SOURCE OF ACETYLCHOLINE IN THE FROG'S HEART ON STIMULATION OF THE VAGUS NERVE

BY INDERJIT SINGH, F.A.SC., F.N.I., SUNITA INDERJIT SINGH, M.D.
AND
O. P. BHATNAGAR, M.D.
(From the Department of Physiology, Medical College, Agra and Lady Hardinge Medical College, New Delhi)

Received December 12, 1959

THE heart is known to contain endogenous acetylcholine (Burn, 1956). If the vagus nerve to the stomach of frog or dog is stimulated, the liberated acetylcholine appears to come not from the nerve but from the stomach muscle itself (Singh, Sharma and Bhatnagar, 1959 *b*). Loewi (1921, 1922) has shown that when the vagus nerve to the frog's heart is stimulated, acetylcholine is liberated, and it has been presumed that it comes from the vagal nerve endings. But if the heart contains endogenous acetylcholine, then there is the possibility that the acetylcholine liberated on stimulation of the vagus nerve to the heart might originate at least partly, if not wholly, from the heart muscle and not from the nerve endings. It was reported in a previous paper (Singh, Sharma and Bhatnagar, 1959 *a*, *b*), that sometimes stomach muscle of the frog does not release any acetylcholine on stimulation of the vagus nerve, but does so on direct electrical stimulation. In the present research, similar instances were found with the frog's heart. Acetylcholine was not released or released in small quantities on stimulation of the vagus nerves; but it was released on direct electrical stimulation if there was no release on stimulation of the vagus nerves, or in much greater quantity than that liberated on nervous stimulation.

EXPERIMENTAL

These experiments were performed on the heart of the frog, *Rana tigrina*. The heart was dissected out with both the vagus nerves attached. It was washed free of blood by repeated washing with eserinised Ringer's solution for half an hour. It was then placed in a tilted petri-dish containing 15 c.c. of eserinised Ringer's solution for one hour; it usually beats spontaneously. This was done for one hour to note any liberation of acetylcholine by the spontaneously beating heart; the heart beats from about

2 to 3 hours. The Ringer's solution was then replaced afresh and both the vagus nerves were placed on a pair of electrodes and stimulated maximally with induction shocks for one hour. At the end of the period of stimulation, the Ringer's solution was collected and tested for acetylcholine. The heart stops on stimulation of the vagus nerves, and does not resume beating on cessation of stimulation, but responds to direct electrical stimulation.

The nerves were then cut out and the heart placed in a small muscle chamber containing 15 c.c. of eserinised Ringer's solution and stimulated with induction shocks twice every 15 seconds for one hour. The Ringer's solution was then tested for acetylcholine.

The acetylcholine was assayed on sensitive leech preparations, which responded to 1 in 1,000 million acetylcholine. These experiments were performed at room temperature, 22–24° C. The composition of the Ringer's solution was as described previously (Singh, Sharma and Bhatnagar, 1959 *a*, *b*, *c*).

Results

The spontaneously beating heart.—In 26 experiments the spontaneously beating heart of the frog showed no release of acetylcholine (Table I). The frog's heart therefore does not require any release of acetylcholine for its rhythmic activity.

Effect of vagus stimulation.—Both the vagus nerves were stimulated in 26 experiments. In 11 experiments, stimulation of vagus nerves did not show any detectable release of acetylcholine; in 15 experiments, there was release (Table I). The period of maximal stimulation, that is, one hour, is sufficient to fatigue the nerves.

Effect of direct stimulation.—Direct electrical stimulation caused release of acetylcholine in all the 26 experiments (Table I). The amount released was very variable. But as with stomach muscle (Singh, Sharma and Bhatnagar, 1959 *b*), direct electrical stimulation is more potent than nervous stimulation for release of acetylcholine.

Discussion

There is no doubt that acetylcholine may be liberated on stimulation of the vagus nerve to the frog's heart, but there is also the curious fact that the heart may stop on stimulation of the vagus and yet there may be no detectable release of acetylcholine. It may be presumed that the acetylcholine is liberated in very small amounts, so that it is all absorbed by the

TABLE I

Acetylcholine released from frog's heart as a result of vagus and electrical stimulation

Number of experiment	Acetylcholine in nanograms released from 1 gm. of tissue per hour			
	Spontaneously beating heart	Vagus stimulation	Electrical stimulation	Increase %
1	0	0	46	..
2	0	0	14	..
3	0	0	62	..
4	0	0	375	..
5	0	51	166	225
6	0	0	400	..
7	0	26	80	200
8	0	40	120	200
9	0	10	100	900
10	0	75	375	400
11	0	36	180	400
12	0	50	350	600
13	0	0	150	..
14	0	0	230	..
15	0	11	34	209
16	0	8	24	200
17	0	0	40	..
18	0	0	241	..
19	0	0	82	..
20	0	0	23	..
21	0	26	130	400
22	0	34	100	200
23	0	120	300	150
24	0	40	120	150
25	0	60	300	400
26	0	65	300	360

receptors, or the release of acetylcholine is intracellular in amounts too small for external leakage.

The fact that acetylcholine can be liberated by direct electrical stimulation after the vagus nerves have been fatigued, or if they do not release acetylcholine on stimulation, suggest that the substance has originated from the muscle cells and not the nerve endings. The spontaneously beating heart does not release acetylcholine, so that the release on direct electrical stimulation suggests some specific effect of electric current, a phenomenon of importance in release of acetylcholine by the action potential of nerve.

Electrical stimulation of the brain is known to release acetylcholine. If electrical stimulation has a specific effect in releasing acetylcholine, then electrical transmission in the brain and other parts of the nervous system is a possibility. This view is supported by the fact that the cerebellum, which is so richly endowed with synapses, is poor in acetylcholine (Quastel, 1955).

Summary

1. The vagus nerves of the frog's heart were stimulated in 26 experiments. In 11 experiments, there was no detectable release of acetylcholine; in 15 there was release in variable amounts.

2. Direct electrical stimulation caused release of acetylcholine in all the 26 experiments, in amounts greater than that released by nervous stimulation after the fatigue of the vagus nerves, and also in those experiments in which the stimulation of the vagus nerves did not show any detectable release of acetylcholine. It is concluded, therefore, that the acetylcholine liberated on stimulation of the vagus nerves comes from the heart muscle cells and not the nerve endings.

References

Burn, J. H. .. *Functions of Autonomic Transmitters*, 1956, p. 38. The Williams and Wilkins Company, Baltimore,

Loewi, O. .. *Pflügers Arch. f. d. ges. Physiol.*, 1921, **189**, 239; 1922, **193**, 201; 1924, **203**, 408.

Quastel, J. H. .. "Acetylcholine in the Central Nervous System," in *Neurochemistry*, p. 153, by Elliot, K. A. C., Page, I. H., and Quastel, J. H., 1955, Charles C. Thomas, Springfield, Illinois, U.S.A.

Singh, I., Sharma, S. and Bhatnagar, O. P. *Proc. Ind. Acad. Sci.*, 1959 *a*, **49**, 129.

——— .. *Ibid.*, 1959 *b*, **49**, 369.

——— .. *Ind. Journ. Physiol. and All. Sci.*, 1959 *c*, **13**, 31.