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The isolated expansor secundariorum—a smooth muscle preparation from the wing of the domestic fowl

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Some aspects of the pharmacology of an isolated smooth muscle, the expansor secundariorum, of the domestic fowl have been investigated. Adrenaline, isoprenaline, tyramine and 5-hydroxytryptamine caused the muscle to contract. The responses to adrenaline and noradrenaline were blocked by phentolamine but not by propranolol at a concentration of $1{\cdot}7 \times 10^{-4}$M. Tyramine was without effect on the muscle of reserpine treated birds. Cocaine potentiated the effect of noradrenaline but blocked the effects of tyramine. Acetylcholine and histamine had no effect and did not alter the responses of the muscle to noradrenaline. The response to noradrenaline was greater at temperatures below 23° and less at 38°. The muscle contracted rapidly on the addition of Tyrode cooled to 18°. It is concluded that the muscle is wholly innervated by adrenergic postganglionic fibres.

THE attention of pharmacologists was drawn to the expansor secundariorum muscle of birds by George & Berger (1966). This muscle is present in most birds (Berger 1956) although the extent to which it is developed varies in different species. The muscles responsible for the movement of the feathers are innervated by sympathetic fibres (Langley, 1904) and since the analagous pilomotor muscles of the cat are unaffected by acetylcholine it was considered that the expansor secundariorum might provide a large smooth muscle with a purely sympathetic innervation. This report describes some of the properties of this muscle preparation.

Experimental

SETTING UP THE TISSUES

Domestic fowls (Cox 404) between 8–12 weeks and 500 to 1300 g were killed by ether inhalation and the expansor secundariorum muscles dissected immediately. The anatomy of this muscle in the domestic fowl is similar to that described by Berger (1956) for the pigeon (*Columba livia*). The long tendon of the muscle runs beneath the skin on the posterior edge of the wing, from the elbow to the axilla. When the skin in the elbow region is incised and reflected, the expansor secundariorum can be seen embedded in connective tissue. The tendon was separated from the skin and a cotton thread tied around the tendon 2 cm medial to the muscle. A piece of cotton was threaded through the expansor secundariorum so that it passed through the bases of the attached feathers. These were then trimmed level with the muscle and all excess skin removed before detaching the muscle from the surrounding connective tissue. To ensure that the isolated muscle gives consistent contractions, it is essential that the cotton passes through the bases of the feathers.

The muscle preparation was set up in a 10 ml tissue bath containing Tyrode solution gassed with a mixture 5% carbon dioxide in oxygen.

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Muscle contractions were recorded on a smoked drum by means of an isometric lever. This consisted of a short length of watch spring which could be adjusted to allow the muscle a maximum shortening of less than 10% of the resting length. A light lever arm was then attached so as to give a 25x magnification of the contractions. The initial tension in the tissue was about 1 g and the lever was calibrated at the conclusion of each experiment. Except where the temperature was varied as a part of the experimental procedure all experiments were at 23°.

MATERIALS

The Tyrode solution was made up as follows (g/litre): NaCl 8·0, KCl 0·2, $MgCl_2$ 0·1, $NaHCO_3$ 1·0, NaH_2PO_4 0·05, glucose 1·0. The weights refer to anhydrous salts.

Solutions of the following compounds were used freshly prepared in Tyrode solution: acetylcholine chloride, adrenaline hydrochloride, cocaine hydrochloride, histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate, isoprenaline sulphate, nicotine acid tartrate, phentolamine mesylate, propranolol hydrochloride, tyramine hydrochloride. All concentrations are expressed as the final molar concentration in the tissue bath.

Results

Spontaneous activity. When first placed in the tissue bath, the muscle usually showed some relaxation of tension followed by small spontaneous contractions. The degree of activity varied greatly; some preparations developed a tension of 0·7 g (Fig. 1), whereas others were completely quiescent. Some preparations showed markedly decreased spontaneous activity after the addition of several doses of one of the catecholamines (Fig. 1), whereas others maintained spontaneous contractions throughout experiments lasting 7 hr. The results with phentolamine (q.v.) suggest that the muscle retained some inherent tone and that the muscle fibres were not at their true resting length. Muscle tone was less at 38° than at 23°.

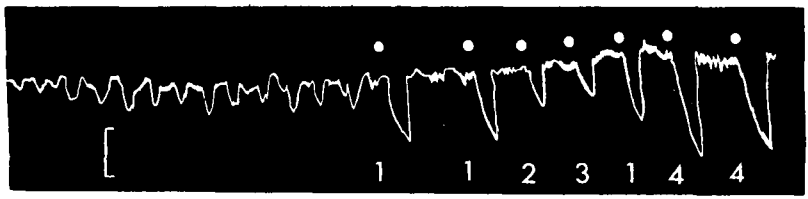

FIG. 1. Spontaneous contractions and the response after addition of tyramine. Four concentrations of tyramine added at 0 to give (1) $5{\cdot}8 \times 10^{-6}$M. (2) $5{\cdot}8 \times 10^{-6}$M. (3) $5{\cdot}8 \times 10^{-7}$M. (4) $2{\cdot}0 \times 10^{-4}$M. Scale 1 g. Contractions are downwards.

Catecholamines. As would be expected from the innervation of the muscles moving the feathers (Langley, 1904), adrenaline, noradrenaline and isoprenaline caused a contraction of the expansor secundariorum. Adrenaline was the most potent of the three catecholamines used, causing

a contraction at $1{\cdot}6 \times 10^{-8}$M. Noradrenaline was approximately 5 times less potent than adrenaline, but was sixty times more potent than isoprenaline. The time to reach the maximum tension for all these compounds at 23° was 3–4 min. It was not possible in most instances to obtain dose response curves for more than one catecholamine on one preparation because the high concentrations caused the muscle to fatigue and the time taken for relaxation was prolonged (15 min). Fig. 2 shows dose-response curves for adrenaline and noradrenaline.

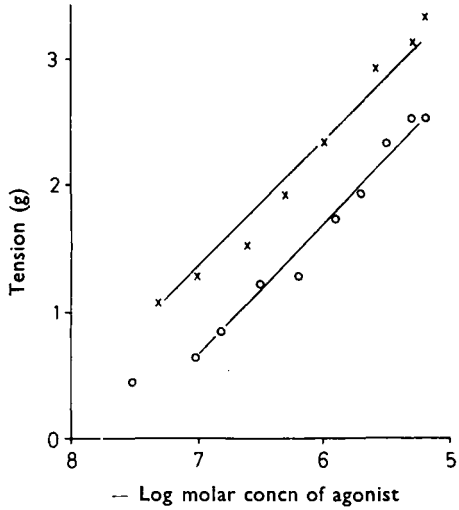

FIG. 2. Dose-response curves for adrenaline (×) and noradrenaline (○).

Tyramine. The expansor secundariorum preparation contracted in the presence of 5×10^{-7}M tyramine and gave greater contractions as the concentration of tyramine was increased (Fig. 1). The effects of reserpine and cocaine on the tyramine-induced contractions are presented under their separate headings.

5-Hydroxytryptamine. The sensitivity of the preparation to this substance was similar to that of noradrenaline. 5-HT did not affect the contractions caused by noradrenaline.

Nicotine. Three out of five preparations tested with nicotine gave contractions at concentrations between 5×10^{-4} and $2{\cdot}5 \times 10^{-3}$M. Fig. 3 shows that more than one contraction can be obtained from a preparation but the response is variable and is not always proportional to dose. Fig. 3A shows that $2{\cdot}5 \times 10^{-4}$M elicited a response but 5×10^{-4}M caused less tension development whilst $2{\cdot}5 \times 10^{-3}$M had the same effect as $2{\cdot}5 \times 10^{-4}$M. The preparation in Fig. 3B gave only two responses to nicotine (5×10^{-5} and $2{\cdot}5 \times 10^{-4}$M) and the preparation in Fig. 3C failed to respond to nicotine. In all instances the addition of nicotine had no effect on the responses to subsequent doses of noradrenaline.

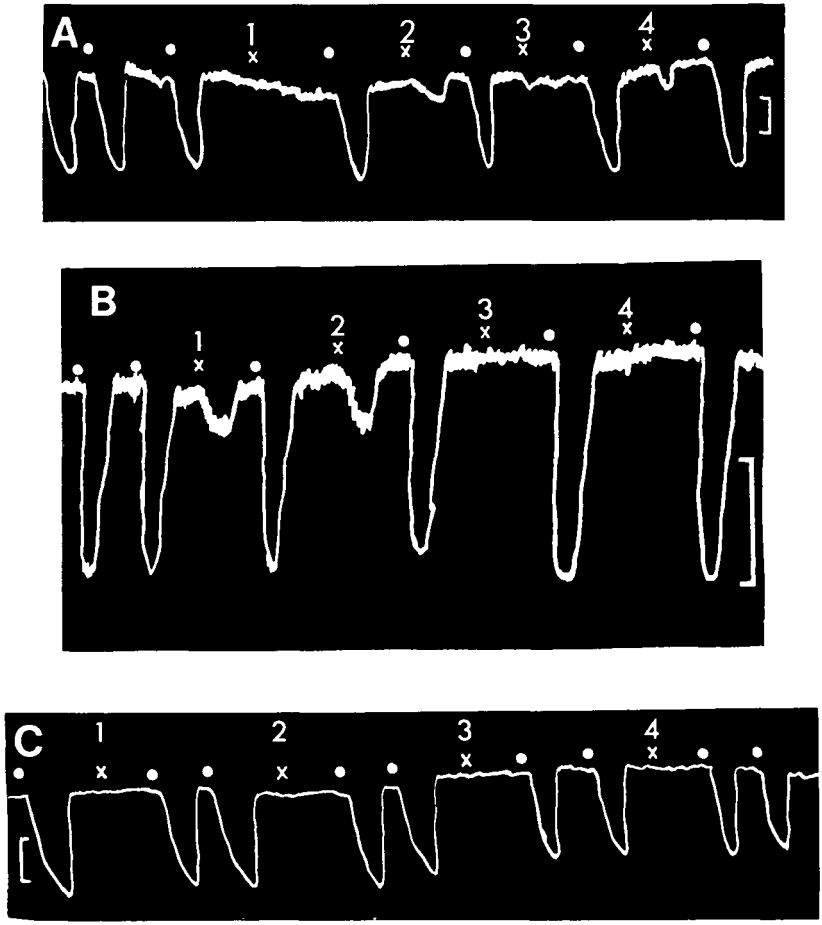

FIG. 3. The response of three preparations to nicotine. Noradrenaline was added at (O) to give a concentration of $3{\cdot}1 \times 10^{-6}$M. Nicotine added at (×) to give the concentrations (1) 5×10^{-5}M. (2) $2{\cdot}5 \times 10^{-4}$M. (3) 5×10^{-4}M. (4) $2{\cdot}5 \times 10^{-3}$M. Scale 1 g. Contractions are downwards.

Antagonists of catecholamines. Phentolamine and propranolol were used in an attempt to determine the nature of the adrenergic receptors in the expansor secundariorum. In these experinents the antagonist was added to the bath 3 min before the addition of the noradrenaline or adrenaline. Propranolol up to a concentration of $1{\cdot}7 \times 10^{-4}$M had no effect and did not influence the contractions produced by adrenaline and noradrenaline. Phentolamine at a concentration of $1{\cdot}3 \times 10^{-6}$M caused some relaxation of the preparation and inhibited the actions of adrenaline and noradrenaline. At a concentration of $1{\cdot}3 \times 10^{-5}$M, phentolamine abolished the response of the preparation to $6{\cdot}3 \times 10^{-6}$M noradrenaline.

Acetylcholine. At concentration $5{\cdot}4 \times 10^{-8}$ to $5{\cdot}4 \times 10^{-3}$M acetylcholine had no effect and did not influence the contractions produced by

adrenaline when this was added to the bath already containing acetylcholine.

Reserpine. Fowls were pretreated by injecting reserpine intramuscularly 1 mg/kg on the first day and on the following day 0·65 mg/kg. After each injection the birds became lethargic and unresponsive to changes in their environment. Expansor secundariorum muscles were used one or two days after the second injection of resperpine. When tyramine was added to the bath at concentrations from $5{\cdot}8 \times 10^{-7}$ to $2{\cdot}9 \times 10^{-4}$M, no effect was observed. Sensitivity to noradrenaline was not different from that of muscles from untreated birds. After the addition of noradrenaline tyramine caused contractions when added in concentrations within the range which caused contractions of muscles from normal birds. Fig. 4 shows the declining effect of successive doses of $5{\cdot}8 \times 10^{-5}$ tyramine to a muscle from a reserpinzied fowl which had been exposed to a noradrenaline at a concentration of $3{\cdot}1 \times 10^{-5}$M. When the response to tyramine had declined to 13% of that produced by the first dose immediately following the noradrenaline, a further dose of noradrenaline ($3{\cdot}1 \times 10^{-5}$M) elicited a normal contraction and restored the ability to respond to further addition of tyramine.

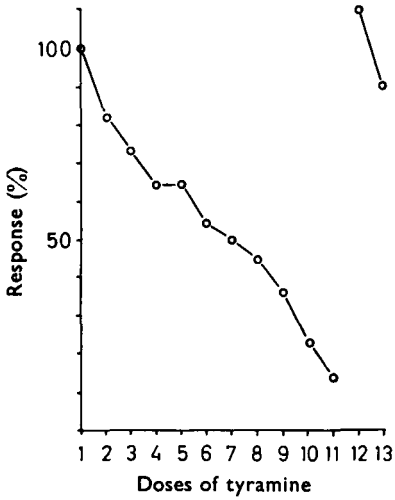

FIG. 4. The response of a muscle from a reserpinized bird to successive doses of tyramine. The muscle was unresponsive to tyramine until the tissue had been exposed to a direct-acting amine. The concentration of tyramine was $5{\cdot}8 \times 10^{-6}$M in each instance, and the response is expressed as a percentage of the response to the first dose of tyramine after obtaining contractions with noradrenaline. A normal response to noradrenaline was obtained between tyramine doses 11 and 12.

Cocaine. Cocaine $2{\cdot}9 \times 10^{-5}$M produced an immediate contraction. After washing, the muscle relaxed and the sensitivity to tyramine was decreased whereas the sensitivity to noradrenaline was increased. In concentrations of $5{\cdot}9 \times 10^{-6}$M cocaine did not cause a contraction but the response of the muscle to tyramine was decreased and the response to noradrenaline was increased. Cocaine did not have an immediate effect

on the responses to tyramine and noradrenaline but required a period of contact of about 10 min before its maximum effect was produced.

Histamine. In concentrations ranging from $3{\cdot}3 \times 10^{-8}$ to $3{\cdot}3 \times 10^{-5}$M histamine neither produced a contraction nor affected the responses to adrenaline.

The Effect of alterations of temperature. The temperature was changed by adding Tyrode solution at the required temperature to the bath. A thermometer in the tissue bath was used to monitor the temperatures. At 38° the response to adrenaline was approximately one third of that produced by the same concentration at 23°. At 17° the response of the muscle to adrenaline was slightly potentiated. The time taken to reach peak tension at 38° and 17° was greater than at 23°. At 18° and below the preparation gave a marked spontaneous contraction reversed quickly by raising the temperature to 23°. When the muscle was partially relaxed from a "cold contraction" it still responded normally to noradrenaline. The maximum tension developed in a "cold contraction" was also developed at 23° when sufficient noradrenaline was added. The amount of tension developed in a cold contraction was related to the temperature inducing the contraction over the range 18–12°. Each cold contraction was followed by a normal response to noradrenaline at 23°. Phentolamine ($1{\cdot}3 \times 10^{-4}$M) did not inhibit the "cold contractions" when added with the cold Tyrode solution.

Discussion

Although there is an overall similarity of pharmacological properties between the pilomotor muscles of the cat (Hellman, 1963a, 1963b) and the expansor secundariorum of the fowl, there are important differences. The most obvious of these is the spontaneous activity present in the expansor secundariorum but absent in the isolated pilomotor muscles of the cat (Hellman, 1963a). It is of interest that cooling can produce spontaneous pilomotor activity in man (Lewis & Marvin, 1927). The spontaneous contractions, and the contraction occasioned by cooling which are properties of the expansor secundariorum, are difficult to ascribe to a physiological function. According to Jollie (1957) the function of this muscle is control of the secondaries and tertiaries during flight. Since phentolamine does not block the cold contraction it is likely that this contraction is not mediated by the nerve endings.

In general, any sudden alteration of temperature can act as a stimulus and provoke a contraction in muscles (Evans, 1926). In the case of the expansor secundariorum this applies only for a fall in temperature, a rise producing a small degree of relaxation. This is in contrast to the guinea-pig ileum which gives a long-lasting contraction on both cooling and rewarming (Innes, Kosterlitz & Robinson, 1957) and the cat isolated iris which develops increased tonus as the temperature falls to 12° and develops further tonus as it is raised again to 20° (Verbitzky, 1923).

Cooling potentiates the actions of catecholamines on the isolated pilomotor muscles of the cat (Hellman, 1963b) and the expansor secundariorum. The rate of catecholamine-induced contraction of the expansor secundariorum at 38° and 15°C is slower than at 23°. The pilomotor muscles contract more slowly as the temperature is raised above 20° but in contrast to the expansor secundariorum the rate is not altered below this temperature (Hellman, 1963b).

The sensitivity of the expansor secundariorum to noradrenaline isoprenaline, phentolamine and propanolol is indicative of α-adrenergic receptors in the tissue. Whilst this is analagous to the suggestion of Hellman (1963a) with respect to the pilomotor muscles, there is a difference between these two muscles in their relative sensitivities to isoprenaline. In the pilomotor muscles of the cat the ratio adrenaline: isoprenaline sensitivity is 1:10,000 (Hellman 1963a) whereas in the expansor secundariorum the ratio is 1:20. The sensivity to phentolamine is similar in the two tissues.

The effects of reserpine and tyramine agree well with the effects of these compounds observed on other tissues. This evidence provides strong support for the tentative conclusion that adrenergic nerve terminals are present in the expansor secundariorum. The lack of effect of nicotine on some of our muscle preparations is not unusual since Bell (1968) and Hellman (1963a) present similar data for the vas deferens and pilomotor muscles.

Potentiation of the action of noradrenaline by cocaine can be explained by an effect of cocaine on the postsynaptic receptors (Reiffenstein, 1968) or by an effect of blocking the uptake of noradrenaline into the nerve terminals (Trendelenburg, 1966). The decreased response of the expansor secundariorum to tyramine in the presence of cocaine is difficult to explain in terms of a postsynaptic effect if tyramine acts on the stores of noradrenaline, as indicated by the lack of its effect in reserpinized muscles. A presynaptic action of cocaine can only potentiate the action of noradrenaline if uptake into the nerve terminals is a factor limiting the action of noradrenaline. In the tissue bath where the amount of noradrenaline is great compared to the capacity of the nerve terminals to take up noradrenaline, it is unlikely that uptake would be important. It thus seems possible that the blocking of the effects of tyramine by cocaine is produced by a blockade of uptake into nerve terminals, whereas the potentiation of the response to noradrenaline is produced by a direct action on the adrenergic receptors.

The lack of effect of acetylcholine provides strong support for the assumption that there is no cholinergic innervation of the expansor secundariorum. The absence of any actions of acetylcholine when added in a wide range of concentrations does not support the hypothesis of Burn & Rand (1965) which requires that acetylcholine releases noradrenaline from sympathetic nerve endings.

The expansor secundariorum is an interesting preparation because it appears to be innervated wholly by postganglionic sympathetic fibres and is completely unresponsive to acetylcholine. The use of this muscle

preparation should make it possible to study the effects of drugs on the assumption that whatever their effects they are unlikely to be mediated via cholinergic receptors or parasympathetic fibres.

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