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A mammalian muscle with the pharmacological characteristics of slow tonic muscle

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The guinea-pig isolated cremaster muscle gave tetrodotoxin-resistant dose-related contractures with acetylcholine, carbachol and the depolarizing neuromuscular blocking agents. The dose-response curve to suxamethonium in tetrodotoxin 2×10^{-7}M could be shifted to the right with tubocurarine 10^{-6}M. KCl, 0·1M, produced slow sustained contractures of the muscle. With the cremaster nerve-muscle preparation tetanic contractions at 20 Hz were maintained over several minutes. Tetrodotoxin eliminated the twitch response to single shock nerve stimulation but not the sustained increase in tension produced by suxamethonium. The results suggest either that there is a component of slow tonic muscle in the guinea-pig cremaster or that the cremaster consists of a type of focally innervated muscle which has pharmacological responses qualitatively different from those of most focally innervated muscles so far described.

The guinea-pig isolated cremaster muscle has been described as giving unusual responses to cholinomimetic agents. Dose-related contractions have been reported not only with acetylcholine (Dale, Evinc & Vine, 1976; Kelkar, Gupta & Gokhale, 1976) but also with depolarizing neuromuscular blocking agents (Dale & Muid, 1976; Kelkar & others, 1976). Increases in baseline tension of about 200 mg with single large doses of acetylcholine and carbachol had previously been recorded by Ninomiya (1975). The receptors involved in these responses are clearly nicotinic as evidenced by the pA_2 values for tubocurarine and atropine obtained by Kelkar & others (1976) and Dale & others (1976). It is unusual to obtain this type of response from striated muscle unless it contains slow tonic muscle. It is not clear what sort of muscle fibre in the cremaster is responsible for the contractions described. This paper presents further information on the responses of this preparation. Some of this material was the subject of a British Pharmacological Society Communication (Dale & Muid, 1976).

MATERIALS AND METHODS

Isolated cremaster muscle preparations. These muscles were dissected out as described by Dale & others (1976) and set up in Krebs Henseleit solution at 32°. Contractions were recorded isometrically with a Pye Ether UFI transducer and a Servoscribe pen recorder.

The cremaster nerve-muscle preparation. The dissection was carried out as for isolated cremaster muscle except that the genito-femoral nerve was included. This nerve can be readily identified on the posterior wall of the abdominal cavity and the proximal part can be easily dissected out whereas the lower part where it crosses the iliac vessels was usually removed by scooping out the whole area. The preparation was set up in Krebs-Henseleit solution at 32°. The nerve was stimulated with a Grass SD5 stimulator and the contractions recorded isometrically with a Pye-Ether UF1 transducer and a George Washington Oscillograph 400 MD/2.

The reagents used were: acetylcholine chloride (Sigma Chemical Co.), suxamethonium chloride (Duncan-Flockhart & Co. Ltd.), tetrodotoxin (Sankyo Co. Ltd.), (+)-tubocurarine chloride (Sigma Chemical Co.), decamethonium iodide, hexamethonium bromide (Koch-Light).

The composition of the Krebs-Henseleit solution was as follows: (g litre^{-1}) NaCl 6·9; KCl 0·35; $MgSO_4.7H_2O$ 0·29; KH_2PO_4 0·16; $NaHCO_3$ 2·1; Glucose 2·0; $CaCl_2$ 0·55.

Histological preparations of cremaster muscle. Cholinesterase at the end plates was shown up by the Karnovsky & Roots technique (1964) in both whole muscle spreads and in frozen sections cut at 5, 20, 40 and 80 μm. Whole muscle spreads were made with both cremasters from 10 guinea-pigs. The spreads were examined at 40× and the tissue carefully dissected with surgical needles. Individual fibres which looked as though they had atypical end plates were teased out and mounted separately in water-soluble mounting medium and then examined under oil immersion at 1000×. Frozen sections were made from muscle tissue from 9 guinea-pigs,

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multiple sections from each muscle being examined at magnifications of 40, 100, 400 and 1000×.

RESULTS

The effect of tetrodotoxin on cholinomimetic drug responses

The nicotinic agonists acetylcholine, carbachol, suxamethonium and decamethonium produced dose-related contractures of the isolated guinea-pig cremaster muscle preparation. In 10 experiments dose-related responses could still be obtained in the presence of tetrodotoxin 2×10^{-7} or 10^{-6} M. One such experiment is illustrated in Fig. 1. That the tetrodotoxin-resistant response was due to stimulation of nicotinic receptors and therefore probably produced by skeletal muscle and not smooth muscle was evidenced by the fact that the curve could be further shifted to the right with tubocurarine 10^{-6} M. In the experiment illustrated, the affinity constant for tubocurarine based on the dose ratio obtained by this second shift was $1{\cdot}9 \times 10^{7}$ M^{-1} ($pA_2 = 7{\cdot}3$) which accords reasonably well with the figures quoted for tubocurarine at the neuromuscular junction in the guinea-pig (Lu, 1970).

The fact that the responses to cholinomimetic drugs are not eliminated by tetrodotoxin also rules out the possibility that the drugs are acting at nicotinic receptors in autonomic ganglia within the tissue, because transmission in postganglionic neurons would be prevented by tetrodotoxin. Furthermore there was little shift in the dose-response curve when hexamethonium was used as antagonist, even at high concentrations. With acetylcholine, the affinity constant for hexamethonium, based on the dose ratio obtained with 10^{-4} M, was $0{\cdot}8 \times 10^{3}$ M^{-1} ($pA_2 = 3{\cdot}9$).

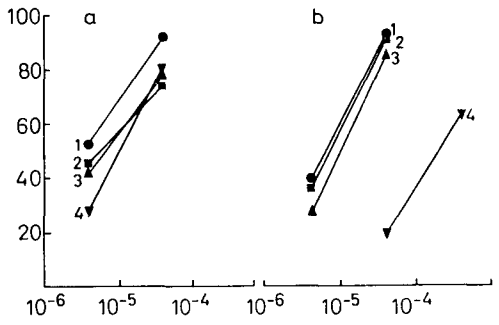

FIG. 1. The effect of tetrodotoxin and tubocurarine on the dose-response curve to suxamethonium chloride in the guinea-pig cremaster muscle. Two preparations, test and control, were set up simultaneously and the responses expressed as the % of the maximum contraction with 0·1 M KCl. The interval between doses was 1 hour. (a) shows four successive dose-response lines in the control preparation. (b) shows four successive dose-response lines in the test preparation in which tetrodotoxin 2×10^{-7} M was added to the bathing fluid immediately after the second dose-response line, and tubocuarine 10^{-6} M was added to the bathing fluid immediately after the third dose-response line. Ordinate: Response as % of 0·1 M KCl contraction. Abscissa: Concentration of suxamethonium mol litre^{-1}.

The maximum response to suxamethonium, elicited by concentrations above 10^{-4} M, gave a mean of 6 g tension in 5 preparations from small guinea-pigs and was as much as 30 g in a guinea-pig over 1 kg in weight. The maximum suxamethonium response was only slightly decreased by tetrodotoxin; in 6 experiments the mean response to these high doses of suxamethonium in the presence of tetrodotoxin 2×10^{-6} M was 80% of the previously obtained control value. Thus substantial responses may be obtained in the presence of tetrodotoxin, although the rate of increase of tension after administration of suxamethonium was slower in the tetrodotoxin-treated muscle.

There is no one particular part of the cremaster muscle which has specific ability to respond to cholinomimetic drugs in the manner described. When muscles were divided up into longitudinal strips and tested separately, all the strips gave responses to suxamethonium. When the band of muscle fibres which curves transversely across the lower portion of the cremaster was dissected out and tested separately, it too responded to suxamethonium.

The contractile response to suxamethonium was invariably seen in all preparations of guinea-pig cremaster and was never obtained with rat cremaster. A response similar to that of the guinea-pig, but much feebler, was seen in the rabbit and the mouse.

The response to KCl

KCl, 0·1 M, which usually elicits only a transient contraction in most focally innervated muscle, produced in the isolated guinea-pig cremaster a sustained contracture. This response varied with the size of the muscle, but could be up to 30 g tension. In some preparations this contracture, after subsiding to 2/3 or 1/3 of its force, was maintained for 30 min or more.

The response of the cremaster nerve-muscle preparation

Set up as a simple nerve-muscle preparation, stimulated through the genito-femoral nerve, the cremaster gave twitches with single stimuli greater than required to produce a maximal response (0·2 Hz,

0·1 ms pulse duration). With repetitive stimulation at 20 Hz a fused tetanus was obtained, the tension rising slowly to approximately 22 g over 2 or 3 s and then being maintained for several min (Fig. 2). Maximum tension (about 40 g in small muscles and up to 75 g in large ones) only developed above 60 Hz.

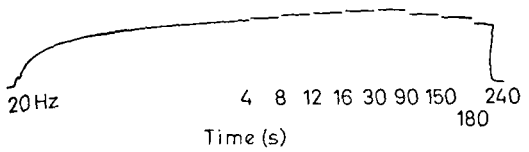

FIG. 2. The tetanic response of the guinea-pig cremaster nerve/muscle preparation stimulated at 20 Hz. Paper speed was changed from 25 to 5 mm^{-1} after 30 s, then to 0·5 mm s^{-1}, after 150 s. Stimulation was discontinued after 4 min.

When suxamethonium was administered the mean maximum contraction was 24% of the maximum tetanic tension. In the presence of tetrodotoxin 10^{-6} M the twitch response was eliminated but the sustained increase in tension remained.

Histological examination. The cremaster was seen to be made up of skeletal muscle fibres. If there was any smooth muscle component it was too small to be visible even at 1000×. Virtually all the fibres examined had typical 'en plaque' end plates. In only a very few instances were several small end plates seen on a single fibre.

DISCUSSION

The responses obtained with cholinomimetic drugs are clearly due to stimulation of nicotinic receptors. From the affinity constants of tubocurarine and hexamethonium and from the results with tetrodotoxin it appears that the nicotinic receptors are those found at the neuromuscular junction and not ganglia. It is therefore presumed that the muscle involved is striated. It is, however, unusual for mammalian striated muscle to give this type of response, which is normally associated with 'slow tonic' skeletal muscle.

The sustained contracture of the cremaster with KCl 0·1 M is similar to that seen with the frog rectus abdominis muscle (Kuffler & Vaughan-Williams, 1953) and the avian anterior latissimus dorsi muscle (Ginsborg, 1960) in which the long continued response is attributed to the presence of slow tonic muscle. The dose-related contractures with acetylcholine, carbachol and the depolarizing blocking agents resemble those seen with the classical frog rectus abdominis preparation and the chick semispinalis muscle (Child & Zaimis, 1960). The slow rise in tetanic tension and the resistance to fatigue seen with the cremaster nerve-muscle preparation occurs with the frog rectus (Kuffler & Vaughan-Williams, 1953) and with sheep extraocular muscle (Browne, 1976). The effect of suxamethonium on the response of the cremaster is similar to that reported in some avian muscle (Ginsborg, 1960) and in extraocular muscle (Bach-y-Rita & Ito, 1966; Eakins & Katz, 1966; Browne, 1976). All the preparations mentioned are believed to contain multiply-innervated slow tonic muscle fibres.

More significant than these similarities is the fact that tetrodotoxin fails to eliminate the response to cholinomimetic agents. This might mean that a propagated action potential is not required for a contraction of the muscle and could imply that there is a type of multiply-innervated slow tonic muscle present. Another possibility might have been that the cremaster was reacting like denervated muscle in that the action potential generating mechanism was resistant to the blocking action of tetrodotoxin (Redfern, Lundh & Thesleff, 1970)—however, it has been found that this concentration of tetrodotoxin blocks the action potential in this muscle (Gustafsson, Suarez-Kurtz & Miledi, 1977, personal communication). The slight reduction in the maximum pharmacological response to suxamethonium which is produced by tetrodotoxin could well be due to some degree of desenitization or fall in sensitivity during the course of the experiment.

The definition of the characteristics required to classify a muscle as a slow tonic muscle is not at all clear. Peachey (1968) concluded that it seemed impossible to generalize about the properties of slow striated muscle fibres. But as regards the extraocular muscles in mammals (which have been considered to contain slow tonic fibres), the following characteristics are involved:— small diameter, multiaxonal innervation, multiple nerve endings of 'en grappe' type, small junctional potentials, large fibrils incompletely surrounded by sarcoplasmic reticulum, and slow mechanical contractions (Peachey, 1968). These features also characterize the slow fibres of the frog, as does the lack of propagated action potentials and the fact that miniature end plate potentials can be recorded whenever a microelectrode is inserted in the fibre (Burke, 1957).

In general, muscle conforming to the above criteria also manifests the type of pharmacological response reported for the guinea-pig cremaster. It might, therefore, perhaps be assumed that the converse

would be true, i.e. that muscle with the pharmacological properties usually seen in slow tonic muscle would manifest both the electrophysiological and the anatomical characteristics of slow tonic muscle. But this does not appear to be so. Firstly, histologically, most fibres in the cremaster have 'en plaque' end plates. Only few motor end plates were seen which might possibly be called 'en grappe'. As the response of the cremaster to suxamethonium was 80% or more of the maximum KCl response and as much as 24% of the maximum tetanic tension, one would imagine that if the fibres giving this magnitude of response were multiply-innervated, it would be possible to find a reasonable proportion of multiply-innervated fibres with 'en grappe' end plates amongst the focally innervated fibres. In sheep extraocular muscle, in which the suxamethonium contracture is only 7% of the maximum tetanic tension (Browne, 1976), multiply-innervated fibres make up 7–33% of the total (Harker, 1972). Secondly, in studies of the electrophysiological characteristics of guinea-pig cremaster no fibres with the features of slow tonic muscle have been found (Gustafsson, Suarez-Kurtz & Miledi 1977, personal communication).

What then is the explanation for the curious pharmacological characteristics of the guinea-pig cremaster? It could still be that it has a component of multiply-innervated slow tonic muscle which is particularly difficult to detect among the more numerous focally innervated fibres.

A further possibility is that the muscle fibres in the guinea-pig cremaster represent a type of fibre different from the focally-innervated and multiply-innervated fibres so far described. Peachey (1968) has questioned the classification of muscle fibres into fast and slow types and has raised the possibility that there may be a continuous spectrum. The guinea-pig cremaster could very well represent a muscle at an intermediate point on this spectrum.

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