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Effects of acetylcholine, vagal stimulation and tyramine on the isolated atria of the tortoise

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In the vagus-atrial preparation of the tortoise (*Amyda japonica*), a cold-blooded animal, acetylcholine and vagal stimulation, in the presence of atropine, produced positive inotropic effects which were inhibited by dichloroisoprenaline or pronethalol. Hexamethonium blocked the excitatory effect of acetylcholine but only slightly inhibited that of vagal stimulation. Tyramine exerted a positive inotropic action in this preparation, but had little effect on atria prepared from reserpine-treated animals. Tyramine was shown to release a substance from the tortoise atria which caused contraction of aortic strips taken from reserpine-treated rabbits, and which appeared to be a catecholamine.

VAGAL stimulation or acetylcholine may produce sympathomimetic effects on the heart or auricle of warm-blooded animals, probably by releasing catecholamines from sites where they are bound (for literature, see Burn & Rand, 1962). However, this effect has not previously been demonstrated in cold-blooded animals. This paper describes the positive inotropic effect of vagal stimulation and of acetylcholine on a vagus-atrial preparation of a cold-blooded animal, the tortoise (*Amyda japonica*). The release of vasoactive material from the same preparation by tyramine was also demonstrated.

Methods

Tortoise vagus-atrial preparation. Tortoises (300–400 g) were killed by severing the cervical spine with a bone cutter and then pithing the spinal cord with a probe. The tortoise was fixed on its back on a board. the plastron was removed and the pericardium cut. The skin covering the neck was cut along the midline and the right vagus nerve was carefully isolated from the right carotid artery and cut at the cranial end. The heart with the right vagus was dissected out and placed in a petri dish containing oxygenated Ringer solution. The ventricle was cut away without injury to the atria. The apex of one atrium was ligated with a thread, and that of the other was clipped with a serffine. The atria were suspended in a 30 ml bath containing oxygenated Frog Ringer solution (NaCl 6·4, KCl 0·3, $CaCl_2$ 0·18, $MgCl_2$ 0·01, $NaHCO_3$ 0·3, glucose 2·0 g/litre) at 25°. The right vagus nerve was placed on platinum electrodes immersed in the bath fluid. The electrodes were raised to the surface of the Ringer solution when stimulation was applied. The atria thus prepared showed regular and spontaneous contractions at a rate of 18 to 30/min. Contractions of the atria were recorded on a smoked drum by a light lever which magnified the contractions 7 times. The experiments were made during the summers of 1962 and 1963.

Rabbit aortic strip. This was prepared by the method of Furchgott (1960), 24–48 hr after reserpine treatment (2-4 mg/kg, intravenously).

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The aortic strips were suspended in a 10 ml bath containing oxygenated Krebs bicarbonate solution at 37°.

Guinea-pig ileum. A piece of ileum, 4 cm long, was suspended in Tyrode solution at 29°.

Drugs. All drugs were dissolved in 0·6% w/v saline solution except noradrenaline which was dissolved in acid saline (pH: about 4·1). Drugs were added to the bath in volumes of 0·5–1·0 ml. The drugs used were acetylcholine bromide, noradrenaline bitartrate, tyramine hydrochloride, atropine sulphate, reserpine phosphate, dichloroisoprenaline, pronethalol, hexamethonium chloride, 5-hydroxytryptamine creatinine sulphate, histamine phosphate, 2-bromolysergic acid diethylamide (BOL-148), mepyramine maleate and EDTA disodium salt.

Results

Experiments with untreated preparations. Vagal stimulation and acetylcholine produced inhibition or arrest of the atrial movement. The threshold stimulus parameters necessary to arrest the movement varied from one preparation to another but usually rectangular pulses of 7 to 10 V strength and of 1 msec duration applied at a frequency of 3 to 6/sec for 10 to 15 sec were adequate for this effect. The minimal inhibitory concentration of acetylcholine ranged from 0·1 to 1 μg/ml.

Noradrenaline produced an increase in contraction height; its effect on the atrial rate was not consistent unless the original rate was distinctly low. The doses of noradrenaline to produce about a 20% increase of the contraction heights were 0·02 to 0·1 μg/ml.

Atropine (5 μg/ml) blocked the inhibitory effects of vagal stimulation and of acetylcholine and in most experiments caused a slight increase in contraction height.

Experiments with atropinized preparations. In the presence of atropine (5 μg/ml) an increase in the frequency and duration of vagal stimulation (25–50/sec for 2 min) produced a positive inotropic effect in most atria (27 out of 31 experiments). The magnitude of this response varied in each preparation but was usually of the order of a 10–20% increase in the contraction height (Fig. 1). The responses of each preparation were

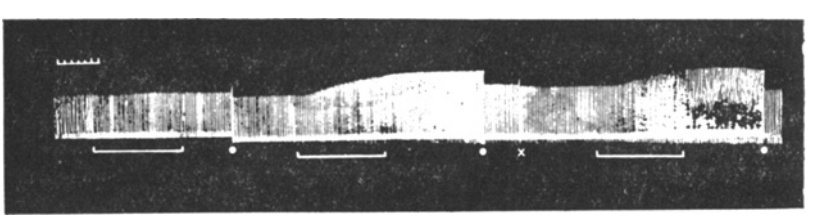

FIG. 1. Effects of vagal stimulation on atropinised isolated vagus-atrial preparation of tortoise. The horizontal lines mark the duration of vagal stimulation (5/sec in the left-hand panel and 25/sec in the other two panels). At the white dots the bath fluid was changed and the kymograph was stopped for 15 min. At x the kymograph was stopped for 5 min. Time marker: 10 sec.

almost consistent when stimulation was repeated at intervals of 15 min for a period of 3 to 4 hr. The chronotropic effect of vagal stimulation was not distinct unless the original atrial rate was low.

Acetylcholine (1 μg/ml) added to the atropinised atria was without inhibitory effect but produced a slight augmentation in some preparations. In 32 out of 37 experiments, an increase in the dose of acetylcholine to 10–100 μg/ml elicited a positive inotropic effect, which was usually of the order of a 20–40% increase in the contraction height. Constant responses were obtained when acetylcholine was added at intervals of 20 to 30 min (Fig. 2).

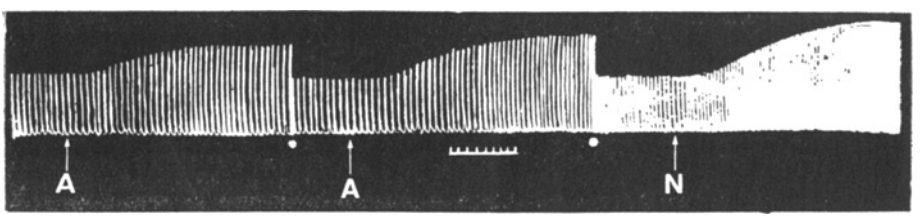

FIG. 2. Effects of acetylcholine and noradrenaline on atropinised isolated atrial preparation of tortoise. At A, 10 μg/ml of acetylcholine and at N, 0·05 μg/ml of noradrenaline were added. At the white dots the bath fluid was changed and the kymograph was stopped for 20 min. Time marker: 10 sec.

Treatment of the atropinised preparation with dichloroisoprenaline (5–10 μg/ml) or pronethalol (1–5 μg/ml), which have been shown to block the action of noradrenaline in the heart (Moran & Perkins, 1958; Black & Stephenson, 1962), markedly reduced or abolished the augmentatory effects of acetylcholine and of noradrenaline. The effect of vagal stimulation was always reduced by both substances but was never completely abolished; i.e. in the presence of dichloroisoprenaline the increase in the contraction height was 13% (average of 5 atria) of the control increase; pronethalol reduced it to 34% (average of 4 atria) of the control increase. These effects of dichloroisoprenaline and pronethalol were reversible.

Treatment of the atria with hexamethonium (100–500 μg/ml) in the presence of atropine abolished the augmentatory effect of acetylcholine, but only slightly inhibited that of vagal stimulation; i.e. in the presence of hexamethonium the increase of the contraction height by vagal stimulation was 79% (average of 6 atria) of the control increase. The hexamethonium effect was reversible. The effect of noradrenaline was not affected by this dose of hexamethonium.

Experiments with preparations from reserpine-treated animals. In some experiments the vagus-atrial preparations were taken from reserpine-treated tortoises (0·1 mg of reserpine phosphate/100 g body weight intraperitoneally 48 to 72 hr previously). The contraction amplitude of atria from reserpine-treated animals (average of 9 atria: 13·3 $\pm$ 2·7 mm) was significantly shorter ($P < 0{\cdot}01$) than that of atria from control animals treated with saline instead of reserpine (average of 10 atria: 26·4 $\pm$ 2·4 mm) when compared under the same conditions (Fig. 3).

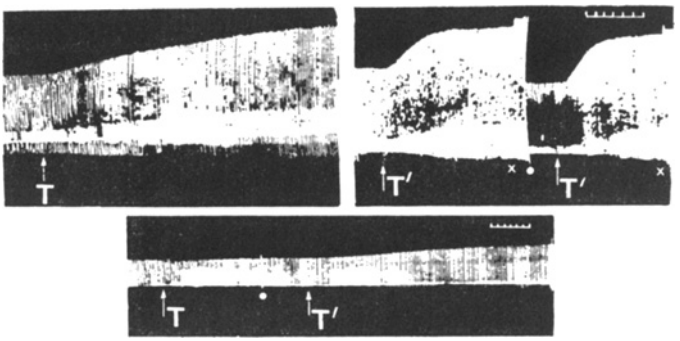

FIG. 3. Effects of tyramine on isolated atrial preparation of tortoise. At T, 2 μg/ml and at T', 20 μg/ml of tyramine were added. The lower panel is a record of a preparation from a reserpine-treated tortoise; the upper panels are records from non-reserpinised preparations. Each panel was taken from a different experiment. At the white dots the bath fluid was changed and the kymograph stopped for 30 min. At x the kymograph was stopped for 2 min. Time marker: 10 sec.

In experiments on nine reserpine-treated atria, five responded to vagal stimulation and 2 responded to acetylcholine with a positive inotropic effect. In the controls, which were given saline instead of reserpine, 14 out of 16 responded to vagal stimulation and 11 out of 15 responded to acetylcholine. The difference was not significant in the case of vagal stimulation but was so with acetylcholine ($P < 0{\cdot}05$).

Response to tyramine. Tyramine (above 2 μg/ml) exerted a distinct augmentatory effect on the tortoise atria. The responses remained fairly constant when tyramine was added every 30 min for 3 to 4 hr. Contrarily, when the same dose of tyramine was added to the atria obtained from reserpine-treated tortoises, the excitatory effect was very slight in all cases (Fig. 3).

Release of vasoactive substance from the tortoise atria by tyramine. In this series of experiments, the tortoise atrial preparations were suspended in 10 ml Ringer solution. Before addition of tyramine the fluid in the organ bath was changed several times and EDTA (10 μg/ml) was added to prevent oxidation of any catecholamine liberated (Crout, Muskus & Trendelenburg, 1962). This dose of EDTA did not affect the atrial movement. The preparation was exposed to tyramine (20 μg/ml) for 10 min. The fluid of the bath was then removed, warmed to 37° and applied to an aortic spiral strip from a reserpinised rabbit.

Tyramine (20 μg/ml) alone or added to bath fluid from an atrial preparation which had not been exposed to tyramine, had little effect on the aortic strip. Neither addition of EDTA nor exchanging the Krebs bicarbonate solution with the hypotonic Frog Ringer caused contraction of the aortic strip.

The application of the bath fluid from an atrial preparation which had been stimulated by tyramine (20 μg/ml) for 10 min always shortened the aortic strip (Fig. 4). When an atrial preparation was exposed to tyramine two or three times at intervals of 50 to 60 min, each sample of bath fluid

contracted the aortic strip. The magnitudes of the contractions of the aortic strip produced by the vasoactive material in the bath fluid (15 applications of tyramine were made to 6 preparations) were almost constant and about equivalent to those produced by 0·005–0·001 μg/ml of noradrenaline.

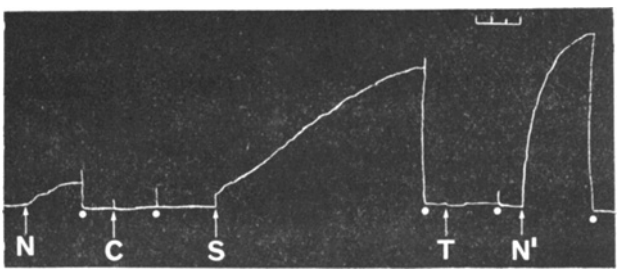

FIG. 4. Responses of an aortic strip from a reserpinised rabbit. At N, 0·001 μg/ml of noradrenaline, at N', 0·005 μg/ml of noradrenaline and at T, 20 μg/ml of tyramine were added. At C, the bath fluid was exchanged for warmed Ringer solution containing EDTA and which had bathed a tortoise atrial preparation for 10 min in the absence of tyramine. At S, the bath fluid was exchanged for Ringer solution containing EDTA and which had bathed a tortoise preparation for 10 min in the presence of 20 μg/ml of tyramine. Note that this concentration of tyramine itself (T) was without effect. At the white dots the bath fluid was exchanged for fresh Krebs solution at 37°. Time marker: 1 min.

In the presence of BOL-148 (0·05 μg/ml), which abolished the vasoactive property of 5-hydroxytryptamine (0·02 μg/ml) but had little effect on that of noradrenaline, the contractions of the aortic strip produced by the bath fluid from the atrial preparations stimulated by tyramine were unaltered. Mepyramine (0·01 μg/ml), which abolished contractions produced by histamine (0·01 μg/ml) but not those produced by noradrenaline, was also without effect on contractions produced by the vasoactive substance in the bath fluid.

Guinea-pig ileum which is known to be highly sensitive to histamine, 5-hydroxytryptamine and vasoactive polypeptides did not contract in response to the bath fluid from atrial preparations stimulated by tyramine.

Discussion

The experiments showed that acetylcholine or vagal stimulation may exert positive inotropic effects in atropinised atria from the tortoise, as they do in those of warm-blooded animals. Inhibition of the inotropic effect by dichloroisoprenaline or pronethalol suggests participation of adrenoceptive receptors on the atrial tissues in producing this effect. Thus the finding is against the opinion of Hashimoto, Kumakura & Hashimoto (1963) that differentiation of adrenergic and cholinergic receptors is incomplete in phylogenetically underdeveloped vertebrates.

The significant decrease in the number of preparations showing the positive inotropic effect in response to acetylcholine after reserpine treatment suggests that liberation of catecholamines is responsible for the

effect. As in other species, the ability of acetylcholine to release catecholamines, but not the action of noradrenaline itself, was blocked by hexamethonium. Acetylcholine may cause the release of catecholamine from chromaffin cells or it may stimulate adrenergic nerve terminals as suggested by Pathak (1958) for the frog heart.

It seemed that the mechanism underlying the inotropic action of vagal stimulation was different from that of applied acetylcholine, as reserpine pretreatment or hexamethonium did not much affect the response to vagal stimulation. However, the difference may be quantitative rather than qualitative; larger doses of reserpine and hexamethonium may be necessary for inhibition of the responses to vagal stimulation.

The present study supplied direct evidence that tyramine liberates vasoconstrictor material from the atria of the cold-blooded tortoise, as it does in warm-blooded animals (Hall, 1963). The control experiments with specific blocking agents suggested that the vasoactive material was a catecholamine. Reserpinization decreased the contraction height of the atria of the tortoise, an effect which has also been described for warm blooded animals.

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