Splanchnic-to-vagal Reflex in Cats

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In lightly anesthetized cats splanchnic nerve stimulation was effective to activate the vagus nerve reflexly. Such effect was hardly seen in somatic nerve stimulation except for the state of systemic strychninization. The A gamma-delta fibers of the splanchnic nerve were responsible for the vagal reflex. The reflex discharges, appearing in the vagi of both sides independently of the side of peripheral stimulation, were mainly conducted to the recurrent laryngeal branch, though some activity could be traced in the broncho-visceral branch, too. Using dual shocks of varying shock-intervals the excitability cycle of the reflex was determined. The reflex inhibition was observed by activating the somatic nerves. This was explained, at least partly, as due to sensory block at the spinal level. The phenomenon of post-tetanic potentiation could be demonstrated by using threshold stimulation. Effects of central nervous stimulation upon the reflex were examined; the inhibitory points were found in the brainstem reticular formation and the hypothalamus while the facilitation was obtained from some restricted points of the brainstem and the cortical surface. The limbic system showed no apparent effects upon the reflex.

The splanchnic-to-vagal reflex means the reflex appearing in the vagus nerve as the efferent in response to stimulation of the splanchnic nerve as the afferent. It may be one of the basic types of the viscerovisceral reflex of which only insufficient studies have been made with the electrophysiological method. Ozawa¹⁰⁾ was the first who studied this type of the viscerovisceral reflex with special attention to the reflex inhibition by bladder distension or its equivalent. The purpose of the present paper is to describe some of the important properties of the reflex which have been left unrevealed.

METHODS

Cats were used. Most of the animals were prepared under pentobarbital sodium anesthesia. Since it was shown in the preliminary observations that the

good reflex activity was maintained only in light anesthesia, the dose of the anesthetic administered during the preparation was kept as small as possible. Some animals were prepared under ether or chloralose anesthesia. In all experiments the prepared animals were fixed to a Horsley-Clarke apparatus with immobilization by Flaxedil and maintained on artificial respiration.

The large splanchnic nerve was exposed retroperitoneally. It was crushed at a point as close as to the abdominal ganglion. Proximally to the crushed point, the nerve was laid on buried electrodes separated by about 3 mm. The nerve stimulation was conducted with a short electric pulse with variable intensities at a rate of 0.5–0.6 c.p.s.

The vagus nerve was exposed in the cervix and cut at the level of entrance to the thorax. After carefully isolating the nerve from the surrounding tissues, two wire electrodes, separated by about 5 mm, were attached to it in order to pick up the nerve impulses bipolarly. The vagal impulses were amplified with a suitable amplifier and displayed on the screen of a cathode ray oscillograph.

The methods for recording the action potential from the sympathetic trunk and the spinal cord and those for stimulating the central nervous system will be discussed in the related sections of this paper.

RESULTS

Single shock stimulation of the splanchnic nerve of one side caused reflex discharges in the vagi of both sides without showing any marked differences between them. Also there were found no appreciable differences in the responsiveness between the both sides of the splanchnic nerve. The reflex volley appeared as massive synchronous discharges of 10–15 msec in duration with an amplitude of 50 μ V or more. The latency of the reflex discharges was usually 15–20 msec and it was shortened by 1–2 msec when maximum stimulation was used. With deterioration of the animal the reflex latency tended to prolong up to 30 msec. The intensity of splanchnic nerve stimulation needed to elicit the vagal reflex response was 1–2 volts when a single square pulse was used with a duration of 0.3–0.5 msec. When the reflex could not be elicited with ease, stimulation with a train of 2–3 pulses of 100–200 c.p.s. was effective. Usually the maximum response obtained by pulse train stimulation was larger than that by single pulse stimulation.

Peripheral source of the vagal reflex. Since the stimulating electrodes were buried so deeply that it was difficult for us to isolate from stimulation the tissues near the splanchnic nerve. However, the following control experiment could provide evidence that spread of the stimulating current is not a serious problem. After a good reflex response of the vagus nerve was found to appear with the routine method of electrode placement, the splanchnic nerve was crushed at a point just proximal to the electrodes. This resulted in a complete abolition of the

vagal response. Then the electrodes were shifted a few millimeters proximally to the crushed point. The vagus nerve under examination again showed the reflex response with the same strength as before.

The splanchnic nerve was found much more potent to activate the vagus nerve reflexly than the somatic nerves. This will be seen in Fig. 1 where the effect of

Fig. 1. Vagal reflex responses to single shock stimulation of the splanchnic (SPL), the sciatic (SCI) and the inferior alveolar nerve (TRI) in normal and systemically strychninized conditions. A, controls. B, 3-4 min. after strychninization (0.2 mg/kg). C, 5 min. after pentobarbital sodium injection (6.0 mg/kg). The anesthetic was given 5 min. after strychninization. The time mark in this and all subsequent figures is 10 msec unless otherwise mentioned. Voltage, 50 μ V.

nerve stimulation was compared among the splanchnic (SPL), the sciatic (SCI) and the inferior alveolar nerve (TRI). Records of column A were obtained under the usual condition of the animal. The vagal reflex response appeared with a sizable amplitude only to splanchnic nerve stimulation, whereas the response to sciatic nerve stimulation was very small and that to alveolar nerve stimulation was insignificant. Then the animal was given strychnine of 0.2 mg per kg of body weight through an intravenous route. Following this procedure stimulation of the somatic nerves, particularly that of the sciatic nerve, acquired some effectiveness. But it still could not exceed the effect of splanchnic nerve stimulation (column B). The heightened excitability of the reflex due to systemic strychninization was again reduced to the control level by administering a small dose of pentobarbital sodium (6.0 mg per kg of body weight) (column C).

Finally it was attempted to determine which component of the splanchnic nerve was responsible for the vagal reflex response. To identify the type of the fibers activated, the sympathetic trunk was exposed by resecting several of the lower ribs and mounted on bipolar electrodes to pick up action potentials in response to splanchnic nerve stimulation. An example of the simultaneous recordings of the vagal reflex response and the sympathetic action potential is reproduced in Fig. 2. When the splanchnic shock was weak, the sympathetic

Fig. 2. Simultaneous recording of the vagal reflex response (upper tracing) and the action potentials of the sympathetic trunk (lower tracing) to stimulation of the splanchnic nerve. Conduction distance in the sympathetic recording was about 6 cm. Stimulus intensities were 2 volts in A, 8 volts in B and 15 volts in C. Time, 10 msec for upper tracings and 1 msec for lower ones.

action potential was a single monophasic wave which had a conduction velocity of 70–80 m per second. Increasing the stimulus intensity to 2 volts, the wave acquired the maximum amplitude, but there were no vagal responses at all (record A). When the stimulus intensity was 8 volts, the sympathetic trunk showed an additional small activity having a conduction velocity of 30–35 m per second (record B). It was this new wave that was associated with the reflex response of the vagus nerve. When a very intense stimulus was applied to the splanchnic nerve, the third deflection appeared in the sympathetic recording with a conduction velocity of 13–15 m per second, but there occurred no changes in the vagal activity (record C). According to the previous workers^{2,9}, the three waves of the sympathetic action potential are ascribed to the A beta, A gamma-delta and the B groups, respectively, in the order of conduction velocity. It is thus concluded that the fibers of the splanchnic nerve contributing to the splanchnic-to-vagal reflex belong to the A gamma-delta group.

Distribution of the vagal reflex discharges. The vagus nerve sends its branches to many organs in the cervix and the thorax. To know what structure is most seriously affected by the reflex discharges descending through the cervical vagus would be of some interest. Since dissection of the fine branches is difficult to perform in the thorax, our approach was limited to a comparison of the activity between the recurrent laryngeal and the broncho-visceral branch. An example of the results shown in Fig. 3 clearly demonstrates that most of the impulses of the main vagus seem to be transferred to the recurrent laryngeal branch. The activity of the broncho-visceral branch could be detected only with a high amplification. The physiological significance of this finding will be discussed in the later section.

Excitability cycle. Aiming at determining the excitability cycle of the splanchnic-to-vagal reflex, two identical stimuli of maximum strength, spaced at varying intervals, were applied to the splanchnic nerve as the conditioning and

Fig. 3. Simultaneous recording of the reflex discharges from the main vagus trunk (A), the recurrent laryngeal branch (B) and the broncho-visceral branch (C). Note high amplification in C.

testing stimuli. There were many instances where no clearcut refractoriness was found to follow the conditioning stimulus. In some cases, however, there appeared some suppression of the test response at the conditioning-testing intervals less than 10 msec. In this case the complete recovery of the reflex excitability was obtained about 40–50 msec after the conditioning shock. We have found no good explanation why the refractoriness can be detected in some cases and it cannot in other cases.

When the stimulus was of submaximum strength, a summation of the conditioning and testing responses was seen at the shock-intervals less than 10 msec, and then there occurred a facilitation sustaining from 10 to 30 msec after the conditioning shock. In some cases there appeared a phase of inhibition starting about 60 msec and the control level of the reflex excitability was restored about 100 msec after the conditioning shock.

The above-mentioned complexity of the excitability cycle of the splanchnic-to-vagal reflex is partly illustrated in Fig. 4. In this experiment the vagus nerve was split into thin filaments and one of them was subjected to the recording. The filament examined in this case was found to contain two elements which were distinguished from each other by the spike size. The intensity of splanchnic nerve stimulation was adjusted to activate only the large spike but not the small spike. When the testing shock was preceded by the conditioning one with the interval of 30 msec, the testing response was such that the latency of the large spike was slightly reduced and the small spike was concomitantly activated, thus suggesting that there is acting some facilitation. Such facilitation, however, was soon replaced by the inhibition. When the conditioning-testing interval was set at 80 msec, the testing shock now completely failed to activate the large spike. The last record of Fig. 4 indicates that the inhibition had disappeared and the reflex

Fig. 4. Unitary responses of the splanchnic-to-vagal reflex. Two identical stimuli of submaximum strength were applied with time intervals indicated below each tracing. The stimuli are marked with arrows. Among the two elements activated reflexly, the small spike appeared sometimes spontaneously. Voltage, $100\mu\text{V}$.

excitability has been restored to the control level within 120 msec after the conditioning stimulus.

Post-tetanic potentiation. The phenomenon of the post-tetanic potentiation is very familiar in the study of the somatic nerve reflex. This could be demonstrated with the splanchnic-to-vagal reflex as illustrated in Fig. 5. After the control reflex responses were recorded by stimulating the splanchnic nerve every 1.5 seconds with a threshold intensity (column A), the tetanic stimulation was made with the same stimulus intensity at a frequency of 85 c.p.s. for 30 seconds. Immediately following this, there was observed a great enhancement of the reflex response (column B). The reflex potentiation so established, however, was hardly maintained for a long time. It was found 30 seconds after the tetanic stimulation that

Fig. 5. Post-tetanic potentiation. Records in A, B and C were obtained, respectively, before, immediately after and about 30 seconds after tetanic stimulation of the splanchnic nerve lasting for 30 seconds. The stimulus intensity was just liminal and was maintained at the same level throughout this experiment. Small dots at the beginning of the sweep mark the splanchnic shocks. Voltage, $20\mu\text{V}$.

the reflex excitability had been reduced to the level slightly higher than the control. It seems important to note that the post-tetanic potentiation as demonstrated here was difficult to obtain with the use of maximum stimulation.

Reflex inhibition by somatic nerve stimulation. Ozawa found that the splanchnic-to-vagal reflex was inhibited by bladder distension or by high frequency stimulation of the pelvic nerve. A similar but more profound reflex inhibition was obtained by activating with 100–200 c.p.s. stimulation of the somatic nerve such as the sciatic, the ulnar or the inferior alveolar nerve. Among the three nerves mentioned above, the inhibition originating from the sciatic nerve was more powerful than that from the remaining nerves. The ulnar or the inferior alveolar nerve required much stronger stimulation to produce the inhibition than did the sciatic nerve. In addition, there was a tendency for the inhibition produced by alveolar nerve stimulation to subside gradually while the stimulation was continued.

The time course of the reflex inhibition of somatic nerve origin was studied by using single shock stimulation of the sciatic nerve applied at varying time intervals preceding the testing reflex. Fig 6 shows a typical experiment. First, it is noted that the inhibition became evident 25 msec after the sciatic shock and then it was strengthened until the maximum was attained at the shock-interval of about 40 msec. Since the decay of the inhibition progressed rather slowly, it was about 800 msec after the conditioning shock that the testing reflex appeared with a size approximately the same as the control.

Fig. 6. Reflex inhibition by single shock stimulation of the sciatic nerve. A control response is in top left. Remaining records are to show reflex responses preceded by sciatic shocks with time intervals indicated in msec. Shock artifacts due to splanchnic nerve stimulation are marked with small dots. Voltage, 50 μ V.

Fig. 7. Recording of the spinal ascending volley. A, a control record of the spinal sensory volley due to splanchnic nerve stimulation. B, complete abolition of the splanchnic volley by a preceding conditioning shock to the sciatic nerve. Splanchnic shocks were marked in the time scale. Recordings were made from the ventrolateral column of C_{2-3} . Voltage, $50\mu V$.

The reflex inhibition may occur at any levels of the central nervous system. The experimental results shown in Fig. 7 may be taken as evidence that the reflex inhibition, at least a part of it, is produced at the spinal level. In this experiment the spinal cord was exposed at the level of C_{2-3} and a tungsten microelectrode, varnished except at the tip (diameter, $5-10\mu$), was introduced to the ventrolateral column in order to pick up the spinal ascending volley. Since it has been established by Ozawa and confirmed by us that the spinal afferents for the splanchnic-to-vagal reflex ascend through the ventrolateral column, excluding the dorsal column, the sensory impulses picked up from this portion in response to splanchnic nerve stimulation may be considered as representing activity of the central sensory pathway of the reflex at issue. An example of the records is shown in Fig. 7, A. Upon single shock stimulation of the splanchnic nerve there appeared in the ventrolateral column of the spinal cord the discharges of a considerable magnitude with a latency of about 20 msec. In contrast to this, record B shows the effect of a conditioning shock which was applied to the sciatic nerve about 120 msec preceding the splanchnic shock. While there appeared large discharges to the sciatic shock, there were no responses to the splanchnic shock. This finding suggests that at least a part of the reflex inhibition is explained as due to blocking of the sensory input to the reflex center.

Effects of central nervous system stimulation. Several experiments were performed to clarify the regulatory effects of the central nervous system upon the splanchnic-to-vagal reflex. The explored structures were as follows; the cerebral cortex, the pyriform lobe, the hippocampus, the amygdaloid nuclei, the hypothalamus including the preoptic region, and the reticular formation of the midbrain and of the medulla. For stimulating deep structures, concentric electrodes with tips separated by 0.1–0.5 mm were introduced stereotaxically (sites of stimulation were checked in the later histological examination). When the cerebral cortex was explored, stimulation was made with bipolar electrodes having ball tips. In both cases of stimulation a short electric pulse repeated at a frequency of 100–200 c.p.s. was used.

It was a consistent finding that the mesencephalic and the bulbar reticular formation and the hypothalamus exerted the heavy inhibitory effects upon the reflex. The effects originating from the hippocampus, the amygdaloid nuclei and the pyriform lobe were of some interest. High frequency stimulation of these limbic structures had no apparent effect upon the reflex. However, when these structures received single shock stimulation, there appeared some efferent discharges in the vagus nerve of the side ipsilateral to the central stimulation. This finding agrees well with that by Akert and Gernandt¹¹.

There were occasionally found in the brainstem some restricted loci exerting an extreme facilitatory effect upon the reflex. The sites of stimulation were located in or near the cerebral peduncle and the nucleus reticularis tegmenti.

Most of the cortical areas, except for the somatosensory area, were found to exert no obvious effects upon the reflex. Within the somatosensory area there seems to exist some complex organization. It was sometimes found that high frequency stimulation of a point within the trunk region of the somatosensory area I was effective to augment the vagal reflex response. This is exemplified by Fig. 8. In contrast to this, stimulation of a point slightly medial to the facilitatory

Fig. 8. Facilitation of the splanchnic-to-vagal reflex by high frequency stimulation of a somatosensory cortical point. A, B and C were recorded before, during and after cortical stimulation, respectively. Upper and lower tracings are the responses on the sides ipsi- and contralateral to cortical stimulation.

point was found to produce a weak inhibition. Concerning the somatosensory area II, only a weak facilitatory effect was very rarely observed by stimulating the region below the ectosylvian sulcus.

DISCUSSION

As has been shown by Gernandt and Zotterman⁷), it is the A gamma-delta fibers in the splanchnic nerve that causes viscer-enteric nociceptive reaction. That the A gamma-delta fibers of the splanchnic nerve serve as the reflex origin is

confirmed in the present experiment where the vagal outflow is used as the index.

The reflex discharges of the vagus nerve evoked by splanchnic nerve stimulation have been found to be conducted mostly to the organs innervated by the recurrent laryngeal branch. This suggests that the reflex has some relation with vocalization like growl as well as with visceral reaction such as vomiting. In fact, Borison and Wang⁴⁾ described in the study of localizing the vomiting center that vomiting often accompanied vocalization and the vagotomy delayed vomiting. Herrin and Meek⁹⁾ also demonstrated that intestinal distension caused vomiting and the afferents for this reaction were the sympathetic including the splanchnic nerve.

The excitability of the splanchnic-to-vagal reflex pathway was determined by using a single shock or repetitive ones as the conditioning stimulus. Apart from some minor points, we have found no particularity in the splanchnic-to-vagal reflex in comparison with the somatic nerve reflex of an ordinary type. However, the problem has remained unattacked at which level, spinal or medullary, the excitability of the reflex is determined principally.

Studying the visceromotor reflex Downman⁵⁾ has reported that afferent impulses from the hind limb inhibit the splanchnic-to-intercostal reflex. Ozawa has shown inhibition of the splanchnic-to-vagal reflex by bladder distension or by high frequency stimulation of the pelvic nerve. With the same reflex we have found a strong inhibition by activation of the sciatic nerve. As to the inhibitory mechanism, Ozawa supposed that it resides within the medulla. Downman and Evans⁶⁾ have found that the splanchnic and the hind limb afferents do not interact with each other in the spinal cord, though they intermingle there extremely. Ammasian³⁾ has maintained that the mutual interaction between the A beta fibers of the splanchnic nerve and the somatic afferents occurs above the thalamic level. Thus, the interaction, if any, between the splanchnic afferents and the somatic ones has been considered as taking place at the medullary level or a level higher than that. However, a high degree of occlusion has been observed to exist between the splanchnic and the somatic nerve in regard to the dorsal column potential¹²⁾. In addition, the present experiment has provided evidence that the sensory impulse originating in the splanchnic nerve is completely stopped by sciatic nerve stimulation from ascending through the spinal cord. From these facts it is admitted that the mutual interaction between the somatic and the visceral afferents occurs at the spinal level as well.

We have pointed out the importance of the spinal sensory block as one of the factors for explaining the reflex inhibition due to somatic nerve activation. Another likely factor would be that the somatic afferent impulses ascend to the upper brain, for example, to the brainstem reticular formation, and the inhibitory effect is in turn triggered therefrom. This is plausible because we have shown that a wide area of the brainstem causes the reflex inhibition upon high frequency

activation. The notion that the reflex inhibition of somatic nerve origin is realized under the support of the upper brain would be applied to the case of activation of one branch of the trigeminal nerve. In considering the mechanism of the reflex inhibition of central nervous system origin, however, it seems necessary to call attention again to blocking of the sensory side of the reflex pathway, because Tolle *et al.*¹¹⁾ have shown that the spinal cord potential by splanchnic nerve stimulation is inhibited by activation of the bulbar reticular formation.

We do not yet know what physiological significance is ascribed to the reflex inhibition which is obtainable from a wide area of the brainstem. In connection with this, the finding is noted by Yamamoto and Araki¹³⁾ that the intra-abdominal pressure reflex of pelvic nerve origin is inhibited by brainstem activation. Though the reflex studied by these authors is effected on the somatic muscles of respiration, it is common with the reflex studied here in the point that both are of visceral origin. Whether the reflex regulation originating from the brainstem is of predominantly inhibitory nature in so far as the visceral reflex is concerned is a problem to be solved in future experiment.

In accordance with Akert and Gernandt¹⁾ single shock stimulation of the limbic structure is effective to elicit the vagal outflow, but high frequency stimulation of the same structure has none of the apparent effects upon the vagal response elicited reflexly. This is in contrast with the fact that the splanchnically evoked vagal response is greatly enhanced by high frequency stimulation of the splanchnic nerve itself (post-tetanic potentiation). It is supposed that the pathway descending from the limbic structure is somewhat different from that ascending from the splanchnic nerve in the mode of connection to the origin of the efferent vagal fibers. For this problem further experiment seems to be required.

The somatosensory cortical area has been found to contain some complex organization to regulate the splanchnic-to-vagal reflex. In view of the fact that the vagal reflex discharges are mainly destined to bombard the organs innervated by the recurrent laryngeal nerve, the somatosensory cortical control of the reflex might be supposed as a trace of the cortical regulation of vocalization.

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