developed to capsaicin, histamine and SP still induced contractions. An enkephalin analogue, D-Met^2,Pro^5-enkephalinamide, also reduced the atropine-resistant contractions of the ileum induced by capsaicin. Chahl (40) conducted similar experiments with capsaicin in the in vitro guinea pig ileum preparation and also observed that atropine or desensitization to SP reduced capsaicin-induced contractions. The combination of these treatments abolished the capsaicin response in the ileum. Bjorkroth (20) used the SP antagonist, D-Arg^1,D-Pro^2,D-Trp^{7,9},Leu^{11}-SP, to abolish the remaining portion of capsaicin-induced ileal contraction after atropine antagonized 70% of this contraction. The noncholinergic contraction produced by 20 Hz transmural electrical stimulation of the isolated ileum was inhibited dose dependently by the SP antagonist, but the relaxation produced by 5 Hz transmural stimulation was unaffected. The response of the preparation to 5-hydroxytryptamine was pharmacologically identical to that of capsaicin, and Chahl (41) speculated that both agents directly induce SP release which then produces acetylcholine release. 5-Hydroxytryptamine, however, was still active in a preparation in which there was tachyphylaxis to capsaicin, indicating that the two agents act via different neuronal cells or different types of release processes.

Extending their observations with capsaicin in isolated intestinal smooth muscle, Szolcsanyi and Bartho (240) found that the guinea pig taenia caeci was also innervated by a capsaicin-sensitive extrinsic mesenteric nerve component. Stimulation of the nerve in preparations taken from guanethidine-treated animals was antagonized by muscarinic blockade, but not by ganglionic blockers. Similar to the effects seen in the guinea pig ileum, capsaicin caused a contraction of the taenia caeci after which tachyphylaxis developed. The tachyphylaxis also blocked contraction produced by mesenteric nerve stimulation, but direct cholinergic neural excitation produced by field stimulation was not altered by capsaicin. The relaxation induced by stimulation of noradrenergic enteric fibers was similarly unaffected. These results were consistent with a SP innervation of the guinea pig caecum similar to that of the ileum, except that there appeared to be less of a direct action of SP on smooth muscle cells in the former.

Bartho and coworkers (11) characterized the noncholinergic contractions evoked by short trains of transmural stimuli administered to the isolated guinea pig ileum. These contractions were totally abolished by tetrodotoxin and reduced in magnitude by 90% by SP desensitization. Addition of capsaicin to the bath inhibited the effects of transmural trains of stimuli, but this inhibition was apparently nonspecific, since histamine- and nicotine-induced contractions were antagonized as well. After capsaicin was washed from the bath, electrical stimulation and the two contractile agents were again able to excite the tissue. Morphine, D-Met^5,Pro^5-enkephalinamide, and long-train transmural stimulation each inhibited the contractions produced by short-train stimulation, and each was blocked by naloxone. These investigators concluded that noncholinergic contraction of the guinea pig ileum was mediated by SP release and that this release could be inhibited by activation of opioid receptors. Tsou et al. (245) also provided evidence for a link between opioid receptors and SP in the gut when they observed that naloxone-induced contraction of the guinea pig ileum from morphine-tolerant-dependent animals could be blocked partially by muscarinic antagonists and essentially totally by the combination of these antagonists with either SP desensitization or preincubation with capsaicin. These investigators added that preliminary findings indicated that the capsaicin pretreatment alone could completely inhibit the naloxone-induced contractions in ileal strips from tolerant-dependent animals.

Holzer and Lembeck (99) made the unusual observation that rapid cooling (from 37°–27°C) of the tissue bath or rapid warming of it (from 27°–37°C) induced contraction of the guinea pig ileum. The contractions were blocked by a muscarinic antagonist and reduced in a naloxone-sensitive manner by opioid agonists. Interestingly, both cooling- and warming-induced responses were abolished after capsaicin tachyphylaxis was induced in the isolated tissues. Treatment of the tissues with indomethacin had no effect on capsaicin-induced ileal contraction or on the ability of capsaicin tachyphylaxis to block the temperature-induced contractions. Bartho and Szolcsanyi (13) found a periarterial nerve stimulation-induced contraction of rabbit isolated ileum that was nearly abolished after the induction of capsaicin tachyphylaxis. Hexamethonium blocked the residual contraction, in contrast to what was seen in the in vitro guinea pig ileum (vide supra). The capsaicin-sensitive contractions could also be antagonized by muscarinic blockade. Similar to observations in guinea pig tissues, capsaicin tachyphylaxis had no effect on nicotine-induced contraction, on cholinergic contractions induced by transmural electrical stimulation, or on adrenergic relaxation in the rabbit ileum. Capsaicin itself also produced an initial contraction of the rabbit gut which was blocked by tetrodotoxin and by muscarinic blockade. A long-lasting tachyphylaxis to this contractile effect rapidly developed.

Tsunoo and coworkers (246) have used capsaicin to implicate SP as the mediator of the noncholinergic slow excitatory postsynaptic potential (EPSP) in isolated prevertebral ganglia of the guinea pig. Intracellular recordings from neurons of the inferior mesenteric ganglion revealed that application of SP onto the cells produced an EPSP with a time course similar to that of the noncholinergic slow EPSP. SP-induced membrane resistance changes were similar to those produced by the EPSP as well. Stimulation of attached lumbar dorsal roots also produced the EPSP in cells of the inferior mesenteric ganglion. In vitro incubation of the ganglion with 6 μM capsaicin for 10 min induced a short-lived excitation of ganglionic cells followed by a gradual return to base-line membrane potential. Subsequently, the slow EPSP induced by intermesenteric nerve stimulation and by dorsal root stimulation was completely blocked, whereas the cholinergic fast EPSP component of the nerve stimulation was normal. These results were consistent with the investigators' postulate that SP contained in sensory afferents was the mediator of the noncholinergic EPSP in the guinea pig inferior mesenteric ganglion. Dun and Kiraly (59) reached the same conclusion in their electrophysiological studies of the guinea pig inferior mesenteric ganglion. They made the additional observations that the depolarizing effect of capsaicin on ganglionic cells was Ca^{2+} dependent, but tetrodotoxin resistant. At times when capsaicin superfusion had abolished the presynaptic stimulation-induced slow EPSP, ganglionic cells were still sensitive to exogenously applied SP. These investigators also made the interesting discovery that some ganglionic cells that exhibited a stimulation-induced slow EPSP did not respond with an EPSP to capsaicin application. The induced EPSP was not affected by high, desensitizing concentrations of SP, thus raising the possibility that it

was due to presynaptic release of another neurochemical mediator, perhaps another peptide.

In studies pursuing their observations that capsaicin can activate adenylate cyclase activity in homogenates of cerebral cortex, Horvath et al. (105) found that Ca^{2+} was required for the prolonged cyclase activation induced by capsaicin. The metabolically stable guanine nucleotide, guanylyl-5′-imidodiphosphate (GppNHp), produced a similar activation of adenylate cyclase that was not Ca^{2+} dependent. GppNHp potentiated cyclase activation by capsaicin in the absence, but not in the presence, of Ca^{2+}. The investigators reported that these membrane effects of capsaicin were clearly due to an action on cyclase, since no effect on phosphodiesterase activity was observed. The possibility that capsaicin was producing the release of some adenylate cyclase-stimulating neurotransmitter from the membrane fragments was briefly mentioned by Horvath and colleagues (105), but no attempt was made to pharmacologically investigate this. Capsaicin has also been reported to stimulate adenylate cyclase in the spinal cord (see section IX).

Superfusion of rat parotid gland fragments with capsaicin produced a small, transient increase in amylase secretion. This was suspected to be due to SP release from the noncholinergic, nonadrenergic innervation of the gland (69). Direct evidence for a membrane effect of capsaicin was provided by Foster and coworkers (66). They observed small decreases in membrane potential and input resistance caused by capsaicin (200 μM) and by N-vanillyl-nonanamide (200 μM) in the giant ameoba, *Chaos carolinense*. The potencies of the two capsaicinoids were, however, substantially less than that of the chemical irritant, dibenzoxazepine, in this preparation. In mammalian tissues, all three compounds are apparently equipotent in decreasing input resistance, so these investigators expressed caution about extrapolating from their findings to mammalian sensory neurons. It is interesting, nevertheless, that an action of the irritants on membrane K^+ channels was suspected (see ref. 56).

IX. Capsaicin-induced Release of Neurochemicals

Considerable evidence for a role of SP in the actions of capsaicin has come from studies of neuropeptide release (see ref. 108). Theriault et al. (243) were the first to report that capsaicin caused release of SP from slices of newborn rat spinal cord. Capsaicin in a concentration of 1 μM produced a 4-fold increase in SP concentration in the perfusion buffer, and this was completely blocked by lowering the Ca^{2+} and increasing the Mg^2 concentrations in the buffer. Capsaicin also produced a depolarization of the ventral root in the spinal cord preparation that resembled the depolarization produced by SP itself. The capsaicin-induced electrophysiological responses were Ca^{2+} dependent, whereas those produced by SP were not. Baclofen in a concentration of 2 μM reversibly reduced the SP-induced depolarizations and abolished those due to capsaicin. Gamse et al. (75) observed a similar Ca^{2+}-dependent SP release produced by 0.3 to 33 μM capsaicin in spinal cord slices from adult rats. In contrast to release of SP evoked by a high K^+ buffer, capsaicin did not release SP from slices of hypothalamus or of substantia nigra. Capsaicin-induced SP release in the cord was not affected by tetrodotoxin, suggesting that capsaicin was having a direct action on SP-containing terminals. A second successive application of capsaicin to spinal cord slices did not evoke SP release, whereas application of high K^+ after an initial capsaicin pulse was still able to evoke substantial SP release. These investigators interpreted their results to indicate either that the capsaicin tachyphylaxis was highly specific for capsaicin or that K^+ produced SP release from capsaicin-resistant SP neurons.

Akagi and coworkers (2) used isolated intact spinal cords from newborn rats to confirm that capsaicin released SP. The authenticity of the released peptide was determined by HPLC which revealed a single peak eluting identically to synthetic SP. Interestingly, capsaicin did not induce a release of γ-aminobutyric acid, glutamic acid, or glycine from the spinal cord, although high K^+ produced a Ca^{2+}-dependent release of all three amino acids and of SP. Gamse and coworkers (73) separated upper dorsal horn tissue from more ventral areas of the cord and found that superfusion with capsaicin evoked Ca^{2+}-dependent release of SP and of somatostain from the upper dorsal horn primarily. High K^+ released both peptides from the dorsal horn tissue and from the more ventral tissue. The amount of capsaicin-stimulated SP release was substantially greater than the stimulated somatostatin release. Pretreatment of rats with a systemic capsaicin dose of 125 mg/kg depleted SP in the dorsal horn by 37% but had no detectable effect on somatostatin levels (see section II A). However, capsaicin-evoked SP and somatostatin release was reduced by 80% in dorsal horn tissue from the pretreated rats. The release of the two sensory neuropeptides induced by high K^+ was not altered by the in vivo capsaicin treatment. Helke et al. (92) demonstrated that capsaicin could also produce a Ca^{2+}-dependent SP release from slices of the nucleus tractus solitarius and of the spinal trigeminal nucleus. Again, no such SP release occurred in hypothalamic tissue slices. These investigators noted that the capsaicin-induced release of SP from the solitary nucleus was evidence that capsaicin could have actions on unmyelinated SP fibers other than those mediating nociceptive stimuli, since the nucleus receives sensory input from nonnociceptive chemo- and baroreceptors, some of which contain SP. The results do indicate that these viscerosensory neurons share some aspect with nociceptive somatosensory neurons that renders them sensitive to capsaicin, in contrast to some other apparently capsaicin-resistant primary afferents (vide supra; see section XII C).

Bucsics and Lembeck (29) compared the ability of

capsaicin and three analogues to release spinal cord SP to their ability to produce irritation when applied topically onto the cornea. In slices of the spinal dorsal horn, capsaicin (0.27 μg/ml, nonanoyl-vanillylamide (0.48 μg/ml), homovanilloyl-dodecylamide (13.8 μg/ml), and homovanilloyl-octylester (18.9 μg/ml) released equivalent amounts of immunoreactive SP. In a quantitative eye-wiping test after corneal application, the ED_{50} concentrations of solutions of these agents were 19.5 μg/ml for capsaicin, 50.5 μg/ml for nonanoyl-vanillylamide, 260 μg/ml for homovanilloyl-dodecylamide, and 340 μg/ml for homovanilloyl-octylester. The investigators estimated a correlation coefficient of 0.98 between potency in releasing dorsal horn SP and potency in the wiping test. The small number of compounds tested, however, weakens the conclusion that the irritation caused by capsaicin is linked to SP release from primary afferents. Bucsics and Lembeck (29) pointed out that the order of potency of the capsaicinoids in their tests was the same as the order of effectiveness of these compounds in producing sensory neuron degeneration in neonates (see 114).

Gamse (70) examined the release of spinal cord SP by capsaicin in adult and neonatal rats and mice. Treatment of neonatal rats with a single 50-mg/kg systemic dose of capsaicin reduced capsaicin-induced SP release from slices of the dorsal horn by 93% when the animals were 4 mo old. Two wk after the same capsaicin dose was given to adult rats, the capsaicin-induced SP release was reduced by 70%. Capsaicin perfusion of spinal cord tissue from mice resulted in a Ca^{2+}-dependent SP release. Treatment of neonatal mice with capsaicin (50 mg/kg) reduced this SP release by 80 to 85% when the animals were 2 mo old. There was some inconsistency between the effects of capsaicin pretreatment on SP release and the effects on nociceptive chemical irritation. Treatment with capsaicin on days 2, 4, or 7 of life resulted in comparable reduction of capsaicin-induced spinal SP release, whereas treatment on day 2 had substantially less effect than at the other two ages on irritation produced by topical capsaicin and i.p. acetylcholine injection. Capsaicin-induced spinal SP release was decreased by 90% 2 wk after treatment of adult mice with capsaicin (50 mg/kg). Gamse (70) also reported that the pretreatment of adult mice reduced basal SP release from the dorsal cord by 56%, but perfusion of the tissue with 60 mM K^+ still evoked a substantial release of the peptide.

Perfusion of the spinal cord in vivo with capsaicin can also release SP into the cerebrospinal fluid. In anesthetized cats, perfusion of the cord with 300 mM capsaicin produced a 10-fold increase in perfusate SP levels (262). Capsaicin administered similarly had no significant effect on perfusate levels of VIP or CCK or on levels of either peptide in the dorsal horn in cats or rats (260). The intrathecal administration of capsaicin in anesthetized cats did, however, evoke a marked hypertension and pupillary dilatation, actions that were similar to those induced by stimulation of Aδ- and C-fibers in the sciatic nerve. Bombesin, another neuropeptide found in certain sensory afferents, was also not released from spinal cord tissue by concentrations of capsaicin which produced a marked SP release (175). Additional evidence for the specificity of the releasing action of capsaicin was provided by Singer and coworkers (227) who confirmed the findings of Akagi et al. (2; vide supra) that concentrations of capsaicin which release large amounts of dorsal spinal cord SP have no effect on the amount of glutamic acid released. Bergstrom et al. (16) provided similar evidence when they observed that superfusion of the rat spinal cord in vivo with capsaicin stimulated SP release, but not release of spinal 5-hydroxytryptamine. Superfusion with *p*-chloroamphetamine, on the other hand, resulted in 5-hydroxytryptamine release, but no release of SP into the spinal perfusate.

One of the more interesting aspects of capsaicin-induced SP release in the spinal cord is the report by Northam and Jones (189) that capsaicin stimulates cyclic adenosine monophosphate (cAMP) accumulation in slices of rodent cord. This stimulation occurred within 4 min and lasted for at least 30 min. The capsaicin 50% effective concentration (EC_{50}) was 0.5 μM rat tissue and 0.05 μM in guinea pig spinal slices, and the maximum stimulation was twice as high in the guinea pig as in the rat. The capsaicin effect on cAMP accumulation did not occur in ventral cord tissue from either species. That the stimulation resulted from an action on adenylate cyclase was indicated by the finding that the capsaicin-stimulated nucleotide accumulation also occurred in the presence of phosphodiesterase inhibitors. The stimulation by capsaicin was totally abolished in Ca^{2+}-free medium, but it was not affected by β- or α-adrenergic blockade or by inactivation of adenosine via the inclusion of adenosine deaminase. Another intriguing aspect of this work is the finding that SP, which has not consistently been observed to stimulate adenylate cyclase in CNS tissue (see ref. 122), also stimulated cAMP accumulation in a dose-dependent manner in rat dorsal cord slices. This action of SP was not seen in tissue from the ventral cord. Interestingly, the maximum stimulation of nucleotide accumulation by SP was identical to the capsaicin maximum. In addition, capsaicin-induced cAMP accumulation was additive with accumulation induced by norepinephrine, adenosine, or 5-hydroxytryptamine. The stimulation by capsaicin was also additive with that produced by high K^+ (50 mM), a finding that could indicate that capsaicin is releasing some neurochemical mediator from other than a functionally releasable pool (vide supra). Northam and Jones (189) speculated that SP was the mediator of capsaicin-induced cAMP accumulation in their preparation. Two important aspects of this hypothesis were not reported: specifically, whether or not the capsaicin effect (and the SP effect) was blocked by SP

antagonists; and the mandatory lack of additivity of the capsaicin stimulation with that of SP. It would be of interest to know what prior dorsal rhizotomy or in vivo capsaicin pretreatment would do to the capsaicin-induced nucleotide accumulation in spinal cord slices.

Juan and coworkers (126) used the "pain-reflex ear" preparation to examine prostaglandin E (PGE) release induced by capsaicin. In the perfused rabbit ear connected to the body only by the great auricular nerve, intraarterial injection of microgram amounts of capsaicin produced reflex decreases in carotid arterial blood pressure. For short periods after these injections, there was tachyphylaxis to the hypotensive actions of capsaicin, bradykinin, and acetylcholine injected into the ear. At the same time, there was no effect on the reflex fall in systemic blood pressure induced by squeezing the ear. Continuous infusion of the ear artery with a low concentration of capsaicin (1 μg/ml) potentiated the hypotensive effect of bradykinin injections and slightly reduced the hypotensive effect of acetylcholine injections. Infusion of capsaicin (10 μg/ml) abolished the hypotensive effects of both bradykinin and acetylcholine, although it was not clear if this was due only to a sustained hypotension induced by capsaicin itself. Both infused concentrations of capsaicin elevated the levels of PGE in the perfusate in rabbit ears completely isolated from the body. The PGE release was markedly inhibited in Ca^{2+}-free medium. These investigators concluded that the stimulation of PGE synthesis by capsaicin occurred in extraneuronal tissue, since the stimulated PGE release was identical in freshly isolated ears and ears which had been denervated 10 days prior to isolation. The possibility that SP release might be involved in the reflex hypotension induced by capsaicin in the perfused neurally innervated ear was not addressed nor were the effects of prostaglandin synthetase inhibition in either rabbit ear preparation.

In their studies of neurotransmission in sympathetic ganglia of the guinea pig, Konishi et al. (136) found that capsaicin releases SP in the inferior mesenteric ganglion. Perfusion of the tissue with 1 μM capsaicin evoked a Ca^{2+}-dependent, 5-fold increase in perfusate concentration of SP. At the same time, perfusate levels of VIP, which were similar to those of SP under basal conditions, were not affected by capsaicin. Capsaicin also produced a depolarization of ganglionic neurons that was not blocked by the combination of hexamethonium and atropine, but which was abolished in Ca^{2+}-free medium. After the capsaicin-induced depolarization had subsided, a long-lasting depression of the nerve stimulation-induced slow EPSP was observed even after the capsaicin had been removed from the medium. These results suggested that SP was the mediator of the slow EPSP in guinea pig inferior mesenteric ganglia and that capsaicin released SP in this tissue to produce depolarization (see section VIII; 246). A possible difference in the SP innervation of the guinea pig sympathetic ganglion compared to other SP-innervated tissues was suggested by the fact that nerve stimulation after capsaicin treatment was apparently unable to release very much SP. In investigations using other preparations, electrical stimulation or depolarization with high K^+ is still able to induce substantial SP release (vide supra; see section X H). Perfusion of guinea pig ureter slices with capsaicin also induced SP release after which depolarization with high K^+ was no longer effective in releasing the peptide (216; see section X I). Capsaicin at concentrations greater than 10 nM or K^+ produced Ca^{2+}-dependent SP release in bovine pia arachnoid that was presumably from trigeminal afferent neurons (177).

Surprisingly, perfusion with SP, but not with capsaicin, induced histamine release in the perfused rat hindquarter (228). Perfusion with capsaicin, but not with SP, released 5-hydroxytryptamine as well. Since dose-response studies were not carried out, these data are difficult to interpret. Neonatal treatment with capsaicin appeared to inhibit the SP-induced histamine release in adulthood, while the capsaicin-induced 5-hydroxytryptamine release was actually enhanced. These latter observations and the fact that adulthood histamine and 5-hydroxytryptamine levels increase in the skin of rats treated neonatally with capsaicin (100) presumably indicate that mast cells do not degenerate after neonatal deafferentation of SP-containing neurons. An alteration in the mast cell responsiveness to SP after neonatal deafferentation cannot be ruled out. More rigorous pharmacological evaluation of the effects of capsaicin and SP on histamine release is needed (see section X I).

X. Other Pharmacological Effects of Capsaicin

A. Thermoregulation

1. Treatment of adult animals. The physiology laboratory of the Obals in Hungary has been the site of intensive investigation of the thermoregulatory effects of capsaicin. Obal et al. (192) desensitized adult rats with a cumulative dose of 250 mg of capsaicin per kg and observed their responses compared to controls at high ambient temperatures. Exposure to 34°–36°C for 6 to 12 h resulted in a decrease in the ratio of salivary gland to body weight in the capsaicin-treated animals. No such change was seen at 32°C. Desensitized animals also showed reduced grooming activity when kept at 36°C for 30 min. Water intake was reduced as well in the desensitized animals kept at 34°C or 36°C for prolonged periods of time. In control animals, but not in capsaicin-treated rats, surgical removal of the submaxillary-sublingual and parotid glands prior to exposure to these temperatures reduced water intake during the exposure. Desensitized rats exposed to ambient temperatures of 32°, 34°, or 36°C exhibited higher body temperatures than control rats, and surgical desalivation had no effect on this difference. In a modified hot-plate test in which

animals had to step up onto a platform above a heated floor, the capsaicin-treated rats did not escape onto the platform as fast as controls at floor temperatures of 42°–46°C. At 48°C and 50°C, however, there was no difference between the groups in escape latency. Surprisingly, in light of these observations, when a standard hindpaw lick test was administered to the rats, no differences between control and desensitized animals was seen at hot-plate temperatures of 48°–58°C. These investigators concluded that some mechanism other than salivation, possibly vasodilatation, was impaired in the capsaicin-treated rats. In addition, even though evidence of impaired heat perception was seen, Obal and coworkers (192) raised the possibility that some capsaicin-insensitive heat receptors exist in the rat. An altered tail skin vasodilatation response to heat in desensitized rats was confirmed in a subsequent study (193). When placed in a warm environment, the capsaicin-treated rats did not exhibit tail skin vasodilatation as fast as controls. Control animals with tails amputated developed hyperthermia at 32°C that approached that in the desensitized rats, although the nondesensitized animals were still more tolerant to prolonged heat and higher heat exposure than treated animals. The capsaicin-treated rats also failed to use a water bath, in contrast to controls, when one was present to protect them from the high ambient temperature. Obal and coworkers (191) continued to investigate the capsaicin-altered tail skin vasodilatation and found that the alteration was different at different ambient temperatures. Under 39°C, the tail skin vasodilatation response was reduced in magnitude in capsaicin-treated rats, while above 39°C the response was normal in magnitude, but delayed in appearance. These same investigators assessed the effects of high and low doses of capsaicin on the thermoregulatory actions of preoptic heating after adult or neonatal drug administration (195; see section X A 2). Rats treated as adults with systemic doses of capsaicin of 50 or 300 mg/kg exhibited the same exaggerated hyperthermia during a 1-h exposure to 38°C, but only the latter treatment attenuated the rise in tail temperature and decline in rectal temperature produced by a 3°C elevation of the temperature of a probe in the anterior hypothalamus-preoptic area. These investigators suggested that low doses of capsaicin in adult rats produced deficiencies in some type of extrahypothalamic or peripheral thermoregulatory structure, while high doses of the compound also affected hypothalamic thermoreceptors. These results raise the possibility that high doses of capsaicin may have effects in other parts of the CNS than the areas of primary afferent neuron input (see section XII A).

Benedek et al. (14) observed that raising the ambient temperature to 32°C increased the time spent in sleep in capsaicin-desensitized animals, but decreased the amount of deep slow-wave sleep in control animals. At 34°C, the sleep-waking stages returned to room temperature levels in the desensitized animals, whereas a greater decrease in sleep and increased wakefulness were seen in the control animals. These investigators postulated that warm environments produced a capsaicin-sensitive behavioral activation and a capsaicin-insensitive behavioral deactivation in the rats. In a more detailed study of the effects of temperature on the sleep-wake cycle in rats, Obal et al. (196) observed that pretreatment with capsaicin abolished the decrease in amount of time spent awake and the increase in non-rapid eye movement (REM) sleep produced by an ambient environment of 29°C. Both treated and control groups of animals had an increased slow-wave sleep fraction of non-REM sleep, and both exhibited slight increases in amount of REM sleep at 29°C.

In an interesting test of heat discrimination learning in rats, Obal and coworkers (194) used a T-maze with one goal arm maintained at 34°C and another at 45°C containing access to a water bottle to obtain additional evidence for an impaired thermal sensitivity. All rats were water deprived for a period prior to testing. Control rats learned to discriminate between the two temperatures and to find the water substantially faster and with fewer trials than rats that had been treated 2 mo earlier with a cumulative dose of capsaicin (300 mg/kg). The running times of the capsaicin-treated animals to the water once they were in the correct arm were also increased compared to controls. When a lighted bulb was used as the cue for the water-containing arm of the maze, there was no difference between controls and capsaicin-treated rats in ability to learn the task or in running time in the arm. The investigators concluded that, although there was some impairment, capsaicin-treated rats were still able to use some capsaicin-insensitive cue to eventually learn to distinguish between the two temperatures in the T-maze. Unfortunately, in this investigation other doses of capsaicin and rats treated as neonates were not tested in the temperature discrimination paradigm.

Szikszay and coworkers (236) did carry out an investigation of the dose-response characteristics of several of the thermoregulatory effects of capsaicin in adult rats. Single injections of capsaicin (1 to 50 mg/kg) produced a hypothermic response that lasted for up to 5 h. The response was greatest at 5 to 10 mg/kg and was reduced in magnitude and duration at lower and at higher doses. Six to 12 h after dosing, there was a 1°–2°C hyperthermic response that increased with dose over the entire 1- to 50-mg/kg range. At 24 to 32 h after dosing, the hyperthermia was still evident in animals treated with 10 to 50 mg/kg, but the response had subsided in animals treated with lower doses. When a test hypothermic dose of capsaicin (2 mg/kg) was used, rats treated with 1 to 2 mg/kg 2 wk earlier showed no desensitization, rats treated with 5 to 20 mg/kg exhibited a slight to moderate desensitization, and rats treated with 30 or 50 mg/kg

showed almost no hypothermic response to the test dose. When animals were treated with capsaicin, 30 mg/kg, 50 mg/kg, 10 × 5 mg/kg, 5 × 10 mg/kg, or 20 + 30 mg/kg, all treatment groups were less able to thermoregulate at 34°C and eventually reached rectal body temperatures of 40°C at 1.5 to 4.5 h after being placed in the heat. The 10 × 5-mg/kg group was somewhat better able to tolerate the heat than the other treatment groups, as evidenced by the extreme value of 4.5 h to reach the 40°C rectal temperature. The single s.c. dose mortality rate ranged from 26% at 5 mg/kg to 69% at 50 mg/kg in animals that had been subjected to no other manipulations than the capsaicin injection itself. Animals receiving capsaicin (20 to 50 mg/kg) sometimes died as long as 32 h after dosing, at times when the basal rectal temperature was substantially elevated. Based on the similar dose-response characteristics, these investigators concluded that there was a correlation between the hyperthermic and desensitizing actions of capsaicin.

The thermoregulatory effects of morphine were also observed to be altered in rats pretreated with capsaicin. Szikszay et al. (233) found that, in freely moving rats pretreated with capsaicin (300 mg/kg), the hyperthermic response to s.c. injection of 8 mg of morphine sulfate per kg was delayed in appearance, but identical to magnitude to that in controls. When rats were restrained, the morphine injection produced a fall in body temperature that was greater in the capsaicin-pretreated animals. The effects of other doses of capsaicin or of other morphine treatments were not investigated. Unlike morphine, the opioid peptide D-Met^2,Pro^5-enkephalinamide produced only hyperthermia in freely moving rats and in restrained rats. In the latter, pretreatment with capsaicin resulted in the peptide producing an exaggerated hyperthermic response (235). These results clearly point out the complexity of the thermoregulatory effects of opioids and of their alteration by capsaicin.

Cormareche-Leydier (44) treated adult rats with capsaicin (75 mg/kg) and determined the effects on body temperature in a warm environment and on food intake and body weight at various ambient temperatures. As expected, capsaicin-treated animals were unable to thermoregulate, but when a water bottle was provided, they were able to regulate their body temperature at 34°C and at 36°C as well as control animals. This is contrary to findings by Obal et al. (193; vide supra) and may have been a reflection of the lower dose of capsaicin used in this study. Food intake and body weight gain were reduced at room temperature for a few days following capsaicin administration, but both soon rebounded to surpass for a time and then equalize with food intake and weight gain of controls. These effects of capsaicin were no doubt due to the condition of apparent sickness that is evident in rats for a few days after treatment with doses of capsaicin above 25 to 50 mg/kg. The surprising finding was made that capsaicin-desensitized rats ate significantly less and lost more body weight than controls at 0°C and 5°C, were comparable to controls during exposure to 20°C, and ate more and lost less body weight than controls at 34°C. These results suggested more than just a CNS effect of capsaicin on warm detectors (see section X J). Of relevance to these findings are the studies of Hori (101) who recorded from single units in the anterior hypothalamus-preoptic area in adult rats that had been pretreated with a cumulative dose of capsaicin (280 mg/kg). This investigator found that the number of units responding to hypothalamic heating was reduced by 50% as was the number responding to hypothalamic cooling in the capsaicin-treated rats.

Using a similar dose of capsaicin, Benedek and coworkers (15) also found evidence that thermoregulation to cold was impaired in addition to thermoregulation to heat in treated animals. They suggested that very early reports of capsaicin having no effect on cold sensation (see ref. 120) could have resulted from failure to control for stress-induced hyperthermia which is exaggerated in capsaicin-treated rats (233). Although the thermoregulatory effects of capsaicin have not been as intensively studied in guinea pigs, the apparent lack of alteration of cold sensation in this species suggests a difference from rats (see section II C).

Rabe and coworkers (209) reported that a single s.c. dose of capsaicin (5 mg/kg) produced a marked 4°C hypothermia in rats and that this dose repeated 4.5 h later produced a substantially attenuated response. Miller et al. (169) found that dihydrocapsaicin also induced a hypothermia in adult rats after systemic administration. There was some indication that the magnitude of the thermoregulatory effects produced by dihydrocapsaicin was greater than those produced by capsaicin at maximal doses of 5 to 10 mg/kg. Desensitization to these hypothermic effects of capsaicin developed, and there was cross-desensitization to capsaicin. The capsaicin congener produced only slight increases in tail-flick latency, but abolished sensitivity to corneal irritation and markedly depleted sensory neuron SP (see section II C). Dib (50) made the interesting observations that 23 μg of capsaicin injected into a lateral ventricle decreased rectal temperature, increased tail skin temperature, and increased bar-pressing behavior to turn on a cooling fan. The hypothermic effects were most marked at 20°C and declined in magnitude progressively at 30°C and 35°C. These decreases in responsiveness were due in part to the different basal temperatures at these different ambient temperatures. When hypothalamic temperature was monitored at 20°C, the intraventricular injection of capsaicin produced a fall in hypothalamic temperature that paralleled in time the fall in rectal temperature and rise in tail skin temperature. At 30°C, no such change in hypothalamic temperature occurred, although the skin temperature change was still present. Dib (50) concluded that, rather than raise hypothalamic temperature, intra-

ventricular capsaicin excited central neurons that in turn activated behavioral and autonomic heat-loss mechanisms.

Donnerer and Lembeck (54) found that i.v. injection of 15 μg of capsaicin in rats produced a substantial rise in tail skin and paw pad temperature and a small decline in rectal temperature that were abolished in rats that had been given injections of 50 mg/kg of the compound on day 2 of life (see section III, A to C). Pretreatment of rats with atropine or propranolol had no effect on the capsaicin-induced temperature changes, but guanethidine or phenoxybenzamine pretreatment prevented the changes. Local application of capsaicin to the saphenous and sciatic nerves of a hind leg did not block the paw pad temperature respone to i.v. capsaicin, but section of both nerves prevented the response in the paw pad of the same leg. These investigators speculated that the rapid temperature changes produced by i.v. capsaicin were due to stimulation of peripheral heat receptors. These receptors may have been present in all parts of the body, and their widespread activation by i.v. capsaicin may have produced a reflex withdrawal of sympathetic vasoconstrictor tone. The results of this investigation suggest the interesting possibility that there is some similarity between peripheral, capsaicin-sensitive thermoregulatory sensors and pain-sensitive primary afferents that are also stimulated by capsaicin (see section XII, C and F).

Szikszay and coworkers (234) observed that adult rats made morphine tolerant developed less hyperthermia than nontolerant rats when placed in environmental temperatures fo 31°, 35°, or 38°C. Pretreatment of the rats with a cumulative dose of capsaicin (300 mg/kg) resulted in the expected increased hyperthermia at 31°C and at 35°C that was not affected substantially by the induction of morphine tolerance. At 38°C, however, the induction of morphine tolerance reduced the hyperthermia induced by the capsaicin pretreatment. When naloxone-induced withdrawal wet dog shakes were precipitated in morphine-tolerant rats, the intensity of the behavior was increased if the animals were treated with capsaicin before the induction of morphine tolerance. These investigators reported that repeated treatment with capsaicin induced the shaking behavior after several days in adult rats. This observation is surprising in view of the desensitization to subsequent doses that would be expected from the initial doses used. The results of this investigation suggest a possible role of the opioid system in some of the actions of capsaicin (see sections III A and VI).

Treatment with capsaicin s.c. also disrupted thermoregulatory behavior in birds. However, the birds apparently did not become insensitive to the topical irritation produced by the compound itself. Some did not exhibit the taste aversion for capsaicin that rats show and actually developed a preference behavior for capsaicin-containing water solutions compared to vehicle-containing solutions (160; see ref. 209a).

2. *Treatment of neonatal animals.* Direct evidence that peripherally administered capsaicin can activate anterior hypothalamic preoptic neurons was obtained in neonatal rats by Hori and Shinohara (102). They found that neurons which responded to heating of the hypothalamus with increased firing also responded to systemic capsaicin injection with marked increases in firing rate. Neurons which were unresponsive to the hypothalamic heating were also unaffected by the capsaicin treatment. A tachyphylaxis was observed to subsequent capsaicin injections in neurons that initially responded to the compound. Similar results were reported by these investigators in adult rats (187).

Hori and Tsuzuki (103) examined in detail the thermoregulatory effects of capsaicin administered to neonatal rats. Intrahypothalamic or s.c. injection of the compound produced a prompt fall in rectal temperature by as much as 4°C. In the hypothalamus, this hypothermic effect occurred only after injections into the anterior and preoptic areas. A tachyphylaxis developed to the effects of both centrally and peripherally administered capsaicin. Adult rats that had been given injections of high peripheral doses of capsaicin as neonates were unable to thermoregulate when placed in an ambient temperature of 41°C. Salivation was reportedly absent under these conditions in the capsaicin-desensitized rats, but it was not clear whether this was due to a defect on the afferent or the efferent side of the thermoregulatory loop. The insensitivity of salivary gland SP to capsaicin suggests the former (see section III A). The capsaicin-treated rats were able to adequately thermoregulate when placed in an ambient temperature of 10°C. These animals also exhibited marked deficiencies in learning skin-cooling operant behavior when placed under various degrees of heat stress. In a skin-warming paradigm during cold exposure, the desensitized rats performed as well as controls in learning the required bar-pressing behavior. Rats treated with capsaicin (463 mg/kg) as neonates were totally insensitive to the thermoregulatory effects of capsaicin administration during adulthood. These investigators concluded that, similar to the situation in adult rats (see section X A 1), treatment of neonatal rats with capsaicin results in an initial stimulation of hypothalamic and peripheral warm receptors that is succeeded by a life-long insensitivity of these receptors to capsaicin and to heat. It was also suggested that the occasional effects of capsaicin on cold-sensitive neurons that had been reported (see section X A 1) could be explained by hypothesizing that these cells were interneurons inhibited by warm-sensitive cells.

Hajos and coworkers (83) treated neonatal rats with capsaicin (50 mg/kg) and found that they responded with normal thermoregulatory behavior to capsaicin injected directly into the preoptic area. Adult rats treated system-

ically with as little as 20 mg of capsaicin per kg, however, were unresponsive to preoptic capsaicin. Both adults and animals treated as neonates still responded with reduced colonic hypothermia to s.c. capsaicin. The failure to observe an effect on preoptic capsaicin responses in rats treated as neonates suggests that adults were more sensitive than young animals to the hypothalamic desensitizing actions of the compound. This seems unlikely, and the difference could have been due to systemic injection problems in the neonates (see section XII C). This investigation, like many others, suffered from the lack of dose-response and time-course considerations for capsaicin.

Obal and coworkers (195) found that systemic treatment of rats with capsaicin (50 mg/kg) on day 2 of life had no effect on the body and tail skin temperature responses of these animals when they were subjected to intrahypothalamic heating as adults. In view of the findings of others with capsaicin treatment of neonates (vide supra) and of the marked effect of this capsaicin treatment on peptide-containing primary afferent neurons (see section III, A to C), this suggests that the hypothalamic capsaicin-sensitive neurons are less sensitive to the compound than peripheral sensory neurons. A similar differential sensitivity was suggested by the experiments of Dib (51). He treated rats on days 2 to 4 of life with a cumulative capsaicin dose of 735 mg/kg and assessed their responsiveness as adults to central and peripheral capsaicin administration and to hypothalamic heating. In comparison to rats treated as neonates with vehicle, the capsaicin-treated rats did not exhibit the fall in rectal temperature or the rise in tail skin temperature elicited by s.c. or i.p. capsaicin injection at an ambient temperature of 20°C. However, when 25 μg of capsaicin were injected intracerebroventricularly, the animals treated neonatally with capsaicin exhibited the same 1½°C fall in rectal temperature and the same 2°C rise in tail skin temperature as controls. Hypothalamic heating to 41°–42°C also evoked the expected drop in rectal and rise in skin temperature in capsaicin-treated rats, but since control data were not included it is impossible to compare these animals to vehicle-treated controls. Dib (51) observed that the capsaicin-treated rats had identical bar pressing behavior for cooling as controls when the hypothalamus was heated in ambient temperatures of 20°, 30°, 35°, and 40°C. This finding is contrary to that of Hori and Tsuzuki (103) (vide supra) who reported defective learning of operant behavior for skin cooling in rats treated neonatally with capsaicin. It is not clear if these differences in results with respect to capsaicin-induced behavioral changes were due to methodological differences, for example, the range of doses of capsaicin from 50 mg/kg to 950 mg/kg that have been used by experimenters in these reports to treat neonatal rats. It should be borne in mind that treatment of neonatal rats with 750 mg of capsaicin per kg has recently been reported to result in a lifelong depletion of β-endorphin in the hypothalamus (201; see section III A). Dib (51) concluded that neonatal treatment with capsaicin results in defective heat loss responses induced by peripheral stimuli, but has no effect on those induced by central mechanisms.

B. Anesthesia and Pyrexia

Jancso-Gabor (121) reported that i.p. doses ranging from 100 to 400 mg/kg of the chemical irritant, zingerone, in rats produced an anesthesia-like condition consisting of rapid ataxia, loss of the righting reflex, and insensitivity to external stimuli. The compound also produced a 3–4°C decrease in rectal temperature. The anesthetic and hypothermic effects of zingerone were dose dependent. Pretreatment of rats with high doses of capsaicin days or months before markedly attenuated these biological effects of zingerone. Ketone congeners of zingerone produced a similar anesthesia that was also blocked by capsaicin desensitization. This investigator also observed that i.p. administration of capsaicin (1.5 mg/kg) 5 min before hexobarbital (70 mg/kg) produced a doubling of the hexobarbital sleep time. The same dose of capsaicin given immediately after rats recovered from a 40-mg/kg dose of hexobarbital resulted in a 3-fold increase in sleeping time, but it is not clear if this was a case of hexobarbital potentiating capsaicin or vice versa. A 50-mg/kg dose of zingerone, which had no anesthetic effect itself, similarly increased sleeping time after animals had awakened from hexobarbital-induced sleep. Since zingerone-like ketones and capsaicin stimulate hypothalamic warm receptors (see section X, A 1 and 2), Jancso-Gabor (121) speculated that the anesthetic-like effect of these agents might be through hypothalamic neurons to inhibit the mesencephalic reticular formation. Both agents also obviously stimulate peripheral sensory neurons and can produce severe respiratory distress in rodents. Although respiratory effects were not mentioned as being present in this study, it is assumed that the signs considered as anesthesia in the study did not result simply from severe respiratory depression. Pretreatment of rats with low doses of capsaicin can prolong pentobarbital sleeping time via inhibition of hepatic microsomal metabolizing enzymes (170), so it appears that capsaicin-like agents can alter CNS arousal by direct and indirect mechanisms.

Szekely and Szolcsanyi (232) administered *Escherichia coli* endotoxin (10 μg/kg) to rats i.v. and found that the febrile response was greater in capsaicin-desensitized animals. The biphasic nature of the temperature response time course was similar in desensitized and control rats, but the capsaicin-treated animals exhibited an exaggerated response regardless of the initial ambient temperature. It was observed that, whereas the febrile response in controls was due primarily to a decrease in heat loss, in capsaicin-treated rats the response even at higher ambient temperatures was accompanied by increased oxygen consumption and brown fat thermogen-

esis. These investigators concluded that impairment of warm receptors by capsaicin permitted the abnormal participation of certain thermogenic mechanisms in the response to endotoxin.

C. Muscle Reflexes

Capsaicin-sensitive sensory nerve endings exist in skeletal muscle. Crayton and coworkers (45) used a neurally intact, donor-perfused dog hindlimb preparation to observe the effects of capsaicin on cardiovascular and respiratory function in the recipient animal. Injection of 1 to 10 μg of capsaicin per kg into the hindlimb resulted in a reflex increase in mean aortic pressure, heart rate, cardiac output, and respiratory minute volume. Renal blood flow decreased, but flow in other regions was not affected. Cutaneous sensory fibers were ruled out as the source of the reflex responses when skinning of the limb preparation failed to abolish the responses. Deafferentation of the limb abolished these responses to intraarterial capsaicin. The pattern of cardiovascular changes in response to capsaicin was similar to that seen during induced isometric exercise of the hindlimb. It was concluded that small myelinated or unmyelinated sensory fibers in the hindlimb muscle mediated the actions of capsaicin, but the possibility that these fibers were located on the vasculature was not eliminated. In a subsequent study of sensory fibers with endings in either the gastrocnemius or the gracilis muscle, Kaufman et al. (129) observed that capsaicin activated 24 of 34 C-fibers and 5 of 19 Aδ-fibers. There was no effect on thick, myelinated fibers innervating the muscles. The majority of the C-fibers that were identified responded only to noxious mechanical manipulation of the muscle, while most of the A-neurons responded to gentle stroking of the tissue. Repeated intraarterial injection of capsaicin at 7-min intervals did not produce tachyphylaxis in C-fibers, although a wide range of doses was not used. Similar to the previous investigation (vide supra), a 20-mm Hg increase in mean arterial blood pressure was seen within a few seconds of the capsaicin injection. The responses evoked by capsaicin differed from those caused by bradykinin which activated 17 of 33 C-fibers and 9 of 19 Aδ-fibers and resulted in a decrease in mean arterial blood pressure.

In the dog hindlimb preparation, intraarterial injection of 2.5 to 10 μg of capsaicin per kg also resulted in reflex relaxation of the trachea (131). This response, as well as the concomitant cardiovascular changes (vide supra), was reported to be resistant to the development of tachyphylaxis, but it was not clear that this was the case after very high doses of capsaicin. The tracheal and cardiovascular effects were seen with injection of capsaicin into the femoral or gracilis arteries, but not with injection into the femoral vein. Section of the sciatic, femoral, and gracilis nerves markedly attenuated the responses to capsaicin. In two dogs, administration of phentolamine and propranolol attenuated the pressor effect of intraarterial capsaicin, but had no effect on capsaicin-induced reflex tracheal relaxation. Intraarterial injection of bradykinin in the same series of experiments also produced tracheal relaxation although less consistently, of lower magnitude, and of slower onset than that produced by capsaicin. These investigators concluded that the three studies from their laboratory indicated that sensory fibers in skeletal muscle receptive to algesic substances and/or to mechanical stimulation were activated during static exercise resulting in the reflex physiological responses seen during exercise. In cats, capsaicin and bradykinin appeared to stimulate only group IV afferents which were not the same as those stimulated by static contraction. It was suggested that these afferents could be metabolic receptors that responded to products which accumulate when blood flow is unable to keep up with the demand of vigorously contracting skeletal muscle (130). This same metabolic signal could be responsible for pain sensation from the skeletal muscle.

Reflex activation of the rat masseter muscle by application of K^+ to the periodontal surface was abolished by systemic or local periodontal application of capsaicin. This presumably resulted from inactivation of chemogenic pain-sensitive afferents which are thought to be activated by K^+ and other algesic substances. On the other hand, activation of mechanoreceptors by veratrine produced the muscle reflex in a capsaicin-resistant manner (231). Although peptide levels in the oral tissue were not determined, the effect of capsaicin may have been due to an action on SP-containing sensory neurons. The results are also consistent with capsaicin having little or no effect on afferents mediating pressure sensation (see sections II B and III B).

D. Visceral Reflexes

Cervero and McRitchie (38) provided evidence that treatment of neonatal rats with capsaicin reduces the function of visceral reflexes without altering the function of autonomic efferent neurons. When the treated animals were 4 mo old, the majority were completely insensitive to the irritation produced by i.p. injection of hypertonic saline. The capsaicin-treated animals also exhibited a 40% reduction in amplitude and a similar reduction in the duration of the inhibitory gastric motility reflex elicited by i.p. injection of bradykinin, an activator of several classes of visceral nociceptive receptors. The interesting observation was also made that the inhibitory gastric motility reflex produced by infusion of 0.1 N HCl into the jejunum was reduced both in magnitude and in duration by 60% in adult rats treated neonatally with 50 mg of capsaicin per kg. In the same animals, electrical stimulation of the vagus and of postganglionic branches of the splanchnic nerve produced gastric contraction and inhibition of gastric motility, respectively, that were unchanged compared to control animals. Lembeck and Skofitsch (143) observed an effect of neonatal treatment

with capsaicin on the reflex cardiovascular changes induced in rats by distension of the jejunum. Normal and solvent-treated animals responded to the distension with a triphasic change in blood pressure: (*a*) a small, short-lived increase followed by, (*b*) a longer lasting fall in pressure followed by, (*c*) a further, transient drop in blood pressure upon release of intraluminal pressure. Capsaicin-treated rats, which had a lower basal blood pressure and a lower heart rate than controls (see section X G), exhibited only the initial hypertensive phase of the reflex. Stimulation of periarterial mesenteric nerve C-fibers also produced a fall in blood pressure, and this response was converted to a small rise in pressure in the capsaicin-treated animals. Afferent vagal and saphenous nerve stimulation produced the normal fall and rise in blood pressure, respectively, in the pretreated rats. The reflex fall in blood pressure induced by intestinal distension was abolished by topical application of a local anesthetic or of capsaicin to the mesenteric nerve, by systemic administration of morphine or of hexamethonium, or by section of the spinal cord, but not by atropine, phentolamine, or vagotomy. These investigators hypothesized that the depressor response to intestinal distension was mediated primarily by capsaicin-sensitive sensory afferents innervating the intestine via the mesenteric nerves. This distension-induced reflex involved nociceptive afferents to some degree and supraspinal regions of the CNS.

The liver contains an afferent neuronal input that is capsaicin sensitive. Ashton and coworkers (6) injected 500 μg of capsaicin into the portal circulation of anesthetized dogs and found rapid cardiovascular changes consisting of a 10% decrease in left ventricular systolic pressure, a 12% decrease in mean arterial pressure, a 4% decrease in heart rate, a 7% decrease in renal vascular resistance, a 12% decrease in rate of left ventricular pressure rise, and a 15% decrease in this rate at developed pressure. Section of the vagus nerve at the level of the diaphragm had no effect on the capsaicin-induced cardiovascular responses, but section of the anterior hepatic nerve eliminated these responses. There was no significant reduction in the responses with three successive injections of capsaicin in the same animal. It was concluded that the cardiovascular reflex responses to intrahepatic capsaicin injection were the result of activation of primary afferent neurons innervating the liver. However, in view of the high dose of capsaicin used and the lack of tachyphylaxis to the compound, some caution should be considered when proposing activation of specific types of sensory neurons in the liver as being responsible for the reflex.

In cats, Ordway and Longhurst (197) determined that application of small amounts of capsaicin or of bradykinin to the serosal surface of the gallbladder evoked reflex cardiovascular responses. These responses included small increases in mean arterial pressure, heart rate, ventricular pressure rise rate, and systemic vascular resistance. No such responses were seen when either agent was applied to the serosal surface of the liver. Vagotomy had no effect on the responses to capsaicin, but removal of the celiac and superior mesenteric ganglia or denervation of the gallbladder abolished the reflex cardiovascular effects. Repeated application of either bradykinin or capsaicin did not produce tachyphylaxis. Application of as little as 500 ng of bradykinin to the mucosal surface of the bile-drained gallbladder produced cardiovascular effects similar to those induced by serosal application. No mention of any effects of mucosal capsaicin was made. Distension of the gallbladder with pressures up to 100 mm of Hg did not produce any detectable cardiovascular responses, suggesting that capsaicin and bradykinin activated sensory neurons other than those responding to stretch in this organ. Capsaicin and bradykinin produced similar cardiovascular responses that were blocked by celiac and superior mesenteric ganglionectomy when applied to the surface of the cat pancreas (198).

Capsaicin and bradykinin applied to serosal surfaces of stomach, small intestine, and gall bladder of the dog decreased tracheal smooth muscle tension. Gall bladder application was least effective. Section of the splanchnic nerves prevented this effect, whereas cutting the vagi did not alter this reflex tracheal relaxation (212). The reflex was also blocked by atropine as was that induced by intraarterial injection of capsaicin into hindlimb skeletal muscle (211).

SP-containing primary afferent neurons may mediate the micturition reflex in the urinary bladder of rats. Sharkey et al. (224) found that the bladder of adult rats treated on day 2 of life with capsaicin (50 mg/kg) contained substantially higher amounts of urine than vehicle-treated litter mate controls. When the marker, True Blue, was injected into the bladder of rats, it was transported in a retrograde direction to two levels of spinal cord dorsal root ganglia: T12-L2 and L6-S1. The latter group of sensory ganglia was labeled in more cells than the former. Capsaicin pretreatment partially reduced the number of cells that accumulated the injected dye. Ten to 16% of the True Blue-labeled cells were also immunohistochemically positive for SP. Capsaicin pretreatment essentially abolished the detection of SP-positive cells. Treatment of neonatal animals with the compound also resulted in obliteration of SP-positive fibers in the bladder wall. Somatostatin-positive cell bodies were also observed in dorsal root ganglia, but none of these was labeled by True Blue injection into the urinary bladder. After capsaicin treatment, no somatostatin-containing somata were visible (see section III, A and C). Acetylcholine- or field stimulation-induced contraction of the in vitro urinary bladder was not altered in tissue taken from animals treated neonatally with capsaicin. The authors concluded that the urinary retention induced by

capsaicin in rats was due to an effect of the compound on afferent innervation of the bladder. This effect could be mediated by alteration of SP-containing sensory neuron function, as these investigators speculated, or it could involve an action of neonatally administered capsaicin on other peptide-containing afferents (VIP, CCK, bombesin).

E. Ocular Function

In the growing interest in the neuropharmacology of the eye of recent years, capsaicin has become a popular pharmacological tool. In 1980, Camras and Bito (34) detailed an interesting investigation in which the effects of prior capsaicin treatment were assessed on the neurogenic ocular hypertension produced by topical nitrogen mustard in rabbits. Retrobulbar injection or intracranial injection of capsaicin via the superior orbital fissure completely blocked the ocular hypertension compared to only partial blockade by alcohol denervation or indomethacin pretreatment. Prior topical instillation of a 1% solution of capsaicin onto the cornea was less effective than injection of the compound in blocking the nitrogen mustard-induced inflammation. The iris-ciliary body complex of rabbits was found to contain 7.9 ± 0.3 pmol of SP per g, and this level was reduced by 50% within 1 h of topical nitrogen mustard application. The amount of SP immunoreactivity in the aqueous humor was simultaneously increased 3-fold, suggesting that the chemical irritant had induced SP release. These investigators observed that the capsaicin-pretreated eyes, although insensitive to the inflammatory effects of nitrogen mustard, had normal pupillary and corneal reflexes. They suggested that nonirritating congeners of capsaicin would be preferable to the nonspecific effects of ethanol denervation in blocking the non-PG-mediated effects of chemical irritation or trauma of the eye. The species-specific inflammatory reaction of the rabbit eye to X-irradiation is mediated to a greater degree by PGs, since it was markedly inhibited by PG synthetase inhibitors, but not by ethanol denervation or by capsaicin pretreatment (18). Duffin and coworkers (58) used iris massage in the rabbit as a model of surgical trauma-induced miosis. PG synthetase inhibitors, topical local anesthetics, or retrobulbar injection of capsaicin antagonized the miosis to a small extent, but the combination of these three treatments blocked miosis by 70%. Injection of capsaicin itself into the rabbit eye induced an immediate, intense miosis that suggested SP release (vide infra). The third of three successive retrobulbar capsaicin injections no longer produced miosis.

Butler and Hammond (31) simultaneously perfused through separate circuits the eyes of rabbits and monitored intraocular pressure and pupil diameter. When capsaicin or bradykinin was perfused through the anterior chamber of an eye which had previously undergone diathermic coagulation of the trigeminal nerve, the intraocular hypertension and miosis produced by these agents was abolished compared to the nondenervated eye. The responses to infusion of PGE_1 were also markedly attenuated. When SP was infused into the eyes, there was a marked, prolonged miosis and occasional intraocular hypertension that were identical in denervated and normal eyes. These investigators concluded that the ocular responses to capsaicin, PGE_1, or bradykinin involved stimulation of sensory nerve endings, whereas the responses to SP were initiated at a site peripheral to the sensory neurons. Mandahl and Bill (157) induced acute experimental uveitis in the rabbit eye by antidromic trigeminal nerve stimulation. Intracameral injection of tetrodotoxin blocked the stimulation-induced miosis and reduced the rise in intraocular pressure. Tetrodotoxin blocked the hypertensive and mitotic effects of PGE_1 and of PGE_2, except at high doses of the PGs where the neurotoxin was ineffective against the pressure effects. The pressure and miotic effects of intracameral capsaicin injection were reportedly blocked by tetrodotoxin as well except at high doses of capsaicin where the miotic effect was resistant to blockade. Tetrodotoxin had no effect on SP-induced miosis, but produced a slight delay in the appearance of SP-induced ocular hypertension. These experimenters speculated that, while the majority of the ocular inflammatory effects of PGs required nerve conduction, the effects of high doses of capsaicin did not. The presence of sensory nerve endings, however, was required for the actions of capsaicin (vide supra). It is unfortunate that Mandahl and Bill (157) did not assess the effects of capsaicin pretreatment on trigeminal nerve stimulation-induced uveitis. Bynke (32) studied the actions of retrobulbar capsaicin administration on disruption of the blood-aqueous barrier produced by infrared irradiation of the cornea, s.c. α-melanocyte-stimulating hormone (MSH), or topical application of PGE_2 onto the cornea in rabbits. Retrobulbar injection of 0.5 ml of a 1% solution of capsaicin into one eye produced a prompt, strong inflammatory reaction consisting of conjunctival hyperemia, chemosis, miosis, and a dense aqueous flare. These effects subsided within 2 days. After the capsaicin pretreatment, the aqueous flare reaction to infrared rays was markedly reduced for several days in the ipsilateral eye, was subsequently markedly reduced in both eyes for up to 6 wk, and then was exaggerated in the ipsilateral eye compared to the contralateral eye at 3.5 mo after capsaicin. A similar time course and pattern of inhibition of the inflammatory response to s.c. MSH was observed, except that no final hyperreactivity was obtained in the ipsilateral eyes. The aqueous flare reaction to corneal PGE_2 was strongly inhibited in the ipsilateral eye 2 days after capsaicin treatment, but by 3 wk after capsaicin, there was no difference between the acute effects of PGE_2 in both eyes. Bynke (32) speculated that the antiinflammatory effects of capsaicin were caused by interference with neurogenic mechanisms, but it is doubtful that

"degeneration of SP-containing trigeminal nerve fibers" was responsible for these reversible capsaicin actions.

Ueda and coworkers (248) have used capsaicin in their studies which implicate SP in contraction of the rabbit iris sphincter muscle. In vitro transmural stimulation of the muscle produced tetrodotoxin-sensitive fast and slow components of contraction. The fast component appeared to be cholinergic, since it was enhanced by physostigmine and abolished by atropine. The slow component was not affected by cholinergic agents nor by adrenergic or ganglionic blocking agents. Capsaicin produced a strong contractile response in the muscle to which tachyphylaxis developed with repeated application. During the tachyphylaxis, the fast component of stimulation-induced contraction was normal, but the slow component was abolished. SP or acetylcholine produced strong contraction of the muscle preparation, whereas adenosine triphosphate, histamine, 5-hydroxytryptamine, or norepinephrine had little effect. In a follow-up investigation, these same investigators (247) studied the effect of prior trigeminal nerve denervation on the noncholinergic, nonadrenergic contraction of the rabbit isolated iris sphincter muscle. Denervation abolished the slow noncholinergic, nonadrenergic component as well as the contractions induced by capsaicin or bradykinin. SP and carbachol each induced contraction that was not affected by trigeminal denervation. Somatostatin, VIP, or enkephalin had no effect in the normal in vitro muscle preparation. These investigators concluded that SP-containing trigeminal neurons were responsible for the slow component of the iris sphincter contraction. Although this conclusion was reached in a species in which the peptide neuropharmacological effects of capsaicin have not been extensively characterized, later studies using a SP antagonist supported a role for SP (vide infra).

Nishiyama and coworkers (188) established that the dose-dependent contraction of the rabbit isolated iris sphincter induced by SP was a direct action on smooth muscle as it was not antagonized by tetrodotoxin, cholinergic antagonism, α- or β-adrenergic antagonism, histamine H_1 or H_2 antagonism, 5-hydroxytryptamine antagonism, or by baclofen. Zhang et al. (263) confirmed that trigeminal denervation abolished the contractile effects of capsaicin and of bradykinin without affecting the actions of carbachol or SP in the isolated rabbit iris sphincter. PGE_1, PGE_2, or $PGF_{2\alpha}$ had no effect in the normal isolated sphincter muscle. They reported the interesting finding that a tachyphylaxis developed to the contractile effects of bradykinin, more so than with repeated applications of capsaicin. It was suggested that this was due to bradykinin-induced SP release from sensory neurons. Unfortunately, the useful information of whether or not bradykinin and capsaicin exhibited cross-tachyphylaxis and whether or not bradykinin-induced contraction was affected by a SP antagonist was not obtained.

Bjorkroth (20) used the SP antagonist, D-Arg[1],D-Pro[2],D-Trp[7,9], Leu[11]-SP, to provide convincing evidence that SP is the mediator of the slow component of electrically induced rabbit iris sphincter contraction. The antagonist at a concentration of 50 μM blocked the slow component of contraction elicited by 30-Hz stimulation, but had no effect on the atropine-sensitive twitch component. The SP antagonist at 10 μM and 50 μM also resulted in a concentration-dependent antagonism of the iris sphincter contraction induced by 10 μM capsaicin and by 10 nM SP. Bynke et al. (33) applied D-Pro[2],D-Trp[7,9]-SP topically onto the cornea and observed a marked attenuation of the miosis and the aqueous flare response induced by retrobulbar injection of PGE_2 or capsaicin in the rabbit. These results strongly implicate SP or a similar peptide in the manifestations of the ocular inflammation produced by prostaglandins and by excitation of capsaicin-sensitive neurons. Mandahl and Bill (158) found a more complicated picture when they evaluated the effects of D-Pro[2],D-Trp[7,9]-SP on ocular function in the anesthetized rabbit. When injected into the eye, the peptide behaved primarily as an agonist at high doses resulting in miosis, breakdown of the blood-aqueous barrier, and a rise in the intraocular pressure. At a low dose, D-Pro[2],D-Trp[7,9]-SP induced a slight miosis and antagonized the miotic responses to PGE_1 and antidromic trigeminal nerve stimulation without affecting the miotic responses to SP or capsaicin. It is possible that these experiments were complicated by the choice of putative SP antagonist and by the in vivo use of the peptide.

Tervo (242) complicated the findings with capsaicin in the rabbit eye when she reported that high s.c. doses of capsaicin, intracameral capsaicin injection, or neonatal capsaicin treatment had no effect on SP immunoreactivity detected with a monoclonal SP antibody in the rabbit cornea and iris sphincter-ciliary body. The capsaicin treatments reportedly blocked the acute effects of intracameral capsaicin injection. In view of the above findings with capsaicin in the rabbit eye, the fact that the actions of capsaicin on SP in the rabbit have not been well characterized, and the fact that Tervo (242) did not consider various time points after capsaicin treatment and did not convincingly confirm capsaicin-induced SP depletion in the rabbit dorsal spinal cord after systemic treatment, her negative results have to be regarded as equivocal.

Capsaicin treatment of neonatal and adult animals clearly induces an insensitivity to chemical irritation of the eye (vide supra; see sections II, B and C, and III, B and C). Keen and coworkers (132) observed that treatment of neonatal mice also led to increased vascularization of the cornea and a 43% decrease in corneal SP content. The corneal reflex in response to tactile stimu-

lation was also markedly reduced, but not abolished as it was in animals with lesions of the trigeminal ganglia. The degree of SP depletion, however, was not correlated with the reduction in corneal reactivity. Furthermore, an effect of capsaicin on corneal responses to tactile stimulation in the rat has not been reported, and capsaicin does not affect the pupillary light reflex or corneal tactile reflex in the rabbit eye (32) or the corneal tactile response in the guinea pig (S. H. Buck and T. F. Burks, unpublished observations). The reported antagonism of the corneal tactile reflex in mice may have thus been due to nonspecific effects of capsaicin.

F. Gastrointestinal Function

In addition to actions in in vitro gastrointestinal tissue preparations (see section VIII), capsaicin can affect gut function in intact animals. In an assessment of intestinal transit in rats in which the distribution of ^{51}Cr was determined after intraduodenal instillation, capsaicin in a s.c. dose of 10 mg/kg markedly retarded the propulsion of ^{51}Cr (173). This inhibition of intestinal transit by systemic capsaicin was not investigated further, but since the animals were not anesthetized when treated with the compound, the inhibition may have resulted from strong irritation produced by the capsaicin injection.

Longhurst and coworkers (144) used a characteristically elegant anesthetized dog preparation to demonstrate that the stomach contains capsaicin-sensitive neural elements that can influence systemic cardiovascular function. When capsaicin doses ranging from 25 to 500 μg were injected into the gastroepiploic artery of the autoperfused in situ canine stomach, there were increases in systolic, mean, and diastolic systemic arterial pressures, in heart rate, and in maximal ventricular pressure rise rate. There was no change in left ventricular filling pressure or in ascending aortic blood flow. Repeated intraarterial injection of 30- to 50-μg doses of capsaicin produced no evidence of tachyphylaxis, but higher doses were not tested. As a control, capsaicin was injected into the inferior vena cava to determine effects downstream from the stomach. Administration of capsaicin i.v. resulted in cardiovascular responses that were the opposite of those seen after gastric intraarterial administration and possibly originating in the pulmonary tree (see section X G). Afferent denervation of the stomach by celiac and/or vagus nerve section indicated that the afferent limb of the capsaicin-induced reflexes existed in both the sympathetic and parasympathetic nerves to the stomach. These investigators noted that the similarities between cardiovascular responses to food ingestion and to gastric intraarterial capsaicin ingestion suggested that the compound was stimulating gastric distension receptors. However, an interesting alternative possibility is that there are afferent noxious chemoreceptors in the stomach (and intestine) that are sensitive to capsaicin and that mediate gastric acid-induced pain.

G. Pulmonary Function

Russell and Lai-Fook (210) evaluated the effects of capsaicin on pulmonary airway diameter in anesthetized, paralyzed, mechanically ventilated dogs. A dose of 20 μg of capsaicin per kg injected into the right ventricle resulted in a 20% decrease in airway diameter as well as decreases in heart rate, aortic pressure, and pulmonary artery pressure. Vagotomy abolished the actions on airway diameter and heart rate and converted the vascular pressure responses to slight increases. These investigators proposed that capsaicin activated a reflex arc that normally served to regulate the driving pressure between the alveolar interstitium and the peribronchial space.

Coleridge et al. (42) recorded smooth muscle tension in a segment of the trachea of anesthetized dogs and determined the effects of capsaicin activation of pulmonary, bronchial, or somatic afferent neurons. Right atrial injection and injection of microgram quantities into the bronchial arterial system caused tracheal contraction which was prevented by cooling or sectioning the cervical vagus nerves. Injection of capsaicin into the femoral artery, however, resulted in tracheal relaxation that was blocked by hindlimb denervation. Injection of 1.5 μg of bradykinin or of 3 μg of capsaicin into a bronchial artery produced a marked increase in tracheal submucosal gland secretion in anesthetized dogs (47). The tracheal secretory effects of these compounds, but not those of mechanical stroking of the laryngeal mucosa, were abolished by cooling or cutting the lower cervical vagus nerves.

McCaffrey and Kern (165) used capsaicin to distinguish pulmonary "J-receptor" stimulation from stimulation of pulmonary stretch or irritant receptors. J-Receptors are pulmonary juxtaalveolar capillary, vagal C-fiber afferents that produce apnea and variable bronchoconstriction. Stimulation of stretch receptors in the anesthetized dog by lung inflation decreased laryngeal airway resistance. Pulmonary J-receptor stimulation by injection of capsaicin into the right atrium produced apnea and an increase in laryngeal resistance. Administration of histamine i.v. to activate irritant receptors resulted in tachypnea and a decrease in laryngeal airway resistance. Vagotomy below the level of the recurrent laryngeal nerves abolished the responses to all of these receptor-activating maneuvers. The distinction between capsaicin-sensitive J-receptors and pulmonary irritant receptors supposedly sensitive to smoke as well as to mechanical and chemical irritation is not supported by recent investigations of capsaicin-induced desensitization of the rat trachea (vide infra). Injection of 10 μg of capsaicin per kg into the canine femoral vein markedly stimulated phasic electromyographic activity in the facial dilator nares muscle presumably in concert with respiratory stimulation (21).

Activation of C-fibers by capsaicin in the dog lung is antagonized by the antiasthmatic agent, sodium cromoglycate (52). Electrophysiological recordings were made

from fibers of the left cervical vagus nerve that responded to hyperinflation and mechanical stimulation of the lung. When capsaicin was injected i.v. in a dose of 10 μg/kg, all of the sensory endings increased in rate of firing. When the capsaicin injection was preceded 10 min by a 100-μg/kg injection of sodium cromoglycate, the capsaicin-induced increase in neuronal firing rate was 40% of the control response. This antagonism by a single dose of the antiasthmatic agent persisted for 45 min, and the compound had no effect on the basal firing rate of the C-fibers. Dixon and coworkers (52) speculated that sodium cromoglycate was interfering with C-fiber endings in the lung that are sensitive to histamine, PGs, veratrum alkaloids, and capsaicin, but that were distinct from irritant receptors (but vide infra). The physiological role of these chemosensitive fibers in the lung is not known. It would be of extreme interest to know if capsaicin or a related compound could produce a desensitization of these pulmonary receptors that resulted in antiasthmatic activity.

The major portion of field stimulation-induced contraction of guinea pig tracheobronchial smooth muscle is mediated by capsaicin-sensitive neuronal fibers (241). The initial rapid contraction is blocked by cholinergic antagonism, while the subsequent sustained contraction is abolished by the induction of capsaicin tachyphylaxis. Tetrodotoxin abolished both phases of the smooth muscle contraction. It is tempting to speculate that the capsaicin-sensitive fibers are SP-containing sensory neurons, but much additional evidence would be needed to allow this conclusion. Szolcsanyi (238) noted a positive correlation between pain-producing ability and the ability of piperine, capsaicin, and some capsaicin analogues to produce contraction of the guinea pig trachea in vitro. Cross-tachyphylaxis experiments suggested that piperine and the capsaicinoids were acting on the same neuronal cells in the trachea.

Lundberg's laboratory at the Karolinska Institute has reported some interesting results with capsaicin and tracheobronchial function in the rat. Lundberg and Saria (150) found that capsaicin-sensitive neurons in the vagus nerve were involved in the control of tracheal vascular permeability. Treatment of neonatal or of adult rats with capsaicin abolished vagal stimulation-induced Evans blue dye extravasation in the trachea. Local capsaicin injection in the trachea resulted in extravasation that was almost abolished in capsaicin-pretreated animals. Local injection of SP induced the same reaction in the trachea, but this response was not altered after capsaicin treatment of adult rats. These investigators hypothesized that capsaicin-sensitive SP-containing vagal sensory fibers mediated the vascular permeability changes induced by vagal nerve stimulation in the rat trachea. Capsaicin pretreatment or D-Arg^{1},D-Pro^{2},D-Trp^{7,9},Leu^{11}-SP prevents cigarette smoke-induced dye extravasation in rat tracheobronchial tissue (149, 151, 153). Exposure of rats to 12 puffs of a Kentucky reference cigarette in a smoking machine induced a marked increase in dye extravasation that was not blocked by glass-fiber filtration of the smoke, but was partially antagonized by prior ganglionic blockade and completely absent in animals treated as neonates with a single 50-mg/kg dose of capsaicin. Instillation of nicotine into the tracheal lumen also induced a profound increase in Evans blue dye extravasation that was blocked by capsaicin or hexamethonium pretreatment. The effects of SP on tracheal vasculature extravasation or of capsaicin itself in capsaicin-pretreated animals were not assessed in this investigation, although it was reported that hexamethonium pretreatment did not alter permeability responses to capsaicin itself. Lundberg and Saria (151) subsequently observed that capsaicin treatment of neonatal rats desensitized respiratory tract mucosa to a variety of mechanical and chemical irritants as well as to cigarette smoke. Exposure to the smoke or to ether produced an increase in tracheal edema that was atropine resistant, but abolished in animals treated neonatally with capsaicin. Mechanical irritation of the trachea, stimulation of the vagus nerve, or the mucosal application of capsaicin, histamine, bradykinin, or formalin also resulted in a capsaicin-sensitive, marked increase in tracheal vasculature permeability. Interestingly, local application of SP itself to the tracheal mucosa induced an increase in permeability that was not blocked by atropine. These investigators speculated that capsaicin could activate and then desensitize tracheal C-fibers, possibly containing SP, that were involved in certain respiratory pathophysiologies including hyperreactive airway and asthma.

SP and capsaicin each induced contraction of human bronchi in vitro (148). SP at a concentration of 1 to 10 μM produced a slowly developing contraction which was resistant to atropine and an antihistamine. Capsaicin at a concentration of 10 μM induced a similar response to which tachyphylaxis developed on repeated application. Both SP and capsaicin were less potent in the human preparation than in an in vitro guinea pig trachea preparation. Systemic administration of capsaicin also results in intense bronchospasms in guinea pigs (28). Transmural electrical stimulation of both preparations induced a contractile response that was nearly abolished by atropine. These investigators postulated that bronchial smooth muscle in man is innervated by a capsaicin-sensitive, SP-containing neuronal system that is capable of local noncholinergic control of muscle tone. The evidence for this would have been more convincing had Lundberg et al. (148) shown that SP was still active in the presence of capsaicin tachyphylaxis or that capsaicin was ineffective after SP tachyphylaxis in their preparations.

Capsaicin i.v. or infranodose electrical stimulation caused a marked increase in pulmonary insufflation pressure in guinea pigs that was substantially reduced after

systemic capsaicin treatment. The capsaicin-sensitive SP afferents innervating the trachea and stem bronchi were observed to be primarily of vagal origin, while those innervating the lung were thought to originate in part in dorsal root ganglia (147). In the anesthetized guinea pig, D-Arg[1],D-Pro[2],D-Trp[7,9],Leu[11]-SP reduced the vagal stimulation-induced increase in respiratory insufflation pressure as well as that produced by injection i.v. of SP or capsaicin. In vitro bronchial contraction (noncholinergic component) induced by field stimulation, SP, or capsaicin was also blocked by the SP antagonist. The cholinergic component of contraction and the noncholinergic relaxation were not altered by the antagonist. The SP antagonist also inhibited vagal stimulation- and SP-induced extravasation of Evans blue dye in respiratory tract and esophagus. After prolonged vagal stimulation, bronchial levels of immunoreactive SP were reduced presumably due to reduction of releasable stores. This thorough investigation from Lundberg's laboratory (152) leaves little doubt that SP-containing afferents are important in tracheobronchial function in the guinea pig.

H. Cardiovascular Function

Capsaicin-sensitive afferent neurons in various regions of the body can influence systemic cardiovascular function. Longhurst and coworkers (145) found that the sciatic nerve of the cat contained sensory neurons capable of influencing cardiovascular function and that entered the spinal cord via both the dorsal and ventral spinal roots. Electrical stimulation of the sciatic nerve produced an increase in arterial blood pressure that was blocked only by sectioning both the dorsal and ventral roots of the lower cord. Stimulation also induced a tachycardia that was abolished by dorsal root section alone. Injection of 1 to 250 μg of capsaicin into the femoral artery resulted in identical cardiovascular responses as sciatic nerve stimulation. The effects of capsaicin were blocked by spinal root section in the same manner as those produced by stimulation. Kaufman et al. (128) recorded impulses in vagal afferents from the heart and aorta in anesthetized dogs and observed a small population of specifically chemosensitive afferents in the left ventricle, left atrium, and aorta that were activated by bradykinin, capsaicin, or phenyldiguanide. These sensory endings were not sensitive to mechanical stimulation or to hypoxia. It was concluded that these afferents were vagal C-fibers the stimulation of which resulted in reflex bradycardia and hypotension, whereas sympathetic chemosensitive C-fibers were responsible for the mediation of cardiac pain.

Virus and colleagues (250) compared the effects of capsaicin treatment on cardiovascular function in WKY and SHR rats. Treatment of adult rats of both strains with a total systemic dose of 150 mg of capsaicin per kg resulted in a 10 to 15% decrease in mean systemic arterial blood pressure 9 days later. There was a slight shift rightward of the dose-response curve for the depressor effect of SP in both strains after capsaicin treatment. In rats of the WKY strain, the capsaicin pretreatment had little effect on the pressor response to angiotensin II, whereas in the hypertensive SHR strain, the pretreatment resulted in a marked shift to the right of the angiotensin II dose-response curve. The capsaicin treatment increased the sensitivity of the WKY strain to the pressor effects of norepinephrine, but decreased the sensitivity of SHR rats to the biogenic amine. While these results are not easily explained by a single unifying hypothesis, they do suggest a difference between WKY and SHR rats with respect to capsaicin-sensitive neural mechanisms. Such possible differences are worthy of further investigation to determine the implications toward the development of hypertension. In the same investigation, Virus et al. (250) observed that the capsaicin treatment elevated tail-flick and hot-plate escape latencies in SHR, but not in WKY, rats. These results also suggest some alteration in capsaicin-sensitive nociceptive neurons in the hypertensive rats (see section III, B and C).

Haeusler and Osterwalder (81) have pursued investigation of the possibility that capsaicin-depletable SP in brainstem nuclei important in baro- and chemoreceptor control of blood pressure is contained in the primary afferent neurons that mediate baro- and chemoreception. Application of SP-soaked filter paper to the obex region of the medulla oblongata in anesthetized rats and cats produced a decrease in blood pressure and heart rate. Microinjection of SP into areas of the solitary nucleus in both species produced similar results. In rats, when capsaicin was similarly injected, the effects mimicked those of injected SP. The intermediate part of the nucleus, which contains the majority of the baroreceptor afferent terminals, was reportedly the only portion responsive to the microinjection of SP. These investigators extended their observations in a subsequent study (82). They observed that, whereas repeated local application of SP to the solitary nucleus region in cats produced cardiovascular responses of constant magnitude, repeated application of capsaicin resulted in rapid development of tachyphylaxis to the hypotensive and bradycardia actions. In addition, the hypotension and bradycardia produced by carotid sinus nerve stimulation in cats were not affected by the capsaicin tachyphylaxis. Similarly, the effects of locally applied SP were not altered during the tachyphylaxis. Haeusler and Osterwalder concluded that SP had a neurotransmitter or neuromodulator role at the first synapse of the baroreceptor reflex. They also hypothesized that capsaicin releases SP from a "limited, functionally unimportant pool" in baroreceptor afferents in the nucleus tractus solitarius (see sections IX and XII A).

Indirect evidence for a role of SP in the reflex was also provided by Donnerer and Lembeck (55) who reported that D-Pro[2],D-Trp[7,9]-SP injected intrathecally reversi-

bly blocked the reflex fall in blood pressure produced by intraarterial capsaicin in rats. This reflex was definitely of brainstem origin, since it was absent in rats pithed intraorbitally down to the occipital foramen, but not in animals that had undergone intercollicular decerebration. A modulatory role of SP was also postulated in the nucleus tractus solitarius of the rat when direct injection of the peptide into the nucleus produced a marked increase in blood pressure. Interestingly, this effect was only seen in rats treated neonatally with capsaicin and not in untreated animals (36).

In a further series of experiments, Lorez and coworkers (146) assessed cardiovascular and sensory neuron function in adult rats treated with either 50 mg or 600 mg of capsaicin per kg at 20 h after birth. Systolic blood pressure after both doses was not altered in the treated animals. Heart rate was slightly lower after treatment with the lower dose of capsaicin, but not after the higher dose. The hypertension and bradycardia produced by i.v. injection of phenylephrine were not affected by either capsaicin treatment. The tail-withdrawal threshold was not altered by either capsaicin treatment, but both treatments significantly increased the response time in the hot-plate test. This alteration in hot-plate response was identical in male and in female rats (see section III, B and C). Both capsaicin pretreatments markedly reduced the nociceptive responses elicited by capsaicin applied topically to the cornea and by formaldehyde injected into a forepaw. These reductions occurred in both male and female animals. RIA determination of SP levels revealed that the 50-mg/kg dose of capsaicin had depleted the peptide by 55% in the dorsal spinal cord and 62% in the trigeminal substantia gelatinosa. SP was not depleted in the rostral or caudal solitary nucleus or in the reticular formation of the medulla oblongata. These investigators also used immunohistochemistry to evaluate SP depletion by the lower capsaicin dose. This technique revealed a reduction in the number of fibers in laminae I and II of the dorsal horn and in the dorsomedial lateral funiculus. SP-positive cell bodies were observed in the solitary nucleus, and the number of these was not altered by neonatal capsaicin treatment. Lorez et al. (146) also made the interesting observation that there appeared to be two distinct populations of SP-positive fibers in the nucleus tractus solitarius. The first of these was many small SP-positive dots, presumably terminals, throughout the nucleus that appeared possibly only slightly depleted by capsaicin in the caudal portion of the nucleus. In addition, coarser, more distinct dots and fibers were observed primarily in the intermediate planes of the nucleus solitarius. These dots and fibers were markedly depleted by capsaicin. They originated from rootlets of cranial nerves IX and X and traversed lateral to the solitary tract to reach the nucleus. Capsaicin also markedly reduced SP-positive immunofluorescence in the spinal tract and substantia gelatinosa of the trigeminal nerve and in the cuneate fasciculus. SP immunofluorescence was not altered by the neonatal capsaicin treatment in the reticular formation, the inferior olive, the motor nuclei of X and XII, the nuclei gracilis and cuneatus, and in the area postrema (but see ref. 113). These investigators concluded that the effects of capsaicin on certain sensory functions, including baroreceptor function, were complex and did not allow unequivocal conclusions to yet be drawn about the role of SP in baroreceptor control of cardiovascular function.

Two additional laboratories have considered cardiovascular function in animals treated systemically with capsaicin. Furness et al. (67) treated adult guinea pigs with a cumulative dose of 785 mg of capsaicin per kg and determined cardiovascular responsiveness 2 wk later. Capsaicin had no effect on resting mean arterial blood pressure or heart rate. In addition, the changes in cardiac function induced by the hypotension resulting from glyceryl trinitrate injection or the hypertension resulting from phenylephrine injection were not altered. This capsaicin treatment reduced immunohistochemically detectable SP by 90% or more in pericardium, atria, superior mesenteric artery, carotid artery and sinus, and in the ureter. It was hypothesized that the capsaicin-sensitive SP-containing fibers of the guinea pig cardiovascular system were not essential for baroreceptor reflex function. In contrast, evidence for a role of SP in baro- and chemoreceptor function in the rat was presented by Bond and coworkers (24). In rats treated with 50 mg of capsaicin per kg a few days after birth, the reflex pressor response to bilateral carotid occlusion was significantly less than in vehicle-treated animals. Resting mean blood pressure was somewhat lower in the treated animals as well when they were breathing air or 100% oxygen. The response to carotid occlusion was also lower in the capsaicin-treated animals in 100% oxygen. Basal respiratory minute volume was lower in capsaicin-treated animals in air or in 100% oxygen, and the occlusion-induced increase in respiration was lower under both conditions in the animals treated neonatally with capsaicin. Dose-response analysis of the pressor effects of i.v. norepinephrine did not reveal any significant difference between vehicle-treated and capsaicin-treated rats. The increase in respiratory minute volume after i.v. injection of the arterial chemoreceptor stimulant, sodium cyanide, was markedly reduced in the capsaicin-treated rats. In the presence of a hypoxic 10% oxygen atmosphere, the increase in minute volume was substantially less in rats treated neonatally with capsaicin. Bond et al. (24) concluded that capsaicin treatment of neonatal rats altered the sensitivity of peripheral chemoreceptors and/or the central component of the chemoreceptor reflex without altering the vascular component of the baroreceptor reflex arc. They could offer no explanation for why their results differed from those of Lorez and coworkers (146; vide supra). Appropriate caution was expressed about

concluding that effects on SP-containing unmyelinated fibers were responsible for the observed reflex alterations by capsaicin, since the compound can deplete several sensory neuron neuropeptides in neonatal rats (see section III, A and C). It would be of interest to determine if the same chemoreceptor functions analyzed by Bond and coworkers (24) in rats were similarly altered in adult guinea pigs treated with capsaicin.

Donnerer and Lembeck (53) studied the acute cardiovascular effects of i.v. capsaicin injection in rats. Injection of 1 μg or 3 μg of capsaicin resulted in a triad of cardiovascular effects: (*a*) an initial, short fall in blood pressure; (*b*) a return to normal or slightly above normal pressure; and (*c*) a delayed, longer-lasting hypotension. Repeated injection of the 3-μg dose for 5 times produced identical effects each time. Pretreatment of animals with atropine, phentolamine, or propranolol had no effect on the cardiovascular responses to capsaicin. Sprague-Dawley rats, in contrast to Wistar rats, did not exhibit apnea or bradycardia after the capsaicin injections. Rats treated neonatally with capsaicin had a lower mean arterial blood pressure and a lower heart rate compared to controls in adulthood (vide supra). In the capsaicin-pretreated animals, the initial fall in pressure was unchanged, the subsequent pressor effect was exaggerated, and the final hypotension was completely abolished. Bilateral cervical vagotomy attenuated the initial hypotension, increased the subsequent pressor effect, and did not alter the final hypotension. In the pithed rat, capsaicin caused only a rise in blood pressure that was not altered by neonatal capsaicin treatment. Intraarterial injection of nanogram quantities of capsaicin in the femoral artery resulted only in a 36-mm Hg fall in blood pressure. The same injection in capsaicin-pretreated rats resulted only in a 19-mm Hg rise in pressure that was not blocked by phentolamine. Both the fall and the rise in blood pressure after femoral artery injection were absent after section of the saphenous and sciatic nerves (see section X C). Donnerer and Lembeck (53) concluded that the initial and delayed hypotension induced by capsaicin were reflex responses to stimulation of capsaicin-sensitive primary afferent fibers, while the intervening hypertensive effect was due to a direct vasoconstriction by capsaicin. They also speculated that the responses remaining in rats treated neonatally with capsaicin were due to the survival of a population of capsaicin-sensitive neurons. This could indicate, as might the effects of injected capsaicin to which tachyphylaxis does not develop (vide supra; see section X, C, D, and G), that the compound may have acute effects on nonsensory neurons. Alternatively, there could be a dissociation between the degeneration and loss of primary afferents induced by capsaicin and the activation of sensory neurons by the compound (see section III D).

Lembeck and Donnerer (141) also found that the splanchnic nerve itself contains capsaicin-sensitive neurons that can mediate cardiovascular effects (see section X D). Stimulation of the splanchnic nerve distal to a transection produced a rise in blood pressure in both Sprague-Dawley and Wistar rats that was not affected by pretreatment of the rats 4 days previously with 50 mg of capsaicin per kg. Stimulation of the proximal part of the sectioned nerve caused a fall in blood pressure in both strains that was markedly reduced by capsaicin pretreatment or by pretreatment with guanethidine. The basal mean arterial blood pressure was lower in both the capsaicin-pretreated and guanethidine-pretreated rats, but this lower initial pressure was ruled out as the cause of the inhibition by the compounds of stimulation-induced hypotension. Injection i.v. of 300 ng of norepinephrine produced a bradycardia in Wistar rats, but not in Sprague-Dawley rats, that was not affected by the capsaicin pretreatment (250). Electrical stimulation of the proximal portion of the sectioned splanchnic nerve did not affect heart rate in the Wistar rats. Depletion of splanchnic nerve SP was confirmed in rats treated neonatally with capsaicin, but not in adult rats treated in a similar manner to those used in the functional studies. These investigators concluded that the reflex fall in blood pressure induced by proximal stimulation of the splanchnic nerve was mediated by capsaicin-sensitive afferent neurons and was the result of vasodilatation from reduced sympathetic tone. A similar mechanism was suggested for the vasodilatation resulting from some of the thermoregulatory effects of capsaicin (see section X A 1). Unfortunately, this investigation and the lack of other detailed characterization of the peptide neurotoxicity of capsaicin in adult rats do not permit the assumption that the afferent neurons involved are SP-containing cells. The cardiovascular and pulmonary reflexes activated by i.v. capsaicin in rats presumably originate from different sensory afferents than those which are sensitive to phenyldiguanide (229).

I. Inflammation

One of the most convincing discoveries to come from the use of capsaicin as a pharmacological tool is the role of SP in the inflammatory process. Lembeck and Holzer reported in 1979 (142) that the antidromic vasodilation and neurogenic plasma extravasation induced by antidromic stimulation of the saphenous nerve in the rat were reduced by 85% in rats that had been pretreated with 50 mg of capsaicin per kg on day 2 of life. SP mimicked the inflammatory effects of nerve stimulation, and the effects of both were blocked by a combination of histamine H_1 and H_2 blockade or by pretreatment with compound 48/80. Treatment with indomethacin had no effect on the vasodilation and the extravasation produced by SP or by nerve stimulation. These results suggested that SP-containing unmyelinated afferents were intimately involved in the inflammatory process and that SP-induced histamine release was partially responsible for inflammation. In a subsequent study, Gamse et al.

(72) compared the capsaicin-induced SP depletion in neonates of various ages with the capsaicin inhibition of neurogenic plasma extravasation. Pretreatment of rats on day 2 or on day 10 of life with capsaicin (50 mg/kg) resulted in SP depletion in sensory neurons and in tissues innervated by primary afferents. In addition, these treatments resulted in an 80% inhibition of extravasation of Evans blue dye. Pretreatment of rats on day 20 of life with the same capsaicin dose resulted in a reversible SP depletion and inhibition of extravasation in contrast to the irreversible nature of the effects seen when capsaicin was administered on the second or tenth days of life. Treatment of adult rats with a total dose of capsaicin (100 mg/kg) produced 4 days later a marked decrease in plasma extravasation and in SP levels in the skin. Intraarterial infusion of SP or physalaemin produced a dose-dependent extravasation of dye. Infusion of caerulein, VIP, somatostatin, or the enkephalin analogue, FK 33-824, did not induce extravasation. SP was equally effective in inducing this change in vascular permeability in vehicle-treated and in neonatally capsaicin-treated rats. In addition to supporting the role of primary afferent SP in inflammation, these results indicated that there was a critical age in newborn rats after which capsaicin did not produce a degeneration and loss of SP-containing sensory neurons, even though SP depletion still occurred (see section XII F). Morton and Chahl (176) confirmed these effects of capsaicin treatment of adult and of neonatal rats on neurogenic plasma extravasation. They postulated further that SP-induced histamine release was involved in a later phase of the response with the early phase being due primarily to the release of a neuronal vasoactive factor, presumably SP. The assumption that all of the effects of capsaicin are due to an action solely on SP-containing neurons that underlies the above investigations is, as stated elsewhere (see sections III, A and C, and XII, B and C), equivocal.

The inflammatory processes that can be induced by certain experimental pathological models are substantially reduced by capsaicin treatment. Lembeck and Donnerer (139) induced postocclusive cutaneous vasodilatation (reactive hyperemia) in the rat hindpaw by clamping shut the common iliac artery for a 3-min period. Upon release of the clamp, venous outflow from the femoral vein increased by 200 to 250%. This postocclusive vasodilatation was inhibited by 60 to 70% in rats treated with capsaicin as adults or as neonates. Section of the saphenous and sciatic nerves or pretreatment of animals with compound 48/80 resulted in a comparable reduction of the vasodilatation. Intraarterial infusion of SP produced a femoral vein dilatation, but CCK-8, somatostatin, caerulein, neurotensin, or bradykinin had little or no vasodilatory effects. VIP induced an increase in femoral vein outflow at doses several orders of magnitude higher than those for SP. Lembeck and Donnerer (139) suggested that postocclusive cutaneous vasodilatation and the axon reflex should be considered a peripheral, neurogenic phenomenon with no involvement of higher reflex control of the peripheral circulation.

Colpaert and coworkers (43) made the interesting observation that capsaicin treatment of adult rats markedly ameliorated the arthritic condition induced by inoculation with *Mycobacterium*. Treatment with a total dose of capsaicin of 140 mg/kg reduced the inflammatory swelling of paws and joints and markedly reduced the body weight loss in the arthritic animals. This antiinflammatory effect of capsaicin was apparent within 24 h of administration of the compound, occurred when capsaicin was given either before or after the onset of inflammation, and persisted for at least several weeks. The capsaicin treatment also antagonized the increase in primary afferent SP levels that occurs in the endotoxin-induced arthritis model. Aside from implicating SP in the inflammation of arthritic conditions, these investigators speculated that the antagonism of weight loss by capsaicin might be an indication that the compound reduced chronic pain in rats with adjuvant arthritis. Weight loss in humans is frequently seen accompanying chronic pain. Thus, the observations in the experimental model of arthritis may have important therapeutic implications (see section XII H).

Immersion of a hindpaw of a rat in 48°C water induces an immediate exudation of Evans blue dye in the skin that is markedly attenuated in rats treated neonatally with capsaicin (215). The dye content of the skin increased 5-fold after a 5-min paw immersion in the hot water. Treatment of rats with capsaicin (50 mg/kg) on day 2 of life reduced the increase in dye content by 74%. Saria and Lundberg pointed out that the reported excitation threshold of polymodal nociceptors to heat-induced pain of 45°C was identical to the threshold immersion temperature for inducing dye extravasation in rat paw skin. Their results suggested an important role of capsaicin-sensitive sensory neurons in local edema. In addition, these results imply a similarity in capsaicin sensitivity between those primary afferents mediating the heat-induced edema and the thermal nociceptive sensation in the hot-plate test that is modified to some degree after neonatal capsaicin treatment (see sections III, B and C, and XII C).

Capsaicin or SP induced plasma extravasation of Evans blue dye in many tissues of the body of rats and guinea pigs with the notable exceptions of adrenal medulla, heart, salivary glands, dental pulp, CNS, gastrointestinal tract, and skeletal muscle. In animals pretreated with capsaicin, the compound no longer produced extravasation, and ovalbumin-induced anaphylaxis was inconsistently reduced. 5-Hydroxytryptamine produced extravasation in the same tissues as SP and capsaicin, whereas histamine or bradykinin was effective in these tissues as well as in the gastrointestinal tract. The inflammatory action of SP was not antagonized by classical

antihistamines, although that of capsaicin was not evaluated (217). These observations indicate that histamine release does not mediate all of the inflammatory effects of SP (see section IX) and that different mediators can be responsible for inflammation in various tissues.

The capsaicin-sensitive, SP-containing neural fibers in the nasal mucosa (see sections II D and III D) are capable of inducing increased vascular permeability in the tissue (156). Electrical stimulation of the maxillary aspect of the trigeminal nerve resulted in increased Evans blue dye accumulation in nasal interstitial tissue. In adult rats treated with capsaicin (50 mg/kg) on the second day of life, basal as well as electrically induced dye accumulation was markedly reduced. The topical application of 25 μl of saline containing either 100 nmol of capsaicin or 7.5 nmol of SP onto the mucosal epithelium also evoked a substantial dye extravasation. This local effect of capsaicin, but not that of SP, was abolished in animals that had been treated neonatally with capsaicin. These investigators reported that the local application of VIP, CCK-8, somatostatin, or bombesin in normal animals did not increase nasal vascular extravasation. Lundblad and coworkers (156) concluded by speculating that SP release may be involved in the local vascular responses and nasal swelling in patients suffering from allergic and hyperreactive disorders of the nose.

Capsaicin-sensitive, SP-containing neurons in the guinea pig ureter mediate changes in vascular permeability in that tissue as well (216). Treatment of adult animals with a systemic capsaicin dose of 125 mg/kg produced a total loss of SP in the ureter as determined by RIA. Treatment of animals with 6-hydroxydopamine, a noradrenergic neurotoxin, had no effect on SP levels in the ureter. Removal of the inferior mesenteric ganglion also depleted SP, but this effect was more pronounced in the rostral than in the caudal ureter. Electrical stimulation of the inferior mesenteric ganglion resulted in Evans blue dye leakage that was greater in the rostral than in the caudal ureter. This stimulation-induced extravasation was reportedly absent in capsaicin-pretreated animals. Exposure of slices of the guinea pig ureter to 10 μM capsaicin evoked a strong release of SP that was not blocked by tetrodotoxin. Interestingly, 120 mM K^+ was able to induce SP release even when applied 2 times in succession. After a pulse with 10 μM capsaicin, however, K^+ at the 120 mM concentration no longer induced release of the neuropeptide (see section IX). This report is the first to directly demonstrate capsaicin-induced SP release in a peripheral organ innervated by SP-containing primary afferent neurons. The results raise the possibility that SP could be involved in inflammation in the ureter as well as in obstruction within this passageway.

J. Food Consumption

South and Ritter (230) made the interesting observation that injection of capsaicin into the area postrema and adjacent nucleus tractus solitarius of the rat resulted in overconsumption of preferred foods. Capsaicin in a dose of 25 μg in 5 μl was used. Even more striking with capsaicin treatment was the absence of additional deficits that do accompany electrolytic lesions of the same brainstem region (e.g., chronic body weight loss, excessive angiotensin II-induced drinking, and diminished food consumption in response to glucose deprivation). These observations suggest that capsaicin-sensitive afferents in the brainstem are critically important to the satiety response. It is unfortunate that determination of neuropeptide levels was not also made after this localized capsaicin application.

XI. Effects in Humans

The recent experimental results with the actions of capsaicin on sensory function in laboratory animals have prompted several laboratories to assess the actions of topical capsaicin on sensory function in humans. Carpenter and Lynn (35) applied a 1% solution of capsaicin several times to an area on the forearm. The initial two or three applications produced a burning-stinging sensation and a widespread flare reaction. These responses eventually subsided on further capsaicin treatment. Touching the treated area with a probe heated to 55°C or gently crushing a fold of skin in the area resulted in a flare that was markedly attenuated after topical capsaicin treatment. The blockade of the flare response persisted for 10 days. Interestingly, these investigators noted that the area directly under the disc-shaped heat probe became red in both normal and capsaicin-treated skin areas even when the drug treatment had strongly reduced the surrounding flare response. From 0.4 to 10 h after the last topical capsaicin treatment, the thresholds to heat pain and to pinprick were reduced (hypersensitivity). However, 2 to 10 days after the treatment, the threshold of the treated skin area to heat pain was increased by 1.9°C (analgesia). Intradermal injection of small quantities of SP in human skin resulted in a long-lasting flare, edema, and itch. In the capsaicin-treated skin, only the flare and itch response were reduced. Foreman and coworkers (65) similarly observed that, on the forearm previously painted with capsaicin in ethanol and washed, only the flare (not the wheal) response to intradermal SP or histamine was reduced. Contrary to speculation by these investigators, it is likely that the capsaicin-induced desensitization involves neither SP nor histamine depletion, but results from a direct deleterious effect on the membranes of those unmyelinated fibers involved in the neurogenic flare. Horn and Enge (104) attempted to implicate catecholamines in inflammation by measuring urinary excretion of vanillylmandelic acid and homovanillic acid during inflammation. The excretion of each of these was reduced by 10 to 20% after a UV-induced mild erythema was produced on the backs of volunteers. Application of a plaster containing capsaicin to the back had no effect on urinary amine

excretion, possibly because the conditions used resulted in only a weak erythema of the skin. Since the mechanism of the reduction of amine metabolite excretion was not determined, the results of this study are not readily interpretable.

Bernstein et al. (17) determined that topical application of capsaicin to human skin abolished histamine-induced axon reflex vasodilatation. Several applications of a 0.1% solution of capsaicin were made to forearm skin. The capsaicin solution produced a delayed erythema and burning sensation that subsided with each successive application. Intradermal injection of histamine in untreated skin produced a flare, wheal, and itch, but in capsaicin-treated skin, only a wheal and itching sensation occurred. These investigators observed that sensation to pin-prick, touch, or temperature was not altered by the capsaicin treatment of skin, but the axon reflex accompanying mechanical injury was reduced. The capsaicin-treated skin area was hyperreactive with regard to the erythema induced by ultraviolet irradiation. The lack of analgesia observed in this study compared to that of Carpenter and Lynn (35; vide supra) may have been due to the lower capsaicin dose used and/or to the shorter time of testing after capsaicin treatment in the investigation of Bernstein and colleagues (17).

Topical capsaicin also inhibits the axon reflex vasodilatation caused by neuropeptides in human skin. Anand and coworkers (3) treated an area of forearm skin with a cream containing 1% capsaicin in successive applications over a 3-day period. As expected, the applications produced a successively diminished erythema and burning sensation. Intradermal injection of 10, 30, or 60 pmol of SP, somatostatin, or VIP produced dose-dependent wheal and flare reactions. Bombesin did not have these effects. Histamine injection also produced the inflammatory responses with the longest and most intense flare reaction of all the agents. Histamine, SP, and somatostatin occasionally produced itching as well. The capsaicin pretreatment of skin nearly abolished all flare and itch reactions without altering the drug-induced wheals. This antiinflammatory effect of topical capsaicin lasted 3 to 4 wk. These investigators speculated that histamine release might be involved in the flare response to the neuropeptides and that capsaicin inhibited the reflex by depleting a vasodilatory substance from the effector side.

Toth-Kasa and coworkers (244) investigated the action of topical capsaicin in patients with acquired heat or cold urticaria. A 1% solution of capsaicin in dimethyl sulfoxide (DMSO) was painted on an area of forearm skin 10 times at 20-min intervals. The burning sensation produced by the application subsided after the fourth or fifth application as did the redness and edema produced by capsaicin. A short-lived hyperesthesia to mechanical stimulation also occurred after the capsaicin application. After the topical treatment, neither heat nor cold stimuli induced substantial flare or wheal reactions in patients previously sensitive to either or both maneuvers. The capsaicin effect lasted for up to 1 wk. The patients were reportedly still able to feel either warmth or cold in the treated area, although the former was often described as less intense in the capsaicin-treated compared to a DMSO-treated control patch of skin. Intradermal injection of compound 48/80 in the capsaicin-treated skin produced a normal wheal, but the flare reaction was abolished around the edema. These investigators postulated that the urticaria was caused by a pathological reaction of chemosensitive sensory neurons and that capsaicin inhibited this reaction. The suggestion that, while inhibiting chemogenic sensory function, capsaicin seemed to interfere with the ability to detect warmth more than the ability to detect cold is strikingly similar to what has been observed in adult guinea pigs treated with capsaicin (see section II C).

XII. Conclusions

A. Neuronal Specificity

There can be little doubt that capsaicin is remarkably specific for primary afferent neurons. This holds for the initial direct excitatory effects of the compound as well as for the subsequent desensitization and biochemical changes. That capsaicin has no general nonspecific neuronal effects in doses that deplete sensory neuron SP is demonstrated by the consistent lack of neurochemical alteration in the ventral spinal cord and ventral roots. Sensory neuron specificity is also supported by the lack of effect of the compound on numerous CNS and peripheral neurochemical parameters that are not associated with these neurons. The alterations in levels of biogenic amines and enkephalin immunoreactivity that have been reported after capsaicin administration most likely reflect secondary changes in response to inflammation or to loss of primary afferent input. This applies to brainstem nuclei, spinal cord (252), and peripheral tissues (19, 100).

There have been a few reports suggesting that capsaicin has effects on neurons other than primary afferents, but most of these must be regarded with caution. For example, the 30-μg dose of capsaicin which produced 5-hydroxytryptamine depletion after direct injection into the substantia nigra (48) must be considered as very high. This action on nigral serotonergic terminals may reflect their relatively high sensitivity to a toxic effect of capsaicin that is unrelated to the compound's action on sensory neurons. The same can be said for the lifelong hypothalamic β-endorphin depletion reported after systemic administration of 750 mg of capsaicin per kg in neonatal rats (201). The possibility that capsaicin may alter intrinsic neurons of the gastrointestinal tract is intriguing, but since this effect was observed after treatment of newborn cats with a dose of 200 mg/kg (61), the possibility of nonspecific effects cannot be ruled out. An indirect effect resulting from altered extrinsic sensory

neuron function is also possible. The interesting report that 10 μg of capsaicin administered twice intraventricularly selectively depletes hypothalamic β-endorphin (201) suggests that there are some very sensitive cells in this brain region. It is possible that these β-endorphin-containing cells are similar to primary afferent neurons. They may also be the source of the potent central thermoregulatory effects of capsaicin (see section X A).

B. Peptide Specificity

Capsaicin is a potent depletor of sensory neuron SP. In adult animals, systemic or local injection of capsaicin may also produce small, short-lived reductions in CCK and somatostatin levels which may be highly dose dependent. Capsaicin does not seem to affect VIP or bombesin, two additional neuropeptides found in primary afferents. The reductions in acetylcholinesterase staining and acid phosphatase activity seen in adult animals presumably indicate the association of these enzymes with capsaicin-sensitive sensory neurons. Given that doses of capsaicin ranging from 50 mg/kg to hundreds of mg/kg have been used to treat adult animals, it is up to each investigator to choose an appropriate dose and to determine exactly what sensory neuropeptides are being depleted under the experimental conditions used.

In neonatal animals, the sensory neuropeptides other than SP appear to be more susceptible to depletion by capsaicin than in adult animals. SP, CCK, somatostatin, and VIP are all consistently depleted by moderate doses of capsaicin in neonates. Since unmyelinated C-fibers are affected before myelinated A-fibers and the capsaicin ED_{50} for SP and somatostatin depletion is 10 to 20 mg/kg in neonatal animals (184), it is probably best to avoid doses such as 750 mg/kg (201) or 950 mg/kg (134) in neonatal animals. Most investigations in neonatal rats have used the dose of 50 mg/kg, and this dosage would seem to be an appropriate maximum (184).

It is also important to consider that most of the work with capsaicin has been carried out in rats. It would be careless to assume that there are qualitatively and quantitatively similar actions in all other species. It is incumbent on investigators using other species to determine the profile of the actions of capsaicin.

C. Sensory Specificity

One of the more controversial aspects of the neuropharmacological actions of capsaicin has been the specificity of these actions for certain sensory modalities. In rats, capsaicin clearly induces a marked loss of sensitivity to nociceptive chemical stimuli. There also is a reduction in sensitivity to nociceptive heat, but it is far from complete in this species. The tail-flick test is apparently more resistant to capsaicin-induced changes than is the hot-plate test. The reason for this difference is unknown, but it may simply reflect that the skin of the paws is innervated in a manner different from the skin of the tail.

While many investigations have been unable to detect an effect of capsaicin on nociceptive mechanical stimuli, some studies in rats treated as neonates or with capsaicin applied directly to nerve tracts have found a reduction in sensitivity to nociceptive pressure (see sections III, B and C, and VII). Nagy and vander Kooy (185) concluded, in fact, that mechanical sensitivity was the most susceptible of all sensory modalities to capsaicin with significant reductions after neonatal doses of capsaicin as low as 5 mg/kg. They also observed substantial variability in animal responsiveness to capsaicin. It seems reasonable to conclude that in rats capsaicin does produce some reduction in nociceptive mechanical sensitivity. The detection of this alteration may be complicated by magnitude, animal variability, and the methodology used to measure the change. We have also noted that in neonatal animals the contents of a s.c. injection are much more likely to leak out the injection hole in the skin than in adult animals. This is presumably due to thinner skin and a smaller subcutaneous space volume in the neonates. It is possible that this factor has also complicated studies in neonatal rats.

In guinea pigs treated with capsaicin, sensitivity to all thermal stimuli and to nociceptive chemical stimuli is essentially abolished. Pressure sensitivity does not appear to be altered, but this has not been studied in great detail. It is safe to assume that, in guinea pigs, nociceptive pressure sensitivity is affected much less than thermal and chemical sensitivity. In this species, sensitivity to cold also appears to be unaffected, whereas in rats some reports of altered thermoregulatory responses to cold have appeared (see section X A). There have also been infrequent observations of reduced responsiveness to pinprick and to corneal touch after capsaicin treatment, but the significance of these cannot be determined. It is possible that they are linked to the aforementioned variable effects of capsaicin on mechanical modalities.

The lack of effect of capsaicin applied directly to nerves on mechanical sensitivity in rats is especially difficult to reconcile with the results that have been obtained with systemic treatment of animals. These studies with direct application have not consistently revealed a reduction in nociceptive mechanical sensitivity. Possible reasons for this include time course considerations, differential proportions of capsaicin-sensitive and capsaicin-insensitive mechanoceptive afferents in different nerves, and a relative sensitivity of different modalities on direct nerve application of capsaicin that is different from that seen with systemic treatment with the compound. Additional research is needed to clarify these aspects of the neuropharmacology of capsaicin.

Unfortunately, it is not possible to say with certainty what neuropeptides are associated with any particular sensory modality based on the data obtained with capsaicin. It is likely that SP is intimately involved in some manner with those neurons which mediate thermal sen-

sation in both rats and guinea pigs. The nature of this involvement cannot be precisely defined at present. It is possible that there are species differences and that the same capsaicin-sensitive SP-containing neurons may mediate other sensory modalities as well (i.e., polymodal). It is also possible that the other peptides affected by capsaicin in neonatal rats are important in some of the sensory changes that occur. Capsaicin has allowed the conclusion that sensory neuron function and neuropeptide levels are linked in some manner, but it has not yet provided a complete picture of this link (see section XII F).

D. Effects in Neonates versus Adults

The effects of capsaicin on sensory neurons are clearly more profound in neonates than in adult animals. Aside from SP, CCK, VIP, and somatostatin all being markedly depleted, the neurotoxic effects of capsaicin on C-fibers and A-fibers are lethal and permanent when neonates are treated with the compound. When adult animals are treated, there does not seem to be as widespread an effect on sensory neuropeptides nor is it clear that there is a permanent degeneration of primary afferents. This latter point is important and is in need of additional investigation. Furthermore, in rats treated with capsaicin as adults, the SP depletion and sensory deficits that ensue are sometimes reversible. This does not seem to be the case in animals treated while neonates, where the effects are lifelong. Compared to adult rats, treatment of adult guinea pigs with capsaicin produces SP depletion and sensory deficits that are much longer lasting. This may indicate a fundamental difference between these species in primary afferent innervation. Much additional work is needed to clarify the potential role of neuronal degeneration in capsaicin's actions in adult animals and to ascertain that the compound has similar neurotoxic actions in neonates of all species.

Since capsaicin definitely has effects on sensory neurons in adult animals when it is applied directly onto central roots and terminals, onto spinal ganglia cell bodies, or onto peripheral processes, the milder neurotoxicity compared to that in neonates is not a consequence of the resistance of mature sensory neurons to the neuropharmacological effects of the compound. Rather, the difference may be linked to the relative dependence of certain mature and immature sensory neurons on trophic factors the actions of which may be secondarily altered by capsaicin (see section XII F).

E. Effects in Various Species

Capsaicin produces sensory alterations and depletes SP in rats, guinea pigs, and mice. Sensory nerve trunks from these species and from rabbits, dogs, and cats are sensitive to the neuropharmacological actions of capsaicin applied directly to the nerve. The dose-response characteristics of peptide depletion and desensitization induced by capsaicin have only been carefully investigated in rats treated as neonates and in adult guinea pigs. The latter appear to be somewhat more sensitive to the sensory deficits produced by capsaicin than adult rats or mice. The reduction in heat sensitivity induced by capsaicin is more marked in adult guinea pigs than in rats or mice treated as neonates or as adults (see section XII C). It has yet to be determined if capsaicin administered to neonates of species other than rats produces the widespread depletion of all primary afferent neuropeptides. It is possible that there are species differences in the sensitivity of A-fibers to the effects of capsaicin (see refs. 184 and 220), but this has not been investigated in sufficient detail. It seems safe to assume that capsaicin has acute and desensitizing effects on sensory C-fibers in most mammalian species. It is dangerous to assume, however, that these effects occur at the same doses, over the same time course, to the same magnitude, or through SP-containing sensory neurons in all species.

F. Mechanism of Action

The following is an attempt to construct a unifying hypothesis of the mechanism(s) of action of capsaicin on sensory neurons. A particular population of sensory neurons with unmyelinated C-fiber-type processes (and presumably some sensory neurons with A-type processes) possesses a unique sensitivity to capsaicin. These neurons occur as primary afferents in spinal ganglia, as afferents to certain brainstem nuclei, and possibly in the hypothalamus. The capsaicin-sensitive neurons, especially those with C-fibers, contain SP and may be polymodal nociceptive detectors presumably with free nerve endings peripherally. There are probably some sensory neurons containing SP that are capsaicin resistant, and some of the primary afferents altered by capsaicin may contain neuropeptides other than SP.

The unique sensitivity of the capsaicin-sensitive neurons is most likely due to the functional makeup of their plasma membrane structure. The uniqueness may also be linked to the free endings of these cells. The occurrence of this membrane structure is such that the free ending (and perhaps all of the peripheral process) is most susceptible to capsaicin followed by the central terminal and then by the remainder of the cell. Capsaicin is a relatively lipophilic molecule, and its dissolution in lipid structures probably contributes to its action on sensory neuron membranes. The compound presumably affects membrane fluidity and/or ion permeability of the plasma membrane. There is a resulting change in the permeability such that Ca^{2+} and possibly other cations stream across the membrane. At the free ending, this results in initiation of an impulse, the release of SP, and ultimately the acute painful, burning sensation associated with capsaicin. When applied to the central process, capsaicin also induces SP release (and possibly other neuropeptides from different populations of capsaicin-sensitive cells). The SP release is mediated through a normal vesicular mechanism since it is Ca^{2+} dependent. The fact

that K^+-induced depolarization is still able to evoke peptide release after tachyphylaxis to capsaicin has developed suggests that capsaicin is not able to induce complete release. This may be important in terms of the mechanism whereby capsaicin is able to produce a marked SP depletion (vide infra). Capsaicin also produces these membrane effects along the peripheral process, but here the cell may be more resistant to the compound, and depolarization apparently cannot be initiated by capsaicin.

In addition to these acute effects of capsaicin on sensitive sensory neurons, the compound also results in a long-lasting plasma membrane perturbation that locks the membrane in a depolarized state or otherwise prevents subsequent depolarizations from being initiated and propagated. It is this membrane effect that produces the sensory deficits in the treated animals. There is also a disruptive effect on intracellular organelles in the peripheral process (and possibly in other locations in the cell). This disruptive effect could occur through a mechanism similar to that in the plasma membrane, or it could be secondary to the capsaicin-induced alteration in plasma membrane ionic fluxes. This intriguing aspect of capsaicin's actions deserves further attention. While the plasma membrane may remain morphologically intact, the intracellular organelles that are altered lose their integrity and begin to disintegrate (see ref. 203). Among the affected structures is the microtubule system, and its destruction results in a loss of anterograde (76) and retrograde (172) axoplasmic transport. The long duration of capsaicin's actions on sensory neurons is apparently not due to a low rate of clearance from the body (218). It has been postulated that the compound binds covalently to intracellular macromolecules (171).

There are probably a number of biochemical consequences of the disruption of the sensory neuron microtubule system. One of these is the inability to replenish the partially depleted peripheral and central SP stores. In addition, the remaining vesicular SP may also be lost through a capsaicin-induced disruption of the storage vesicles that may be similar to or related to the disruption of other organelles. Another consequence is probably the loss of the affected cell's ability to transport in an anterograde manner molecules necessary for repair of the capsaicin-induced membrane perturbations and damage. It is this loss which is most responsible for the long-lasting effects of capsaicin. The reversibility of capsaicin's actions that has been reported presumably reflects a lesser amount of damage to the repair system such that it is able to still function.

Yet another consequence of the damage to the microtubule system is interference in the retrograde transport of trophic factors in the capsaicin-sensitive primary afferents. One of these molecules is nerve growth factor (NGF) on which SP-containing sensory neurons are highly dependent for development and maintenance (see refs. 87 and 134). Treatment of adult guinea pigs with capsaicin produces a marked reduction in the amount of locally injected iodinated NGF that is transported back to the dorsal root ganglia (172). This reduction in transport of a critical trophic factor brings about a decrease in SP production in the cell body which further hinders the cell's ability to replenish the depleted peptide in its more distal portions. A similar phenomenon probably occurs in adult and neonatal rats treated with capsaicin, although in this species, the damage to the transport system or the proportion of NGF-dependent afferents that are also capsaicin sensitive may be less than in guinea pigs (see refs. 124 and 166). It is likely that there are trophic factors other than NGF also affected by capsaicin and that the processing of SP is not the only biochemical process to be affected by capsaicin's actions on trophic factors. It is not clear if capsaicin produces actual cell death in adult animals, and it is possible that only severe atrophy results in some cases without disappearance of the cell body. The role of NGF in the biochemical actions of capsaicin is consistent with growing evidence that this trophic factor is important to the well-being of some mature sensory neurons (125).

In rats treated while neonates, the results of capsaicin's actions on the above systems are presumably more deleterious to the sensory cells, and more types of sensory cells are apparently susceptible to the compound than in adults. In this case, there is definite loss of peripheral and central cell processes, and there may also be a loss of cell bodies in sensory ganglia. It is likely that an effect of capsaicin on NGF is involved in neonates (199), but since perturbations of only the NGF system in neonates do not necessarily result in cell death, it is likely that additional factors are important in newborn animals. These may include effects of capsaicin on other trophic molecules or actions of the compound that are more drastic than a simple reduction of NGF availability. In view of the more widespread peptide depletion and the irreversibility of capsaicin's effects when administered to neonates, it is likely that different populations of sensory neurons lose some or all of their dependence on certain trophic factors as they mature (see ref. 125).

It is not clear if capsaicin produces its effects by more than one mechanism of action. Based on limited and very indirect structure-activity studies, some investigators have suggested that the initial excitation and SP release in primary afferents are one action and that the subsequent desensitization and sensory deficits produced by the compound are a separate action. This conclusion, however, is hampered by the fact that such studies have used few compounds, failed to consider dose-response relationships, ignored likely differences between adult and neonatal animal sensitivity, and/or overlooked potential differences between in vivo and in vitro use of the compounds. The unequivocal resolution of the number of mechanisms involved in capsaicin's actions on sensory

cells will require elucidation of the biochemical bases of the compound's effect on neuronal membranes.

G. Capsaicin as a Pharmacological Tool

The sensory neuron specificity of capsaicin makes the compound an extremely valuable tool for investigating the roles of sensory neurons in biological function. When the appropriate doses of the compound are used, its actions seem to be limited to primary afferents with C-type fibers. Most of these afferents contain SP and are presumably polymodal nociceptive neurons. The results obtained with capsaicin indicate that these neurons are involved with certain sensory modalities and to varying degrees in different species. Capsaicin provides the opportunity to investigate the link between SP (and possibly other neurochemicals) and these sensory functions.

It is clear from the results with capsaicin that there are sensitive afferents throughout the periphery. These neurons may be involved in monitoring the chemical milieu in the innervated regions and in detecting and signaling functional abnormalities to the CNS. This monitoring role probably extends from the skin (e.g., pain and inflammation) to the visceral organs (e.g., pain from mucosal ulceration or intestinal muscle dysfunction). That most or all of these capsaicin-sensitive afferents are naturally chemosensitive could indicate that capsaicin is chemically similar to a naturally occurring pain-producing substance. Investigation of this possibility may provide intriguing information on the origin of pain in compromised tissues.

As indicated by the large number of neuroanatomical studies that have made use of capsaicin, the compound is an indispensible tool for determining the extent of sensory neuron innervation in the periphery. The activation and functional deficits induced in these neurons allow the determination of the existence of novel biological reflexes. Discovery of the mechanism of capsaicin's actions on primary afferents may provide important clues about the physicochemical character of the membranes of these unique sensing cells.

The probable involvement of trophic factors in the mechanism of the neuropharmacological effects of capsaicin provides an opportunity for new approaches to the study of these factors. Work with capsaicin has already strengthened the growing evidence that trophic factors are more important than previously thought in the maintenance (and possibly survival) of mature sensory neurons. Capsaicin promises to be of great value in the further investigation of this importance. The compound may lead to the discovery of new trophic factors of critical importance to neuronal survival.

H. Therapeutic Implications

The possibility that capsaicin might mimic an endogenous pain-producing substance could have important implications for human therapeutics. An antagonist to capsaicin might be a novel analgesic agent at peripheral sites. In addition, a capsaicin analogue that does not produce pain, but retains the ability to desensitize C-fiber-type afferents, would be potentially useful as an analgesic agent devoid of substantial effects in CNS neurons. Such a compound might also have applications as an antiinflammatory agent or an antiasthmatic agent. The presence of capsaicin-sensitive SP-containing neurons in many tissues may signal new vistas toward control of pain in these locations (e.g., gastrointestinal tract, heart, cerebral vasculature, and skeletal muscle). This control might be approached either through the use of capsaicin analogues or through the continued development of SP antagonists. Future research in this area will undoubtedly shed light on the feasibility of these novel therapeutic interventions.

The specificity of capsaicin for certain sensory modalities could raise the possibility that an analogue of the compound would produce a specific functional denervation of localized tissues when applied directly to dorsal root ganglia cells or to dorsal roots. If the capsaicin-sensitive cells in humans mediate chronic pain sensation, then the analogue might be able to produce alleviation of intractable pain without disrupting other sensory functions in the affected area. This possibility is worthy of consideration, and it would be of extreme interest to know the nature and duration of capsaicin's effects when the compound is applied directly to ganglia or roots.

The syndrome of sensory deficits produced by capsaicin in animals resembles in some ways genetic disorders in humans and in animals. In familial dysautonomia (Riley-Day syndrome), there is a loss of certain sensory and autonomic neuronal cells, and SP is markedly depleted in the spinal substantia gelatinosa (205). Among other signs, patients with dysautonomia frequently suffer from severe sensory deficits and from peripheral epithelial lesions (see section II C). Nerve growth factor from fibroblasts of patients with the disease has only a small fraction of the amount of biological activity as that from controls, suggesting the involvement of the factor in the neuronal loss (223) (see section XII F). A strain of mutant rats known as mutilated foot has reduced numbers of sensory ganglia cells and suffers from ataxia, reduced sensitivity to certain types of pain, and self-mutilation. These animals also exhibit a reduced amount of SP immunoreactivity in the spinal dorsal horn (221). Thus, capsaicin may be useful in providing insight into these conditions and toward developing therapeutic approaches for the treatment of familial dysautonomia and other human sensory neuropathies. As pointed out by Hanley (84), the epithelial lesions seen in animals with capsaicin treatment (see section II C), as well as recent evidence of a mitogenic action of SP, suggest that the neuropeptide may have a trophic role in some types of cells. Additional work with capsaicin could provide information about this possibility. Such information would

be of value in understanding epidermal (i.e., psoriasis) and other hyperproliferative disorders.

Supplemental Information

In the period since 1983, the number of investigations dealing with capsaicin has declined substantially. However, the compound continues to be a unique pharmacological agent for probing the roles of C-fibers in biological function. Results from several international groups, most notably the University of Graz Institute for Experimental and Clinical Pharmacology headed by Fred Lembeck, have provided important new information. It is clear that capsaicin is capable of depleting additional neuropeptides from primary afferents. These include corticotropin releasing factor (CRF), calcitonin gene-related peptide (CGRP), galanin, and substance K (neurokinin A). Some of the putative SP antagonists that have been used may not be specific for SP and may have pharmacological effects unrelated to peptide antagonism. It has been shown that capsaicin can alter the micturition reflex and some endocrine functions. Capsaicin clearly also has some acute effects which are not mediated by primary afferents. It will be extremely interesting as the link between each of the various capsaicin-sensitive peptides and specific sensory functions is unraveled. The unique effects of capsaicin on certain biological membranes continue to offer important clues to membrane function. Selected supplemental references highlighting these more recent findings are listed after the reference section.

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