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PHARMACOLOGICAL STUDY OF THE ANOCOCCYGEUS MUSCLE OF THE DOG

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1 The response of the dog anococcygeus muscle to field stimulation and to some drugs has been studied. The results are compared with those reported previously in the rat, cat and rabbit.

2 Field stimulation produced frequency-dependent contractions which were inhibited by guanethidine and phentolamine. When the tonus of the muscle was increased with guanethidine, field stimulation always produced frequency-dependent relaxation. The relaxation was not prevented by propranolol.

3 The muscle was contracted by noradrenaline, tyramine, acetylcholine, histamine (H_1), 5-hydroxytryptamine, prostaglandin E_2 and vasopressin. Phentolamine, atropine, promethazine (but not cimetidine) and methysergide inhibited the effect of the respective agonists.

4 After increasing the tonus of the muscle, it was relaxed by low concentrations of isoprenaline. The relaxation was antagonized by propranolol.

5 The response to adenosine triphosphate (ATP) was variable. In some preparations, it relaxed the muscle, in others it contracted the muscle prior to relaxation, in others still it only contracted the muscle. Indomethacin did not prevent ATP-induced contraction.

6 It is concluded that the anococcygeus of the dog, like that of rat, cat and rabbit, has an adrenergic motor innervation and an inhibitory innervation, the transmitter of which is not identified.

Introduction

The effects of agonists and nerve stimulation on the anococcygeus muscle have been studied in the rat (Gillespie, 1972), cat (Gillespie & McGrath, 1974) and rabbit (Creed, Gillespie & McCaffery, 1977). In these three species, the motor nerve is adrenergic. There is also an inhibitory nerve supply to the muscle of the three species, the transmitter of which is unknown. There are differences in the effects of drugs in these species. For example, the rat muscle is contracted by most drugs except histamine, while the cat and rabbit muscles are relaxed by most drugs (Creed *et al.*, 1977). Also the response of the rabbit muscle to histamine differs from that of the cat. In the present experiments, the effects of drugs and nerve stimulation have been studied in the dog anococcygeus muscle. A preliminary account of some of these findings has been published (Dehpour, Khoyi, Koutcheki & Zarrindast, 1978).

Methods

Mongrel dogs, 3 to 6 kg, were anaesthetized with sodium pentobarbitone (35 mg/kg) and bled to death. The two anococcygeus muscles were isolated and mounted in organ baths as described for rat muscle by Gillespie (1972). The nutrient medium was oxygenated Tyrode solution (37°C) of the following composition (mM): NaCl 136.8, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, NaH_2PO_4 0.4, $NaHCO_3$ 11.9 and glucose 5.5. Tension was adjusted to 1 g. For field stimulation, the muscle was drawn through a pair of platinum ring electrodes. Impulses of 0.5 ms duration, 2 to 50 Hz and supramaximal voltage from a Grass S88 stimulator were applied for 1 s every 60 s. Drugs were added to the medium in a volume not exceeding 1% of bath volume.

The following drugs were used: acetylcholine chloride (E. Merck), adenosine triphosphate (ATP, Sigma), atropine sulphate (E. Merck), carbamylcholine hydrochloride (Sigma), cimetidine (SK&F), desipramine hydrochloride (Ciba-Geigy), guanethidine sulphate (Ciba-Geigy), hexamethonium bromide (Koch-Light), histamine hydrochloride (Sigma), 5-hydroxytryptamine creatine sulphate (Sigma), indomethacin (Merck, Sharp & Dohme), isoprenaline hydrochloride (Sigma), methysergide hydrogenmaleinate (Sandoz), morphine hydrochloride (E. Merck), neostigmine (Roche), noradrenaline bitartrate (Sigma), phentolamine methanesulphonate (ampoules Regitine, Ciba-

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Figure 1 The response of dog anococcygeus muscle to field stimulation of 1 ms duration, supramaximal voltage and at 2, 5, 10, 20 and 50 Hz, for 1 s every 30 s: (a) before and (b) after incubation with 4×10^{-6} M guanethidine. In (c) the tonus of the muscle was increased by exposure to 10^{-4} M guanethidine and field stimulation exposed the inhibitory response. Horizontal calibration is 2 min in (a) and (b) and 1 min in (c). Ordinate scale shows increase in tension (g), zero equals resting tension i.e. 1 g.

Geigy), promathazine (Phenergan, Specia), propranolol hydrochloride (ICI), prostaglandin E_2 (PGE_2, Upjohn), tyramine hydrochloride (Sigma) and vasopressin (Parke, Davis).

Results

Anatomically, the dog anococcygeus muscle is similar to that of the rat, although the dog muscle is thicker, larger and heavier. It arises from the upper coccygeal vertebrae in the midline of the pelvic cavity and passes caudally where it is attached to the colon. In a dog of 5 kg, the colonic end is about 3 mm in diameter and the coccygeal end spreads fan-like vertically in a 12 to 15 mm length on the bone. The distance between the two muscles at the coccygeal end is about 1 mm. Usually, the muscle has no spontaneous activity. In some of the experiments, small spontaneous contractions were observed which did not interfere with the experiments.

Response to field stimulation

Field stimulation of the muscle produced frequency-dependent motor responses (Figure 1a). In some instances, the contractions had two components (Figure 2a). The motor response was inhibited by guanethidine (4×10^{-6} M, Figure 1b) or phentolamine (2×10^{-6} M, Figure 2). The motor response was unaffected by hexamethonium (6×10^{-5} M), atropine (3×10^{-8} M), promethazine (3×10^{-8} M), methysergide (2×10^{-6} M) or morphine (3×10^{-7} M). When the tonus of the muscle was increased by guanethidine

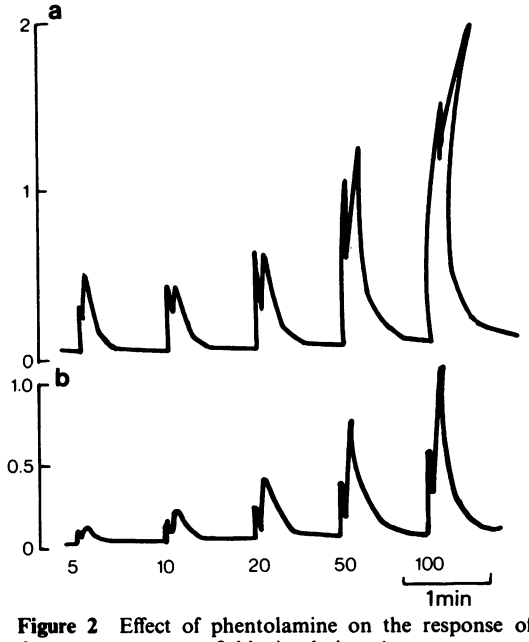

Figure 2 Effect of phentolamine on the response of dog anococcygeus to field stimulation: 1 ms, supramaximal voltage, at indicated frequencies for 1 s every 60 s: (a) before and (b) after incubation with 2×10^{-6} M phentolamine. Ordinate scale shows increase in tension (g); zero equals resting tension i.e. 1 g.

(10^{-4} M), field stimulation caused the muscle to relax (Figure 1c). When the tonus was increased by 5-hydroxytryptamine to a submaximal level, the relaxation was preceded and/or followed by a small contraction.

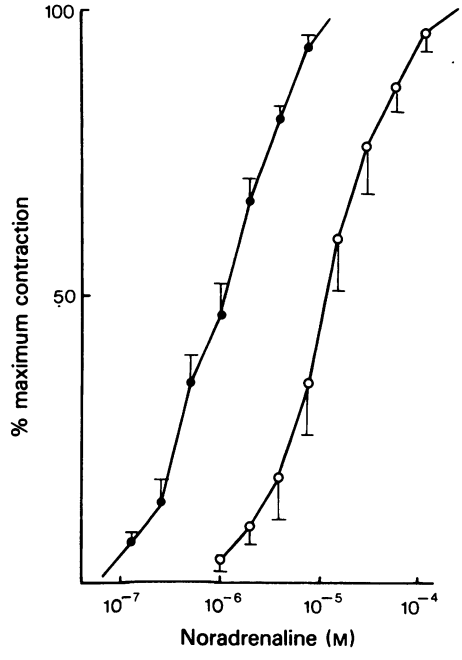

Figure 3 Dose-response curves for noradrenaline before (●) and after (○) incubation for 10 min with 2×10^{-6} M phentolamine. Each point is mean of at least 6 experiments. Vertical lines show s.e. means. Abscissa scale: dose of noradrenaline; ordinate scale: % maximum contraction of the muscle.

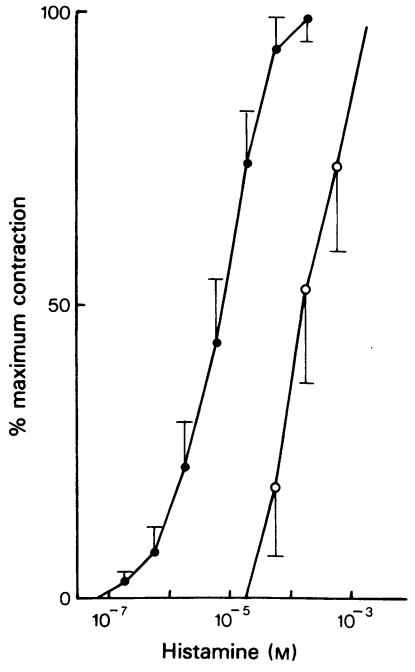

Figure 4 Dose-response curves for histamine before (●) and after (○) incubation for 10 min with 3×10^{-9} M promethazine. Each point is mean of 5 experiments. Vertical lines show s.e. means. Abscissa scale: dose of histamine; ordinate scale: % maximum contraction of the muscle.

The relaxations were frequency-dependent (1 to 20 Hz) and were not blocked by propranolol (3×10^{-6} M).

Effect of drugs

The muscle was contracted by noradrenaline (Figure 3), tyramine (10^{-6} to 10^{-4} M), guanethidine, acetylcholine (10^{-6} to 10^{-4} M), carbamylcholine (10^{-6} M), histamine (Figure 4), 5-hydroxytryptamine (Figure 5), PGE_2 (from 5×10^{-7} M) and vasopressin (from 20 μu/ml). The sensitivity of the muscle to acetylcholine and carbamylcholine decreased markedly after 1 to 2 exposures. To find out whether the response to acetylcholine had a relaxant component, the tonus of the muscle was increased by 5-hydroxytryptamine. Under these conditions, no relaxation was observed with acetylcholine. The responses to PGE_2 and vasopressin developed very slowly and were well sustained. Higher doses of isoprenaline (10^{-6} to 10^{-4} M) contracted the muscle. When the tonus of the muscle was increased by guanethidine or 5-hydroxytryptamine, lower doses of isoprenaline (10^{-9} to 10^{-7} M) relaxed the muscle. This relaxation was antagonized by propranolol (Figure 6). ATP was effective at 10^{-5} M or higher concentrations in 8 of 11 preparations. PGE_2, 5-hydroxytryptamine or guanethidine were used to increase the tonus of the preparation before adding ATP; at a concentration of 10^{-5} M, ATP relaxed the muscle slightly (Figure 7a) while 10^{-4} M and higher concentrations showed variable effects in different preparations: in some preparations, it relaxed the muscle, in others it contracted the muscle transiently prior to sustained relaxation (Figure 7a, b), and in others it contracted the muscle without showing any relaxation (Figure 7b, c). The agonists used to increase the tonus of the muscle did not affect the response of the tissue to ATP. Incubation of the tissue with indomethacin (10 μg/ml, for 1 h) did not abolish the contractile component.

The contractile response to noradrenaline, tyramine, guanethidine and higher doses of isoprenaline was inhibited by phentolamine. Neostigmine (10^{-7} M) potentiated the effect of acetylcholine. Contractions caused by acetylcholine and carbamylcholine were antogonized by atropine (3×10^{-8} M) but not by hexamethonium or phentolamine. The effects of histamine and 5-hydroxytryptamine were inhibited by promethazine (but not by cimetidine) and methysergide respectively (Figures 4 and 5).

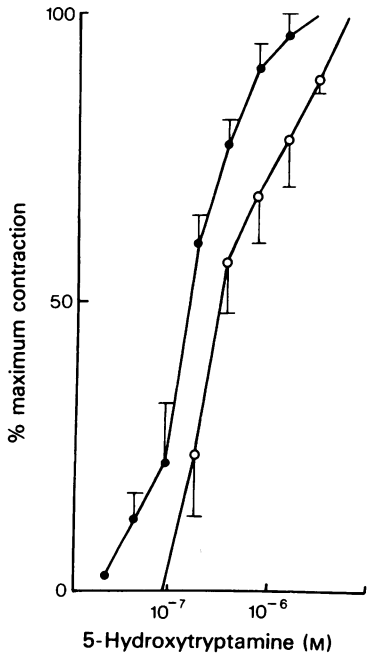

Figure 5 Dose-response curves for 5-hydroxytryptamine before (●) and after (○) incubation for 10 min with 2×10^{-6} M methysergide. Each point is mean of at least 5 experiments. Vertical lines show s.e. means. Abscissa scale: dose of 5-hydroxytryptamine; ordinate scale: % maximum contraction of the muscle.

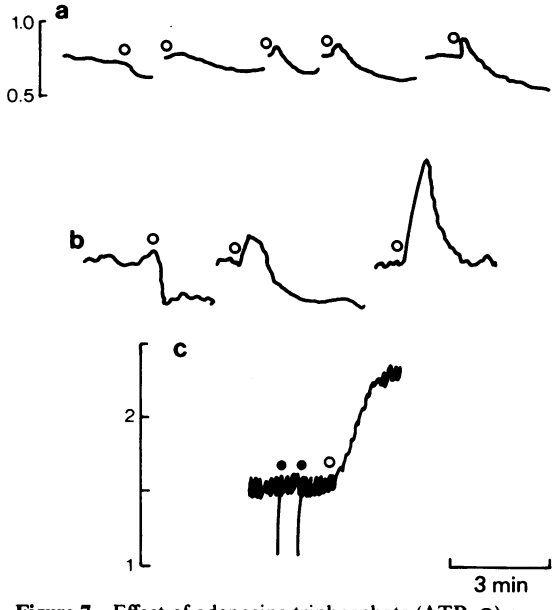

Figure 7 Effect of adenosine triphosphate (ATP, ○) on dog anococcygeus muscle. In (a) the tonus of the muscle was increased with prostaglandin E_2 (6×10^{-6} M). From left to right the effect of 10^{-5}, 2×10^{-5}, 10^{-4}, 2×10^{-4} and 4×10^{-4} M ATP is shown. (b) shows the responses of three different preparations to 10^{-4} M ATP. In (b) the tonus was increased with 2×10^{-7} M 5-hydroxytryptamine to at least +1 g. In (c) the tonus was increased with 2×10^{-5} M guanethidine; (●) field stimulation: 1 ms, 10 Hz, supramaximal voltage, for 1 s. Dose of ATP was 4×10^{-4} M. Horizontal calibration is 3 min. Ordinate shows increase in tension (g).

Discussion

Comparison of the results obtained in dog anococcygeus muscle with those found previously for the rat, cat and rabbit muscle, indicates the similarities and variabilities in these species (Table 1). In all four species, noradrenaline contracts the muscle (α-receptor) and this is prevented by phentolamine. Also, 5-hydroxytryptamine contracts the muscle in the four species. With regard to other agonists, the rat muscle contracts in response to all the drugs tested in the present experiments except for histamine which is

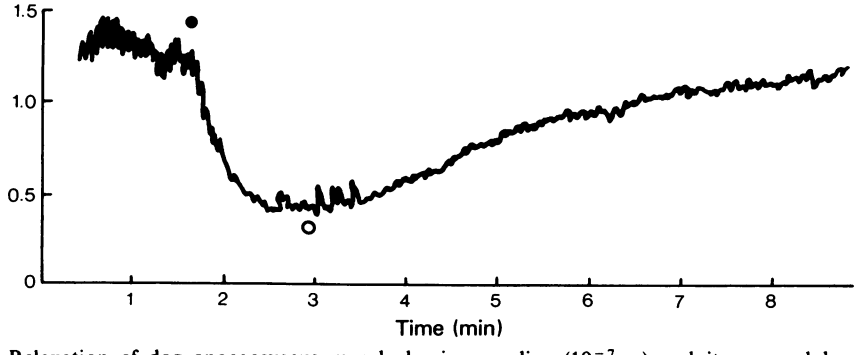

Figure 6 Relaxation of dog anococcygeus muscle by isoprenaline (10^{-7} M) and its reversal by propranolol (6×10^{-6} M). The tonus of the muscle was increased with guanethidine (10^{-4} M). At (●) isoprenaline and at (○) propranolol was added. Time in minutes. Ordinate scale shows increase in tension (g); zero equals resting tension i.e. 1 g.

Table 1 The responses of the rat, cat, rabbit and dog anococcygeus muscles to drugs

Drug	*Receptor*	*Rat*	*Cat*	*Rabbit*	*Dog*
Catecholamines	α	Motor	Motor	Motor	Motor
Catecholamines	β	0	Inhibitor	Inhibitor	Inhibitor
Acetylcholine	Muscarinic	Motor	Inhibitor	Inhibitor	Motor
Histamine	H_1	0	0	Motor	Motor
Histamine	H_2	0	0	Inhibitor	0
5-Hydroxytryptamine		Motor	Motor	Motor	Motor
ATP		Inhibitor*	Inhibitor	Inhibitor	Variable
Prostaglandin E_2		Motor	Inhibitor	Not tested	Motor
Vasopressin		0†	Inhibitor	0†	Motor

The information on the rat, cat and rabbit is from Gillespie (1972), Gillespie & McGrath (1974) and Creed *et al.* (1977)

* From Burnstock *et al.*, 1978.

† Personal observation.

ineffective in the rat and ATP which has a relaxant effect in addition to its contractile component (Gillespie, 1972; Burnstock, Cocks & Crowe, 1978). The cat muscle is relaxed by most of the drugs but has no receptor for histamine (Gillespie & McGrath, 1974). The muscle of the rabbit responds to drugs similarly to the cat muscle except that it has H_1- and H_2-histamine receptors (Creed *et al.*, 1977). As indicated in Table 1, the response of dog anococcygeus to drugs has similarities to the rat and rabbit muscle. The response to acetylcholine and PGE_2 are motor in the rat and dog. The responses to isoprenaline and histamine (H_1) are similar in the dog and rabbit. The effect of ATP on the dog muscle has similarities to the responses of the rat and rabbit muscles.

In all four species, the contractile response to nerve stimulation is abolished or inhibited by both guanethidine and the α-adrenoceptor blocking agent, phentolamine. Therefore, it can be concluded that the motor nerve is adrenergic. On the other hand, the transmitter for the inhibitory response to nerve stimulation has not been identified. From the present experiments, the involvement of β-adrenoceptors is excluded. The relaxation caused by isoprenaline is prevented by propranolol, while the inhibitory response to nerve stimulation is not. Similar observations have been made in the cat and rabbit (Gillespie & McGrath, 1974; Creed *et al.*, 1977) and the rat muscle does not show relaxation in response to isoprenaline (Gillespie, 1972). ATP is another possible candidate responsible for the inhibitory response. The cat and rabbit muscles are relaxed by ATP (Gillespie & McGrath, 1974; Creed *et al.*, 1977). The small motor response of the rat muscle to ATP is changed to relaxation by previous incubation of the tissue with the prostaglandin synthesis inhibitor, indomethacin (Burnstock *et al.*, 1978). The contractile component of the response of the dog muscle to ATP is also observed with field stimulation when the tonus of the muscle is low. These observations are consistent with the view that the anococcygeus muscle is innervated by purinergic inhibitory nerves (Burnstock *et al.*, 1978). The acceptance of this view is made difficult by the observations that: (a) in the rabbit, incubation of the muscle with ATP, for 10 min, diminishes the response of the tissue to the following dose of ATP without affecting the response to inhibitory nerve stimulation (personal observation) and (b) in preparations of dog anococcygeus which contract in response to ATP, inhibitory nerve stimulation still relaxes the muscle (Figure 7c) and the contraction induced by ATP is not inhibited by preincubation of the tissue with indomethacin.

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