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STIMULATION OF ISOLATED RABBIT AURICLES BY SUBSTANCES WHICH STIMULATE GANGLIA

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Although it is generally accepted that the ganglia of the sympathetic fibres to the heart are situated at some distance from it, in the stellate ganglion, there have been experiments reported from time to time suggesting that there are ganglia in the heart itself capable of liberating appreciable quantities of an adrenaline-like substance.

An investigation into this question was conducted by Hoffmann, Hoffmann, Middleton, and Talesnik (1945). These workers showed that, in the presence of atropine, acetylcholine had an adrenaline-like action on mammalian hearts, producing under these conditions a stimulation of force and rate of contraction as well as an increase in the coronary flow. They found that this stimulant action of acetylcholine was abolished by nicotine, by curare, and by ergotamine. By testing the coronary perfusate on the frog heart, on the rectal caecum of the chicken, and on the rabbit's gut they demonstrated that an adrenaline-like substance was released into the coronary outflow when this stimulant action of acetylcholine was observed. They concluded that either sympathetic ganglia or chromaffin tissue is present in the heart and thought that these probably play a role in the regulation of its activity. Hoffmann and his coworkers did not speculate on the nature of the preganglionic fibres to these structures. McDowall (1946) found that the slowing and weakening of isolated hearts produced by acetylcholine in small doses were invariably followed by a period of increased activity. In a few hearts examined by him this inhibition was preceded by a few very forcible contractions. Like Hoffmann and his coworkers, McDowall found that, after atropine, acetylcholine produced stimulation of the isolated heart; but according to him the stimulation was usually confined to the force of contraction, though sometimes the rate was also increased. McDowall noted that this action of acetylcholine might simulate that produced by adrenaline on the heart in affecting the rate and force of contraction simultaneously. He found that after ergotoxine the stimulant action of acetylcholine on the atropinized heart was abolished or even reversed. McDowall, however, was unable to suppress the stimulation by adding paralysing doses of nicotine. He thought it unlikely that acetylcholine produced its stimulant action under these conditions by the stimulation of sympathetic ganglia in the heart.

During the course of examining some choline derivatives prepared by Dr. H. R. Ing in this laboratory, it was found that one of these had a nicotine-like action on the blood pressure of the cat; but when this compound was tested on the isolated rabbit auricle and the rabbit intestine it exerted a muscarine-like action. On studying the actions in the presence of hexamethonium, it was found that the apparent muscarine-like action disappeared and therefore was really a nicotine-like action. This discovery led to an investigation of the action of nicotine and other drugs on the isolated rabbit auricle.

METHOD

Freshly dissected rabbit auricles were suspended in a bath of 40 ml. volume containing Ringer-Locke solution at 29° C., aerated with oxygen. Some preparations were kept overnight; they were placed in an evaporating dish containing about 10 ml. of Locke solution, covered with a wad of cotton-wool, and stored in the refrigerator at a temperature of 2–4° C. When testing the effect of nicotine it was found that several washes were necessary to remove all the nicotine afterwards. Care was taken to wash out the bath the same number of times after every application of a drug.

RESULTS

When a dose of 0.4 mg. nicotine was applied to the isolated rabbit auricles a mixed effect was noted (Fig. 1). There was inhibition followed by some stimulation of the auricular beat. When the same dose of nicotine was applied in the presence of 10 μg. atropine, stimulation alone was seen.

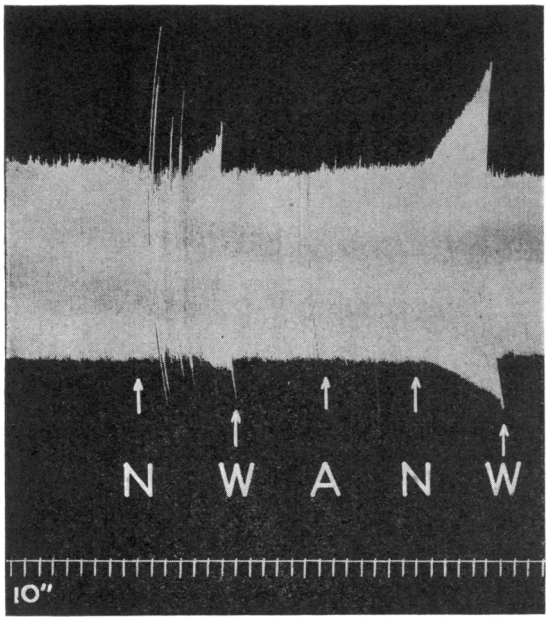

FIG. 1.—Spontaneous contractions of rabbit auricles in a bath of 40 ml. At N 0.4 mg. nicotine hydrogen tartrate was added Note the mixed effect of inhibition and stimulation. After 1 min., at W, the bath was changed 8 times, during which the drum was stopped. At A 10 μg. atropine sulphate was added. In its presence the addition of nicotine as before caused stimu'ation on!y.

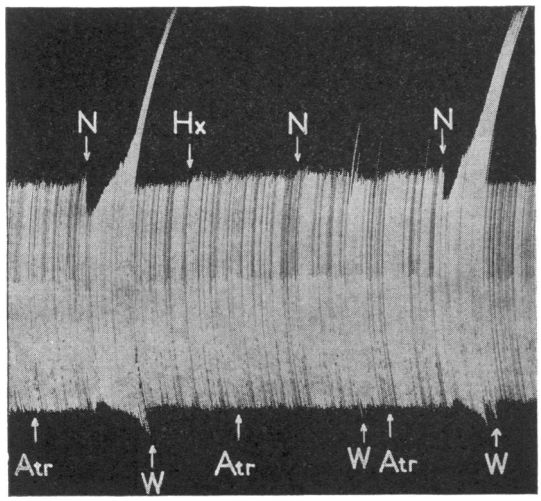

FIG. 2.—Isolated rabbit auricles. The record shows the mainly stimulant action of 0.8 mg. nicotine hydrogen tartrate (N) in the presence of 10 μg. atropine sulphate (Atr). At W the bath was changed 15 times. At Hx 5 mg. hexamethonium bromide was added, followed by 10 μg. atropine sulphate. Nicotine was then without effect. After washing out the bath, the stimulant action of nicotine returned.

In Fig. 2 is seen the effect of 0.8 mg. nicotine in the presence of 10 μg. atropine. There was initially a small inhibition followed by stimulation. On this preparation 0.4 mg. nicotine applied earlier had no effect. After washing out several times, 0.8 mg. nicotine was applied after atropine in the presence of 5 mg. hexamethonium. It is seen that hexamethonium suppressed the action of nicotine completely. On washing out the hexamethonium, nicotine again stimulated the auricles in the presence of atropine. When the same auricles were examined after they had been kept for about 18 hours at 2–4° C., a dose of 0.4 mg. nicotine, which had no effect on the previous day, stimulated the auricular beat. This action was again abolished by hexamethonium.

Fig. 3 shows the action of the phenyl ether of choline (kindly supplied by Dr. P. Hey) on the auricles. This substance has a strong nicotine-like action. In the presence of 50 μg. atropine, 0.2 mg. of the phenyl ether of choline caused an initial transient inhibition followed by a strong stimulation. As with nicotine this stimulant action was absent when hexamethonium was present in the bath. After washing out the hexamethonium the choline compound again caused stimulation.

Acetylcholine was similarly applied to the auricles (Fig. 4). In the presence of atropine a large dose of acetylcholine stimulated the auricles, and, as before, this action was not seen if hexamethonium was present. In a similar experiment Hoffmann and his co-workers also found that comparatively large doses of acetylcholine were necessary to produce stimulation after atropine. In Fig. 4 it is seen that as the hexamethonium was gradually washed out the stimulant action of acetylcholine returned.

It was conceivable that the stimulant action of nicotine and other drugs recorded above could be due to their acting more peripherally, like adrenaline, and that hexamethonium was able to block this peripheral action. To test this, a dose of 5 μg. adrenaline was applied to the auricles, and this dose was repeated in the presence of increasing concentrations of hexamethonium. A dose of hexamethonium as high as 80 mg. failed to suppress the action of 5 μg. adrenaline.

DISCUSSION

The results which have been described show that nicotine, the phenyl ether of choline, and a large dose of acetylcholine exert in the presence of atropine a strong stimulant action on the isolated auricles of the rabbit, which is blocked by hexamethonium.

These findings recall the observations of Ambache (1951) on the gut. He found that botulinum toxin, which selectively paralyses the cholinergic nerve endings, reversed the action of nicotine

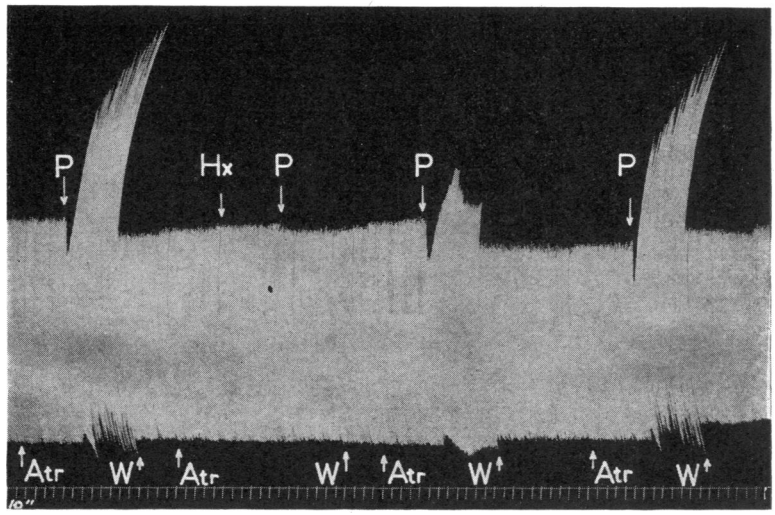

FIG. 3.—Isolated rabbit auricles. The stimulant action of 0.2 mg. phenyl ether of choline (P) in the presence of 50 μg. atropine sulphate. The action was absent when 2.5 mg. hexamethonium bromide (Hx) was added to the bath. The action returned gradually after washing out the hexamethonium.

on the gut, so that in its presence nicotine caused a relaxation. This effect was suppressed by hexamethonium and by paralysing doses of nicotine. Ambache and Edwards (1951) also showed that in the presence of atropine the action of nicotine on the gut was reversed. Hoffmann and his colleagues (1945) noted that it was not unusual for the atropinized rectal caecum of the chicken to relax after acetylcholine; they thought that this was probably due to nervous and chromaffin tissue in these preparations which released adrenaline when stimulated by acetylcholine or nicotine. Ambache postulated the presence of ganglion cells in the gut which, when stimulated, liberated an adrenaline-like substance. He thought that the pre-ganglionic fibres to these cells were vagal.

It is possible that the same situation exists in the heart. Dale, Laidlaw, and Symons (1910) showed that certain substances, nicotine, tropine, and curare, reversed the effect of vagal stimulation in the cat's heart. They thought that this was due to a selective paralysis rather than to a reversal of action. By eliminating all sympathetic nerves they came to the conclusion that the accelerator fibres were probably in the vagus. They considered that the presence of these accelerator fibres in the

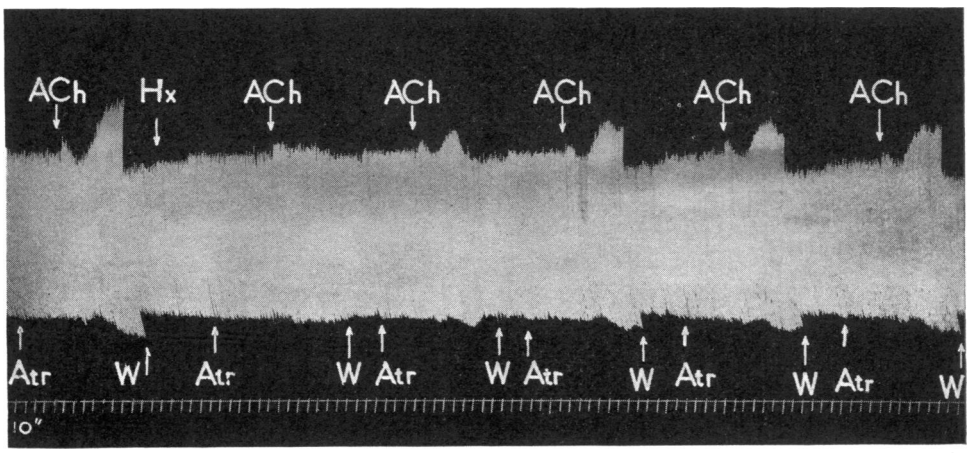

FIG. 4.—Isolated rabbit auricles. At Atr 0.5 mg. atropine sulphate was added to the bath. At ACh 2 mg. acetylcholine bromide was added. At Hx 5 mg. hexamethonium bromide was added. Note that the stimulant action of ACh in the presence of atropine was abolished by hexamethonium.

vagus might explain the readiness with which the cat heart escapes from inhibition by vagal stimulation.

One explanation, therefore, of the action of nicotine on the atropinized auricles is that there are ganglia or chromaffin tissue present which nicotine stimulates with consequent liberation of an adrenaline-like substance. This is in agreement with Ambache's hypothesis concerning the intestine. There are, however, other explanations, such as that nicotine may stimulate adrenergic nerves in continuity. Furthermore, actions of nicotine which are blocked by hexamethonium may not all be actions on ganglia.

Summary

1. The application of nicotine to the isolated rabbit auricle causes a mixed inhibitory and stimulant action.

2. When nicotine, the phenyl ether of choline, or large doses of acetylcholine are applied to the isolated rabbit auricle in the presence of sufficient doses of atropine to block the " muscarine " effects, there is a marked stimulation of the auricular beat.

3. In the presence of hexamethonium the stimulant action of these drugs after atropine is suppressed, but the stimulation can be demonstrated again when the hexamethonium is washed out.

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