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THE RESPONSE OF THE CAT ANOCOCCYGEUS MUSCLE TO NERVE OR DRUG STIMULATION AND A COMPARISON WITH THE RAT ANOCOCCYGEUS

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1 The cat anococcygeus muscle is shown to possess a dual innervation similar to the rat anococcygeus, with a motor adrenergic innervation and an inhibitory innervation whose transmitter is unknown. The pharmacological properties of the cat muscle were investigated and compared with those of the rat muscle.

2 The cat muscle contracts to noradrenaline, 5-hydroxytryptamine, tyramine, amphetamine, guanethidine, cocaine and lysergic acid diethylamide (LSD). The effects of noradrenaline and 5-hydroxytryptamine are blocked by phentolamine and methysergide respectively.

3 The cat anococcygeus is relaxed by acetylcholine, carbachol, isoprenaline, ATP, prostaglandins E_1, E_2 and $F_{2\alpha}$ and vasopressin, all of which contract the rat muscle. The effects of acetylcholine and carbachol are blocked by atropine and those of isoprenaline by propranolol.

4 Field stimulation produces contraction of the cat anococcygeus, which is blocked by phentolamine and guanethidine but unaffected by hexamethonium, atropine or neostigmine.

5 In the presence of guanethidine (10^{-5} M), the tone of the muscle is raised and field stimulation produces relaxation of the muscle. These inhibitory responses are unaffected by phentolamine, hexamethonium, atropine or neostigmine.

6 Neostigmine potentiates the effects of acetylcholine, but not of carbachol in relaxing the cat anococcygeus and in contracting the rat anococcygeus, but has no effect on either motor or inhibitory responses to field stimulation.

7 Cold storage for up to eight days had little effect on either the motor response to noradrenaline or the motor or inhibitory response to field stimulation of the cat anococcygeus. Beyond eight days, the response to field stimulation diminishes more rapidly than the response to noradrenaline.

Introduction

The anatomy and responses to nerve and drug stimulation of the rat anococcygeus have been described (Gillespie & Maxwell, 1971; Gillespie, 1972; Gillespie & McGrath, 1973; Gibson & Gillespie, 1973). The muscle has a dense adrenergic motor innervation, but there is no evidence of cholinergic nerves; it does, however, possess a powerful inhibitory innervation mediated by an unknown transmitter (Gillespie, 1972; Gillespie & McGrath, 1972).

Recently, Garrett, Howard & Lansdale (1972) have examined the similar muscle in the cat and shown that it too has a dense adrenergic innervation but, unlike the rat, it also stains for acetylcholinesterase. It is tempting to interpret this staining as evidence of a cholinergic innervation and to associate the latter with a possible inhibitory mechanism.

The purpose of the present experiments was to determine whether a cholinergic innervation exists in the cat muscle and if so, whether its properties resemble those of the inhibitory innervation in the rat. In the course of the work, certain responses to drugs in the cat muscle were found to differ so radically from those in the rat that a simultaneous comparison was made on muscles from the two species; and these results are described here.

Methods

The arrangement of the cat anococcygeus muscles is similar to that in the rat. The two muscles arise from the upper coccygeal vertebrae close to one another in the mid-line of the pelvic cavity. The muscles run caudally, lying first behind and then to one side of the colon. In the female, the muscles terminate at the sides of the colon. In the

male they continue ventrally to form a bar in front of the colon.

The muscles used in these experiments were from cats primarily intended for experiments with the perfused spleen. For this latter purpose they were anaesthetized with a mixture of nitrous oxide and halothane and given (intravenously) 70 μg of prostaglandin E_1 and 20,000 i.u. of heparin, before removal of the spleen (Blakeley, Brown, Dearnaley & Woods, 1969). The anococcygeus muscles were then immediately dissected out. The animal was first killed by cardiac incision, the abdomen opened in the mid-line as far as the anal margin, the pelvis split and the bladder, urethra and vasa deferentia or uteri removed. The colon was then cut through at the pelvic brim, the pelvic portion pulled forward and the connective tissue behind carefully cleared until the anococcygeus muscles came into view. The muscles were isolated and in females the portion running from the vertebrae to the colon, or in males the entire length to the ventral bar, was removed to a 10 ml bath containing Krebs saline at 36° C and gassed with a mixture of 95% O_2 and 5% CO_2. Rat anococcygeus muscles were removed as described by Gillespie (1972). Tension was measured with Grass FT03 isometric transducers and displayed on a Grass Polygraph. Field stimulation of the intramural nerve fibres was applied after drawing the muscles through a pair of electrodes similar to those described by Burn & Rand (1960). Stimulation of intramural nerves was with 1 ms pulses and supramaximal voltage, at the frequencies indicated in the text.

Most muscles were set up as described above within minutes of removal. On other occasions, this was inconvenient and the muscles were stored in Krebs solution at 4°C for some time before use. For this reason, the effect of cold storage on the responses to field stimulation and to drugs was examined. Muscles were quickly removed from the animal to 100 ml of oxygenated Krebs in a sealed vessel and kept at 4° C for 1-18 days.

The distribution of adrenergic nerves within the muscle was examined histochemically on freeze dried tissue with a slightly modified version of the technique of Hillarp & Falck (Gillespie & Kirpekar, 1966).

Noradrenaline assays were carried out by the trihydroxyindole method of Euler & Lishajko (1961) after absorption on alumina.

Drugs used were: acetylcholine chloride (Koch-Light); (−)-adrenaline bitartrate (B.D.H.); dexamphetamine sulphate; adenosine 5′-triphosphate disodium (Sigma); atropine sulphate; carbachol (Burroughs Wellcome); cocaine hydrochloride; guanethidine sulphate (CIBA); hexamethonium bromide (Koch-Light); histamine acid phosphate (B.D.H.); 5-hydroxytryptamine creatinine sulphate (Koch-Light); (±)-isoprenaline hydrochloride (Sigma); (+)-lysergic acid diethylamide tartrate (Sandoz); mepyramine maleate (May & Baker); methysergide bimaleate (Sandoz); neostigmine methylsulphate (Roche); (−)-noradrenaline bitartrate (Koch-Light); pancuronium bromide (Organon); phentolamine mesylate (CIBA); propranolol sulphate (Sigma); prostaglandins E_1, E_2, $F_{2\alpha}$; tyramine hydrochloride (Sigma); vasopressin (Parke-Davis). Doses refer to the base, with the exception of prostaglandins and vasopressin.

Results

Anatomically, the rat and cat muscles are similar. Both are about 3 cm in length; in both, the female muscle is shorter than the male because of the absence of the ventral extension. The one macroscopic difference is that the cat muscle is considerably thicker and consequently heavier. Representative weight ranges for a pair of muscles are: cat, male 100-150 mg; cat, female, 50-100 mg; rat, male 25-50 mg. The Falck technique shows that the cat, like the rat, has a terminal plexus of brightly fluorescent fibres scattered uniformly throughout the muscle. Assay of the noradrenaline content gave a value of 2.1 μg/g for the cat compared with 2.56 ± 0.48 μg/g for the rat. Suspended in Krebs solution at 36°C, the cat anococcygeus differs from the rat in developing both spontaneous tone and rhythmic acitivity, greatest immediately after setting up the preparation but usually present to some extent throughout the experiment (e.g. Figures 5 and 8). Papaverine (10^{-5} M) abolished both tone and rhythmic activity.

Effects of drugs

Drugs causing contraction Noradrenaline, adrenaline and 5-hydroxytryptamine all caused a dose-related contraction of the cat anococcygeus. Noradrenaline and adrenaline were approximately equipotent, producing contractions which were antagonized by phentolamine (10^{-6} M). Responses to adrenaline and to a lesser extent noradrenaline, were potentiated by propranolol (10^{-6} M). 5-hydroxytryptamine produced contractions which were antagonized by methysergide (2×10^{-7} M). Dose-response curves for noradrenaline and 5-hydroxytryptamine and for the effects of phentolamine and methysergide, are shown in Figure 1. Muscles from male cats produced larger responses to noradrenaline and 5-hydroxytryptamine than those from females, but the dose-response curves (drawn as a percentage of the

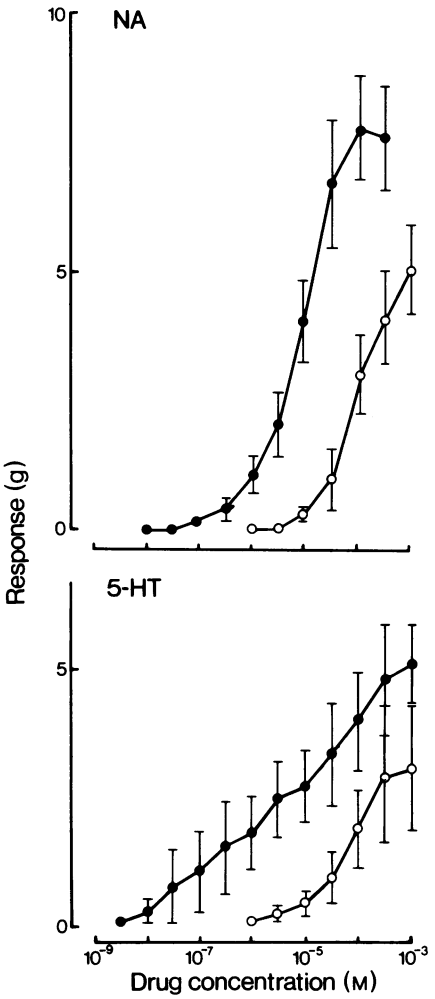

Fig. 1 Dose-response curves of the cat anococcygeus to noradrenaline (NA) and 5-hydroxytryptamine (5-HT) and the effect of phentolamine (10^{-6} M) and methysergide (2×10^{-7} M). Control (○), phentolamine or methysergide (●). Bars represent ±s.e. mean. Muscles from male and female cats.

maximum) were similar. Noradrenaline produced a larger maximum response than 5-hydroxytryptamine which had still not reached its maximum at a concentration of 10^{-3} M. This difference was observed in each individual experiment.

The sensitivity of the cat anococcygeus to noradrenaline is similar in the rat, but that to 5-hydroxytryptamine is approx. 100 times greater, as indicated by the contraction produced by it at the start of each panel in Figure 3. The response to histamine as in the rat, was small, was seen only at high concentrations (10^{-4} M) of the drug, produced marked tachyphylaxis and was completely abolished by mepyramine (10^{-6} M).

Four drugs known to act as indirect sympathomimetics on other tissues were examined: tyramine, amphetamine, cocaine and guanethidine. All produced contraction, tyramine and amphetamine at or above 3×10^{-7} M, cocaine at or above 10^{-5} M (Fig. 2) and guanethidine at or above 10^{-6} M (Figures 2 and 4). All four drugs produced contractions slower in onset than those to noradrenaline (Fig. 2) and all were reduced or abolished by phentolamine (3×10^{-7} M). One distinguishing feature was that the contraction to tyramine, amphetamine and cocaine was complete within 5 min, whereas guanethidine required up to 30 min to develop its full motor effect even though its blocking effect on the adrenergic motor response developed rapidly and the final tension achieved was equal to that of the other drugs (Figures 2 and 4). Cocaine (3×10^{-5} M) also potentiated and prolonged the motor response to noradrenaline and to field stimulation of the adrenergic nerves.

One unexpected finding was the ability of lysergic acid diethylamide (LSD) to cause contraction. Concentrations as low as 10^{-8} M caused contractions which characteristically were prolonged and persistent even after repeated washing (Figure 2).

In the rat anococcygeus, all five drugs caused contraction and the only minor difference was that the rise in tone induced by guanethidine developed more rapidly than in the cat. LSD was equally effective in the rat and the contraction equally resistant to washing. In both species, some degree of tachyphylaxis was observed with all five drugs.

Drugs causing inhibition A major qualitative difference in the responsiveness to drugs of the cat and rat anococcygeus was the number causing inhibition in the cat. In the rat, no drug capable of causing inhibition was found. In the cat, acetylcholine, carbachol, isoprenaline, ATP, prostaglandins E_1, E_2 and $F_{2\alpha}$ and vasopressin, all of which were motor in the rat, produced relaxation. Because of the presence of spontaneous tone, this inhibitory effect could be demonstrated directly in the cat muscle, but the response was more dramatic if tone was first raised by the addition of 5-hydroxytryptamine, tyramine or guanethidine. Figure 3 contrasts this inhibitory effect on the cat anococcygeus with a simultaneous motor action on the rat for carbachol, isoprenaline, ATP and prostaglandin E_1. Acetylcholine had a similar action. This inhibitory effect on the cat of acetylcholine and carbachol was abolished by

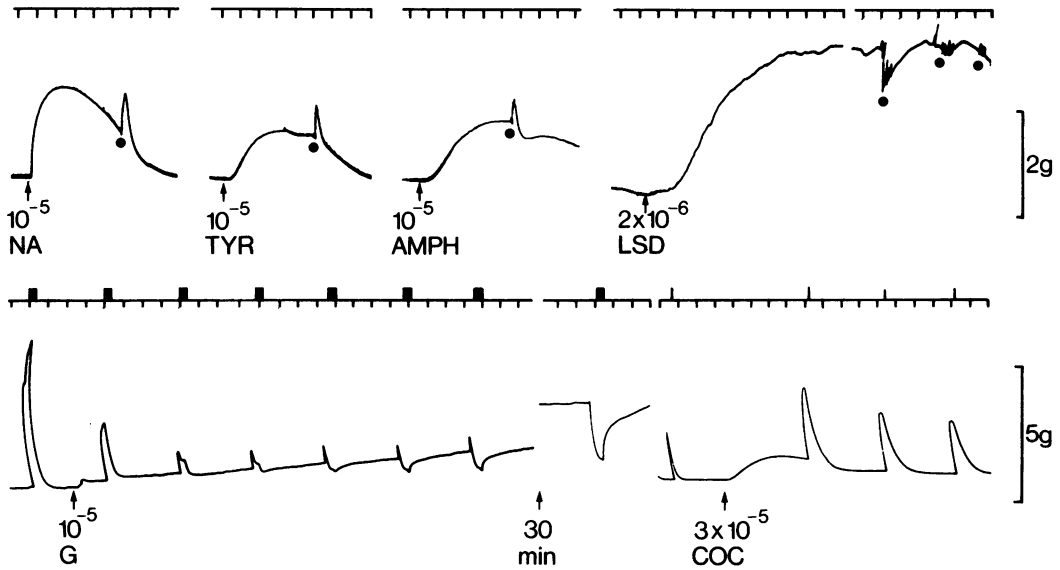

Fig. 2 The effect on the cat anococcygeus of a series of indirect sympathomimetics compared with the action of noradrenaline (NA). Noradrenaline (NA) (10^{-5} M) produced a rapid motor response. Tyramine (TYR) (10^{-5} M), dexamphetamine (AMPH) (10^{-5} M) and lysergic acid diethylamide (LSD) (2×10^{-6} M) produced responses slower in onset. Responses to LSD were persistent even after several washes. Wash at dots. Guanethidine (G) (10^{-5} M) raised the tone slowly, reaching its maximum after 40 min and reversed the response to field stimulation (10 Hz, 20 s) from motor to inhibitory. Cocaine (COC) (3×10^{-5} M) produced a motor response and prolonged and potentiated the motor response to field stimulation (10 Hz, 1 s). Time: 1 minute.

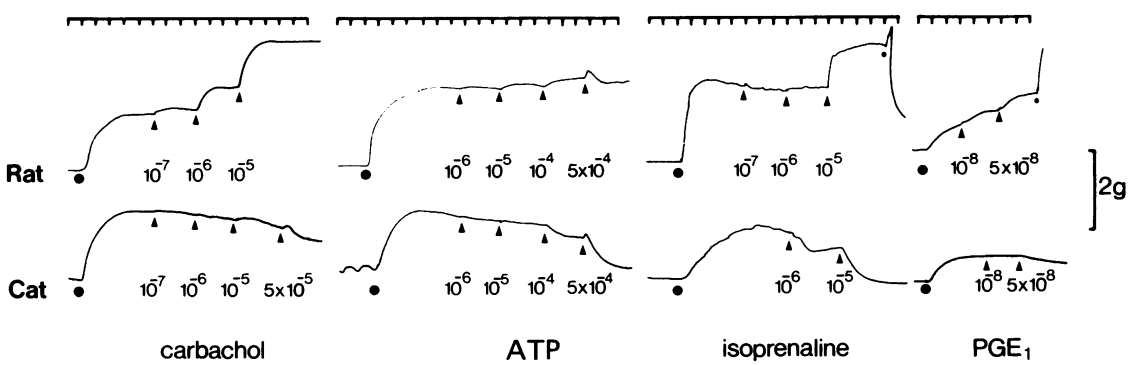

Fig. 3 Comparison of the effects of carbachol, ATP, isoprenaline and prostaglandin E_1 (PGE_1) on the rat and cat anococcygeus. In order to detect inhibitory responses, the tone of the muscles was first raised with 5-hydroxytryptamine (5-HT), •. Because the cat is more sensitive to 5-HT, the dose of 5-HT given was 10^{-7} M compared with 10^{-5} M for rats. In the final rat trace, carbachol (10^{-6} M) replaced 5-HT for this purpose. All four substances contracted the rat muscle but relaxed the cat muscle. Time: 1 minute. Concentrations shown are molar for carbachol, ATP and isoprenaline but g/ml for prostaglandin E_1.

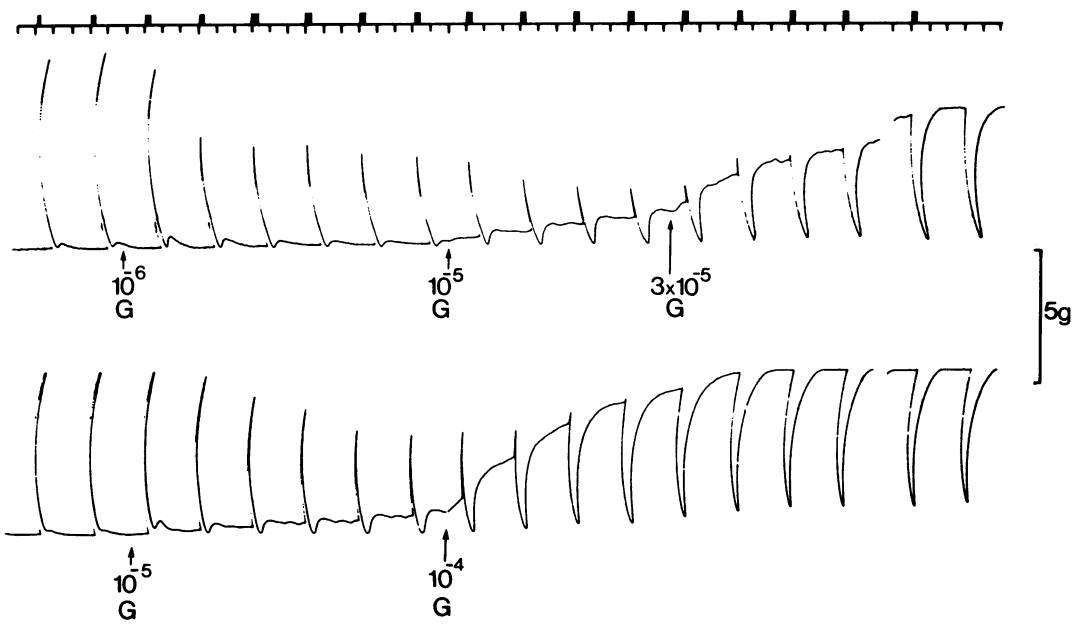

Fig. 4 The effect of guanethidine on the response to field stimulation in the cat anococcygeus muscle. In the upper trace, guanethidine (G) (10^{-6} M) rapidly produced a 50% reduction in the motor response to field stimulation but caused only a slight increase in muscle tone. Guanethidine (10^{-5} M and 3×10^{-5} M) further reduced the motor response and increased the tone more rapidly thus uncovering a large inhibitory response. The lower trace shows that a still larger dose of guanethidine (10^{-4} M) produced a faster increase in tone and reversal of the response from motor to inhibitory. The contractions on the lower trace were truncated by the limits of the recorder, the first two motor responses on the left were 2 g larger than shown and at the right, the maximum tone produced by guanethidine (10^{-4} M) was 1.5 g larger than shown. Stimulation at bars on time trace, 10 s train at 10 Hz. Time: 1 minute.

atropine (10^{-6} M) and of isoprenaline by propranolol (10^{-6} M). Vasopressin (20-200 milliunits/ml) was also examined and found to produce a relaxation in the cat and contraction in the rat muscle.

Response to field stimulation

Field stimulation of the rat anococcygeus produces motor responses due to its adrenergic innervation. When these motor nerves are blocked with guanethidine and the tone of the muscle raised, field stimulation then produces a powerful inhibitory response. Similar motor and inhibitory responses were found in the cat anococcygeus (Figure 4).

Increasing doses of guanethidine gradually depressed the motor response to field stimulation. At 10^{-5} M and above, guanethidine also raised the tone of the muscle and at the same time uncovered an inhibitory response. At 3×10^{-5} M and above, guanethidine completely abolished the motor response leaving a pure inhibitory response which was capable of abolishing over 80% of the guanethidine-induced tone.

Effects of blocking drugs The effects of phentolamine, atropine, hexamethonium, pancuronium, mepyramine and propranolol were tested on both motor and inhibitory responses in the cat anococcygeus. None of these drugs altered the inhibitory response and only phentolamine blocked the motor response (Figure 5).

In order to examine the effect of phentolamine on the inhibitory responses, the tone was raised with a large dose of LSD. This situation allowed inhibitory response to be seen while the tone of the muscle itself was unaffected by the blocking agent phentolamine.

Effects of neostigmine

The report by Garrett, et al. (1972) of a dense cholinesterase staining, together with the present observation that acetylcholine is inhibitory in the cat anococcygeus, suggested that the inhibitory

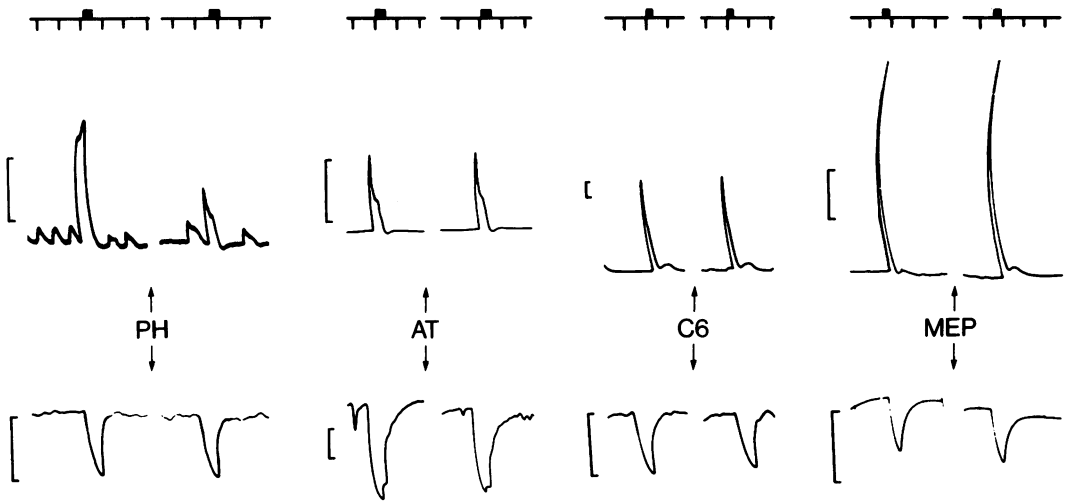

Fig. 5 The effect of phentolamine (PH) (10^{-6} M), atropine (AT) (10^{-6} M), hexamethonium (C6) (10^{-5} M) and mepyramine (MEP) (10^{-5} M) on the motor and inhibitory responses to field stimulation of the cat anococcygeus. Each pair of responses (i.e. before and after drug) was obtained on a different muscle. Motor responses were obtained from stimulation at 10 Hz with the exception of the first pair (30 Hz). Inhibitory responses were produced in the presence of guanethidine (3×10^{-5} M) with the exception of the first pair of responses where the tone was raised by LSD (10^{-6} M). Frequency of stimulation for inhibitory responses was 10 Hz with the exception of the first pair (5 Hz). Phentolamine decreased the size of the motor response but did not affect the inhibitory response. Atropine, hexamethonium and mepyramine had no effect on responses whether motor or inhibitory. Time: 1 minute. Vertical calibrations: 1 g.

response might be due to a cholinergic pathway. We therefore examined the effect of an anticholinesterase, neostigmine, to see if this would potentiate either motor or inhibitory responses to field stimulation at frequencies of stimulation ranging from 1-20 Hz. Figure 6 shows the result for the inhibitory response. Neostigmine (10^{-7}M) had no effect on either motor or inhibitory responses to field stimulation at any frequency.

The same dose of neostigmine did, however, potentiate the motor responses to acetylcholine in the rat and the inhibitory responses to the same in the cat (Figure 7). The corresponding responses to carbachol in both species were unaffected by neostigmine.

Effects of cold storage

Since the anococcygeus muscles used were obtained from cats used in other experiments, some were stored in Krebs solution at 4° C for a variable time before their responses were measured. Observations were therefore made on the effect of cold storage on the responses to field stimulation and to agonist drugs. The action of direct and indirect sympthomimetics and the responses to field stimulation proved remarkably resistant to cold storage. Up to five days, there was little diminution of the motor response to either drug or nerve stimulation.

In another experiment, the effect of noradrenaline and field stimulation was examined in the

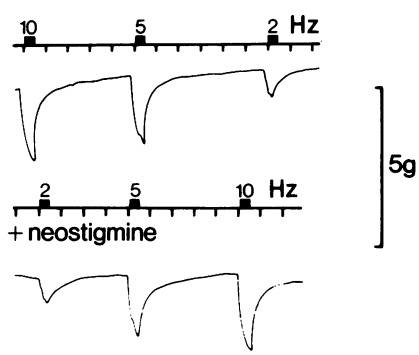

Fig. 6 Effect of neostigmine (10^{-7} M) on the inhibitory response to field stimulation in the cat anococcygeus. In the presence of guanethidine (3×10^{-5} M), stimulation at the frequencies indicated above the time trace produced inhibitory responses. Neostigmine (lower trace) had no effect on the size or duration of responses at 2, 5, or 10 Hz. Time: 1 minute.

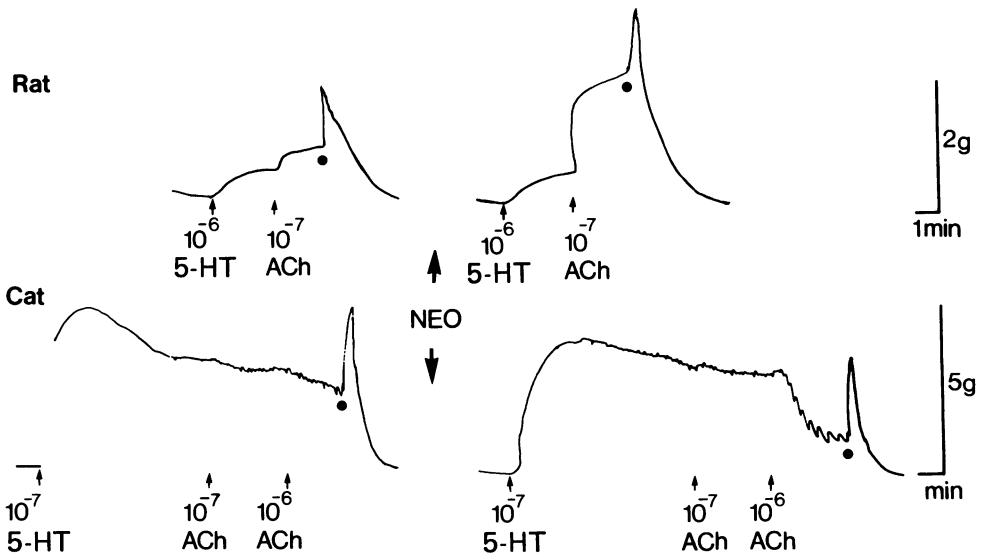

Fig. 7 Comparison of the effects of neostigmine (NEO) (10^{-7} M) on the response of the rat and cat anococcygeus to acetylcholine (ACh). The tone of the muscles was first raised with 5-hydroxytryptamine (5-HT) (10^{-6} M) for rat, (10^{-7} M) for cat. Neostigmine enhanced the motor response to acetylcholine (10^{-7} M) in the rat and the inhibitory response to acetylcholine (10^{-6} M) in the cat but did not affect the response to 5-hydroxytryptamine in either species. Wash at dots.

same muscle after 8, 11, 14 and 18 days cold storage (Figure 8). At eight days, the responses to both noradrenaline and field stimulation were similar to those obtained with fresh tissues. After eight days, the responses to noradrenaline declined gradually but were still present at 18 days, the dose-response curve drawn as a percentage of the maximum remaining unchanged. The motor response to field stimulation, on the other hand, declined more rapidly, the greatest change occurring between 8 and 11 days. Motor responses to field stimulation were just detectable at 10 and 30 Hz at 18 days. The effect of cold storage on the inhibitory response was more difficult to examine since guanethidine was necessary to display inhibition and, once given, further examination of the effect of cold storage on the motor response in that muscle was impossible. For this reason, the effect of cold storage on the inhibitory response was examined in only two cats. In one cat, the response was measured in one muscle immediately after removal and in the contralateral muscle from the same animal after five days cold storage. The muscles from the second cat were stored for 11 and 18 days respectively. The percentage inhibition of induced tone in the fresh muscle was 68%, comparable to the average for all cats; at five days, in the same cat, the inhibition was 60%. In the other animal at 11 days, the maximum inhibition was 27% and at 18 days no response was obtained. The ability of guanethidine (3×10^{-5} M) to raise the tone of the muscle was undiminished at five days, was reduced approximately 50% at 11 days and at 18 days only a small transient response was found.

Histochemical examination of a cat anococcygeus muscle after five days cold storage showed many fluorescent nerve terminals still visible. After 11 days, the terminals were less bright but still present, and at 18 days they were just detectable.

Discussion

The cat anococcygeus muscle shows both similarities and differences with the rat muscle. Both possess a motor adrenergic innervation and an inhibitory innervation whose transmitter is unknown. In both species, the motor responses are blocked by either α-adrenoceptor or neurone blocking agents but, so far, we have found no blocking agent for the inhibitory response. The adrenergic motor nerves seem particularly sensitive to drug-induced release of their transmitter so that not only the classical indirect sympathomimetics, such as tyramine and amphetamine, are effective but also less well known releasing agents, such as guanethidine, cocaine and LSD. The last two are

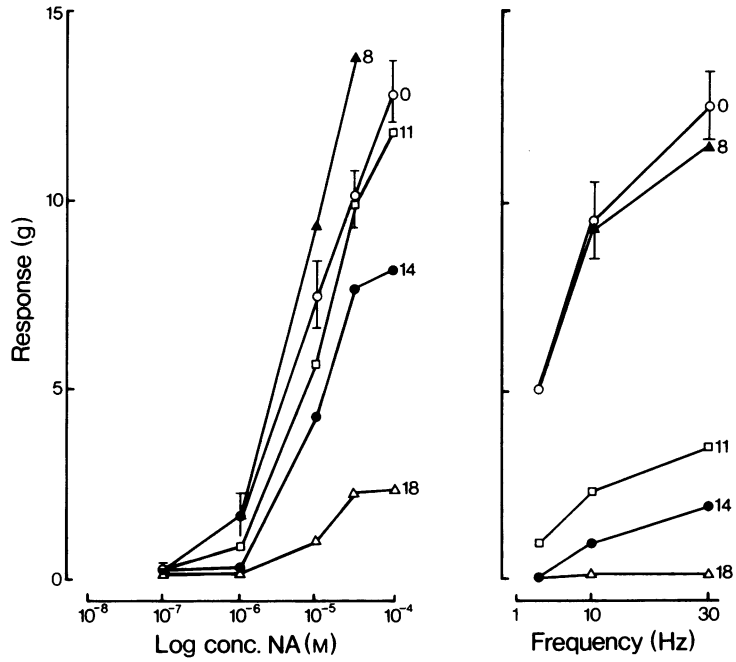

Fig. 8 Effect of cold storage on the response of a cat anococcygeus to noradrenaline (NA) and to field stimulation. The mean of the responses in four fresh muscles (○) is compared with responses of a muscle which was cold stored and intermittently tested between 8-18 days (▲, 8; □, 11; ●, 14; △, 18 days). The responses to noradrenaline declined steadily after the eighth day but survived longer than those to field stimulation. The responses to field stimulation showed a sudden decrease between 8 and 11 days which may correspond to nerve degeneration.

seldom regarded in this light but cocaine has been shown to be an indirect agonist in several tissues, including cat nictitating membrane (Kukovetz & Lembeck, 1962), rat vas deferens (O'Donnell & Hecker, 1967), rabbit aortic strip (Maengwyn-Davies & Koppanyi, 1966) and guinea-pig atria (Trendelenburg, 1968); and LSD contracts the cat isolated nictitating membrane (Thompson, 1958) in a manner similar to that in our findings with the rat and cat anococcygeus. In addition, in rat and cat anococcygeus muscles, after chemical sympathectomy with 6-hydroxydopamine, the contractor response to cocaine or LSD is reduced, suggesting that both drugs act by releasing noradrenaline from nerves within the tissue (Gillespie & McGrath, unpublished observations).

Nevertheless, in terms of receptors, there are clearly both quantitative and qualitative differences. The cat apparently has more types of receptors and several of these induce inhibitory responses. In the rat, there is good evidence for only three receptors (α-adrenoceptors, muscarinic and 5-hydroxytryptamine receptors) and all of these produce motor responses. Other drugs, including histamine, ATP, prostaglandins and isoprenaline, always produce motor effects but are effective only in high doses. With isoprenaline in particular, the evidence in the rat is that this is not an effect on β-adrenoceptors since it is uninfluenced by propranolol. In the cat, however, isoprenaline produces inhibition and the effect is abolished by propranolol, indicating the presence of β-adrenoceptors. Even the response to 5-hydroxytryptamine shows a greater sensitivity by about two orders of magnitude in the cat over the rat, though in this instance the response is motor in both species.

The ability of several potential transmitters to produce inhibition in the cat muscle raises the possibility that they are involved in the inhibitory response to field stimulation. The most likely are acetylcholine and ATP. Since the inhibitory response to field stimulation is quite unaffected by either atropine or neostigmine, in concentrations which respectively abolish or potentiate the response to acetylcholine, it does not appear possible that acetylcholine is the transmitter. Similar evidence to exlude ATP is lacking but in view of the high concentrations needed to produce

a small mechanical inhibition in contrast to the intense inhibition produced by field stimulation, it does not seem a likely candidate. Furthermore, it appears more probable that the inhibitory transmitter will be the same in both species and in the rat only motor responses can be obtained with ATP.

Garrett *et al.* (1972) reported acetylcholinesterase in the cat anococcygeus. No similar staining was observed in the rat muscle, yet neostigmine caused a similar degree of potentiation of the effects of acetylcholine in both. We have no explanation of this discrepancy nor can we suggest a function for the enzyme in this muscle.

These experiments on nerve or drug responses of the cat muscle were carried out on tissues which might be cold stored up to a maximum of two days. The necessary control studies on the effect of cold storage showed that little change in nerve or drug responses occurred over this short period. Less expected was the ability of these nerves, cut off from their ganglion cell body, to be still present and capable of functioning after 11 days, as shown by the histochemical demonstration of adrenergic nerve terminals together with the responses to field stimulation and to the indirect sympathomimetic action of guanethidine. Stimulation of post-ganglionic adrenergic nerves fails to produce responses after two days in mouse, rat and rabbit ileum, three days in guinea-pig colon (Holman & Hughes, 1965) and seven days in guinea-pig taenia caecum (Hattori, Kurahashi, Mori & Shibata, 1972). Adrenergic nerves were not demonstrable histochemically after five days cold storage in guinea-pig caecum (Hattori *et al.*, 1972) or rabbit aortic strips (Shibata, Hattori, Sakurai, Mori & Fujiwara, 1971). The capacity of the anococcygeus muscle to respond to noradrenaline after the nerves had ceased to function is in agreement with the usual finding that in cold stored tissues the smooth muscle cells remain viable longer than the nerves. Thus intestinal smooth muscle from various species remains responsive to acetylcholine for several days after the cholinergic nerves have ceased to function (Ambache, 1946) and in guinea-pig taenia caecum phenylephrine still relaxes the muscle after 18 days cold storage (Fukuda & Shibata, 1972) despite the complete absence of nerve responses after 5 days (Hattori *et al.*, 1972).

It was not possible, however, to differentiate between motor and inhibitory nerves in the cat anococcygeus by their ability to survive cold storage. In previous studies, where cold storage of a tissue has been employed successfully to differentiate between the components of a multiple innervation, the different anatomical arrangement of the nerves has been a major factor contributing to the preferential survival of one type (Holman & Hughes, 1965; Hattori *et al.*, 1972). However, in the isolated cat anococcygeus muscle as in the rat, (Gillespie, 1972), both motor and inhibitory nerves consist purely of post-ganglionic fibres since ganglion blockers have no effect on responses to field stimulation. There is therefore no reason to expect differential survival. Despite the long survival after cold storage of the nerve and muscle elements of the cat anococcygeus, we feel that species variation may be at least as important as the properties of the particular tissue used, since the rat anococcygeus muscle does not respond to either field stimulation or noradrenaline after only five or six days (D. Templeton, personal communication).

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