with Mil rat Chow pellets from Morini Laboratories, S. Polo D'Enza, Italy.

Animals were killed by decapitation: one group at the end of the feeding period (fed animals), the other groups at different intervals thereafter (fasted animals). Stomach and duodenum were quickly dissected, cleaned in distilled water, blotted with filter paper, frozen in liquid nitrogen and stored at −30 °C until analyzed. Tissues were homogenized with a Polytron tissue homogenizer (Kinematica Gmbh., Luzern, Switzerland). Tryptophan and serotonin were assayed fluorometrically as previously described[14,15].

Results. The changes of 5-HT content occurring in the stomach and duodenum after feeding are shown in the table. The levels of 5-HT declined by 31 and 40%, respectively, at the end of the feeding period, remained at this level about 3 h and had returned to the fasting levels 9 h after food removal. In the stomach, the level of tryptophan, the 5-HT precursor, was unchanged in fasted and fed rats. On the contrary, in the small intestine, tryptophan levels rose by 100% at the end of the feeding period and remained elevated for more than 9 h. This increase most likely reflects the absorption of the digested proteins.

Discussion. The present study demonstrates that 5-HT concentrations in the stomach and duodenum are influenced by food intake. Fasted rats have higher levels of gastro-duodenal 5-HT than fed rats. It is possible that the decline in the gastro-duodenal content of 5-HT following food intake might be due to the release of the amine in situ and, therefore, into circulation. Consistently, Buldring and Crema[16] observed both in vitro and in vivo that, by exerting pressure on the gastro-intestinal mucosa, serotonin is released into the intestinal lumen.

On the other hand, a degradation of 5-HT by the action of monoamine oxidase does not seem to occur to a considerable extent in the gastro-intestinal wall, since we found that 5-hydroxyindoleacetic acid levels in this tissue were below the sensitivity of our method of assay (10 ng/g of tissue[14]) both in fasted and fed animals. The observed changes in the serotonin content were not associated with changes in the level of gastro-intestinal tryptophan. In fact, the levels of tryptophan did not change after feeding in the stomach; while, in the duodenum, they underwent changes in the opposite direction to intestinal 5-HT.

Experiments are in progress in our laboratory to clarify whether serotonin levels are affected by gastric distension, by some food constituent, or are controlled by the CNS in a reflex manner; moreover, to evaluate the physiological significance of our findings.

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Effect of nerve stimulation, denervation, and duct ligation, on kallikrein content and duct cell granules of the cat's submandibular gland

M. Schachter, Susanne Barton, M. Uddin, E. Karpinski and E. J. Sanders[1,2]

Department of Physiology, University of Alberta, Edmonton T6G 2H7 (Alberta, Canada), 15 November 1976

Summary. Various procedures which reduce or deplete the kallikrein content of the cat's submandibular gland correspondingly reduce the number of apical granules in the striated duct cells. The kallikrein content is greatly reduced after chronic parasympathetic but not after sympathetic nerve section which suggests that the parasympathetic innervation is required for synthesis or storage of this enzyme.

The kallikreins, or kininogenases, are widespread enzymes belonging to the larger group of serine proteases[3,4]. The cellular and subcellular location of kallikrein has been studied in the salivary gland by several groups of workers[5–8] since it may have a bearing on its physiological significance in this organ where its role has been a subject of much speculation and discussion[9,10]. Whereas all workers agree that kallikrein is located in secretory granules, the earlier results suggested its location in acinar granules[5,11]. More recent work suggests that it is located in granules of the granular tubules and striated ducts in the rat[12] and in striated ducts of the cat[7,8]. The present experiments which correlate the kallikrein content of the cat's submandibular gland with associated microscopic changes, lend support to the thesis that kallikrein is localized in small granules in the apical region of the striated duct cells. They also indicate that the sympathetic innervation which is most effective for the stimulation of kallikrein secretion[13] is unnecessary for the synthesis and storage of this enzyme whereas parasympathetic denervation results in almost complete failure of the gland to synthesize or to store kallikrein.

Methods. For acute experiments involving nerve stimulation, cats of 1.5–4.5 kg and of either sex were starved for 16–20 h, and anaesthetized with chloralose (80 mg/kg^{-1} i.v.) after induction with chloroform. The chorda lingual and cervical sympathetic nerves were exposed and stimulated electrically at 10 and 20 Hz respectively and saliva was collected as described previously[13]. For operative procedures with recovery, sodium pentobarbital

1 We wish to acknowledge the valuable assistance of Dr T. Nihei and Mr J. Wimal in some of the enzyme measurements.
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(30 mg kg^{-1} i.p.) was given as anaesthetic. The chorda lingual nerve was sectioned and stripped under aseptic conditions as described by Schachter et al.[14]. Sympathetic denervation was effected in 18 experiments by removing the superior cervical ganglion along with a 2 cm strip of the preganglionic nerve. In 2 other experiments a 2 cm strip of preganglionic nerve only was removed to produce a decentralization rather than a postganglionic sympathectomy of the submandibular gland. Experiments were performed 1–49 days later on these cats (see figure 1). For duct ligation an incision was made in the mylohyoid muscle exposing the submandibular (Wharton's) duct which was ligated 5–10 mm rostral to the point where the chorda lingual nerve crosses it. The gland was removed 3–4 days later and prepared for electron microscopy as described by Barton et al.[7] except that 0.1 M rather than 0.5 M cacodylate buffer was used for fixation. Kallikrein was measured by its enzymatic hydrolysis of BAEe and protein concentration as previously described[7]. Acid phosphatase and β-glucuronidase were measured according to Bhoola and Heap[15].

Results. Nerve stimulation: In 3 experiments after parasympathetic nerve stimulation for 15, 37 and 40 min at 10 Hz, kallikrein concentrations (esterase units per unit protein) in the gland were 108, 90 and 109% respectively of the contralateral unstimulated gland. This is in accord with previous findings[7,13] that parasympathetic nerve stimulation, particularly at 10 Hz or less, has little effect on the kallikrein content of the gland despite a copious secretion of saliva which contains some kallikrein. After parasympathetic nerve stimulation, in contrast to sympathetic nerve stimulation, the apical granules in the striated duct cells were unaffected in number and appearance, but the acinar granules were largely depleted or clearly mobilized for secretion into the lumen.

In 5 experiments the sympathetic nerve was stimulated for periods of 10–30 min at 20 Hz (see 'methods'). In these experiments, in contrast to parasympathetic nerve stimulation, there was a reduction in the kallikrein content to 5–39% of the control glands (mean 16%). In these instances there was also a marked decrease in apical granules of the striated ducts. The mitochondria, usually confined to the basal and central regions of the cell, were now more dispersed in the cell. The acinar cell granules were indistinguishable in number and appearance from those in the control gland. Effects on demilune cell granules were too variable to permit definite conclusions to be drawn from stimulation of either sympathetic or parasympathetic nerves.

Denervation: Parasympathetic denervation markedly reduced the kallikrein concentration and content of the submandibular gland and resulted in the disappearance of the small granules in the apical position of striated duct cells. Figure 1 shows the ratio of the kallikrein content of the denervated to the control glands at periods of 1–14 days after denervation. It shows that after an initial increase on the first day after denervation, the kallikrein content falls to about 20% in 7 days and to about 8% after 2 weeks. There was no further decrease even 7 weeks after nerve section. In contrast to kallikrein, the lysosomal enzymes, acid phosphatase and β-glucuronidase increased about 2fold after 2 weeks of denervation. This is consistent with the increase in lysosomal enzymes which occurs in denervated striated muscle[16]. 2 weeks after parasympathetic denervation the apical granules in the striated cell ducts have disappeared. The cell has also undergone other degenerative changes and a large portion of the cell is devoid of its organelles and is filled with fine granular material.

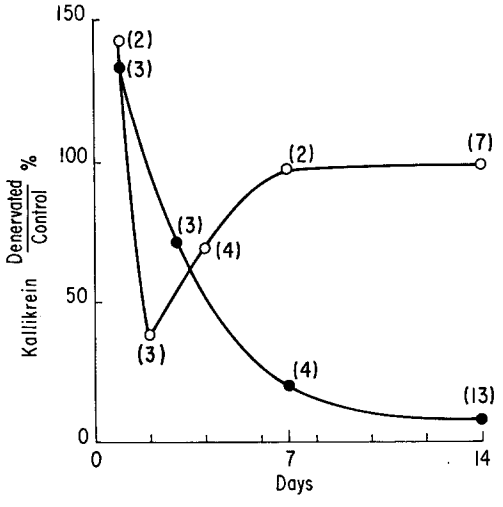

Fig. 1. Effects of parasympathetic ●—● and of sympathetic ○—○ nerve section on kallikrein content (esterase units/unit protein) of the cat's submandibular gland. Numbers in brackets are the number of experiments from which each mean value was plotted. Days, 1–14 days after denervation.

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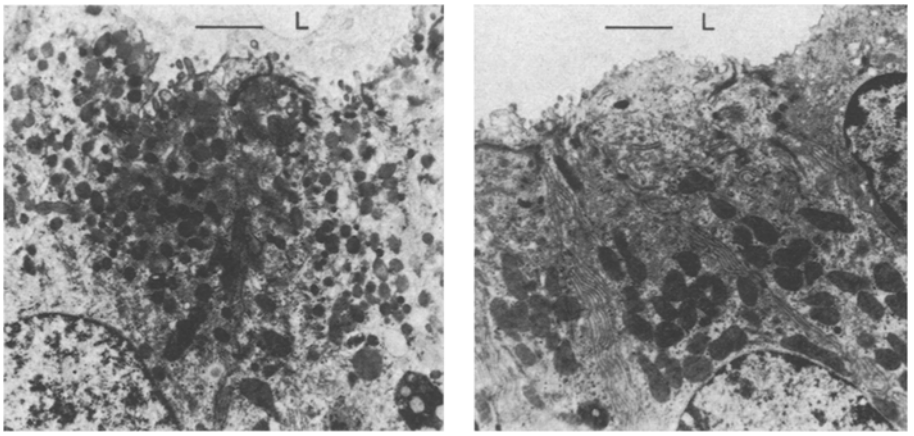

Fig. 2. Left, normal submandibular gland showing numerous small secretory granules in the apical regions of several striated duct cells. Right, ligated gland 3 days after duct ligation showing absence of apical granules and prominence of mitochondria longitudinally arranged in this region. L, Lumen; bar, 1 μm.

Sympathetic denervation (postganglionic; see 'methods') after causing a transitory decrease had no significant effect on kallikrein concentration in the submandibular gland. Figure 1 shows that although there was an increase after 1 day, followed by a decrease after 2–4 days, it returned to normal and remained there 1 week after ganglionectomy. This temporary reduction in kallikrein content did not occur in 2 cats after preganglionic sympathectomy. 2 weeks after pre- and postganglionic sympathectomy there was no change in the number and appearance of the apical granules of the striated ducts, in the concentration of acid phosphatase and β- glucuronidase, nor in the weight of the gland. Also, there were no gross microscopic changes in the acinar or demilune cells.

Duct ligation: Ligation of the submandibular duct for 3–4 days caused a marked decrease in kallikrein content of the gland. In 2 such experiments the kallikrein concentration (esterase units per unit protein) fell to 3 and 4% of the contralateral control gland. Also, there was a complete disappearance of the apical granules in the striated ducts and characteristic dispersion and longitudinal arrangement of mitochondria (figure 2). The larger secretory granules of the acinar cells showed some degenerative features but these were very minor compared with the almost complete disappearance of the apical granules from the striated duct cells. Changes in appearance of demilune cell granules were not noteworthy.

Discussion. 2 main conclusions may be derived from these experiments in the cat: a) The synthesis or storage of kallikrein depends on an intact parasympathetic innervation. It is now well established that sympathetic nerve stimulation rather than parasympathetic strongly mobilizes the secretion of kallikrein in saliva[7,8,13]. In contrast, however, our results clearly demonstrate that kallikrein disappears almost completely from the gland after 1–2 weeks with parasympathetic denervation but that with sympathetic denervation the kallikrein concentration is normal at this time and remains so afterwards (figure 1). It must be concluded, therefore, that the parasympathetic nerve exerts some trophic or other action on the gland cells. We also noted that there was a substantial atrophy of the gland after parasympathectomy but not after sympathectomy. Figure 1 shows that 2–4 days after postganglionic sympathectomy there is a temporary reduction in the kallikrein content of the gland which then returns to normal. This temporary reduction is perhaps due to 'degeneration secretion'[17] since it did not occur after preganglionic sympathectomy which does not result in degeneration of the postganglionic axons. b) Our results support the view[7,8] that kallikrein is located in the striated duct cells of the cat's submandibular gland. They show that there is always a marked decrease in the number of apical secretory granules in these cells when there is a depletion of kallikrein irrespective of whether it is as a result of duct obstruction, parasympathetic denervation, or sympathetic nerve stimulation.

These studies do not establish the physiological role of salivary kallikrein but since they indicate that it is secreted into the ducts rather than into the interstitial fluid and circulation, they lend no support to the view that this enzyme is the regulator of functional hyperaemia in the gland[10]. The presence in the submandibular gland of known active macromolecules like kallikrein, renin, nerve growth factor, and of a new one like sialotonin[18], suggests that the physiological significance of the salivary glands may extend beyond those relatively simple functions which are generally assigned to them.

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Information processing along the course of a visual interneuron

L. J. Goodman, P. G. Mobbs and R. G. Guy

Department of Zoology and Comparative Physiology, Queen Mary College, Mile End Road, London E1 4NS (England), 29 November 1976

Summary. Locust ocellar retinal cells are innervated by giant second order cells, 2 mm long, which show discrete zones of integration along their course, including a major zone in the axonal length of the neuron. The complex synaptic arrangements which exist between higher-order afferent and efferent cells and these second order cells along their course suggests that transmission takes place by the electrotonic spread of slow potentials. The size and accessibility of these visual interneurons offers a unique preparation for examining mechanisms of graded synaptic transmission.

The locust, Schistocerca gregaria, possesses 3 ocelli, 2 lateral and 1 median, each consisting of around 1000 visual cells lying beneath a common cuticular lens. Individual ocelli are innervated by 7 giant interneurons, 10 μm in diameter, and a number of smaller cells whose axons run out from the brain in an ocellar nerve approximately 2 mm long. 4 of the giant interneurons innervate both the median and 1 lateral ocellus. The interneurons are of the classical annelid/arthropod form with anatomically unipolar cell bodies, lying within the brain, whose neurites give rise to a T or a Y shaped axon terminating in dendritic arborisations. In the invertebrate nervous system the integration of information in electrotonic form is generally considered to be confined to the dendritic regions of the cell, the axon transmitting information from point to point usually by means of action potentials. Although presenting a conventional appearance in Golgi and cobalt stained material[1], serial thin sections and semi-thin sections show that the synaptic organisation of these large interneurons is unusual. 3 major zones of integration can be identified along their length, one of which comprises a major section of the axonal region of each fibre. Longitudinal sections through the ocellar tracts show that they resemble elongated neuropilar areas rather than fibre tracts.

The first integration zone, within the ocellus, consists of an extensive network of dendrites onto which the visual cells are highly convergent. The giant fibres are not equivalent in this region, those linking the ocelli synapse with the upper regions of the descending visual cell axons,