THE SOURCE OF ACETYLCHOLINE IN THE FROG'S HEART ON DIRECT ELECTRICAL STIMULATION

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DIRECT electrical stimulation releases acetylcholine from the stomach of dog and frog (Singh, Sharma and Bhatnagar, 1959 *b*) and from the frog's heart (Singh, Singh and Bhatnagar, 1960) after the vagal nerve endings have fatigued. These tissues contain ganglia, and it is possible that the acetylcholine might originate from the ganglion cells and not the muscle fibres. The possibility of origin of acetylcholine from the muscle fibres is suggested by the presence of cholinestrase in the muscle cells as shown by histochemical technique (Koelle, 1950, 1951; Holmes, 1957; Giacobini, 1959). In the present research an attempt has been made to find whether the acetylcholine originates from the ganglion cells or muscle fibres when the above tissues are directly stimulated with electric current. These experiments suggest that the acetylcholine originates from the muscle fibres.

EXPERIMENTAL

Frog's heart on direct electrical stimulation releases acetylcholine (Singh, Singh and Bhatnagar, 1960). The lower two-thirds of the ventricle do not contain ganglion cells (Lovatt Evans, 1956); this we have confirmed in these experiments. The heart of the frog, *Rana tigrina*, was taken out and two pieces were made by cutting the ventricle. One piece consisted of sinus venosus, auricles and the upper half of the ventricle, and the other piece, of the lower half of the ventricle. To get at least one gram of the lower half of the ventricle, tissues from four hearts had to be pooled together for each experiment.

The pieces from the hearts were washed free of blood in eserined Ringer's solution for half-an-hour. After this preliminary washing, they were aerated for one hour in 12 c.c. of fresh eserinised Ringer's solution to study the liberation of acetylcholine by the unstimulated pieces. They were then electrically stimulated with induction shocks for one hour in a muscle chamber

containing 12 c.c. of eserinised Ringer's solution, as described previously (Singh, Singh and Bhatnagar, 1960). These solutions were assayed for acetylcholine on leech muscle, sensitive to 1 in 1,000 million acetylcholine. In some experiments, the acetylcholine content of heart muscle was determined as described previously (Singh, Sharma and Bhatnagar, 1959 *b*).

RESULTS

The unstimulated heart pieces.—The spontaneously beating frog's heart does not release acetylcholine, so that the release of this substance is not necessary for rhythmicity (Singh, Singh and Bhatnagar, 1960). In contrast, cut-pieces of both auricles and ventricles may release acetylcholine (Table I). The release of acetylcholine is therefore clearly due to injury caused by cutting. This shows how necessary it is to avoid injury to tissues in studying release of acetylcholine.

Effect of stimulation.—Electrical stimulation of lower half of the ventricle causes greater release of acetylcholine per g./wt. than similar stimulation of the piece consisting of the sinus venosus, auricles and the upper half of the ventricle (Table I). This was rather surprising, so it was decided to estimate the acetylcholine content of these pieces. It was found that the upper piece consisting of the sinus venosus, auricles and the upper half of the ventricle contained less acetylcholine per g./wt. than the lower half of the ventricle (Table II). This clearly proves that the heart muscle fibres contain acetylcholine, rather more than nervous tissue.

DISCUSSION

The ganglion-free portion of the ventricle releases more acetylcholine per g./wt. than the rest of the heart which contains ganglion cells. It is clear therefore that acetylcholine chiefly originates from the muscle tissues and not the nervous tissue in the heart.

Then what is the function of acetylcholine in the heart? The frog's heart does not release any acetylcholine while beating spontaneously (Singh, Singh and Bhatnagar, 1960). Extracellular acetylcholine is therefore not necessary for rhythmicity. Rabbit's auricle releases acetylcholine while beating rhythmically (Burn, 1956); this is probably an overflow. This is similar to the phenomenon shown by frog's stomach muscle and dog's stomach muscle; the latter always releases acetylcholine while at rest, but the former may not (Singh, Singh and Bhatnagar, 1959 *a*, *b*). It appears that mammalian tissues are rather more delicate than frog's tissues and are thus more liable to release acetylcholine than frog's tissues.

TABLE I

Acetylcholine released from two pieces of frog's heart during electrical stimulation. One piece consisted of lower half of the ventricle and the other, the remaining portion of the heart consisting of sinus venosus, auricles and upper part of the ventricle

No. of Experiment	Acetylcholine in nanograms released from 1 gm. of tissue per hour			
	From sinus venosus, auricles and upper half of ventricle		From lower half of ventricle	
	During resting condition	During stimulation	During resting condition	During stimulation
1	14	320	17	876
2	18	1064	27	1627
3	0	710	0	790
4	110	660	300	1505
5	140	563	0	1216
6	215	646	154	1081
7	300	600	467	93
8	0	180	90	360
9	56	840	10	1421
10	109	875	200	1202
11	0	900	0	1337
12	10	427	300	1000
13	30	140	40	1000
14	0	130	0	941
15	0	208	0	192
16	75	900	39	1186
17	100	900	70	1500
18	0	55	0	80
19	16	64	37	150
20	153	1541	440	3122
21	53	911	0	2051
22	0	888	0	1700
23	51	1770	25	2770
24	100	880	200	1700
Average ..	64	670	101	1240

Acetylcholine has therefore two possible functions in the heart; it is either released during stimulation of the vagus nerve to produce inhibition or it has some other intracellular function. It is quite possible that the acetylcholine released on stimulation of the vagus nerve comes not from the vagal nerve endings but from the muscle fibres. If the frog's heart is immersed in Ringer's solution containing various concentrations of acetylcholine up to 1 in 10,000, it may continue to beat and yet on stimulation of the vagus nerve, complete inhibition is produced (Singh, Sehra and Singh, 1945). The same

TABLE II

Acetylcholine content of two pieces of frog's heart. One piece consisted of lower half of the ventricle and the other of the remaining portion of the heart, consisting of sinus venosus, auricle and upper half of the ventricle

No. of Experiment	Acetylcholine content, μg./g. of tissue	
	Sinus venosus, auricle and upper half of ventricle	Lower half of ventricle
1	6	9
2	7·3	9·2
3	5·8	9·2
4	5·8	9
5	5·4	8
6	6·4	9·2
7	5·6	7·8
8	7·8	10
9	6·5	10·2
10	5·4	8·8
11	5·6	10·8
12	7	11·4
13	7	11
14	6·4	10·6
15	3·2	9
16	3·6	9·2
17	7·8	9
Mean ..	6	9·5

has been found on mammalian heart (Obrink and Essex, 1953); vagal stimulation could stop the heart even when it was beating under the influence of high concentrations of acetylcholine (up to 1 in 100). This can be interpreted in two ways: either the nerve did not liberate acetylcholine, or liberated it within an area into which the acetylcholine from outside had difficulty in penetrating. In the frog's heart this is unlikely, as the tissue is thin and transparent; at least the experiment excludes the extracellular release of acetylcholine by the vagal nerve endings.

The view that nerves may release chemical substances from the tissue cells is not unreasonable, as sympathetic nerves actually release noradrenaline and adrenaline from the adrenal medulla; the cells of the adrenal medulla may be related to nerve cells developmentally but they are not nerve cells. The discovery of many active substances in various organs also supports the above view. Thus, besides acetylcholine, adrenaline and noradrenaline, other substances have been discovered which are suspected to play a role in transmission at nerve endings and synapses; these are histamine, 5-hydroxytryptamine, gamma aminobutyric acid. The chemical theory of transmission by liberation of active substances at nerve endings was reasonable so long it was restricted to two substances, acetylcholine and noradrenaline; and two kinds of nerves were postulated, cholinergic and adrenergic. But with the discovery of more active substances in tissues, more kinds of nerves have to be postulated, such as histaminergic, etc. More active substances are likely to be discovered in tissues and so further classification of nerves will be required. It is likely therefore that there is only one kind of nerve liberating different substances from tissue cells which contain these substances.

In contractile cells, acetylcholine may have some role in contraction. This is suggested by the observation that factors, both physical and chemical, which increase the mechanical response of dog's stomach muscle, also increases its acetylcholine content (Singh, Sharma and Bhatnagar, 1960). Further, if frog's stomach muscle is heated to 60° C., it releases acetylcholine and also relaxes actively, suggesting that acetylcholine had kept the contractile mechanism from relaxing, and is thus, in some way, involved in contraction (Singh, Sharma and Bhatnagar, 1959 *a*). Acetylcholine probably neutralises active relaxation in frog's stomach muscle, and thus enhances the mechanical response, as in this muscle, contraction and active relaxation appear to occur simultaneously (Singh and Singh, 1956). The occurrence of simultaneous active relaxation with contraction will diminish the mechanical response, so that if it is antagonised, the mechanical response will increase. This may explain the poor acetylcholine content of striated muscle, as it

relaxes passively. As the heart muscle fibres contain acetylcholine, they probably relax actively.

SUMMARY

1. In frog's heart, on electrical stimulation, more acetylcholine per g./wt. is liberated from the lower half of the ventricle which is free from ganglion cells, than from the rest of the heart which contains ganglion cells. This suggests that acetylcholine mainly originates from the muscle cells.

2. The acetylcholine per g./wt. content of the ganglion free portion of the ventricle is greater than the rest of the heart which contains ganglia. This shows that acetylcholine is also contained in muscle cells besides nerve cells.

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