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SOME PHARMACOLOGICAL PROPERTIES OF THE CREMASTER MUSCLE OF THE GUINEA-PIG

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1 The tension developed in the guinea-pig cremaster was recorded during spontaneous activity and electrical stimulation. Spontaneous rhythmic contraction was observed in many preparations, particularly in the tip of the cremaster. These contractions were very slow, lasting about 20 s and occurring at about 2 min intervals, but different preparations varied greatly. Twitches produced by electrical stimulation were similar to those in other skeletal muscles, being reduced by (+)-tubocurarine and abolished by tetrodotoxin.

2 A slow contraction could be initiated by electrical stimulation using a pulse longer than 10 ms in spontaneously active preparations and in some quiescent preparations. Spontaneous and evoked slow contractions were not suppressed by tetrodotoxin, but were selectively abolished by verapamil. Histamine increased the basal tension and generated spontaneous contractions in quiescent preparations. Hypertonic solutions also had excitatory effects.

3 Contractions produced by acetylcholine and carbachol were blocked by atropine. Those produced by adrenaline and noradrenaline were stronger than those due to histamine, acetylcholine and carbachol and were abolished by an α-adrenoceptor blocking agent, phentolamine. In the preparations in which the slow contraction was not observed, histamine, acetylcholine or adrenaline had very little effect.

4 It is suggested that the cremaster muscle consists of striated muscle together with some smooth muscle having properties similar to that in the vas deferens.

Introduction

The cremaster muscle has been considered as a striated muscle. In the rat this muscle is composed of two layers which are extensions of the internal oblique and transversus abdominis muscles (Greene, 1955; Grant, 1966). The aim of the present experiments was to study the mechanical and pharmacological properties of the cremaster in an attempt to elucidate possible differences from typical striated muscles.

Methods

Male guinea-pigs weighing 250-350 g were stunned and bled. The sac of cremaster muscle containing the testis was excised. The testis was gently pulled out from the sac, turning the inside of the sac out. The cremaster was mounted under slight tension (about 0.2 g) in an organ bath (3 ml), through which a physiological salt solution (containing (mM): NaCl 120.7, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 15.5, glucose 11.5) flowed at a rate of about 1 ml/minute. The bath was maintained at 36°C and was aerated with mixture of 3% CO_2 and 97% O_2. In some experiments, the cremaster was divided into two, one half containing the neck of the sac and the other half containing the tip of the sac. Tension was recorded by means of a strain gauge. A pen recorder was used only for the slow mechanical responses; when fast twitch responses to electrical stimulation were studied, the response was displayed on a cathode-ray oscilloscope and recorded on film. Electrical stimulation was applied transversely through an assembly of Ag-AgCl electrodes similar to that described by Hill (1949).

Drugs were injected into flowing solution near the inlet of the organ bath to give the stated concentration in the organ bath. This concentration was maintained only briefly after injection and was thereafter progressively diluted by the solution flowing through.

Drugs used were (+)-tubocurarine chloride, tetrodotoxin citrate (Sankyo), verapamil, histamine hydrochloride, acetylcholine chloride,

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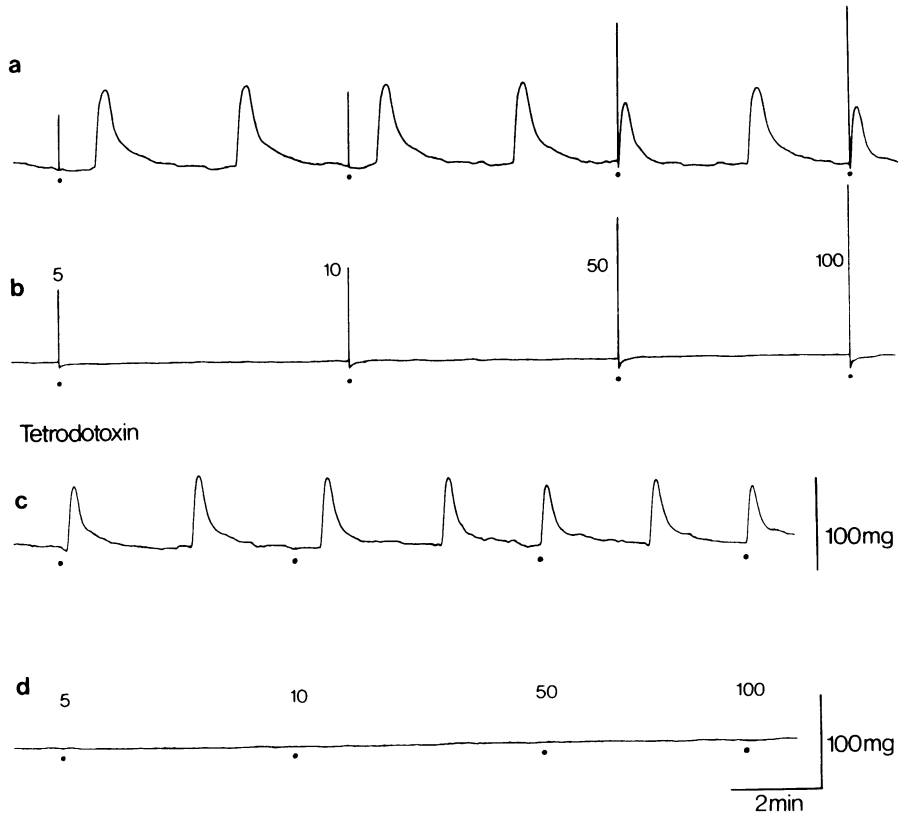

Figure 1 Effects of tetrodotoxin (0.5 μg/ml) on evoked twitches (stimulation is shown by dots and numbers indicate duration of pulse in ms), and on spontaneous and evoked slow contractions. Mechanical responses were simultaneously recorded isometrically from two preparations, divided into tip (a, c) and neck (b,d) of the cremaster but mounted in the same organ bath. Tetrodotoxin blocked the fast twitches but not the slow contractions. Only the tip produced the slow contractions.

carbachol, atropine sulphate (−)-adrenaline hydrochloride, (−)-noradrenaline bitartrate and phentolamine mesylate. The concentrations of the drugs refer to their salts.

Results

Spontaneous activity and mechanical responses to electrical stimulation

In many preparations, spontaneous slow rhythmic contractions were observed. When the tip of the cremaster was used, these contractions were observed in 80% of preparations ($n = 20$). Spontaneous contractions were not so common (20%, $n = 20$) in the neck of the cremaster. The frequency of rhythmic contractions varied greatly in different preparations. The range was 0.006-0.093 Hz ($n = 15$) and the frequency often decreased or the spontaneous activity stopped completely during the course of experiments. The average time to reach peak tension was 7.5 s (range: 4-12; $n = 11$) and to reach 50% relaxation was 6.8 s (range: 3.5-10; $n = 11$). The maximum tension of the slow contraction was very small (usually about 100 mg) compared with that of the fast twitch (about 2 g).

Figure 1 shows the mechanical responses recorded from the tip (a and c) and neck (b and d) of the cremaster. The tip showed spontaneous activity but the neck was quiescent. Single electrical pulses shorter than 10 ms evoked fast twitches only, but a pulse longer than 50 ms evoked fast twitches followed by a slow contraction which was similar to the spontaneous contraction in the tip of the cremaster (a). No

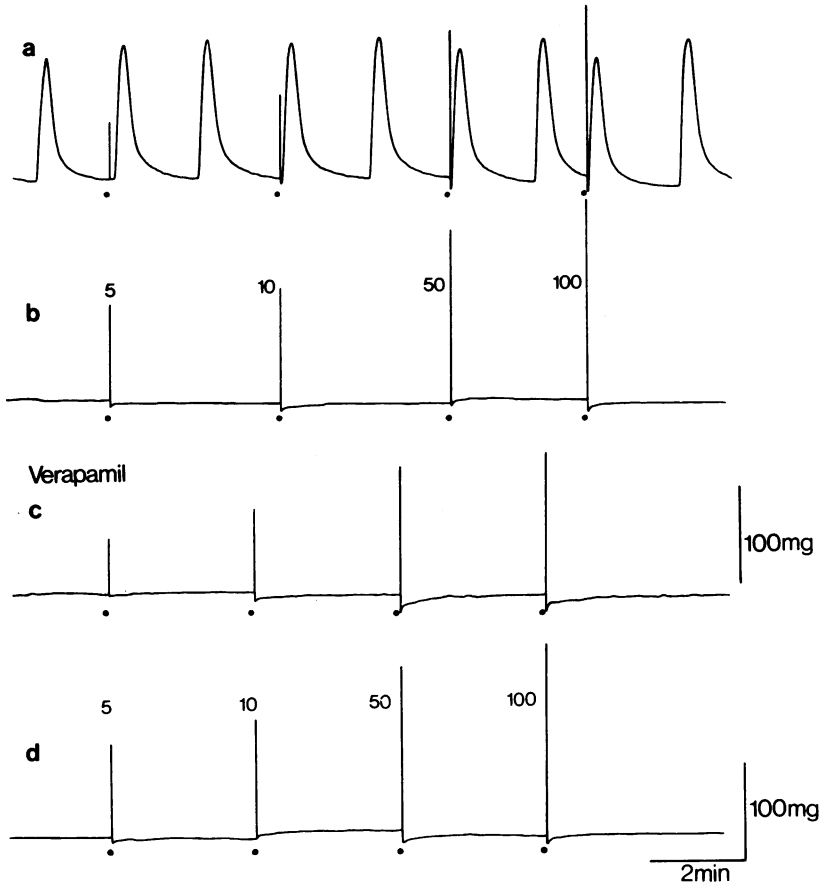

Figure 2 Effects of verapamil (10 μg/ml) on spontaneous and evoked responses simultaneously recorded from the tip (a, c) and neck (b, d) of cremaster. Dots and numbers indicate stimulation and pulse duration in milliseconds. Verapamil abolished the slow contractions only.

slow contraction was evoked in the neck of the cremaster (b). Tetrodotoxin (0.5 μg/ml) blocked the fast twitch responses, but not the spontaneous and evoked slow contractions (c and d) which were also little affected by (+)-tubocurarine (10 μg/ml).

In contrast to the effect of tetrodotoxin, verapamil (10 μg/ml) selectively abolished the spontaneous contractions and evoked slow contractions (Figure 2c) whereas the twitches were little changed. The blocking effect of verapamil was reversible.

Table 1. Twitch tension produced by single maximal shocks in three muscles of the guinea-pig

	Time to peak (ms)			*Time to half relaxation (ms)*		
Pulse duration (ms)	0.5	1	5	0.5	1	5
Extensor digitorum longus	10.8	11.2	17.1	12.7	12.5	9.4
Cremaster	19.7	21.9	28.5	17.9	26.0	32.4
Soleus	47.8	54.0	59.7	51.5	58.5	48.0

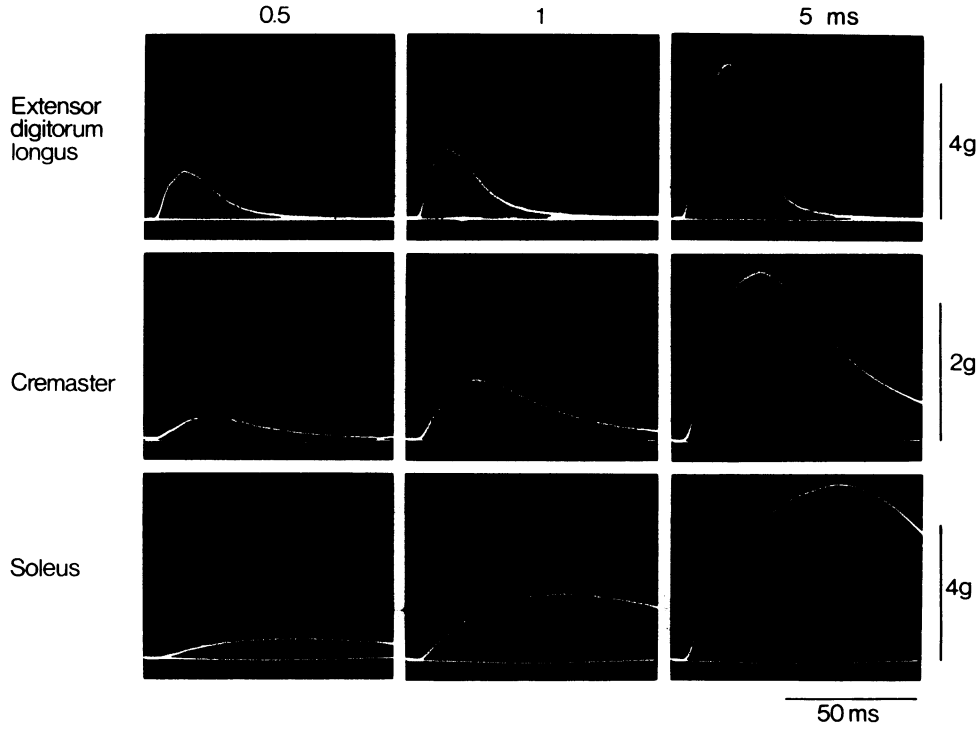

Figure 3 Isometric tension during twitches of extensor digitorum longus (top), cremaster (middle) and soleus (bottom), elicited by electrical stimulation of three different pulse durations (0.5, 1 and 5 milliseconds).

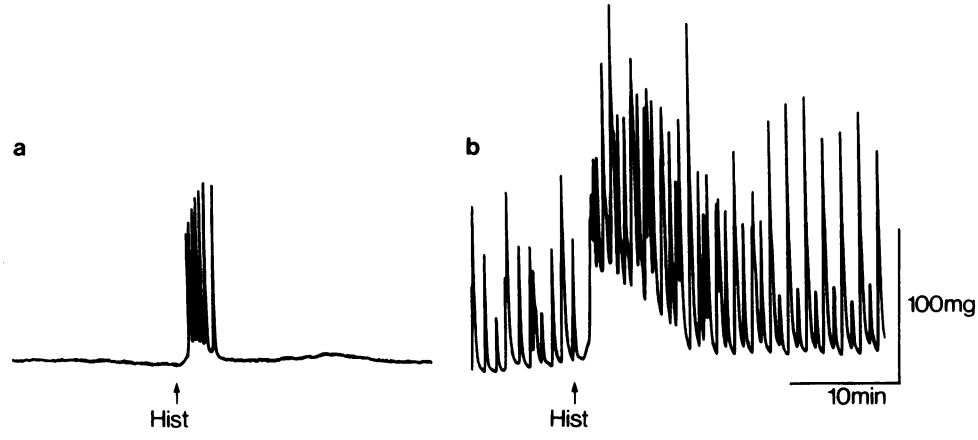

Figure 4 Effects of histamine (Hist), 1 μg/ml (a), and 5 μg/ml (b) in two different, inactive (a) and spontaneously active (b) preparations of the tip of the cremaster.

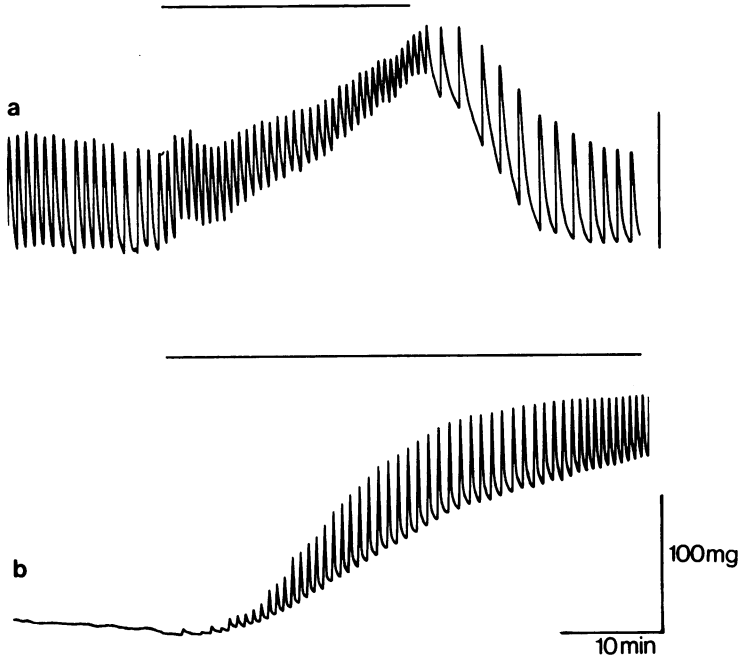

Figure 5 Effects of hyperosmotic solution made by the addition of 5 g sucrose to 100 ml of physiological salt solution. Perfusion of hyperosmotic solution is indicated by horizontal bars. Two different preparations, (a) having spontaneous activity and (b) no activity.

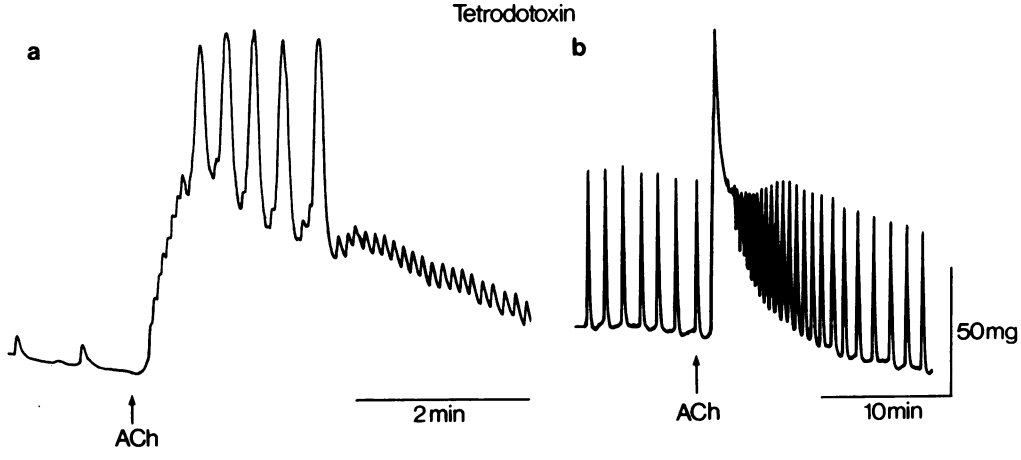

Figure 6 Effects of acetylcholine (ACh), 10 μg/ml, on two different preparations of whole cremaster muscle. The acetylcholine response in (b) was recorded in the presence of tetrodotoxin (0.5 μg/ml).

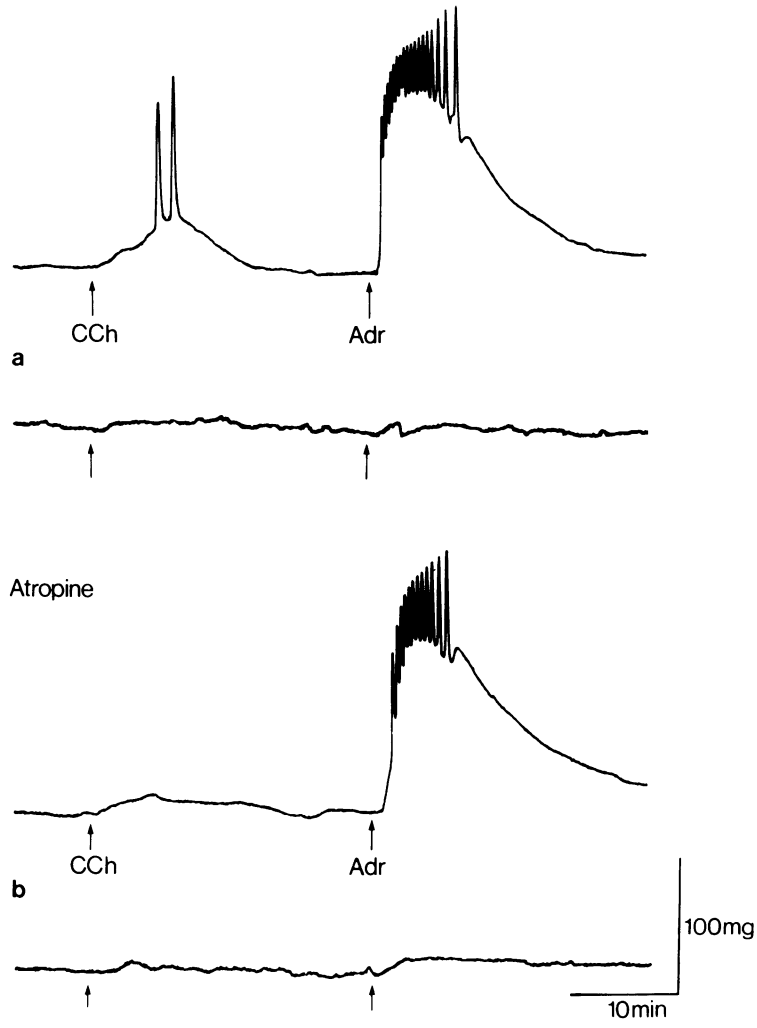

Figure 7 Responses to carbachol (CCh), 1 μg/ml, and adrenaline (Adr), 1 μg/ml, (a) before and (b) after atropine (1 μg/ml). In both (a) and (b) the upper trace recorded from the tip, and the lower trace from the neck of the cremaster.

Twitches produced in the cremaster by single maximal shocks at three different pulse durations were compared with those of the extensor digitorum longus and soleus muscles (Figure 3). The average times to reach the peak tension and half relaxation are summarized in Table 1. The time course of twitch tension of the cremaster was slower than the extensor digitorum longus, which is a typical fast muscle, but faster than the soleus, which is a typical slow muscle. The values for the extensor muscle and the soleus were slightly faster than those previously reported by Tashiro (1973). The difference may be due partly to the higher temperature (36° C instead of 33° C), and partly to the smaller size of the animal (250-350 g instead of 300-400 g) in the present experiments.

Twitch tensions were reduced by (+)-tubocurarine (10 μg/ml), the reduction being 80% with a pulse of 0.5 ms, 39% with 1 ms and 14% with 5 ms pulse. It is likely that the muscle fibre is directly stimulated by long current pulses. Tetrodotoxin (0.5 μg/ml) completely blocked all twitches.

Histamine (1 to 5 μg/ml) had a stimulant action

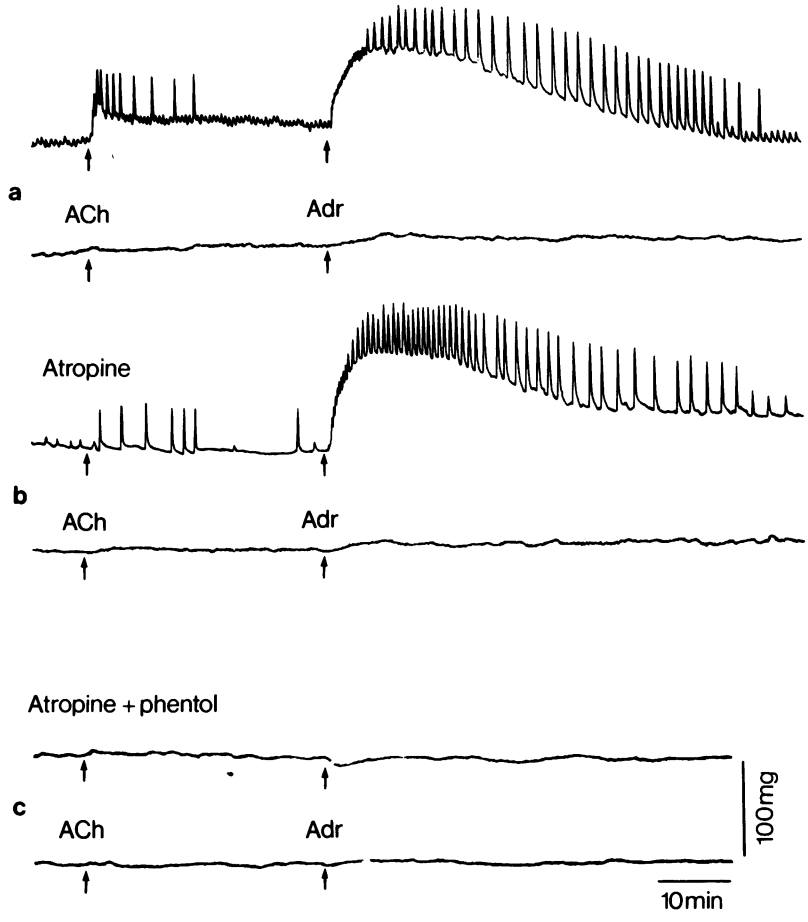

Figure 8 Responses to (a) acetylcholine (ACh), 5 μg/ml, and adrenaline (Adr), 5 μg/ml as modified by (b) atropine, 1 μg/ml, (c) atropine, 1 μg/ml and phentolamine, 1 μg/ml. In (a), (b) and (c) the upper trace is from the tip and the lower trace from the neck of the cremaster.

on the tip, but it usually failed to give a response in the neck of the cremaster. Histamine initiated rhythmic slow contractions in a quiescent preparation (Figure 4a), and it raised the basal tension and increased the frequency of slow contractions in a spontaneously active preparation (Figure 4b).

Figure 5 shows effects of solutions made hyperosmotic by adding sucrose to the salt solution. The hyperosmotic solution increased the basal tension and the frequency of spontaneous contractions (a); it often produced spontaneous slow contractions in quiescent preparations (b).

Effects of parasympathomimetic and sympathomimetic drugs and antagonists

Acetylcholine (1-10 μg/ml) produced a rise in basal tension and increased the frequency of spontaneous contractions. In 20% of preparations ($n = 20$) which had no spontaneous activity or showed small contractions, acetylcholine produced spontaneous activity or increased the magnitude of contractions in the whole cremaster preparation, as shown in Figure 6a. These effects were not abolished by tetrodotoxin (1 μg/ml, Figure 6b). Carbachol produced responses similar to those

with acetylcholine (Figure 7a). The responses caused by acetylcholine or carbachol were suppressed by atropine (1 μg/ml, Figure 7b). In the neck of the cremaster, which did not exhibit the slow spontaneous activity, acetylcholine and carbachol failed to increase the basal tension or a slow contraction (Figure 7).

Adrenaline (1 μg/ml) also had stimulant actions, causing an increase in the basal tension and the generation of spontaneous contraction in the tip of the cremaster (Figure 7). When spontaneous activity existed, increases in magnitude and in frequency of the slow contractions were usually produced by adrenaline. These effects of adrenaline were not affected by atropine (Figure 7b) or tetrodotoxin. The effect of noradrenaline (1 μg/ml) was similar to that of adrenaline.

Figure 8 shows responses to acetylcholine (5 μg/ml) and adrenaline (5 μg/ml). Only the tip of the cremaster (top traces) responded to the drugs (a). The cremaster was always more sensitive to adrenaline than to acetylcholine or carbachol. The response to acetylcholine was suppressed by atropine (1 μg/ml), but that to adrenaline was not (b). The contraction produced by adrenaline was abolished by the α-adrenoceptor blocking drug phentolamine (1 μg/ml), and instead there was often a small relaxation, as shown in the upper trace of Figure 8c.

The preparation shown in Figure 8 produced small spontaneous contractions at the beginning of the experiment, but the spontaneous activity gradually disappeared. In other preparations in which the spontaneous activity persisted, phentolamine abolished the response to adrenaline but did not block spontaneous activity.

Discussion

The speed of a twitch response to electrical stimulation in the cremaster muscle is slower than in the extensor digitorum longus, but faster than in the soleus. The twitch is blocked by tetrodotoxin which is known to abolish the action potential in most nerves and striated muscles (Kao, 1966; Kuriyama, Osa & Toida, 1966). These properties of the cremaster are those of skeletal muscle.

The slow contractions of the cremaster are quite different from the twitch, and are readily stimulated by adrenaline, which also raises the tone of the tissue. It is possible that this peculiarity is attributable to smooth muscle contained in the cremaster. This could also account for the anaphylactic response observed in the guinea-pig cremaster (Alonso-deFlorida, Ninomiya & Paz, 1972). It may be argued that the property of the cremaster is similar to that of denervated striated muscle, since it is known that the anaphylactic response can be observed in the guinea-pig diaphragm after denervation (Alonso-deFlorida, del Castillo, González & Sánchez, 1965; Alonso-deFlorida, del Castillo, García & Gijón, 1968) and that adrenaline produces contracture in the denervated rat diaphragm (Bhoola & Schachter, 1961). The simplest hypothesis is that the cremaster, particularly at its tip, consists of both striated muscle and smooth muscle.

Supporting evidence for the possibility that the slow contraction is generated in smooth muscle is (1) that the speed of slow contraction is too slow to be a response of striated muscle, (2) that tetrodotoxin, which does not affect the action potential in smooth muscle (Kuriyama *et al.*, 1966; Bülbring & Tomita, 1967), has no effect on the slow contraction, (3) that verapamil which blocks the action potential in smooth muscles (Golenhofen & Lammel, 1972) selectively suppresses the slow contraction and (4) that effects of histamine, cholinomimetic and sympathomimetic agents are similar to those observed in smooth muscles such as the vas deferens, which receive adrenergic innervation.

In a preliminary histological study smooth muscle has been seen in close connection with the blood vessels. It seems that this muscle is not part of the wall of the blood vessels, but further studies are necessary to come to a firm conclusion. The density of blood vessels of a medium size (10-50 μm) is much higher in the tip of the cremaster than the neck of the muscle. This corresponds with the observation that the slow contraction is recorded mainly from the tip of the muscle. It has been shown that hyperosmotic solution produces vasodilatation in the vascular bed in skeletal muscle and suppression of spontaneous activity in the rat portal vein (Mellander, 1960; Mellander, Johansson, Gray, Jonsson, Lundvall & Ljung, 1967; Johansson & Jonsson, 1968). However, the spontaneous activity in the cremaster is increased in hyperosmotic solution. Although topical application of adrenaline results in constriction of the blood vessels in the rat cremaster, intravenous application of adrenaline and acetylcholine dilates the blood vessels (Grant, 1964). If the spontaneous activity in the cremaster is generated in the blood vessels, their properties seem to be different from those of the blood vessels in striated muscles. Further experiments are necessary to see whether the slow contractions observed in the present experiments are due to the presence of smooth muscle.

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References

ALONSO-deFLORIDA, F., DEL CASTILLO, J., GONZÁLEZ, C.C. & SÁNCHEZ, V. (1965). On the pharmacological and anaphylactic responsiveness of denervated skeletal muscle of the guinea-pig. *Br. J. Pharmac. Chemother.*, **25**, 610-620.

ALONSO-deFLORIDA, F., del CASTILLO, J., GARCÍA, X. & GIJÓN, E. (1968). Mechanism of the Schultz-Dale reaction in the denervated diaphragmatic muscle of the guinea pig. *J. gen. Physiol.*, **51**, 677-693.

ALONSO-deFLORIDA, F., NINOMIYA, J.G. & PAZ, C. (1972). On the receptor function of antibodies in muscle fibers. *Physiologist*, **15**, 71.

BHOOLA, K.D. & SCHACHTER, M. (1961). Contracture of the denervated rat diaphragm by adrenaline. *J. Physiol.*, **157**, 20-21P.

BÜLBRING, E. & TOMITA, T. (1967). Properties of the inhibitory potentital of smooth muscle as observed in the response to field stimulation of the guinea-pig taenia coli. *J. Physiol.*, **189**, 299-315.

GOLENHOFEN, K. & LAMMEL, E. (1972). Selective suppression of some components of spontaneous activity in various types of smooth muscle by iproveratril (Verapamil). *Pflügers Arch. ges. Physiol.*, **331**, 233-243.

GRANT, R.T. (1964). Direct observation of skeletal muscle blood vessels (rat cremaster). *J. Physiol.*, **172**, 123-137.

GRANT, R.T. (1966). The effects of denervation of skeletal muscle blood vessels (rat cremaster). *J. Anat.*, **100**, 305-316.

GREENE, E.C. (1955). *Anatomy of the Rat.* Pp. 39-40. New York: Hafner.

HILL, A.V. (1949). The abrupt transition from rest to activity in muscle. *Proc. R. Soc. B.*, **136**, 399-420.

JOHANSSON, B. & JONSSON, O. (1968). Cell volume as a factor influencing electrical and mechanical activity of vascular smooth muscle. *Acta physiol. scand.*, **72**, 456-468.

KAO, C.Y. (1966). Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. *Pharmac. Rev.*, **18**, 997-1049.

KURIYAMA, H., OSA, T. & TOIDA, N. (1966). Effect of tetrodotoxin on smooth muscle cells of the guinea-pig taenia coli. *Br. J. Pharmac. Chemother.*, **27**, 366-376.

MELLANDER, S. (1960). Comparative studies on the adrenergic neuro-hormonal control of resistance and capacitance blood vessels in the cat. *Acta physiol. scand.*, **50**, suppl. 176, 1-86.

MELLANDER, S., JOHANSSON, B., GRAY, A., JONSSON, O., LUNDVALL, J. & LJUNG, B. (1967). The effects of hyperosmolarity on intact and isolated vascular smooth muscle. Possible role in exercise hyperemia. *Angiologia*, **4**, 310-322.

TASHIRO, N. (1973). Effects of isoprenaline on contractions of directly stimulated fast and slow skeletal muscles of the guinea-pig. *Br. J. Pharmac.*, **48**, 121-131.

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