DIENCEPHALIC CARDIOVASCULAR CONTROL
IN TOADS

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In mammals, action potentials of the sympathetic nervous system were first studied by Adrian, Bronk and Phillips. In the cervical and abdominal sympathetic nerves, they observed persistent discharges which tended to occur in groups with the frequency of the heart beat or of respiration. Next Bronk, Ferguson, Margaria and Solandt reported similar discharges in the cardiac sympathetic nerves which were markedly affected by electrical stimulation of the sinus or aortic nerve. Thereafter, Pitts, Larrabee and Bronk recorded unit axonal activities mainly in the cervical sympathetic trunk and studied the effect of electrical stimulation of the hypothalamus on sympathetic discharges. Recently, Sell, Erdelyi and Schaefer and Weidinger, Fedina, Kehrel and Schaefer observed that discharges of the various sympathetic nerves were increased nonspecifically by stimulating any sensory nerves from the trigeminal nerve down to the sciatic and that, even without stimulation, sympathetic discharges in the cardiac and renal nerves waxed and waned in parallel.

In earlier papers the present author reported efferent discharges in the splanchnic and cardiac nerves of the toad. There are striking similarities between sympathetic discharges in this animal and those in cats reported by the above mentioned authors: Tonic discharges are also present in the toad's sympathetic nerves, which are frequently grouped with the frequency of the heart beat. Stimulation of almost any sensory nerves evokes a reflexive increase of discharges in the cardiac and splanchnic nerves on both sides which is followed by a transient inhibition of spontaneous discharges. At first the present author imagined that this nonspecific and diffuse response of the sympathetic nervous system was restricted to lower animals such as the toad. It was a great surprise to find that a similar phenomenon is also observed in mammals as reported by Schaefer and others. The centers for sympathetic reflex to sensory nerve stimulation are in the medulla oblongata and the upper spinal cord both in the cat and in the toad. It appears that there is not much difference between both animals for the sympathetic nervous system at least below
In mammals the hypothalamus is supposed to be the principal focus of integration of the entire autonomic nervous system. The aim of the present study was to locate such higher centers controlling the cardiovascular system in toads. The author thought that this would be a necessary step for the understanding of the neuronal mechanism of cardiovascular regulation not only of toads but of mammals considered from the similarity of sympathetic activities in both animals.

METHODS

A total of 46 toads, *Bufo vulgaris*, weighing between 200 and 300 g, were used in this study. After induction with ether the animal was placed supine and 1 cc of a 25% solution of urethane was injected into the abdominal vein. The cardiac nerve was prepared for recording action potentials from its central cut end as described previously. The animal was then turned over and a small window was cut in the lateral abdominal wall through the skin and the oblique muscles to reach the splanchnic nerve retroperitoneally, which was also prepared for recording efferent action potentials. The skin covering the cranial bone was then removed along the median line in a flap and, starting from the foramen magnum, the bone was removed with a bone forceps to expose the brain from the medulla oblongata to the olfactory bulbs. The experimental setup around the animal is briefly sketched in Fig. 1. The stimulating electrode (S) consisted of a pair of 0.2 mm thick silver wires spaced approximately 0.3 mm.

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Fig. 1. S: stimulating electrode, L₁: recording electrode for the cardiac nerve, L₂: for the splanchnic nerve.
apart and coated with lacquer for insulation except at the tips. The stimulating electrode was oriented perpendicularly and introduced into the brain from the dorsal surface by means of a micromanipulator. Square pulses isolated from ground, ranging 1-5 v in intensity, 10-50/sec in frequency and 1-5 msec in duration, were employed for stimulation. Action potentials in the cardiac and splanchnic nerves were picked up with silver-silver chloride electrodes (L₁ and L₂ in Fig. 1), amplified by CR amplifiers of an over all time constant of 0.02 sec and recorded with a 2-channel pen-writer under simultaneous observation with a dual beam cathode ray oscilloscope.

RESULTS

Sympathetic discharges By stimulating the dorsal part of the diencephalon, sympathetic discharges in the bilateral cardiac and splanchnic nerves were inhibited. On the other hand, in the ventral part of the diencephalon, stimulation caused an excitation of discharges in these nerves. One example is presented in Fig. 2. In A of this figure, by stimulating the dorsal surface of the right diencephalon, discharges in the left cardiac nerve disappeared almost completely (The remaining potentials might be vagal discharges which will be described below.), and those in the right splanchnic nerve were slightly diminished. When the tip of the electrode was introduced 1mm deeper into the
ventral part of the diencephalon, the effect of stimulation was quite opposite: profuse increase of discharges occurred in both cardiac and splanchnic nerves as shown in Fig. 2B. In this case inhibition and excitation of the sympathetic discharge were reproducibly evoked several times by alternately withdrawing and introducing the tip of the electrode 1 mm perpendicularly.

Sometimes even single pulse stimulus in the ventral part of the diencephalon evoked an appreciable response in the cardiac and splanchnic nerves. When single-pulse or tetanic stimulation was applied on one side of the median line, the sympathetic response was usually observed in the cardiac and splanchnic nerves of both sides in a similar way. The relatively large size of the tip of the electrode compared to the small diencephalon of this animal on one hand and the lack of precise method of quantitation of sympathetic discharges on the other impeded detection of either segmental or lateral representations, if such were present at all.

It was also difficult, due to the relatively large size of the electrode, to determine the exact border plane between the inhibitory and excitatory areas for sympathetic discharges. It seemed to be variable from animal to animal, however. For example, in the rostral part of the diencephalon, electrical stimulation evoked inhibition of sympathetic discharges in some animals and excitation in others. Even in one and the same animal, at one location of the stimulating electrode, the effects sometimes changed from inhibition to excitation after repeated stimulation. Deterioration of the brain due to electrode penetration and other experimental intervention seemed to abolish the inhibitory effect earlier than it did the excitatory effect. Elements belonging to the two different functional groups are presumably intermingled in the diencephalon and the effects of stimulation are different depending on their thresholds as well as responsiveness of the lower system to the impulses from the diencephalon.

Increases in the sympathetic discharge in the cardiac and splanchnic nerves were also observed when the midbrain or medulla oblongata was stimulated in their ventral halves near the midline. This response was usually followed by a transient inhibition of spontaneous discharges. Stimulation in the olfactory bulbs had no effect on discharges in the cardiac and splanchnic nerves.

Vagal discharges To observe vagal impulses, which were rarer and smaller than sympathetic impulses in the cardiac nerve, the sympathetic chain should be severed just below its confluence to the vagus and amplification of the recording system should be increased. The response of the cardiac vagus to diencephalic stimulation was quite opposite to that of the sympathetic nerves: Stimulation of the dorsal part of the diencephalon evoked vagal discharges and that of the ventral part inhibited it. In Fig. 3A, B and C of each pair the upper tracing shows vagal discharges of the cardiac nerve and the lower tracing those of the splanchnic nerve for reference, because the splanchnic discharge
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FIG. 3. A and B. Excitation of vagal discharges in the cardiac nerve and reciprocal inhibition of sympathetic discharges in the splanchnic nerve by stimulating the dorsal surface of the diencephalon. C. Inhibition of vagal discharges and excitation of sympathetic discharges by stimulating the ventral part of the diencephalon. In these experiments the sympathetic chain had been severed to eliminate sympathetic impulses in the cardiac nerve. For all A, B and C, stimulation parameters were: 50/sec, 3 v, and 1 msec.

usually parallels sympathetic discharges in the cardiac nerve. In A and B, stimulation in the dorsal part of the diencephalon evoked discharges in the cardiac vagus and diminished the sympathetic discharges in the splanchnic nerve. The difference between A and B is that spontaneous vagal discharges were present in B alone. Unlike the sympathetic spontaneous discharge, which was always observed in all animals, the vagal spontaneous discharge was very inconstant. As shown in Fig. 3C, this vagal spontaneous discharge was inhibited by stimulating the ventral diencephalon.

The vagal response was as inconstant as the vagal spontaneous discharge. On deterioration of the animal the vagal responsiveness disappeared very rapidly. And in some animals, diencephalic stimulation had no effect on vagal discharges from the beginning of the experiment, while marked effects were observed on sympathetic discharges.

In the other parts of the brain, electrical stimulation evoked no discharge in the cardiac vagus except in the medulla oblongata close to the origin of the vagus root. There the root itself might have been stimulated.
Effects of diencephalic stimulation on circulatory system

In the experiment reproduced in Fig. 4, the effect of diencephalic stimulation on heart rate was observed by simultaneously recording electrocardiogram and splanchnic discharges. As shown in A of this figure, stimulation in the dorsal part of the diencephalon slowed the heart. Such marked slowing of the heart could be attributed to vagal evoked discharges rather than inhibition of the sympathetic spontaneous discharge. The effect of stimulation of the ventral part of the diencephalon which would inhibit vagal spontaneous discharges, if they were present, and reciprocally cause an increase of sympathetic discharges was usually more obscure. When heart rate was very low, however, long-lasting stimulation of the ventral diencephalon accelerated the heart as shown in Fig. 4B.

Though no observation has been made of the blood flow in the splanchnic bed, it may be safely assumed that the increase of discharges in the splanchnic nerve on stimulation of the ventral diencephalon evokes constriction of the splanchnic vessels and that the inhibition of discharges on stimulation of the dorsal diencephalon causes dilatation. Needless to say, the former leads to an elevation of arterial blood pressure and the latter to a lowering.
DISCUSSION

The greatest disadvantage of the method of localized stimulation is that it cannot be determined by the experiment itself whether the effects of an electric current applied to a small portion of the brain are due to stimulation of a group of efferent nerve cells, a group of internuncial neurons or fibers of passage which may be either afferent or efferent. However, it may be concluded from the present study that, in the toad as in mammals, the diencephalon plays a very important part controlling the integration of the cardiovascular system. Though a similar sympathetic response is evoked in the cardiac and splanchnic nerves by stimulating the medulla oblongata or mid-brain, the diencephalon is quite different from these parts in that here the results of stimulation reversed completely by shifting slightly the tip of the stimulating electrode. Effects on the vagal impulses were another reason for assuming a rather elaborate integrative mechanism in the diencephalon, which were absent when the lower brain was stimulated.

As reported previously, in the toad, the sympathetic reflex to sensory nerve stimulation as well as the tonic sympathetic activity is maintained after removal of the diencephalon. Further transection experiments disclosed that the mechanism of generating sympathetic spontaneous impulses as well as withholding them after reflex discharges is the function of the lower sympathetic centers extending from the medulla oblongata to the upper spinal cord. It is likely that the diencephalic center plays a more elaborate role in the control of the sympathetic discharge with reciprocal effects on the vagal neurons. To solve the interesting problem of the interaction between the lower autonomic centers and the diencephalic centers, observation of activity of the autonomic neurons in the medulla and the upper spinal cord as well as in the diencephalon would be valuable.

SUMMARY

In search for higher cardiovascular centers, the brain of the toad was explored with a pair of stimulating electrodes, separated about 0.3 mm apart, when efferent discharges in the cardiac and splanchnic nerves were being observed. In the dorsal part of the diencephalon tetanic stimulation of 10-50/sec inhibited sympathetic spontaneous discharges in these nerves and excited vagal discharges in the cardiac nerve. On the other hand, in the ventral part of the diencephalon, stimulation caused an increase of sympathetic discharges and a reciprocal decrease in vagal discharges. Thus stimulation of the dorsal diencephalon slowed heart rate and that of the ventral diencephalon accelerated it. It may be concluded that, in the toad as in mammals, there exist in the diencephalon the centers for an integrative control of the cardiovascular system.
through both sympathetic and parasympathetic nerves.

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REFERENCES