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Research Articles

Vagal afferent innervation of the pylorus and the upper small intestine studied in the rat with the horseradish peroxidase technique

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Summary. The neuronal tracer horseradish peroxidase was injected into different segments of the gastrointestinal in the rat, in order to study the vagal afferent innervation. In the nodose ganglia the extent of labeling was greater in the experiments on the gastric antrum and pylorus than in the experiments on the first part of the small intestine. Vagal afferents are scarce in the upper duodenum and originate mainly from the left nodose ganglion.
Key words. Horseradish peroxidase; vagus nerve; nodose ganglion; stomach; pylorus; duodenum; rat.

Recent electrophysiological studies have clearly proved the existence of many kinds of receptors such as mechanoreceptors, chemoreceptors, thermoreceptors and osmoreceptors in the intestinal wall[1]. These receptors probably play important physiological roles. Vagal receptors are particularly abundant in the duodenum of the cat and are distributed in all layers[2-4]. In this animal, the use of the horseradish peroxidase (HRP) technique has shown that the first part of the small intestine receives many vagal afferent fibers; in fact, a few hundred labeled neurons have been found in the nodose ganglia after injections of this neuronal tracer[5]. As for the duodenum, Lundberg et al.[6] have found HRP-positive neurons both in the left and in the right nodose ganglia of cat and guinea pig after injections of the marker at multiple sites in the visceral wall. The occurrence of the HRP reaction product on one side was prevented by crushing the cervical vagal nerve on that side. Unfortunately, these authors did not report either how many neurons were labeled or the number of HRP-positive cells in the left and right nodose ganglia.

The present study was carried out in the rat, employing the HRP tracing technique to provide clearer information on the distribution of duodenal afferents in the nodose ganglia. The pylorus and gastric afferents were also investigated for purposes of comparison with the innervation of the first part of the small intestine. The rat was chosen as the experimental animal because, even if it has been extensively investigated for the afferent and efferent organization of the vagus nerve[8-14], no data are available concerning the vagal afferents in the first part of the small intestine.

Materials and methods

Experiments were performed on 21 male Wistar rats, 300–350 g in body weight, anesthetized with intramuscular injection of 50 mg/kg ketamin chlorhydrate. Following laparatomy various quantities of a 20% (W/v) solution of HRP in H_2O were injected, with the aid of a dissecting microscope, through a Hamilton microliter syringe. The amount of the tracer administered depended on the area to be injected. In each injection, two HRP aliquots of 1 µl each were delivered, withdrawing the needle into nearby sites, to reduce the number of injections and thus to minimize the leakage of the neuronal tracer. For the same reason the zone was covered with a pledget after the injection, to avoid backward spreading

of HRP into the peritoneal cavity. By following these surgical procedures, false positive results due to the uptake of leaked tracer from the nerve terminals of the surrounding tissue were prevented.

– In nine rats, 50–74 µl of HRP were injected in contiguous sites of a 10-cm-long portion of proximal small intestine, gently exteriorized and placed on sterile gauze pads. Injections were made into the ventral and dorsal sides and the side opposite to the mesenteric insertion. The mesenteric side did not receive tracer injections since when injecting at this site it is possible, even with the closest attention, to injure the mesentery.

– In one rat, in order to show the possible presence of vagal afferent terminals, 660 µl of HRP were administered only into the mesenteric side of the duodenum.

– Four rats received 100 µl of HRP in a 15-cm-long segment of the gut immediately distal to the tract injected in the animals described above.

– In five rats, 10–25 µl of HRP were injected into the ventral side of the pylorus, excluding the dorsal part which is covered by the pancreas.

– Finally, in two rats 62 µl of the tracer were administered into the wall of the gastric antrum.

After survival times of one to two days, all animals were perfused transcardially, under ether anesthesia, with saline solution (NaCl 0.9%) followed by 300 ml of fixative (1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4). Both vagi with their sensory ganglia were dissected out and after two hours in the same fixative were stored overnight at 4 °C in the same buffer containing 10% sucrose. Frozen serial sections were then cut longitudinally at 40 µm and processed for HRP histochemistry following Mesulam's technique [15]. Sections from visceral areas injected with HRP were also processed (as controls) together with the vagal ganglia, to verify the histochemical reaction. All sections were examined with bright and dark field microscopy without counterstaining, since it is easier in this way to see the HRP reaction products.

Results and discussion

The table reports the number of labeled neurons found in the left and right nodose ganglia following injections of HRP in different segments of the gastrointestinal wall. As can be seen, the numbers of HRP-positive cells present in the nodose ganglia are not correlated to the total volume of neuronal tracer injected. The labeled neurons were found throughout the ganglion without evidence of a viscerotopic distribution. In some animals no HRP-positive cells were observed in the nodose ganglia on either side. These negative results were not due to a defective histochemistry reaction since the control sections of the visceral areas injected with HRP and processed at the same time were HRP-positive.

In our study of the afferent innervation of the small intestine we were primarily interested in the duodenum,

Number of neurons in the vagal sensory ganglia labeled with the neuronal tracer HRP injected at different sites in the gastrointestinal wall in the rat.

Gastric antrum			Pylorus			Upper duodenum			Lower duodenum		
µl	L	R	µl	L	R	µl	L	R	µl	L	R
62	13	17	10	4	9	50	2	0	100	2	8
60	75	23	10	0	4	50	6	0	100	0	0
			10	18	2	50	5	0	100	1	1
			15	65	13	52	12	1	100	4	2
			25	14	49	54	10	4			
						66	0	2			
						72	0	0			
						72	14	3			
						72	0	0			
						74	0	0			

µl, µl of HRP injected in 1 µl aliquots; L, left nodose ganglion; R, right nodose ganglion.

because of the lack of a precise picture of the vagal afferents. We also believed, on the basis of anatomical studies of the abdominal vagal system, that two different patterns of vagal afferent innervation could be present in the duodenum of the rat. In particular, the upper duodenum, in contrast to the lower segment, should be mostly innervated by the left vagus nerve. In fact, the findings of anatomical dissection indicate that the subdiaphragmatic vagal system is almost the same in the rat, dog and man, and that it includes four truncal divisions (hepatic, celiac, anterior and posterior gastric divisions). Owing to the great difficulty caused by the small size and delicacy of the structure, descending branches leading from the hepatic division to the pylorus, which are also present in dog and man, could not be demonstrated in the rat [16]. The examination of the distribution of the vagus nerve fibers to the stomach in human cadavers revealed that in many cases the major branch of the anterior gastric division (anterior nerve of Latarjet) reaches the pylorus, and in some specimens the initial part of the duodenum as well. On the contrary, in no case does the posterior gastric division of the vagus reach the duodenum [17]. Our data are in agreement with these anatomical studies; in fact, the upper duodenum is mainly innervated by neurons of the left nodose ganglion, whereas its most distal part seems to be equally innervated by both vagi.

The duodenal vagal afferents are scarce compared with those of the gastric antrum. A quantitatively different innervation of these two parts of the gastrointestinal tract has also been shown in the rat by electrophysiological investigations using single fiber dissection techniques in the cervical vagus. Of the 38 single afferent fibers isolated, 34 units were found in the gastric antrum and only four in the small intestine [18]. The vagal afferent endings are both mechanoreceptors and non-specific chemoreceptors, unlike the cat mucosal endings which respond to only one of the several forms of chemical stimuli applied. The presence of several chemoreceptors could partially explain the great number of HRP-labeled neurons found in the cat [5].

As far as the pylorus is concerned, in our study far fewer HRP-positive neurons were found in the nodose ganglia than were reported in a previous study on the guinea pig [19]. The discrepancy can be only partially explained by the species difference. In the study on the guinea pig, HRP injections were made into the whole of the pyloric sphincter, which could have damaged the nearby pancreas, making it easier for tracer to be taken up from nerve terminals of this gland, which is richly innervated by the vagus nerve [14]. Furthermore, the high concentration of HRP used in that study could also have facilitated a backward spreading of the tracer in large quantities, labeling the surrounding tissues and so giving false positive results [20]. With a lower concentration of HRP injected only into the ventral side of the pylorus we believe we have overcome the problem of mislabeling due to diffusion from the injection site. Our results on vagal afferent innervation of the pylorus are of course limited to one side of this sphincter only, but are more reliable.

The number of HRP-positive neurons calculated from our experiments on the vagal gastric afferents is similar to that found in the nodose ganglia of guinea pigs and cats [21]. This study was also one in which mislabeling was avoided, by using a very low concentration of HRP. A study in the rat carried out by injecting a large amount of HRP into the stomach wall resulted in the labeling of the majority of the ganglion cells [11]. Unfortunately, the number of HRP-positive neurons was not specified, so we cannot make a relative comparison; also our investigation was in fact limited to the gastric antrum.

In the rat, the extent of labeling in the nodose ganglia found by studying the vagal afferent innervation of the gastric antrum and pylorus probably indicates that both vagi participate in the reflex regulation of gastric function and reflex control of the pyloric region. In contrast, the vagal afferents are scarcely present in the upper part of the small intestine. Keeping in mind that in the proximal small intestine the vagal efferent projection is also sparse and is limited to the dorsal motor nucleus [11], it is reasonable to suppose that the intrinsic innervation plays a principal role in duodenal function, compared to the extrinsic one of the vagus nerve.

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Vagal afferent innervation in regenerated rat liver

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Summary. The possible presence of neural sprouting in the afferent neurons of regenerated rat liver after hepatectomy was investigated by retrograde transport of horseradish peroxidase. This experiment was carried out to see if the increase in hepatic parenchyma could provide an adequate stimulus for the sprouting process. The study was limited to the vagal afferents, particularly the left ones, because they are the principal contributors to hepatic afferent innervation in the rat. The results show that neural sprouting does not occur in regenerated rat liver after 3 weeks. In fact, the number of intensely labeled neurons in the left nodose ganglia of hepatectomized rats was significantly smaller than in controls. This could be due to a lessened availability of horseradish peroxidase to nerve terminals because of the increased non-innervated hepatic mass. There was no difference between right nodose ganglia neurons in hepatectomized and control animals. This could be a consequence of their possible distribution in hepatic areas not involved in the regenerative process.
Key words. Hepatectomy; neural sprouting; nodose ganglion; horseradish peroxidase.