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ISOLATED RABBIT ATRIA WITH SYMPATHETIC NERVE SUPPLY

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The atria of the rabbit heart have been isolated together with their sympathetic nerves, so that the latter could be stimulated and the effect on the rate and amplitude of beating could be measured. Cocaine and phenoxybenzamine increased the response to stimulation. When the preparation was from rabbits previously given reserpine, stimulation of the sympathetic nerves caused inhibition. This inhibition was increased by eserine and abolished by atropine.

McEwen (1956) has described a preparation of rabbit atria with the vagus nerves attached. An attempt has now been made to set up rabbit atria with the sympathetic nerves and to study the action of cocaine, phenoxybenzamine, acetylcholine, and ganglionic blocking agents upon it. Some rabbits were given reserpine beforehand.

METHODS

The rabbit was killed by cutting off the head with strong shears as high up the neck as possible and was fixed on its back on the operating table. The skin covering the neck was cut along the midline. The carotid artery, vagus, and cervical sympathetic nerves and internal jugular vein were tied and cut at the cranial end as a single bundle, and liberated from the surrounding connective tissue. Holding the caudal end of the tied nerves, artery, and vein laterally, the blunt end of a scalpel was inserted between them and the trachea and oesophagus. This was done on both sides. Holding the bundles together in the midline, a cut was made with strong scissors through the dorsal part of the first rib and continued to the last rib on each side. Then the front of the thorax was removed leaving the thymus and adipose tissue attached to the heart. Lifting the heart upwards, the vertebral column was cut through transversely at the third thoracic vertebra and a second cut was made between the first and second thoracic vertebrae. The preparation was then freed quickly from skeletal muscle, and put into oxygenated Locke solution at 30°.

The preparation was left in the solution for 5 min., then taken out, freed from muscle, and fixed on an L-shaped Perspex rod by tying the remaining piece of vertebral column to the foot of the L-shaped rod. The preparation was returned to the Locke solution. With the preparation immersed and with continuing oxygenation the cervical sympathetic nerve was identified in the bundle containing the carotid artery and vagus nerve and a thread was attached to it so that it could later be passed through the electrode. One nerve was cleaned with forceps as far as the stellate ganglion, without cutting any branches of that nerve. (The other nerve was kept in reserve.) The stellate ganglion was freed from surrounding tissue as far as possible. The trachea and oesophagus were removed; adipose tissue and the thymus were pulled away with the fingers. (If a preparation was required for stimulation of the vagus as well as the sympathetic nerve, the trachea and oesophagus were not removed.) The pericardium was cut and the ventricles were cut away. The aorta and pulmonary artery were cut through close to the auricles. A thread was passed through the apices of both auricles for attachment to a lever. The sympathetic nerve was passed through electrodes similar to those for stimulation of the phrenic nerve in the rat diaphragm preparation (Bülbring, 1946). The nerve passed through two small platinum rings 1 mm. apart embedded in a piece of Araldite cement on the end of a glass tube containing the insulated leads. In this tube there was also a 0.5 mm. polyethylene tube which terminated between the two platinum rings. The other end of the polyethylene tube carried the electrodes out of the glass tube over the edge of the bath, and hung outside so that fluid from the bath syphoned over at about 2 drops/min. thus keeping the nerve oxygenated.

The thread from the atria was attached to a spring lever which carried a platinum wire on its under side. When the lever was pulled down by a contraction, the wire dipped into a small trough containing saline and completed a circuit through a post office counter driven by a 120 V dry battery. The atrial beats were then recorded on the counter and the number of beats/min. was observed.

Stimulation was applied in the early experiments for 2 min., but in the later ones mostly for 15 sec., using pulses of 0.5 to 1.0 mA., and 1 to 2 msec. duration at a frequency of 10/sec. Since hexamethonium (1.2 mg./ml.) did not reduce the atrial contractions, the stimulation was postganglionic.

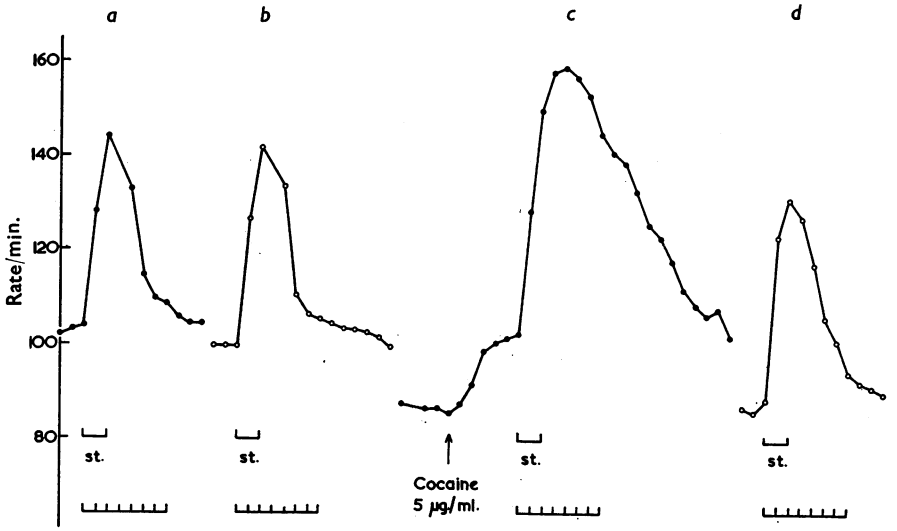

FIG. 1.—Ordinate, atrial rate. Abscissae, time in min. Sympathetic nerves stimulated at st for 2 min. causing rise in atrial rate. *a* and *b*, control observations. *c*, in presence of cocaine (5 µg./ml.). *d*, after washing out cocaine.

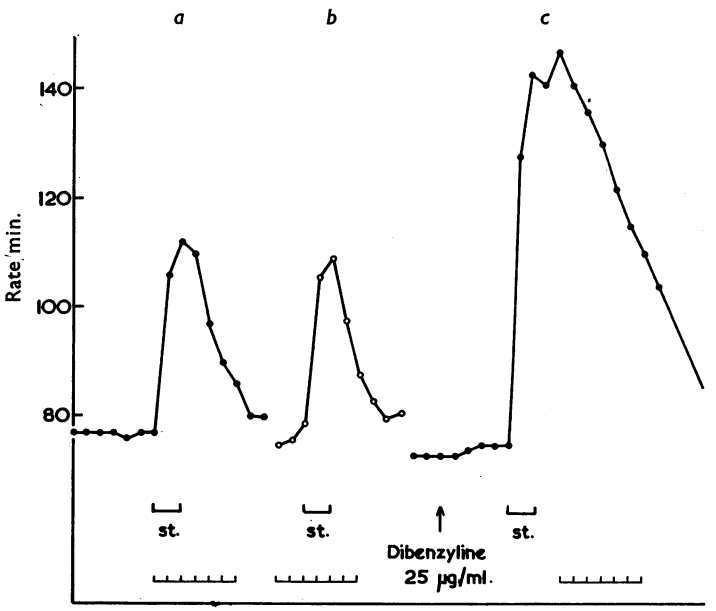

FIG. 2.—Co-ordinates as in Fig. 1. *a* and *b* show rise in atrial rate after sympathetic nerve stimulation for 2 min. *c* shows rise in presence of 25 µg./ml. of phenoxybenzamine (Dibenzyline).

RESULTS

Effect of Cocaine.—The effect of stimulating the postganglionic fibres on the rate of atrial contraction is illustrated in Fig. 1. Stimulation for 2 min. (Fig. 1*a* and *b*) caused an acceleration in which the rate rose from about 100 to 140/min., and returned in the next 2 to 3 min. The two effects were very similar.

In a series of preparations, cocaine (2×10^{-6} g./ml. and upwards) increased the effect of stimulation on the rate. Curve *c* (Fig. 1) shows the much greater effect of stimulation in the presence of 5×10^{-6} g./ml. of cocaine. The increase was almost twice as great as in the control and the duration was about 7 min. When cocaine was removed from the bath, the effect of

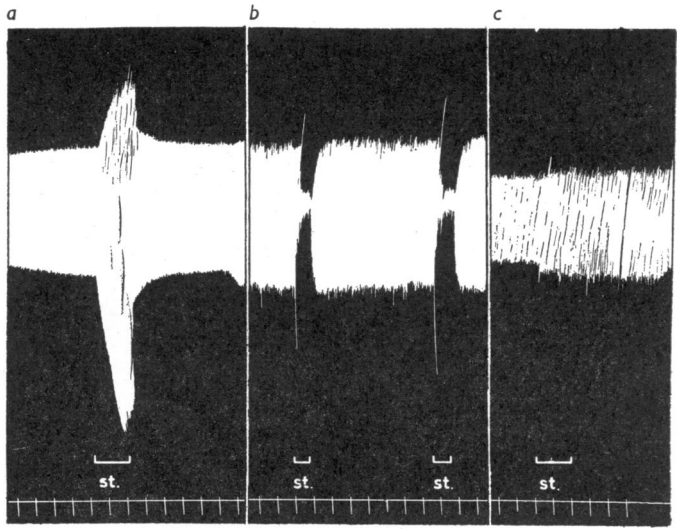

FIG. 3.—Kymograph record of atrial contractions. *a*, Effect of sympathetic nerve stimulation (st) for 2 min. on atria from normal rabbit; *b*, effect of sympathetic nerve stimulation for 1 min. on atria from rabbit treated with reserpine; *c*, effect of stimulation on the same preparation as in *b* after addition of atropine. Time, 1 min.

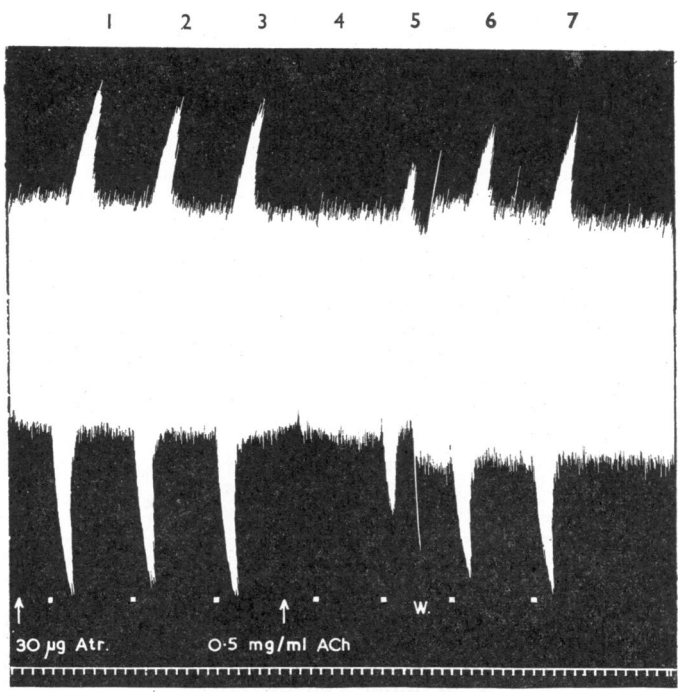

FIG. 4.—Kymograph record of atrial contractions showing the effect of sympathetic nerve stimulation for 15 sec. (at white rectangles) in the presence of atropine (30 µg./ml.). After the addition of acetylcholine (0.5 mg./ml.) to the bath stimulation 4 was without effect, and stimulation 5 had only a small effect. After this stimulation the bath was washed out (W), atropine being replaced. Time, 30 sec.

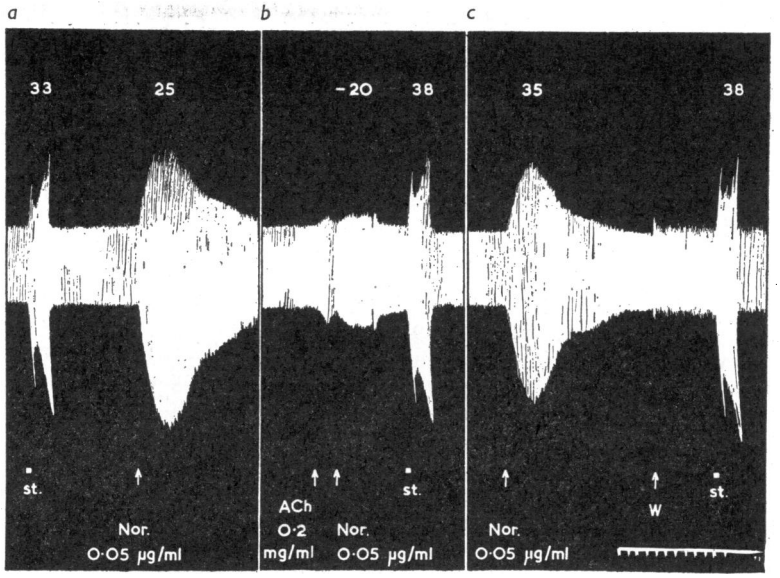

FIG. 5.—Effect of sympathetic nerve stimulation (st) and of noradrenaline (Nor) on the atrial contractions. The numerals at the top of the record show the change in atrial rate. All observations were made in the presence of atropine (30 µg./ml.). In *b* the addition of acetylcholine (0.2 mg./ml.) prevented the increase in rate by noradrenaline but not that due to sympathetic nerve stimulation. In the presence of acetylcholine noradrenaline caused a decrease in rate. Time, 30 sec.

stimulation returned to its initial dimensions (curve *d*).

Effect of Phenoxybenzamine.—Observations were also made with phenoxybenzamine (Dibenyline, Dibenzyline, N-phenoxyisopropyl-N-benzyl-β-chloroethylamine), which modified the effect of stimulation in the same way as cocaine. When 2.5×10^{-5} g./ml. of phenoxybenzamine was added to the bath, the increase in rate was much greater and its duration was longer. The effect persisted when the phenoxybenzamine was removed from the bath. An example of the action is given in Fig. 2.

Effect of Previous Administration of Reserpine.—Preparations of atria were made from rabbits treated for 2 days beforehand with reserpine, 1 mg./kg. on the first day and 2 mg./kg. on the second day intraperitoneally. In all preparations, the increase in rate due to sympathetic nerve stimulation was very small or absent, and in 5 out of 10 stimulation caused a slowing of the rate (Fig. 3). In *a* the effect of stimulation in a normal preparation is shown, in *b* the inhibition produced in a preparation from a reserpine-treated rabbit, and in *c* the absence of effect after adding atropine to the bath. The inhibitory action shown in Fig. 3 *b* was increased by eserine, and was clearly due to the liberation of acetylcholine.

Effect of Quinidine.—Quinidine, which has been shown by many workers to reduce the action of adrenaline on blood vessels, blocked the effect of sympathetic stimulation in a concentration of 20 to 30 µg./ml., the effect being restored when quinidine was removed from the bath.

Effect of Acetylcholine.—Some evidence has been published showing that postganglionic stimulation of sympathetic fibres can be blocked by ganglionic blocking agents (Brücke, 1935; Varagić, 1956) and observations were therefore made on atria. When hexamethonium was added to the bath, there was no diminution of the effect of stimulation even when the concentration was as high as 1.2 mg./ml. Pempidine also showed no evidence of block. With acetylcholine, however, some block was observed. When atropine was first added to the bath to exclude this inhibition, a high concentration of acetylcholine (0.2 to 0.5 mg./ml.), which itself had no effect on the heart rate, blocked the effect of sympathetic stimulation. This is illustrated in Fig. 4, which shows the increased amplitude of contraction caused by three successive stimulations of the postganglionic fibres for periods of 15 sec. in the presence of atropine, 30 µg./ml. When acetylcholine was added in the high concentration of 0.5 mg./ml., stimulation 1 min. later was without effect, and 3 min. later produced only a small effect. Acetylcholine was then removed from the bath, atropine being replaced, and stimulation then produced its original effect.

It was next observed that in the presence of the high concentration of acetylcholine the effect of noradrenaline in causing a rise in the atrial rate was blocked while stimulation was unaffected. This is illustrated in Fig. 5 for which the concentration of noradrenaline was 0.05 µg./ml. In the presence of atropine, acetylcholine (0.2 mg./ml.) blocked the action of noradrenaline (0.05 µg./ml.)

though it did not block the effect of stimulation. After removing the acetylcholine the effect of noradrenaline returned.

Discussion

Observations on tissues isolated together with their extrinsic nerve supply have increased in number in the last few years. Garry and Gillespie (1955) described a preparation of the rabbit colon with sympathetic and parasympathetic nerves, and in this department three different preparations have now been made. Varagić (1956) described a preparation of the rabbit uterus with the hypogastric nerves; McEwen (1956) described the setting up of the isolated heart and of the isolated atria with the vagus nerves, and in the present work the isolated atria has been used with the sympathetic nerves attached.

Certain similarities in the sympathetic innervation of these tissues may first be noted. The addition of cocaine to the bath greatly increased the effect of sympathetic stimulation both on the rabbit uterus and on the atria, and so did the addition of anti-adrenaline substances. Thus Varagić (1956) found that tolazoline increased the effect on the uterus of stimulating the hypogastric, and I found that phenoxybenzamine increased the effect of stimulating the sympathetic nerves to the atria. Recently Brown and Gillespie (1957) have shown that when the splenic nerves were stimulated at a frequency of 10/sec. the amount of noradrenaline liberated in the venous effluent was increased in the presence of phenoxybenzamine. It seems probable that the greater effect of stimulation on the uterus and on the atria was due to a similar increase in the amount of noradrenaline able to act on the uterine and atrial receptors.

The observations on the different tissues have been similar in another respect, namely that they have suggested the presence of cholinergic fibres in the sympathetic nerves. After Dale and Feldberg (1934) had shown that the fibres to the sweat glands were cholinergic, Bülbring and Burn (1935) gave evidence of cholinergic fibres to the muscles of the dog hindleg, and then Bacq and Frédericq (1935) stated that there were cholinergic fibres in the sympathetic supply to the cat nictitating membrane. Recently Varagić (1956) reported that, when he stimulated the hypogastric nerves to the uterus, the addition of eserine increased the size of the contractions and the addition of atropine reduced it. The effect of eserine was, however, seen in only two out of fourteen experiments. Gillespie and Mackenna (1959) have found that, when the isolated colon was prepared from a rabbit treated with reserpine, the inhibitory response to stimulation of the sympathetic nerves was converted to an excitatory response which was abolished by atropine. In the present experiments with atria from rabbits treated with reserpine, stimulation of the sympathetic nerve fibres has caused inhibition in half of them. This inhibition was increased by eserine and abolished by atropine. Thus the number of organs in which some of the sympathetic innervation appears to be cholinergic steadily increases. It thus appears that it is still too early for a final statement concerning postganglionic sympathetic nerve fibres. We do not yet understand the relation between those which are adrenergic and those which are cholinergic.

Finally, it was observed that sympathetic stimulation of the atria could be blocked by very high concentrations of acetylcholine, which, however, also blocked the stimulant action of noradrenaline. The experiments were done because Brücke (1935) found that the pilomotor response in the cat tail to stimulation of the lumbar sympathetic chain was blocked at the site where acetylcholine was injected into the skin, and because Varagić (1956) found that hexamethonium blocked the response of the rabbit uterus to hypogastric stimulation. Since in the atria acetylcholine not only blocked sympathetic stimulation but also the effect of noradrenaline, its action had no relation to block at a synapse.

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