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Review

Effects of capsaicin on visceral smooth muscle: a valuable tool for sensory neurotransmitter identification

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Abstract

Studying the visceral effects of the sensory stimulant capsaicin is a useful and relatively simple tool of neurotransmitter identification and has been used for this purpose for approximately 25 years in the authors' and other laboratories. We believe that conclusions drawn from experiments on visceral preparations may have an impact on studies dealing with the central endings of primary afferent neurons, i.e. research on nociception at the spinal level. The present review concentrates on the effects of capsaicin—through the transient receptor potential vanilloid receptor type 1 (TRPV1) receptor—on innervated gastrointestinal, respiratory and genitourinary smooth muscle preparations. Tachykinins and calcitonin gene-related peptide (CGRP) are the most widely accepted transmitters to mediate "local efferent" effects of capsaicin-sensitive nerves in tissues taken from animals. Studies more and more frequently indicate a supra-additive interaction of various types of tachykinin receptors (tachykinin NK_1, NK_2, NK_3 receptors) in the excitatory effects of capsaicin. There is also evidence for a mediating role of ATP, acting on P_2 purinoceptors. Non-specific inhibitory actions of capsaicin-like drugs have to be taken into consideration while designing experiments with these drugs. Results obtained on human tissues may be sharply different from those of animal preparations. Capsaicin potently inhibits tone and movements of human intestinal preparations, an effect mediated by nitric oxide (NO) and/or vasoactive intestinal polypeptide.

Keywords: Capsaicin; Sensory neurotransmitter; Visceral smooth muscle; Gastrointestinal tract; Bronchial smooth muscle; Genitourinary tract

Contents

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1. Introduction

Capsaicin, the substance responsible for the pungent (hot) character in various chilli peppers, excites specific, ion channel-bound receptors, (capsaicin) vanilloid VR1 receptors or transient receptor potential vanilloid receptor type 1 (TRPV1) receptors (for reviews see Caterina and Julius, 2001; Clapham et al., 2003), located on sensory nerve endings. Upon binding of capsaicin-like drugs to these ion channel receptors the channels open and the nerve ending (varicosity) depolarizes. The burning sensation and redness (erythema) caused by capsaicin on mucous membranes is known to almost everybody. Some people even assume that capsaicin has objective and subjective actions on the gastrointestinal tract, but only a few (mostly scientists) know that it can exert effects in, e.g., the urinary bladder. In this review we would like to demonstrate that capsaicin-induced actions in inner organs have important implications from a neuroscientist's point of view. Because of space limitations, many interesting aspects of the topic (e.g. vascular smooth muscle, heart muscle, smooth muscles of the eye) cannot be dealt with in the present work.

Studying the visceral effects of capsaicin has been used as a tool for identifying sensory neurotransmitters for approximately 25 years (see Barthó and Holzer, 1985, Holzer and Barthó, 1996 for reviews). The first systematic studies were carried out in Hungary, on the small intestine of the guinea-pig and rabbit and in the guinea-pig taenia caeci (Barthó and Szolcsányi, 1978, 1980; Szolcsányi and Barthó, 1978, 1979). It has been established that capsaicin-sensitive neurons mediate the motor (excitatory) actions of this drug. Capsaicin treatment in vitro strongly inhibits the motor effects of electrical stimulation of sensory nerves (Szolcsányi and Barthó, 1978, 1979; Barthó and Szolcsányi, 1980). This kind of in vitro desensitization to capsaicin has become a tool widely used in studying the role of capsaicin-sensitive nerves in isolated organs. Thus, we took the visceral effects of capsaicin a phenomenon analogous with the antidromic vasodilatation and neurogenic plasma extravasation evoked in the skin and mucous membranes. As to the present state of knowledge calcitonin gene-related peptide (CGRP) and substance P are the two most important sensory neurotransmitters that mediate these two vascular reactions (for reviews see Chahl, 1988; Holzer, 1991; Geppetti and Holzer, 1996). Soon after that, bronchial (Lundberg and Saria, 1982; Szolcsányi and Barthó, 1982; Lundberg et al., 1983b), cardiac (for review see Lundberg et al., 1992), and genito-urinary (Lundberg et al., 1984; Holzer-Petsche and Lembeck, 1984; see Maggi, 1995) effects of capsaicin were analyzed with special reference to the possible transmitters involved. Some of these studies utilized earlier descriptions of excitatory and tachyphylactic effects of capsaicin on the guinea-pig isolated ileum, trachea and auricles (Toh et al., 1955; Molnár et al., 1969a,b).

Neurogenic plasma extravasation has been the subject of highly conceptful work in the "classical" period of capsaicin research (Jancsó et al., 1967, 1968), many ideas of which have also facilitated the interpretation of the visceral effects of capsaicin. Among others, topical or systemic capsaicin desensitization (Jancsó and Jancsó-Gábor, 1959) has been found to abolish not only the effects of a subsequent application of the drug, but also those of sensory nerve stimulation. It has also been hypothesized that capsaicin and related compounds act on specific receptor molecules, located at sensory nerve terminals (Szolcsányi and Jancsó-Gábor, 1976). Subsequently, other desensitization techniques (neonatal, perineural) have been developed (Jancsó et al., 1977; Jancsó and Such, 1983).

Another seminal observation was that, in the skin, release of bioactive substances by capsaicin is not dependent on voltage-sensitive Na^+ channels (Jancsó et al., 1968), a feature that is now easy to interpret on the basis that TRPV1 is a voltage-independent cation channel receptor which opens upon agonist binding, leading to depolarization of the nerve ending/varicosity (see Caterina and Julius, 2001). In fact, excitatory actions of capsaicin on bronchial, cardiac, or genitourinary preparations in vitro are resistant to tetrodotoxin or to blockade of neuronal voltage-sensitive Ca^{2+} channels (see Maggi, 1995), but are prevented by chronic (degenerative) denervation. The gastrointestinal tract proved to be a more complicated matter (see below).

Quite naturally, the question arises as to which transmitter(s) participate in the mediation of capsaicin's visceral effects? In this latter context, visceral actions of capsaicin might offer a relatively easy way to the identification of at least some of the biologically active substances released from the endings (varicosities) of capsaicin-sensitive afferent neurons. These substances, according to Dale's principle, are probably also released from central endings of sensory neurons, i.e. in the dorsal spinal cord, where they could play a role in the mediation/modulation of nociception (see below). The present paper mainly concentrates on visceral effects of capsaicin as a tool to identify sensory neurotransmitters.

2. Search for mediators of visceral effects of capsaicin

Of the well-known criteria of neurotransmitter identification, probably the most convincing one is still "identity of antagonism", though others (presence of the neurotransmitter candidate in the tissue, release, and "identity of action" ["mimicry"]) are also important. In other words, a specific neurotransmitter antagonist should inhibit not only the effect of the exogenously applied transmitter (exogenous agonist), but also similar responses to the activation of the nerves in question. Alternative ways of blockade are the use of genetically modified animals, immuno-neutralization, antisense techniques, enzymatic degradation, etc. A careful and judicious use of neurotransmitter antagonists in such studies is essential and sometimes lacking. Also the difficulties in publishing negative results, as opposed to positive ones, sometimes bias public impression as to the importance of one mediator or another. Moreover, capsaicin-sensitive neurons seem to release several biologically active substances (see among others Holzer, 1988, 1991; Kuraishi et al., 1991; Maggi, 1995; Geppetti and Holzer, 1996; Holzer and Holzer-Petsche, 1997; Zhang et al., 1997; Barthó et al., 1999a; Lever et al., 2001), whose contribution to a given response can be different and non-additive (see e.g. Barthó et al., 1999b, 2000; Patacchini et al., 1999) or even qualitatively different (such as that of tachykinins and CGRP, see Maggi, 1995; Holzer and Barthó, 1996).

There is ample morphological and neurochemical evidence for the presence of the neuropeptides substance P, neurokinin A and CGRP in, and release from, primary afferent neurons (see among others Maggi, 1995; Geppetti and Holzer, 1996). Many other putative transmitter substances may exist in these neurons and even be released from them, but they usually fail to fulfill all the necessary neurotransmitter criteria. Here again, space does not allow us to give a detailed survey on morphological data (indicating the presence of sensory neurotransmitters in viscera), neurochemical work (release) or even the effects of exogenously applied transmitter substances ("mimicry"), but see among others Maggi (1995) and Geppetti and Holzer (1996) for reviews. Here we concentrated on the criterion of "identity of antagonism", a point largely brought by pharmacologists to neuroscience and hormone research.

3. Effects of capsaicin on gastrointestinal preparations

3.1. Intestinal preparations

3.1.1. Guinea-pig

The presence and involvement of intrinsic enteric neurons colours the scene of intestinal effects of capsaicin. Most of the substance P-like immunoreactivity (LI) (but less of the CGRP-LI) in the gastrointestinal tract is insensitive to capsaicin desensitization (see Dockray, 1994; Maggi, 1995). Based on a detailed pharmacological analysis of the action of capsaicin and of mesenteric paravascular nerve stimulation in the guinea-pig *ileum*, it has been suggested that capsaicin-sensitive sensory nerve fibres reach the intestine *via* the mesentery. However, the biologically active substances released from them largely act by stimulating intrinsic myenteric neurons that release acetylcholine and, to a lesser extent, tachykinins as final mediators (schematic representations can be found with Barthó et al., 1992, 1994, 1999a). Release of acetylcholine from the ileal longitudinal muscle-myenteric plexus strip by capsaicin has been confirmed by neurochemical methods (Barthó and Vizi, 1985). Similar results have been obtained in the taenia caeci (Szolcsanyi and Barthó, 1979) of the guinea-pig, whereas in the ileum of (young) rabbits (Barthó and Szolcsanyi, 1980) periarterial nerve stimulation elicits an excitatory response that has a nicotinic receptor-mediated (probably parasympathetic) and a capsaicin-sensitive component. Capsaicin contracts these preparations (Barthó and Szolcsanyi, 1978, 1980). A capsaicin-sensitive excitatory action of mesenteric nerve stimulation on the intestinal longitudinal muscle has been confirmed by others; electrophysiological studies also indicated a Ca^{2+}-dependent slow depolarizing effect of mesenteric stimulation on myenteric plexus neurons (Takaki and Nakayama, 1988). Mesenteric stimulation elicits an excitatory response on the circular muscle as well (Takaki et al., 1990).

The inference made from pharmacological studies, i.e. that TRPV1 receptors in the gut are located predominantly on extrinsic afferent neurons, has been proved by immunohistochemical studies in the rat, guinea-pig and mouse gastrointestinal tract in which numerous TRPV1-positive nerve fibres occur in the musculature, enteric nerve plexuses and mucosa (Patterson et al., 2003; Ward et al., 2003). Since in these studies enteric neurons were not found to stain for TRPV1 it follows that the TRPV1-positive nerve fibres in the intestine represent processes of spinal afferents and, in the stomach, of some vagal afferents. It remains to be elucidated whether the TRPV1-like immunoreactivity which other investigators have seen in guinea-pig, porcine and human enteric neurons (Poonyachoti et al., 2002; Anavi-Goffer and Coutts, 2003; Chan et al., 2003) represents

authentic TRPV1 or a closely related member of the TRPV channel family (Clapham et al., 2003). The functional significance of these findings also remains to be elucidated.

The nature of the neurotransmitters that activate intrinsic myenteric neurons in the guinea-pig small intestine in the course of the effect of capsaicin is still a matter of investigation. Logically, the role of tachykinins has been studied and there was evidence for a mediating role of these substances (Chahl, 1982; Barthó et al., 1982; Grbovic and Radmanovic, 1983; Donnerer et al., 1984; see among others Barthó and Holzer, 1985; Maggi, 1995; Holzer and Barthó, 1996). It is, however, difficult to decide if tachykinins originate from sensory or myenteric neurons (cf. Barthó et al., 1982, 1994). A lot of myenteric neurons release tachykinins, partly together with acetylcholine (see Furness and Costa, 1987; Taylor and Bywater, 1989; Dockray, 1994) which act as neuroeffector transmitters to the longitudinal muscle. But as tachykinins (in particular substance P) can stimulate myenteric neurons (see refs. above) they can also mediate the neurogenic excitatory effects of capsaicin-sensitive nerve stimulation. In fact, some tachykinin antagonists (mainly acting at tachykinin NK_1 receptors) have been shown to diminish the capsaicin-induced excitatory response (Maggi et al., 1988b; Legat et al., 1992; Croci et al., 1995; Venkova et al., 2002). In some other studies, however, tachykinin NK_1 receptor antagonists (SR 140 333 or GR 82 334) alone failed to reduce the effect of capsaicin (Barthó et al., 1999b, 2000).

To the authors' surprise, an inhibition of tachykinin NK_3 receptors (a receptor type whose stimulation causes an exclusively neuronal excitatory effect) failed to affect the contractile action of capsaicin (Patacchini et al., 1995; Barthó et al., 1999b). The supra-additive inhibition of the response by a combination of a tachykinin NK_1 and NK_3 receptor antagonists can provide some key to the tachykinin mediation of the activation of cholinergic neurons. The TRPV1 receptor-mediated acetylcholine releasing effect of the endocannabinoid and endogenous TRPV1 receptor ligand anandamide was also diminished by such a combination of tachykinin receptor antagonists (Mang et al., 2001). It should be noted, however, that the inhibition provided by the combined antagonism at tachykinin NK_1 *plus* NK_3 receptors on the capsaicin effect is only partial (Barthó et al., 1999b, 2000).

Efforts have been made to approach the question further (Table 1). It seems that ATP co-mediates with substance P the effect of capsaicin, again in a supra-additive manner. Although ATP release from sensory neurons has long been known, we have no direct evidence that the ATP encountered with the intestinal response originates from sensory nerves (Barthó et al., 1999a, 2000).

Capsaicin also exerts not only an excitatory but also an *inhibitory* action on the longitudinal muscle of the guinea-pig ileum. This effect is insensitive to tetrodotoxin, but is strongly diminished by degenerative mesenteric denervation. There is good evidence that CGRP mediates the

Table 1
Effects of drugs on the capsaicin-induced contraction (A) and the excitatory response to mesenteric nerve stimulation (B) in the longitudinal muscle of the guinea-pig ileum

Drug	Change compared to appropriate control	Ref.
(A) Contraction in response to capsaicin		
Untreated preparations		
CGRP tachyphylaxis plus hCGRP8-37[a]	–	a
CCK receptor antagonist[b]	–	b
NO synthase inhibitor[c]	–	c
VIP/PACAP[d] receptor antagonist	–	c
P_2 purinoceptor antagonist[e]	–	c, d
Endothelin[d] receptor antagonists	–	e
Tachyphylaxis to CRF[d]	–	e
Tachyphylaxis to muscimol (GABA receptor agonist)[d]	–	NP
Bicuculline ($GABA_A$ receptor antagonist)	–	NP
Tachyphylaxis to neuropeptide gamma	–	NP
Tachykinin NK_1 receptor antagonists	–	f
Tachykinin NK_2 receptor antagonist	–	f
Tachykinin NK_3 receptor antagonists	Slight decrease	f
Tachykinin NK_1 + NK_3 receptor antagonists	Decrease	e, f
Preparations pretreated with tachykinin NK_1 plus NK_3 receptor antagonists		
CGRP tachyphylaxis plus hCGRP8-37	–	NP
NO synthase inhibitor	–	c
VIP/PACAP receptor antagonist	–	c
P_2 purinoceptor antagonist	Decrease	c, d
Endothelin receptor antagonists	–	e
Tachyphylaxis to CRF	–	e
Peptidase inhibitors	Increase	NP
Tachyphylaxis to muscimol	–	NP
(B) Contraction in response to mesenteric nerve stimulation		
Tachykinin NK_1 receptor antagonist	Slight inhibition	NP
Tachykinin NK_3 receptor antagonist	Slight inhibition	NP
NK_1 plus NK_3 receptor antagonists	Strong inhibition	NP

a—Barthó et al., 1993; b—Barthó et al., 1987a; c—Barthó et al., 2000; d—Barthó et al., 1999a; e—Lázár et al., 2003; f—Barthó et al., 1999b.
NP—unpublished observation.

[a] The CGRP receptor antagonist hCGRP8-37 is ineffective on the stimulatory effect of CGRP on myenteric neurons. This latter fulfills the criterion of "identity of action".

[b] Cholecystokinin (CCK)-like peptides contract the ileum by stimulating myenteric neurons (for ref. see Dockray, 1994), thus, they "mimic" the action of capsaicin. CCK-like biological activity is released, however, by mesenteric nerve stimulation, in a capsaicin-insensitive manner (Barthó, 1989).

[c] Nitric oxide (NO) is primarily an inhibitory neurotransmitter. Yet, in the guinea-pig ileum it can cause cholinergic contraction (see ref. c) and an elevation of basal acetylcholine release (Hebeiss and Kilbinger, 1996); since NO may appear in sensory neurons, its mediating role was considered in the capsaicin-induced excitatory response.

[d] "Mimicry" is present (these putative neurotransmitters release acetylcholine from the myenteric plexus); there are some data for their presence in sensory neurons (see c, e).

[e] ATP can release acetylcholine from the myenteric plexus (Barthó's team, in preparation).

[f] Perivascular nerve stimulation at 5 or 10 Hz for 10 s in the presence of guanethidine and a CCK receptor antagonist (see the tachykinin NK_1 and NK_3 receptor antagonists used were SR 140 333 and SR 142 801, respectively, both at 2×10^{-7} M. "Weak inhibition" was detected at 5 Hz only (n=6 for each group).

relaxant effect (Barthó et al., 1987b, 1991; Maggi et al., 1988b). If we assume that CGRP is released from capsaicin-sensitive neurons, the lack of inhibition by tetrodotoxin indicates that CGRP exerts its inhibition on the smooth muscle directly (see Barthó et al., 1992). A further type of smooth muscle-relaxant action of capsaicin has been described in this and other systems (Maggi et al., 1987a; Barthó et al., 1987b; Barthó and Holzer, 1995). This type of response appears at relatively high concentrations (below 10^{-5} to 10^{-4} M), does not undergo tachyphylaxis and most probably reflects non-specific depression of smooth muscle contractility. An underlying mechanism may be an inhibition of voltage-gated Ca^{2+} channels (Sim et al., 2001). The non-specific smooth muscle effect of capsaicin, together with the relatively long duration of action of the CGRP released, might account for some "false positive" findings concerning the possible involvement of capsaicin-sensitive nerves in excitatory responses in smooth muscle preparations, i.e. that capsaicin pretreatment (erroneously considered as "desensitization", but actually only a "spasmolytic" effect) inhibits the response of some nerve stimulation. It should be remembered that drugs structurally related to capsaicin, e.g. the TRPV1 receptor antagonist capsazepine, are also able to cause non-specific smooth muscle relaxation (Nocerino et al., 2002).

A specific inhibitory effect of capsaicin has been detected with different methods in the circular muscle of the guinea-pig ileum. Here again, it is suggested that CGRP plays a mediating role (Takaki et al., 1990; Barthó, 1991; Barthó et al., 1991). Low concentrations of capsaicin exert a transient excitatory and specific inhibitory effect on the pressure-evoked peristaltic reflex (a process that mainly involves the circular muscle of the ileum). The CGRP antagonist hCGRP(8-37) or inhibition of nitric oxide (NO) synthase diminishes the inhibitory effect. High capsaicin concentrations cause non-specific inhibition (Barthó and Holzer, 1995).

Several motor effects of capsaicin on the guinea-pig distal *colon* longitudinal muscle have been described (Maggi et al., 1987a). A specific contractile action involves myenteric cholinergic neurons. A more sustained, apparently specific inhibitory effect is partly inhibited by tetrodotoxin, probably indicating an involvement of intrinsic enteric neurons or/and an "axon reflex" arrangement in capsaicin-sensitive extrinsic nerves. The circular muscle of the guinea-pig proximal colon also shows relaxation in response to capsaicin, which seems partly mediated by CGRP (Maggi et al., 1996).

3.1.2. Other animal species

Capsaicin relaxes the *rat duodenum* longitudinal muscle by a neural (partly tetrodotoxin-sensitive) mechanism (Maggi et al., 1986). The role of extrinsic (probably sensory) nerves is indicated by the ineffectiveness of the drug after coeliac ganglionectomy. Tachyphylaxis to CGRP or ATP both reduces the response. Capsaicin (Allescher et al., 1992; Pinna et al., 1995) or mesenteric nerve stimulation (Wali, 1985) was reported to cause a weak excitatory action in the *rat ileum*, but ineffectivity of capsaicin has also been reported (Nocerino et al., 2002). In the *rat colon*, capsaicin and hCGRP8-37 have been reported to inhibit the afferent arm of motor reflexes due to muscle stretch (Grider, 1994). In the rabbit colon, tachykinins and CGRP seem to mediate the excitatory and inhibitory effects of capsaicin, respectively (Mayer et al., 1990).

3.2. Effects of capsaicin on the oesophagus and lower oesophageal sphincter

Capsaicin elicits a contractile response on the *guinea-pig* oesophagus longitudinal muscle (Lundberg et al., 1984; Barthó et al., 1999b). There is also a capsaicin-sensitive (late) cholinergic component of the oesophageal contraction evoked by vagus nerve stimulation (Kerr et al., 1995). Tachykinins may mediate these effects, at least in part. The vagally induced response has been shown to be reduced by a tachykinin NK_3 receptor antagonist, but not by antagonists specific for tachykinin NK_1 or NK_2 receptors (Kerr et al., 2000). The capsaicin-evoked response is largely sensitive to atropine; it is reduced by tachykinin NK_1, NK_2 and NK_3 receptor antagonists in a supra-additive way, i.e. none of these receptor specific antagonists alone produces an inhibition, whereas any combination does so (Barthó et al., 1999b), especially tachykinin NK_1 plus NK_2 or NK_1 plus NK_2 plus NK_3 receptor antagonists, which is a striking similarity with the guinea-pig ileal (see above) or choledochal resonse (see below). The *ferret* lower oesophageal sphincter strip is relaxed by capsaicin (Smid et al., 1998), whereas in *dogs in vivo*, capsaicin applied in the distal oesophagus elicits lower oesophageal sphincter contraction, probably by activation of a local reflex mechanism that may also involve tachykinin receptors (Sandler et al., 1993).

3.3. Effects of capsaicin on the stomach

In the *guinea-pig* isolated stomach capsaicin-sensitive neurons have been shown to mediate adaptive (distension-induced) relaxation, but not that evoked by vagal stimulation. NO is involved in the capsaicin-sensitive response (Uno et al., 1997). An analysis of the effect of capsaicin on the *rat* gastric corpus (circular strips) showed a predominantly excitatory, acetylcholine- and tachykinin-mediated response to a low concentration of capsaicin. The stomach fundus shows similar responses (Pinna et al., 1995). In the corpus, a consistent, apparently specific inhibitory action of capsaicin was observed at higher concentrations, with no evidence for a participation of CGRP or vasoactive intestinal polypeptide (Holzer-Petsche et al., 1989). Stomach preparations, however, release CGRP- and substance P- (Holzer et al., 1990; Kwok and McIntosh, 1990), or CGRP- and neurokinin A-like immunoreactivity (Renzi et al., 1991; Ren et al., 1993) in response to capsaicin. In the *cat*, a non-

parasympathetic excitatory motor response of the stomach to splanchnic nerve stimulation has been described; the effect is probably due to antidromic activation of sensory nerves. This and a similar colonic response were found inhibited by tachykinin antagonists (Delbro et al., 1983; Fändriks et al., 1985).

3.4. Effects of capsaicin on the gallbladder and bile ducts

Capsaicin-sensitive nerve fibres that contain neuropeptides including tachykinins and CGRP have been identified in the gallbladder and biliary ducts in several mammalian species (see Maggi et al., 1989d; Patacchini et al., 1999 for recent papers). A substantial part of the tachykinin content in the biliary system is, however, stored in intrinsic neurons (see Patacchini et al., 1998, 1999) and mediate non-adrenergic, non-cholinergic (NANC) contraction to electrical field stimulation. Capsaicin has been shown to produce smooth muscle contraction in the guinea-pig gallbladder (Lundberg et al., 1984). The capsaicin-induced contractile response is a result of two opposite effects: contraction mediated by tachykinins and relaxation caused by CGRP (Maggi et al., 1989d), another example of physiological antagonism between sensory neuropeptides. Similar results have been obtained in the guinea-pig common bile duct (longitudinal muscle). Capsaicin, administered to resting preparations, produces a neurogenic, acetylcholine-mediated, contractile response; the involvement of tachykinins is indicated by a strong, though partial (50–60%) inhibitory effect of a combination of tachykinin receptor-selective antagonists. Here again, none of the tachykinin NK_1, NK_2 or NK_3 receptor antagonists administered alone is able to significantly affect the response, but any two of them, or especially all three given together provide a reduction of capsaicin-induced contraction (Patacchini et al., 1999). As with the guinea-pig ileum or oesophagus (Barthó et al., 1999b), we interpret these results in terms of a supra-additive involvement of neuronal tachykinin NK_1, NK_2 and NK_3 receptors. CGRP (which causes a neurally mediated contraction in resting preparations) and/or other substances may be responsible for the non-tachykininergic component of the capsaicin-induced response, but no antagonist is available to block the enteric neuronal stimulatory effect of CGRP. On the other hand, capsaicin is able to relax precontracted and atropine-treated common bile duct preparations, an effect mediated by endogenous CGRP via $CGRP_1$ (hCGRP8-37-sensitive) receptors. As to other transmitters, neither ATP (which causes contraction), nor NO (which causes relaxation) were found to be involved in capsaicin-induced excitatory or inhibitory effects in the common bile duct (Patacchini et al., 1999). By studying the effects of tachykinins and capsaicin on neurons in the ganglionated plexuses of guinea-pig gallbladder and sphincter of Oddi Mawe (1995) and Manning and Mawe (2001) have shown that tachykinins (exogenously applied or released by capsaicin) produce slow excitatory postsynaptic potentials, *via* activation of tachykinin NK_3 receptors. Given the tachykinin release from sensory neurons, it could theoretically be assumed that stimulation of these neurons by a gallstone in any of the ducts or in the sphincter of Oddi could aggravate the spasm of the smooth muscle, while CGRP could have the opposite effect.

3.5. Effects of capsaicin on human gastrointestinal smooth muscle

As opposed to the motor effects of capsaicin on guinea-pig gastrointestinal preparations, the action of this drug on human gastrointestinal smooth muscle is mostly inhibitory. Moreover, not even CGRP seems to mediate these responses. Longitudinally oriented preparations of various segments of the human small or large intestine show relaxation or at least an inhibition of contractions in response to small, specific concentrations of capsaicin (Maggi et al., 1988a, 1990a,b); a delayed enhancement of movements was only observed on the jejunal longitudinal muscle (Maggi et al., 1988a). A quick tachyphylaxis caused by capsaicin indicates a specific action *via* the TRPV1 receptor. Only an inhibitory effect of capsaicin has been observed on circular muscle strips (Maggi et al., 1990a; Barthó et al., 2002). This action is resistant to tetrodotoxin; thus, if we accept the mechanism that substances active on smooth muscle are released from capsaicin-sensitive neurons, the inhibitory agent(s) probably reach the musculature directly. Although CGRP relaxes the human intestinal smooth muscle, there is no functional evidence that it could play a role in the induced inhibition. On the other hand, both functional experiments (utilizing immuno-neutralization) and release studies support the assumption that vasoactive intestinal polypeptide or a closely related peptide takes part in the mediation of the effect of capsaicin (Maggi et al., 1989b, 1990a,b).

Subsequently, an inhibitor of NO synthase and an inhibitor of the soluble guanylate cyclase have been found to strongly reduce the relaxant effect of capsaicin on (mucosa-free) circular strips of the human sigmoid colon (Barthó et al., 2002). We confirmed that the capsaicin-induced relaxation is not influenced by tetrodotoxin (Barthó et al., 2002) and that capsaicin is able to release vasoactive intestinal polypeptide-like immunoreactivity from human colonic circular muscle strips (unpublished observations). Preparations taken from the human ascending colon showed similar results as the sigmoid colon (Table 2). A NO-mediated inhibition by capsaicin seems to be a general feature of the circular muscle of the human intestine; we confirmed its presence in the human ileum and appendix as well; moreover, similar results have been obtained in colonic circular muscle preparations from mice and rats, but not guinea-pigs (unpublished observations). These data may implicate a release of NO from primary afferents, in man and rodents. There are morphological data to indicate a

Table 2
Relaxant effect of capsaicin (3×10^{-7} M) on the circular muscle of the human ascending colon in vitro precontracted with acetylcholine and its inhibition by the NO synthase inhibitor N^G-nitro-L-arginine (L-NOARG) and the guanylate cyclase inhibitor ODQ

Pretreatment	Relaxation to capsaicin	*n*
–	48.2±6.8	9
L-NOARG (10^{-4} M)	10.9±3.3[a,b]	8
Tetrodotoxin (10^{-6} M)	49.3±5.8	7
–	51.1±4.6	8
ODQ (10^{-6} M)	26.9±5.3[a]	7

Relaxations in response to capsaicin are expressed as % of isoprenaline-evoked maximal relaxation. Statistically significant differences ($p<0.05$ or less) are indicated by letters in the index; ([a]), significantly less than the control group (–); ([b]) significantly less than the tetrodotoxin-treated group (Mann–Whitney or Kruskal–Wallis tests); *n* denotes the number of preparations taken from at least six operation specimens. Krebs solution of 37 °C and isotonic recording with a load of 1 g was used. ODQ stands for 1H-(1,2,4)oxadiazolo(4,3-*a*)quinoxalin-1-one.

presence of NO synthase-like immunoreactivity in dorsal root ganglia, though its reaction to nerve injury is a complex matter (for references see Thippeswamy and Morris, 2002; Barthó et al., 2002). Alternatively, we could assume that sensory neurotransmitters excite endings of "nitrergic" myenteric neurons in a tetrodotoxin-resistant way, release NO from some other source or that the capsaicin-sensitive (probably TRPV1) receptors themselves are located on non-neuronal NO-releasing cells. Another open question is the relation between the vasoactive intestinal polypeptide- and NO-mediated mechanisms in the effect of capsaicin in the human gut. To date, despite continuing efforts, we have no reliable method to selectively block the relaxation evoked by exogenous vasoactive intestinal polypeptide in the human intestine, which would be necessary to address this question. There is evidence for a release of NO from dispersed smooth muscle cells by vasoactive intestinal polypeptide (see Murthy et al., 1995), but the NO synthase involved may be induced in the course of the preparation procedure (Dick et al., 2000).

Capsaicin has been found ineffective on human lower oesophageal sphincter strips in vitro (Smid and Blackshaw, 2000). In vivo studies with intra-oesophageal capsaicin-containing solutions indicated a stimulatory effect on oesophageal motility and contraction of the lower oesophageal sphincter (Gonzalez et al., 1998; Király et al., 2001) in healthy volunteers. On the other hand, gastric emptying is prolonged but this is compensated for by an increased small intestinal propulsion, so orocoecal transit time is not altered (Horowitz et al., 1992; Gonzalez et al., 1998).

In conclusion, the most prominent stimulatory effect of capsaicin-sensitive neurons can be seen in guinea-pig gastrointestinal preparations, where tachykinins mediate these effects through different types of tachykinin receptors, as well as ATP through P_2 purinoceptors, acting in a supra-additive manner. An activation of intrinsic enteric neurons is involved, hence acetylcholine is the main final mediator of these responses. Another, inhibitory action of capsaicin is mediated by CGRP. Results obtained on human tissues strongly differ from those on animal preparations. There is evidence for a vasoactive intestinal polypeptide- or (and?) NO-mediated relaxation in the circular muscle of human (but also rodent) intestinal preparations. The non-specific smooth muscle-relaxant effect of capsaicin-like drugs is frequently disregarded.

4. Effect of capsaicin on respiratory smooth muscles

A capsaicin-sensitive NANC excitatory motor response of the guinea-pig main bronchi to electrical field stimulation has been described independently by two groups of investigators (Lundberg and Saria, 1982; Szolcsányi and Barthó, 1982). Vagus nerve stimulation also elicits a capsaicin-sensitive response (Lundberg et al., 1983b). Evidence has been presented for a tachykinin mediation of this bronchial response (mainly through tachykinin NK_2 receptors, but with the supra-additive contribution of tachykinin NK_1 receptors). On the other hand, the ineffectiveness of exogenous CGRP makes it unlikely that the latter peptide could play a role (Lundberg et al., 1983b; Martling et al., 1988; Lou et al., 1993; Ellis and Undem, 1994). By contrast, in the guinea-pig trachea and in the presence of airway epithelium CGRP induces contraction and may be involved in the contractile response to capsaicin (Tschirhart et al., 1990). Quite interestingly, inhibitory nerves arising from the oesophagus can modulate the response of the trachea to capsaicin (Canning and Undem, 1994). A combined inhibition of tachykinin NK_1 and NK_2 receptors is required to block the capsaicin-induced depolarization in the tracheal smooth muscle (Girard et al., 1997). A new aspect of airway excitation by capsaicin-sensitive nerves is the functional presence of protease-activated receptors on these nerves, whose activation by trypsin evokes tachykinin-mediated contraction of the guinea-pig bronchus (Carr et al., 2000).

Human bronchi at best moderately contract in response to capsaicin (Lundberg et al., 1983a; Honda et al., 1991). This effect is not influenced by a tachykinin NK_2 receptor antagonist (Ellis et al., 1997). The precontracted mouse and rat bronchus (in the presence of the epithelium) is relaxed by capsaicin (Manzini, 1992; Szarek et al., 1995). Tachykinins and cyclo-oxigenase products may be involved in the capsaicin-sensitive relaxant response to electrical field stimulation in the rat bronchus (Szarek et al., 1995). Another function of sensory neurotransmitters, as indicated by the effect of capsaicin may be a tachykinin-mediated stimulation of mucus secretion (Ramnarine et al., 1994).

In conclusion, capsaicin-induced release of sensory neurotransmitters (mostly tachykinins) induce a strong excitation in guinea-pig respiratory muscles. There is only a weak stimulation of an uncertain transmitter background in human preparations and a variable response (also

including a specific relaxant effect) in other species. The mediation of the inhibitory responses is also still uncertain. Capsaicin-sensitive responses seem to be modulated by the airway epithelium.

5. Effects of capsaicin on genitourinary preparations

All parts of the upper and lower urinary tract and the genital organs of various species, including man, are densely innervated by peripheral projections of capsaicin-sensitive primary afferent neurons that represent the main source of CGRP and tachykinins in noninflamed tissues of this area. In man, the density of such nerves may be lower than that in laboratory animals (Maggi, 1995; Lecci and Maggi, 2001, for review). In addition to tachykinins and CGRP, other neurotransmitters of both peptide and non-peptide nature—among others pituitary adenylate cyclase activating peptide (PACAP) (Fahrenkrug and Hannibal, 1998)—have been detected in capsaicin-sensitive organs of the genitourinary tract, but their significance remains uncertain. (Neurogenic inflammatory responses of this organ system are beyond the scope of the present paper, but see among others Maggi, 1995.)

5.1. Capsaicin-sensitive motor responses of the urinary bladder and urethra

Acute administration of capsaicin provokes a release of tachykinin- and/or CGRP-like immunoreactivity from rat, guinea-pig and hamster urinary bladder tissue (Maggi, 1995; Giuliani et al., 2001), followed by tachyphylaxis. The motor effects produced by capsaicin in the urinary bladder smooth muscle are for the most part ascribable to the release of tachykinins and CGRP, with the net effect being dependent on the species, the region studied (dome vs. base) and the initial tone of the preparation. Thus, the rat urinary bladder detrusor responds with a contraction to capsaicin, an effect prevented not only by capsaicin desensitization, but also by chronic extrinsic denervation of the organ or an administration of tachykinin antagonists. A fine interplay seems to function between tachykinin NK_1 and NK_2 receptors in the course of the capsaicin-induced contraction of the rat bladder, which might reflect a co-release of substance P and neurokinin A (Maggi et al., 1985, 1991b; Benkó et al., 2003). In the guinea-pig bladder, there is a region-specific effect of capsaicin, consisting of tachykinin-mediated excitatory and CGRP-mediated inhibitory components (see Maggi, 1995). The latter is more characteristic for the bladder neck. In the rat detrusor, capsaicin causes no relaxation or inhibition of cholinergic or purinergic responses (Benkó et al., 2003), while in the hamster bladder relaxation prevails, with a participation of CGRP (Giuliani et al., 2001). Neither rabbit, nor human detrusor smooth muscle tone is affected by capsaicin (Maggi et al., 1989a; Maggi, 1995).

Tetrodotoxin-sensitive excitatory motor effects ascribable to endogenous tachykinins in the rat bladder (Meini and Maggi, 1994; Benkó et al., 2003), and inhibitory motor effects ascribable to endogenous CGRP in the hamster bladder (Giuliani et al., 2001) in response to electrical field stimulation have been described. In the rat, an attempt should be made to pharmacologically eliminate cholinergic and purinergic excitatory responses and special parameters of electrical stimulation have to be used for obtaining a capsaicin-sensitive excitatory response to electrical stimulation (Meini and Maggi, 1994; Benkó et al., 2003). Other neurotransmitters that can be released by capsaicin in the lower urinary tract include NO (Nishizawa et al., 1997; Birder et al., 1998), part of which may originate from the urothelium. Another novel transmitter that may be released by capsaicin in the urinary bladder is the 33-aminoacid chemotactic neuropeptide secretoneurin (Kirchmair et al., 1994) which, hypothetically, might play a role in neurogenic inflammation.

Most studies indicate an inhibitory effect of capsaicin in the urethra or the external urethral sphincter in vitro (Maggi et al., 1989c; Maggi, 1990). In the dog (Nishizawa et al., 1997), but not in the rat urethra (Persson et al., 1997), the response to capsaicin is mediated by NO. In the rat external urethral sphincter CGRP seems to mediate the inhibitory action of capsaicin (Parlani et al., 1993).

5.2. Capsaicin-sensitive responses in the renal pelvis and ureter

The renal pelvis and ureter of various species, including man, receive a dense innervation of tachykinin- and CGRP-containing primary afferent neurons sensitive to capsaicin. A release of these peptides has also been reported (see Maggi, 1995). In the spontaneously active guinea-pig renal pelvis, capsaicin or electrical stimulation evoke a neurokinin A-mediated excitatory motor response (enhancement of both the amplitude and frequency of contractions). Tachykinin NK_2 receptor antagonists unmask an inhibitory effect that is mediated, at least partly, by CGRP. Capsaicin pretreatment blocks the entire response (Maggi et al., 1992).

In the ureter of the rat and guinea-pig, a CGRP-mediated (and glibenclamide-sensitive) inhibitory response predominates upon electrical or chemical stimulation of capsaicin-sensitive nerves, although exogenous tachykinins evoke an excitatory response (Maggi et al., 1987b; Maggi and Giuliani, 1991). The inhibitory effect of CGRP is most probably associated with a depression of activity in pacemaker cells. An enhanced activity in the pelvis and a decreased one in the ureter could facilitate the passage of agents that stimulate sensory nerve endings (Santicioli and Maggi, 1998). In the pig intravesical ureter (a portion of ureter richly innervated by neurokinin A-immunoreactive fibres) electrical stimulation elicits contraction that is reduced in the presence of capsaicin (in our view, a non-

specific effect cannot be excluded) or a selective tachykinin NK_2 receptor antagonist (Bustamante et al., 2000).

5.3. Genital organs

Extrinsic, capsaicin-sensitive nerve fibres containing tachykinins and/or CGRP distribute to the vas deferens, uterus and Fallopian tubes of various species (see Maggi, 1995). In the rat vas deferens capsaicin inhibits the twitch contraction elicited by electrical field stimulation of post-ganglionic excitatory nerves (Saito et al., 1987; Maggi et al., 1987c). The inhibitory effect of capsaicin is mimicked by exogenous CGRP and is blocked by a CGRP antagonist (Maggi et al., 1987c, 1991a). On the other hand, exogenous tachykinins exert an excitatory motor effect in the vas deferens of various species (see among others Patacchini et al., 1989). Thus, the effect of endogenously released tachykinins is largerly overcome by that of CGRP in this organ.

In the myometrium, exogenously applied tachykinins produce excitatory motor responses, whereas CGRP exerts a relaxant effect (Samuelson et al., 1985; Patak et al., 2000). These peptides, as well as secretoneurin (Collins et al., 2000) and part of the galanin-like immunoreactivity (Shew et al., 1992) occur and coexist in sensory nerves of the uterus. A functional study with capsaicin seems to indicate an inhibitory influence of sensory nerves on uterine movements (Klukovits et al., 2004). In the rabbit isolated corpus cavernosum, capsaicin produces concentration-related relaxations, unaffected by N^G-nitro-L-arginine methylester (L-NAME) or tetrodotoxin (Teixeira et al., 1998). In contrast, capsaicin does not produce any appreciable motor effect in the human isolated corpus cavernosum or spongiosum (Patacchini et al., 2002), although exogenous tachykinins cause contraction (through tachykinin NK_2 receptors) or relaxation (through tachykinin NK_1 receptors) (Patacchini et al., 2002). The latter effect involves the non-neurogenic formation of NO.

In conclusion, many data point to the functional release of tachykinins and CGRP by capsaicin in the renal pelvis, ureters and urinary bladder of different animal species, but not in man. Except for the vas deferens, the local effects of endogenous sensory neurotransmitters in the genital organs are still poorly understood.

6. Physiological and pathophysiological roles of visceral release of sensory neurotransmitters

In the living animal or in man, visceral capsaicin-sensitive afferents clearly play a role in evoking autonomic reflexes arising from viscera (see Holzer-Petsche and Lembeck, 1984; Holzer et al., 1986; Mizutani et al., 1990). Much less is known of the roles of biologically active substances, released in the viscera from capsaicin-sensitive nerves, in health and disease. Capsaicin-induced sensory neuron blockade fails to alter intestinal transit in the rat in vivo (Holzer, 1986) and the peristaltic reflex in the guinea-pig ileum in vitro (Barthó and Holzer, 1995). However, capsaicin-sensitive nerves might be involved in diseases associated with hypersensitivity and subsequent hypermotility of viscera. This is suggested by an increase in TRPV1-immunoreactivity in the gut of patients suffering from rectal hypersensitivity or inflammatory bowel disease (Yiangou et al., 2001; Chan et al., 2003). The role of capsaicin-sensitive nerves in functional gut disorders is dealt with in the review by Holzer (this volume). Apart from their direct or indirect influence on motility, capsaicin-sensitive nerves are an important component of gastric mucosal protection. This aspect, however, is beyond the scope of this review (but see Holzer, 1998).

In the respiratory system, cigarette smoke-induced bronchoconstriction may be mediated, at least partly, by a release of neuropeptides from capsaicin-sensitive nerves (Lee et al., 1995). Moreover, tracheo-bronchial hyperresponsiveness evoked by virus infection and/or antigen challenge (Ladenius et al., 1995; Herd et al., 1995), as well as a less well-defined asthma-like state, “sensory hyperreactivity” (see Lowhagen, 1999) seem to be mediated by capsaicin-sensitive nerves.

7. A look at the central nervous system

Central endings of primary afferents most probably release the same sensory neurotransmitters as their peripheral endings in the skin, mucous membranes and viscera. The fields of peripheral and spinal capsaicin-sensitive mechanisms are richly interconnected. Current pharmacological tools make it possible to more precisely assess the roles of the known sensory transmitters in nociception, even in human studies, as briefly overviewed below. Various aspects of the involvement of sensory neurotransmitters in nociception at the spinal level have been reviewed by Schaible (1996), Quartara and Maggi (1998), Hill and Rupniak (1999), Hill (2000), Herrero et al. (2000), Sandkühler et al. (2000), Fundytus (2001), Herbert and Holzer (2002), and Pezet et al. (2002).

7.1. Tachykinins

Substance P and neurokinin A are released within the rat spinal cord by noxious cutaneous stimuli or by electrical stimulation of A-delta and C fibres. An involvement of tachykinin (NK_1 and NK_2) receptors in spinal nociceptive processing has been revealed by various elelctrophysiological methods both in vitro and in vivo (see Schaible, 1996; Quartara and Maggi, 1998, Hill and Rupniak, 1999). Dorsal horn neuron responses to noxious thermal, mechanical or chemical stimulation or to inflammation of the cutaneous receptor field were attenuated by intrathecally applied tachykinin NK_1 and/or NK_2 receptor antagonists.

In behavioural studies intrathecally administered substance P or neurokinin A evoke responses indicative of nociception and produce an increased responsiveness (hyperalgesia) to peripheral heat and mechanical stimuli. Interestingly, tachykinin NK_1 receptor antagonists given intrathecally produce either no or minimal inhibitory effect in tests of phasic heat and mechanical nociception but display a consistent inhibitory effect in a tonic pain model. Tachykinin NK_1 receptor antagonists have an anti-hyperalgesic effect, e.g. in traumatic or inflammatory hyperalgesia. Tachykinin NK_2 and NK_3 receptor antagonists have also been shown to exert antihyperalgesic effects (Sluka et al., 1997; Löfgren et al., 1999; Zaratin et al., 2000). In a model of visceral hyperalgesia neither tachykinin NK_1 nor NK_2 receptor antagonists were effective, but their combination produced an inhibition, similar to that caused by a tachykinin NK_3 antagonist (Kamp et al., 2001). In tachykinin NK_1 receptor knockout mice acute nociceptive responses to mechanical and heat stimuli appear unaltered but tonic pain induced by formalin injection is reduced. Although wind-up (see below) is absent in these animals, inflammatory hyperalgesia is not diminished (De Felipe et al., 1998).

The amino acid neurotransmitters *glutamate* and *aspartate* are also stored in, and released from, central terminals of primary afferents by noxious stimulation together with substance P and neurokinin A, suggesting co-transmission. All four subtypes of glutamate receptors—the ionotropic (*RS*)-alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate, *N*-methyl-D-aspartate (NMDA) and the metabotropic receptor—are thought to be involved in nociceptive processing in the spinal dorsal horn, albeit under different conditions (see Fundytus, 2001). In addition, a potentiation between tachykinin and glutamate receptors has been revealed (see Quartara and Maggi, 1998). Co-administration of tachykinin NK_1 and NMDA receptor antagonists exerts an additive or even supra-additive inhibitory effect on spinal nociceptive responses supporting the concept of tachykinin−glutamate cotransmission. Two extensively studied experimental models of dorsal horn neuron hyperexcitability, namely wind-up and long-term potentiation (see Herrero et al., 2000; Sandkühler et al., 2000) depend predominantly on NMDA receptor; activation with wind-up is prevented by tachykinin NK_1 receptor antagonists, while induction of long-term potentiation requires an activation of both tachykinin NK_1 and NK_2 receptors. A contribution of endogenous substance P and neurokinin A (via tachykinin NK_1 and NK_2 receptors, respectively) to synaptic transmission between primary afferent fibres and dorsal horn neurons has been shown in a model in which all glutamate receptors were blocked, thereby any modulatory effect of tachykinins on glutamate release or action was excluded (Li and Zhou, 2001). Surprisingly, the results of the *clinical* studies completed so far with tachykinin NK_1 receptor antagonists are disappointing; these drugs fail to be analgesic in osteoarthritis, peripheral neuropathy and migraine. One compound was found to reduce pain following tooth extraction (see Hill, 2000; Herbert and Holzer, 2002).

7.2. CGRP

This peptide is also released in the rat dorsal horn by noxious stimulation. Exogenously applied CGRP was found to be pro-nociceptive in some but not all studies (see Schaible, 1996). Intrathecal administration of the CGRP antagonist CGRP8-37 was shown to have anti-nociceptive and anti-hyperalgesic effects in various animal models (see Gschossmann et al., 2001; Yu et al., 2002; Sun et al., 2003). In anaesthetized rats intrathecally applied CGRP8-37 reduces responses of wide dynamic range dorsal horn neurons to noxious and innociuous mechanical stimulation, as well as the sensitizing effect of arthritis (Schaible, 1996; Yu et al., 2002). CGRP knockout mice display normal responses to noxious heat stimuli but no signs of secondary hyperalgesia after the development of knee joint inflammation (High, 2001). These data may support a pro-nociceptive role for CGRP in the spinal processing of noxious input under inflammatory and neuropathic conditions.

Brain-derived neurotrophic factor (BDNF), acting on tropomyosin receptor kinase B (TrkB) receptors is also found in nociceptive primary afferent neurons and released in the dorsal horn in response to noxious peripheral stimulation (see Pezet et al., 2002). Intrathecally applied BDNF potentiates the ventral root potential induced by C fibre stimulation, most likely by a phosphorylation and consequent facilitation of NMDA receptors. Although specific antagonists acting at the TrkB receptor are not available, there is some evidence that endogenous BDNF is involved in the long-latency ventral potential elicited by C fibre stimulation in vitro and in both phases of formalin-induced nociception in vivo. In a recent study BDNF knockout mice exhibited diminished wind-up response upon repeated nociceptor stimulation. Thus, BDNF is also a likely candidate for a neurotransmitter/neuromodulator in the spinal cord.

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