INTRAMEDULLARY PATHWAYS OF THE VAGO-VAGAL REFLEXES WITH SPECIAL REFERENCE TO THOSE EVOKED BY STIMULATION OF THE ABDOMINAL VAGUS

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Summary An electrophysiological study has been made on the central relay pathway of the vago-vagal reflexes with special reference to those evoked by afferent stimulation of the abdominal vagus in cats under chloralose (50 mg/kg i.v.) anesthesia.

1. Stimulation of the central cut end of the cervical or thoracic vagus nerve produced three mass reflex discharges in the contralateral cervical vagus nerve. On the other hand, stimulation of the abdominal vagal branch caused only the most delayed mass discharge.

2. Among the three mass discharges caused by cervical or thoracic vagal stimulation, at least the third one was regarded as the same discharge as that caused by afferent stimulation of the abdominal vagus nerve.

3. The maximum conduction velocity of the afferent fibres which caused the third response ranged between 1.0–1.4 m/sec.

4. Transection of the medulla oblongata at the level of 5–6 mm rostral to the obex did not abolish the vagal mass discharges but transection at the level of 4 mm abolished or markedly reduced all of them.

5. The third mass discharge and sometimes the second ones were abolished by a superficial dorsal sagittal section (1–2 mm deep) of the obex with its immediate caudal region. On the other hand, the first and sometimes the second were still recorded after such a section.

6. The possibility of the existence of the specific reflex pathway at least for the mass reflex discharge caused by afferent stimulation of the abdominal vagus nerve was discussed.

The vago-vagal reflex effect on gastric motility has been investigated by

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several authors in the cat (ABRAHAMSSON and JANSSON, 1969; CRAGG and EVANS, 1960; HARPER et al., 1959; JANSSON, 1969) and the dog (OHGA et al., 1969, 1970). According to these investigations, this effect predominantly provided relaxation or inhibition of the stomach. OHGA et al. (1969, 1970) and JANSSON (1969) suggested that this gastric inhibitory response might be mediated through non-adrenergic inhibitory fibres in the efferent vagal pathway to the stomach (CAMPBELL, 1966; MARTINSON, 1965). Although the relation between the reflex activity of the vagus nerve and the reticuloruminal motility of the ruminants was studied by IGGO and LEEK (1967), little is known about the vagal reflex control of the motility of the simple stomach in nonruminants.

NAKAZATO (1968) has previously shown in cats that stimulation of the central end of the cervical vagus nerve evoked three mass reflex discharges in the contralateral cervical and thoracic vagus but only a delayed reflex discharge in the abdominal vagus nerve. It seems possible to assume that the reflex discharge recorded from the abdominal vagus nerve may play a certain role in the vagal reflex control of abdominal visceral functions. The purpose of the present experiment has been to explore the vago-vagal reflex pathway mediating in particular the reflex due to afferent stimulation of the contralateral abdominal vagal branches. Of course, there is also the ipsilateral vago-vagal reflex (RUDOMIN, 1965), but, in the present study, observations were limited to those recorded in the contralateral vagus nerve.

METHODS

Thirty-five adult cats were used. Under ether anesthesia, the trachea was cannulated and the bilateral cervical vagus nerves were exposed. The right thoracic and dorsal vagal trunks (hereafter referred to as the abdominal vagus nerve) were exposed by opening the thorax after removing all the ribs of the right side under artificial ventilation. After these surgical procedures, anesthesia was maintained by an intravenous injection of chloralose (50 mg/kg) and the animal was immobilized by the periodic intravenous administration of gallamine triethiodide (Flaxedil).

Pairs of stimulating electrodes were applied to the cervical, thoracic and abdominal vagus nerves at the middle of the neck, just caudal to the branching of the recurrent nerve and immediately rostral to the diaphragm, respectively. The distance between the pairs of stimulating electrodes was 7–11 cm for those in the cervical and thoracic nerves and 6–9 cm for those in the thoracic and abdominal nerves.

Mass reflex discharges were recorded from the central cut end of the left cervical vagus nerve at the middle of the neck and displayed on a double-beam oscilloscope through C-R coupling preamplifiers (time constant 0.3 sec) and photographed on an X-ray film. These arrangements are shown in Fig. 1.
To examine the central relay pathway of the vago-vagal reflexes, after tracheal cannulation the animal was fixed on a stereotaxic holder in a prone position. Thereafter, the bilateral cervical vagus nerves were exposed by making an incision in the dorsal neck skin and separating *M. clavotrapezius* from *M. splenius* and were prepared for recording and stimulation. The lower brain stem was also exposed by removing the occipital skull and by suction of the cerebellum. Serial transections and sagittal sections of the medulla oblongata were carried out with a thin metal spatula or a piece of safety-razor blade mounted on the stereotaxic apparatus. All the dissected nerves and the exposed medulla oblongata were protected by a pool of warm liquid paraffin.

A pair of silver wire electrodes of 8–10 mm interpolar distance were used for stimulation and recording. Stimulation consisted of a rectangular pulse of 1 msec in duration and of various intensities and was applied at a rate of 1 pulse every three seconds.

At the end of each experiment, the brain stem was removed and the level, length and depth of the section were verified. Thereafter, it was immersed in a 10% formalin solution.
RESULTS

Vagal reflex discharges produced by stimulation of the cervical, thoracic or abdominal vagus nerve

Electrical stimulation of the central cut end of the cervical vagus nerve evoked three mass reflex discharges in the contralateral cervical vagus nerve. The latencies of these mass discharges were $7 \pm 2$ msec, $71 \pm 18$ msec and $107 \pm 13$ msec (means $\pm$ S.D. $n=5$ hereafter), respectively. Stimulation of the thoracic vagus nerve also produced three mass discharges although in some cases the second and third ones fused into a complex form. The latencies were $11 \pm 5$ msec, $138 \pm 12$ msec and $192 \pm 15$ msec, respectively. On the other hand, stimulation of the abdominal vagus nerve caused only one mass discharge, the latency of which was $251 \pm 17$ msec. In Fig. 2, an example of these responses is shown.

Fig. 2. Mass reflex discharges recorded from the left cervical vagus nerve. A, B and C: the mass discharges produced by stimulation of the cervical, thoracic, and abdominal vagus nerves, respectively. 1, 2 and 3 represent the first, second and third mass reflex discharges. Numerals on the right side indicate the distance between each stimulating electrode. White dots indicate artifacts of stimulation.
As evidenced by this figure, the shapes of each mass discharge evoked by stimulation of the cervical vagus nerve resembled those caused by the thoracic vagal stimulation. In most cases, the amplitude was highest and the threshold was lowest in the first mass discharge. In order to evoke the second and third mass discharges, the stimulus intensity had to be raised to between five and ten times that of the first one (Fig. 3). In general, the amplitudes of the second and third were approximately the same. The amplitude and threshold of the mass reflex discharge evoked by afferent stimulation of the abdominal vagus nerve were
close to those of the second and third ones evoked by the cervical and thoracic vagal stimulation. As shown by an interrupted line in Fig. 2, the latencies of the third mass discharges and the mass discharge caused by abdominal vagal stimulation shifted on a straight line.

From all these results, it seems most likely that the third mass discharges evoked by stimulation of either cervical or thoracic vagus nerves are the same in origin as those caused by afferent stimulation of the abdominal vagus nerve. On the basis of this assumption, the maximum conduction velocity of the afferent fibres causing the third mass discharge was estimated from the difference in the latencies and the distance of each pair of stimulating electrodes. As a result, it ranged between 1.0–1.4 m/sec in four cats.

**Effect of transection of the lower brain stem on the mass reflex discharges**

It has been shown in the previous paper that the vagal reflex discharges could still be recorded even after the transection of the brain stem at the uppermost level of the medulla oblongata (NAKAZATO, 1968). In the present study, more detailed analysis has been made on pathways of the vago-vagal reflexes. Frequently the mass reflex discharges were reduced by removing the occipital skull and cerebellum alone. However, in most cases, they could be partially recovered after a certain time lag if a brief tetanic stimulation was applied. Therefore, the tetanic stimulation with frequency at 50 Hz was invariably applied for 10–15 sec prior to the observation of the response to a single pulse stimulation.

![Summary of the transection study in nine cats. One sign means one transection. Numerals indicate the distance from the obex. The plus sign represents the presence and the minus sign the disappearance of the mass reflex discharges after the section at each level. The drawing is a semidiagram of the dorsal view of the lower brain stem.](image)

Using nine animals, twenty-six transections of the medulla oblongata were made. The results are summarized in Fig. 4. As shown in this figure, the mass reflex discharges caused by afferent stimulation of the cervical vagus nerve were still recorded after transection of the brain stem at the level of 5–6 mm rostral to the obex. When transection, however, was carried out at the level of 4 mm rostral
Fig. 5. Effects of transections of the lower brain stem on the mass reflex discharges. (a), Control after removal of the cerebellum; (b), after transection at 6 mm; (c), at 4 mm rostral to the obex. The levels of transection are indicated with heavy black lines in the drawing of the dorsal view of the lower brain stem.

to the obex, the mass discharges disappeared. An example is illustrated in Fig. 5. On the other hand, the spinal transections at C1 or C2 levels did not affect them significantly. These results indicate that the mass reflex discharges may be derived from the region of the medulla oblongata caudal to the level of entrance of the vagal rootlets.

**Effect of sagittal section of the lower brain stem on the mass reflex discharges**

In order to determine transmedullary pathways involved in the mass reflex discharges, successive longitudinal sections of the medulla were made along the midline from the level of the entrance of the vagal rootlets to 5–6 mm caudal to the obex. At first, the region rostral to the obex was sectioned along the midline of the floor of the fourth ventricle. Even when the section reached closely adjacent to the obex, the mass discharges did not disappear (Fig. 6 A). The results in four animals are summarized in Table 1. On the other hand, when the obex along with its immediate caudal region was severed, all mass discharges were completely eliminated. Therefore, the most important transmedullary pathway for the mass reflex discharges may be located in the small restricted region near the obex. Finally, in order to determine the caudal limit of the effective region, serial sagittal sections were made in the part caudal to the obex along the midline.
Fig. 6. Effects of sagittal sections of the lower brain stem on the mass reflex discharges. A, sagittal section of the rostral region and B, of the caudal region of the obex along the midline. (a), Control; (b), after the section. The parts of the section are indicated with heavy black lines in the drawing.

Until the section reached 1–2 mm caudal to the obex, the mass discharges could still be recorded (Fig. 6 B, Table 1).

The question has been raised whether or not the reflex pathways of each mass discharge are different. In order to gain some insight into this problem, the obex with its immediate caudal region was severed at various depths along the midline. When the sagittal section was restricted to the dorsal superficial layer (1–2 mm deep), the third mass discharge disappeared in all seven animals (Fig.
Table 1. Effects of sagittal section of the lower brain stem on the vagal mass reflex discharges. a, extension of the unsectioned region from the obex along the midline; b, longitudinal length of the section; c, depth of the section; →, unchanged; ↓, decreased; ↓↓; abolished; 1, 2, 3, numbering of the mass discharges.

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>a (mm)</th>
<th>b (mm)</th>
<th>c (mm)</th>
<th>Reflex discharges</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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<tr>
<td>104</td>
<td>1.5</td>
<td>3</td>
<td>total</td>
<td>↓    ↓↓    ↓</td>
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<tr>
<td>126</td>
<td>1</td>
<td>6</td>
<td>total</td>
<td>→    →    →</td>
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<tr>
<td>127</td>
<td>1</td>
<td>6</td>
<td>total</td>
<td>→    →    →</td>
</tr>
<tr>
<td>132</td>
<td>1.5</td>
<td>6</td>
<td>total</td>
<td>→    →    →</td>
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<tr>
<td>Caudal region to the obex</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>2</td>
<td>3</td>
<td>total</td>
<td>↓↓    ↓↓    ↓</td>
</tr>
<tr>
<td>105</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>→    →    →</td>
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<tr>
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<td>4</td>
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<tr>
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<td>1</td>
<td>5</td>
<td>total</td>
<td>→    →    →</td>
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<tr>
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<td>1</td>
<td>4</td>
<td>total</td>
<td>↓↓    ↓    ↓</td>
</tr>
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Table 2. Effects of superficial sagittal section of the obex along with its immediate caudal region on the vagal mass reflex discharges.
See abbreviation at Table 1.

<table>
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<th>Cat No.</th>
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<th>b (mm)</th>
<th>c (mm)</th>
<th>Reflex discharges</th>
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<td>1.5</td>
<td>→    ↓↓    ↓↓</td>
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<tr>
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<td>1.7</td>
<td>1.5</td>
<td>↓    ↓↓    ↓↓</td>
</tr>
<tr>
<td>111</td>
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<td>2</td>
<td>1</td>
<td>↓    ↓    ↓↓</td>
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<tr>
<td>113</td>
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<td>1.5</td>
<td>↓    ↓    ↓↓</td>
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<tr>
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<td>1.5</td>
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<tr>
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<td>↓    →    ↓↓</td>
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</table>

7 A). In three cases, the second one also disappeared. On the other hand, the first mass discharge remained following this section, although the amplitude was decreased to some extent. Details are shown in Table 2. These results were further confirmed in three cats in which only the deeper layer at the level of the obex was severed. After this section, the first and second discharges disappeared and only the third one remained (Fig. 7 B).

In view of these results, it seems likely that at least the third mass discharge is derived from the dorsal superficial nervous structures (within 1 or 2 mm in depth) and the other ones from the deeper structures than this in the small restricted region surrounding the obex.
Fig. 7. Effects of sagittal section to various depths of the obex with its immediate caudal region on the mass reflex discharges. A: superficial section (1–2 mm deep). B: section of the deeper structure. (a), Control; (b), after the section. Sectioned regions are indicated by black areas in the drawing of the lateral view of the lower brain stem.

DISCUSSION

A single pulse stimulation of the central cut end of the cervical or thoracic vagus nerve elicited three mass reflex discharges in the contralateral cervical vagus nerve. On the other hand, stimulation of the abdominal vagus branch produced only a delayed mass discharge. In general, the first mass discharge evoked by afferent stimulation of the cervical or thoracic vagus nerve had a much lower
threshold and a higher amplitude than those of the second and third ones. The threshold and the amplitude of the second and third discharges were approximately equal to those caused by abdominal vagal stimulation.

In the previous report (NAKAZATO, 1968), it was shown that among the cervical vago-vagal mass reflex discharges due to contralateral cervical vagal stimulation, the first mass discharge was mainly conveyed to the recurrent nerve and the third one was the only discharge which reached the abdominal vagal branch. In the present experiment, afferent stimulation of the abdominal vagus nerve caused only the delayed mass discharge in the contralateral cervical vagus nerve. This delayed discharge corresponded to the third discharge produced by afferent stimulation of the cervical or thoracic vagus nerve. Accordingly, it seems likely that the delayed mass discharge caused by stimulation of the abdominal vagus nerve is due to the reflex activation of the contralateral vagal efferent fibres to the abdominal organs.

The maximum conduction velocity of the afferent fibres which were involved in the initiation of the third mass discharge was about 1 m/sec. This value ranged within those reported by Iggo (1958), who stated that the conduction velocities of the vagal afferent fibres from gastric and intestinal stretch receptors ranged from 0.5 to 2.3 m/sec and that these fibres belong to C fibres.

According to Agostoni et al. (1957), about 16% of the total number of fibres in the cervical vagus nerve are myelinated in the cat. Among them, most of the large myelinated fibres above 10 μ in diameter pass to the recurrent nerve and are efferent (Murray, 1957). The number of myelinated fibres decreases to about 10% in the upper thoracic level and to about only 1% in the abdominal branches. Therefore, especially in the abdominal vagus nerve, almost all fibres are non-myelinated irrespective of whether they are efferent or afferent. This fibre distribution of the vagus nerve may support the authors' interpretation for their experimental results. Although the functional significance of the mass reflex discharges in the present experiment are, of course, unknown, it may be possible that at least the third mass discharge is partly participating in the vago-vagal reflex control of gastric motility.

The main central nervous structures involved in the vago-vagal reflexes are probably present in the small restricted region near the obex. The sagittal section of the obex with its immediate caudal region along the midline abolishes all the mass reflex discharges completely. However, when the section is restricted to 1–2 mm in depth from the dorsal surface along the midline, the third mass discharge is selectively abolished and in some instances the second one also.

Histologically, the pathway of the primary afferent fibres of the vagus nerve in the medulla oblongata has been investigated (Cottle, 1964; Foley and Dubois, 1934; Ingram and Dawkins, 1945; Kerr, 1962). According to previous authors, the primary afferent fibres enter the tractus solitarius and descend in the tract giving off collaterals and terminals to the nucleus of the tractus solitarius. Some
collaterals also reach the commissural nucleus of Cajal bilaterally at the level just caudal to the obex. Furthermore, Kerr (1962) reported that after giving off some collaterals to the contralateral commissural nucleus, a moderate number of afferent fibres of vagal origin could be followed upward into the contralateral nucleus of the tractus solitarius. On the other hand, evoked potentials in response to cervical or thoracic vagal afferent stimulation have been recorded from the tractus solitarius, the nucleus of tractus solitarius, the commissural nucleus and in their vicinity in the medulla oblongata (Anderson and Berry, 1956; Hellner und von Baumgarten, 1961; Lam and Tyler, 1952). Porter (1963) has written that evoked unit responses recorded from the vicinity of the nucleus ambiguus are probably an antidromically activated response and those from the tractus solitarius, nucleus of tractus solitarius and dorsal motor nucleus of the vagus are possibly a synaptically activated response to vagal afferent stimulation. Furthermore, some units recorded in the region immediately posterior to the nucleus of the tractus solitarius and in the commissural nucleus could be excited by both the ipsilateral and contralateral vagal stimulation. On the basis of these previous findings, the following assumption was made in the present experiment on the medullary pathway of the vagal reflexes. That is, after entering the medulla through the vagal rootlets, the vagal afferent impulse descends in the tractus solitarius as far as the levels of the obex. At the level just caudal to the obex, the impulse traverses the midline toward the contralateral side and then it ascends via a certain course to the level of the contralateral vagal rootlets and flows out through them as the efferent impulse. In particular, the pathway for the third mass reflex discharge at the level of the obex may exist in the more superficial dorsal structures at the midline than the pathways for the other mass discharges. This superficial structure might be the commissural nucleus of Cajal.

The excitatory and inhibitory points for the motility of the stomach were located by stimulation of the medulla oblongata (Kuru and Sugihara, 1955; Semba et al., 1964). It is uncertain, however, whether the excitatory as well as inhibitory responses are produced by direct stimulation of the vagal motoneurons or by stimulation of their axons. Recently, Kerr (1969) and Kerr and Preshaw (1969) emphasized by their chronic degeneration experiment that the dorsal motor nucleus of the vagus does not supply the motor fibres to the smooth muscle of the bronchioles, esophagus and duodenum and the cardio-inhibitory fibres, but it does supply the secretory ones. Accordingly there is much less information on the sources of the motor fibres especially those innervating the abdominal organs in the medulla oblongata. In the present experiment, the transmedullary pathways for the vagal reflex discharge were roughly distinguished by surgical section of the lower brain stem. Further detailed studies have to be carried out in order to clarify the vagal pathways in the central nervous system and these will contribute to the further understanding of the vagal reflex control mechanism of gastrointestinal motility and secretion.
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