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THE DENERVATED CREMASTER MUSCLE OF THE GUINEA-PIG AS A PHARMACOLOGICAL PREPARATION

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1 The pharmacological responses of both denervated and innervated cremaster muscle preparations from the guinea-pig have been investigated and compared. Acetylcholine (ACh) dose-response curves were obtained in both preparations. The affinity constants for (+)-tubocurarine and for atropine were calculated and indicated that the ACh receptors in both preparations were nicotinic.

2 Histamine dose-response curves could be obtained only in the denervated preparation. The response was unaltered by metiamide, and the affinity constant for mepyramine fitted in with those previously obtained by others on ileum and trachea, indicating that the histamine receptors are H_1 in type.

3 Sustained contractions were obtained to adrenaline and noradrenaline but not isoprenaline, with the denervated preparations.

4 Schultz-Dale responses were obtained with denervated muscle in sensitized guinea-pigs. This preparation did not respond to 5-hydroxytryptamine, bradykinin, or the slow-reacting substance of anaphylaxis.

Introduction

On denervation the pharmacological properties of skeletal muscle change, the receptor areas sensitive to acetylcholine (ACh) increase and, in the guinea-pig, sensitivity to other substances such as histamine and adrenaline may appear. Anaphylactic contractions, normally seen only in smooth muscle, have been described in guinea-pig denervated skeletal muscle and attributed to direct stimulation of the muscle by the antigen-antibody reaction (Alonso-de Florida, del Castillo, Gonzales & Sanchez, 1965). The exact mechanism of this anaphylactic contraction has not been clarified, and the receptors involved in the histamine responses have not previously been investigated.

In this preliminary study, the usefulness of an isolated cremaster muscle for studies on guinea-pig denervated skeletal muscle has been investigated and some of the responses obtained with denervated muscle have been compared with those of innervated preparations.

The effects of cholinomimetic drugs, anticholinesterases and ACh antagonists have been assessed and compared in denervated and innervated preparations, and the responses of denervated preparations to histamine, catecholamines and a number of other pharmacological agents have been investigated. Experiments on the nature of the histamine receptors have been carried out.

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While this manuscript was in preparation, a paper appeared outlining some pharmacological properties of the cremaster muscle of the guinea-pig (Ninomiya, 1975). Ninomiya found that the guinea-pig cremaster manifested substantial contractions (2 g) with transmural stimulation and adrenaline 1 μg/ml produced an increase in tension of 300 mg. ACh and carbachol increased baseline tension by 200 mg and also increased spontaneous contractions in the tip of the muscle.

Methods

Hartley guinea-pigs of 250 to 300 g were used for denervation and the cremaster muscle was used for experiments 2 to 6 weeks afterwards.

Denervation

The abdomen was shaved and cleaned with alcohol, the guinea-pig anaesthetized with halothane and a midline incision made. The abdominal contents were expressed, wrapped in warm wet sterile gauze and pulled to one side to expose the posterior abdominal wall. The genito-femoral nerve was cut. In later operations a 5 mm portion was removed in order to be sure that regeneration would not occur. In some animals both cremaster muscles were denervated.

Dissection of the cremaster muscle preparation

The skin was reflected from the abdominal wall and both testes. A midline incision was made through the abdominal muscles, extending caudally as far as the symphysis pubis. The testis was pushed back through the inguinal canal into the abdomen, the cremaster muscle being everted in the process. The spermatic cord was cut and the cremaster muscle was carefully dissected free by cutting through the abdominal wall at its base. Finally the testis was cut free from its attachment to the cremaster sac leaving a small tag of testicular tissue for the attachment of a ligature. At no stage was the cremaster muscle itself cut, but rather the structures adjacent to it, so as to minimize damage to the muscle.

Recording apparatus

The muscle was set up in a 5 ml organ bath at 37°C in oxygenated Tyrode solution of the following composition (g/l): NaCl 8, KCl 0.2, $MgSO_4.7H_2O$ 0.26, $CaCl_2$ 0.396, $NaH_2PO_4.2H_2O$ 0.65, glucose 1.0 and $NaHCO_3$ 1.0. Isotonic contractions were recorded with a Servoscribe pen recorder, 0.5 g of tension being applied to the muscle. The recording apparatus was calibrated after each experiment by depressing the lever by measured amounts (2 mm, 5 mm, 10 mm) and recording the corresponding deflections of the pen recorder. Because there is a linear relationship between the excursion of the lever and the deflections on the pen recorder, the contractions of the muscle could also be expressed as a percentage of its length. With some agents, isometric contractions were recorded, with a Pye-Ether UFI transducer and Servoscribe recorder.

In general, contractile responses to drugs were expressed as a percentage of the maximum contraction produced by 0.1 M KCl.

Drugs

The following drugs were used: acetylcholine chloride (Sigma Chemical Co.), adrenaline bitartrate (BDH), atropine sulphate (BDH), bradykinin (Sandoz), carbachol chloride (BDH), histamine dihydrogen phosphate (BDH), 5-hydroxytryptamine creatinine sulphate (Sigma Chemical Co.), isoprenaline sulphate (Burroughs Wellcome & Co.), mepyramine maleate (May & Baker), methacholine chloride (Sigma Chemical Co.), metiamide (Smith, Kline & French), neostigmine methanesulphonate (Koch-Light), noradrenaline hydrogen tartrate (levophed; Winthrop), ovalbumin (BDH), physostigmine sulphate (BDH), slow reacting substance of anaphylaxis (SRS-A)—an extract was obtained from Dr W.E. Brocklehurst (Biology Department, Lilly Research Centre, Erl Wood Manor, Windlesham, Surrey), (+)-tubocurarine chloride (Sigma Chemical Co.).

The catecholamine solutions were made freshly just before use.

Calculation of affinity constants of competitive antagonists

In some instances the affinity constant (K_I) of an antagonist was calculated from the dose-ratio (A/a) using the equation

$$A/a-1=K_I[I]$$

where A/a is the ratio of two concentrations of agonist which produce the same response with and without the antagonist, and [I] is the concentration of antagonist (Schild, 1949).

In some cases, where it was possible to obtain a series of parallel log dose-response curves with increasing concentrations of antagonist, the affinity constant was calculated from a Schild plot: a graph of log (A(a−1) against log [I] (Arunlakshana & Schild, 1959). In these cases it was possible to obtain a measure of the slope of the line, which equals 1 in a simple competitive antagonism.

Results

Response to acetylcholine

Denervated preparations The response to ACh was tested in seven denervated preparations. All responded with graded and readily reproducible contractions to concentrations of ACh in the range 2×10^{-7} M to 10^{-5} M. An example is given in Figure 1a. This range is similar to that reported for the response of the denervated hemi-diaphragm of the rat (Elmqvist & Thesleff, 1960) and the gracilis muscle of the rat (Turkanis, 1969). The response and recovery after each dose was rapid, and a 2 or 3 min time cycle with a 20 s drug contact period gave satisfactory results with little loss of sensitivity except at high concentrations of the drug.

The maximum isotonic contractions produced by ACh were generally about 70% of the contraction produced by 0.1 M KCl. The maximum isometric contractions represented 2–5 g tension in different preparations which constituted 40–70% of the maximum tension obtained with 0.1 M KCl. The preparations were most sensitive between 2 and 4 weeks after denervation.

Innervated preparations Eight preparations were tested in the same way as the denervated preparations. All responded to ACh with graded and readily reproducible contractions to concentrations usually within the range 5×10^{-6} to 5×10^{-4} M (an example is given in Figure 1b). The muscles were about fifty times less sensitive than denervated preparations. The

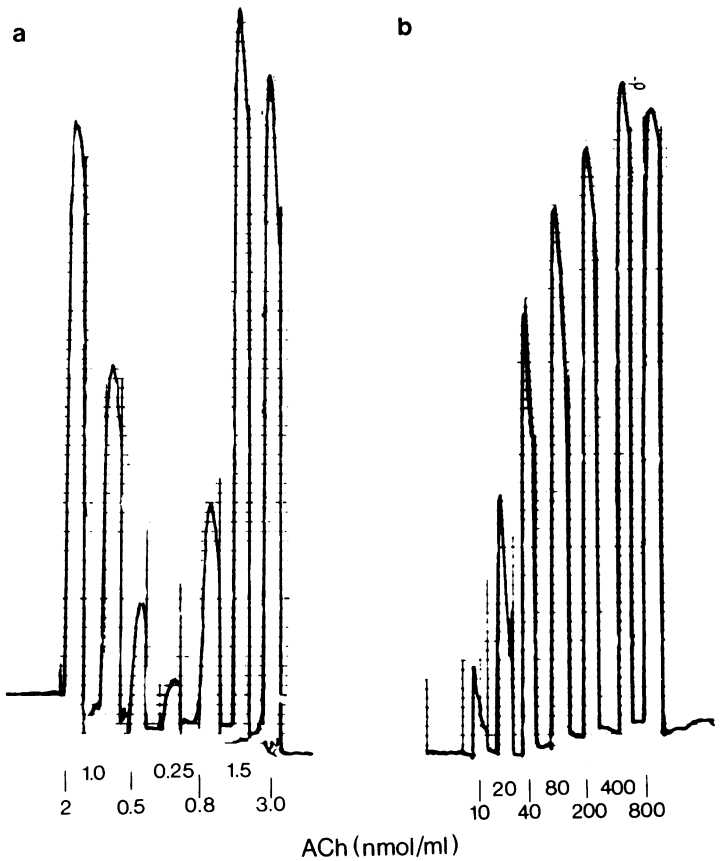

Figure 1 Responses of the isolated cremaster muscle to acetylcholine (ACh). (a) The denervated preparation responded to concentrations from 2.5×10^{-7} to 3×10^{-6} M (0.25–3 nmol/ml), and (b) the innervated preparation to concentrations ranging from 10^{-5} to 8×10^{-4} M (10–80 nmol/ml). The maximum ACh contraction was about 80% of the maximum contraction with 0.1 M KCl (which caused a 50% shortening of the muscle length).

magnitude of the contractions was similar to that obtained with denervated preparations although the innervated preparations gave a slightly less prolonged response than those that had been denervated. Desensitization was minimal except when very high doses of ACh were used, or when anticholinesterases were added to the bathing fluid.

The effect of anticholinesterases on acetylcholine responses

Denervated preparations Langley & Kato observed in 1915 that anticholinesterases did not potentiate the effects of ACh on denervated frog gastrocnemius muscle. In the denervated cremaster muscle some potentiation was observed with physostigmine sulphate, 6×10^{-7} M. A 6-fold potentiation was found in one experiment though in others the potentiation was only 3-fold. The fact that any potentiation at all was observed indicates that some cholinesterase is present.

Innervated preparations Physostigmine sulphate 6×10^{-7} M and neostigmine methanesulphonate 6×10^{-7} M both produced a 3- to 7-fold potentiation of the response to ACh. This was equivalent to the potentiation produced in the denervated preparations, but less consistent dose-response curves were obtained in the presence of the anticholinesterases, since marked desensitization occurred.

The effect of acetylcholine antagonists

Denervated preparations Although most studies support the view that the new ACh receptors in denervated mammalian muscle are nicotinic (Elmqvist

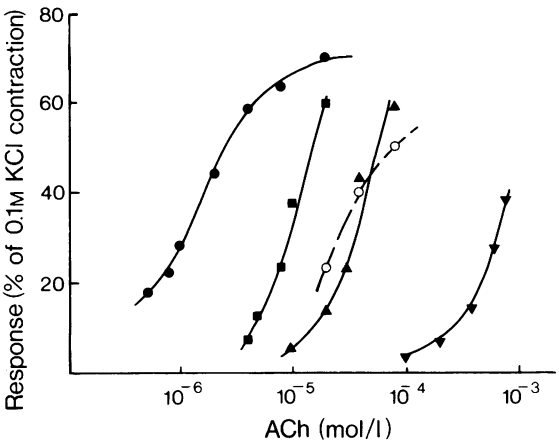

Figure 2 Effect of (+)-tubocurarine (Tc) on the acetylcholine (ACh) response of denervated cremaster muscle: (●) ACh alone, (■)ACh+Tc 10^{-7} M, (▲) ACh+Tc 10^{-6} M, (▼) ACh+Tc 10^{-5} M, (○) partial recovery after washing out Tc.

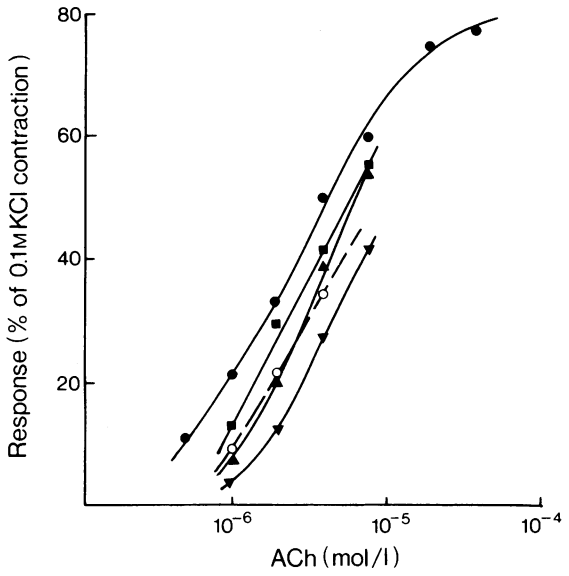

Figure 3 Effect of atropine on the acetylcholine (ACh) response of denervated cremaster muscle: (●) ACh alone, (■) ACh+atropine 10^{-7} M, (▲) ACh+atropine 10^{-6} M, (▼) ACh+atropine 10^{-5} M, (○) recovery curve after washing out atropine.

& Thesleff, 1960; Beránek & Vyskočil, 1967), some evidence indicates that muscarinic receptors might also be present (Turkanis, 1969). The effects of a competitive antagonist for nicotinic receptors ((+)-tubocurarine) and a competitive antagonist for muscarinic receptors (atropine) were tested on the denervated cremaster muscle. When the log dose-response curves to ACh alone and in the presence of (+)-tubocurarine were plotted, there was a marked reversible parallel shift to the right with increasing doses of antagonist from 10^{-9} M to 10^{-5} M (see Figure 2). Atropine, on the other hand, had only a minimal effect even at 10^{-5} M, when tested concurrently on the contralateral denervated cremaster muscle of the same guinea-pig (see Figure 3).

There was some loss of viability of the preparations towards the end of the experiments as evidenced by the fact that recovery, after washing the antagonist out of the bath, was not complete. Using the values from Figure 2, a Schild plot was constructed, graphing log (A/a−1) against log [I]. The slope of the line which best fitted the points was 0.95 and the affinity constant for (+)-tubocurarine was 2×10^7 M^{-1} ($pA_2 = 7.3$). This value is fairly similar to the figures reported for innervated guinea-pig skeletal muscle, which have been in the region of 9×10^6 M^{-1} ($pA_2 = 7.0$) for diaphragm and 11×10^6 M^{-1} ($pA_2 = 7.0$) for latissimus dorsi (Lu, 1970).

The affinity constant for atropine calculated from the dose-ratio given by the highest concentration of atropine was 2×10^5 M^{-1} ($pA_2 = 5.3$) which compares with the pA_2 value of 4.2 for atropine on the frog rectus (see Arunlakshana & Schild, 1959).

These data indicate that in the denervated guinea-pig cremaster muscle, the ACh receptors are very similar to those in innervated guinea-pig skeletal muscle and are mainly if not entirely nicotinic.

Innervated preparations (+)-Tubocurarine produced reversible, dose-related shifts of the dose-response curve to ACh, whereas atropine had little or no effect. Examples are given in Figures 4 and 5. The affinity constant for tubocurarine obtained from a Schild plot was 8×10^7 M^{-1} ($pA_2 = 7.9$), and the slope of the line was 1.12. This pA_2 value is rather higher than the figures reported by Lu (1970) for innervated guinea-pig skeletal muscle. The difference could again be due to the fact that our preparations lost sensitivity during the course of the experiment. This would be expected to give rather high dose-ratios.

The effect of other cholinomimetic agonists

Methacholine The denervated muscle gave repeatable graded responses to metacholine in the concentration range 4×10^{-4} to 4×10^{-3} M. The responses were of similar size and time course to those elicited by ACh but methacholine was approximately 400

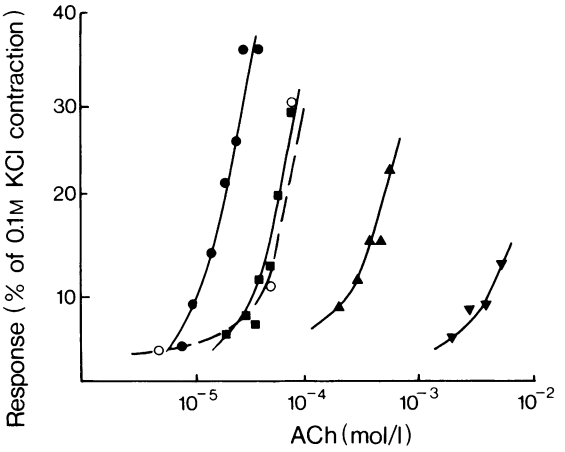

Figure 4 Effect of (+)-tubocurarine (Tc) on the acetylcholine (ACh) response of innervated cremaster muscle: (●) ACh alone, (■) ACh+Tc 10^{-7} M, (▲) ACh+Tc 10^{-6} M, (▼) ACh+Tc 10^{-5} M, (○) partial recovery after washout of tubocurarine.

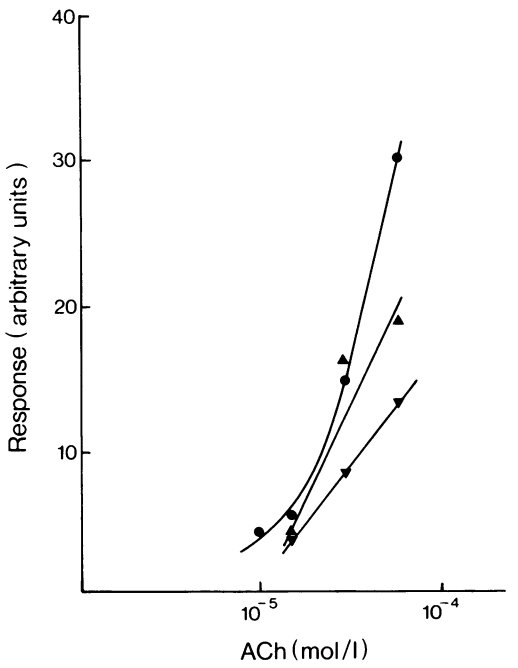

Figure 5 The effect of atropine on the acetylcholine (ACh) response of innervated cremaster muscle: (●) ACh alone, (▲) ACh + atropine 4×10^{-7} M, (▼) ACh + atropine 2×10^{-6} M. Neostigmine 2×10^{-7}M was present in the bath fluid throughout the experiment. Responses in arbitrary units: 30 units is equivalent to approximately 60% of the contraction with 0.1 M KCl.

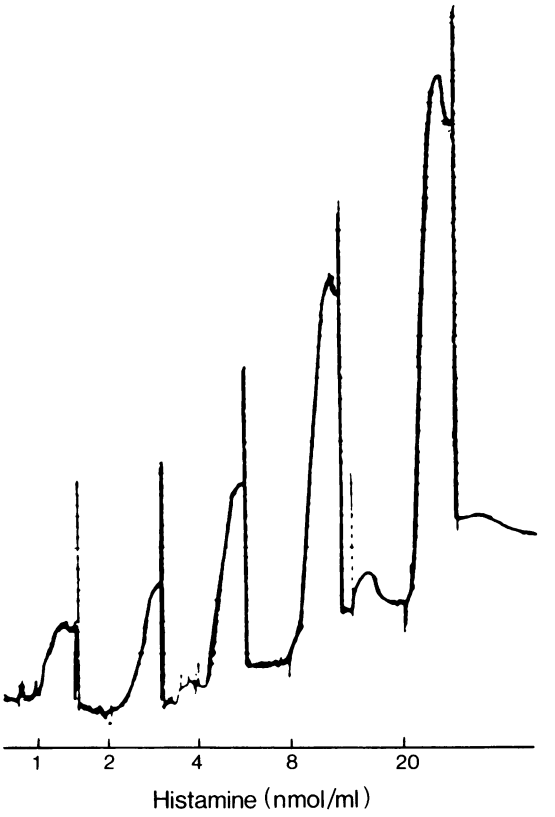

Figure 6 Response of the denervated isolated cremaster muscle preparation to histamine. The maximum histamine contraction was about 40% of the maximum contraction obtained with 0.1 M KCl (which gave about 50% shortening of the muscle length).

times less potent. Methacholine has been reported to have an activity 180 times less than that of ACh on frog rectus (Barlow, 1964).

Carbachol Carbachol in the concentration range 10^{-6} to 10^{-5} M produced repeatable graded responses. It was roughly 3 times less potent than ACh on this preparation.

The response to histamine

Contractions of the denervated cremaster muscle could be elicited with concentrations of histamine in the range 10^{-7} to 10^{-5} M. No responses were observed in innervated controls at concentrations up to 10^{-3} M. The response to histamine was slower than that to ACh, and smaller. The ACh contraction began

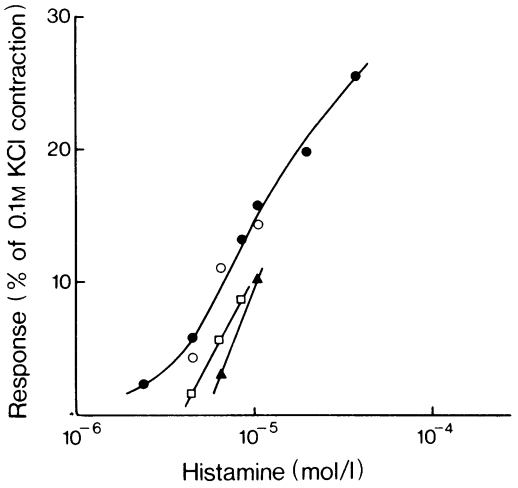

Figure 7 The effect of metiamide on histamine-induced contractions of denervated cremaster muscle: (●) histamine alone, (○) histamine + metiamide 10^{-6} M, (□) histamine + metiamide 10^{-5} M, (▲) histamine + metiamide 10^{-4} M.

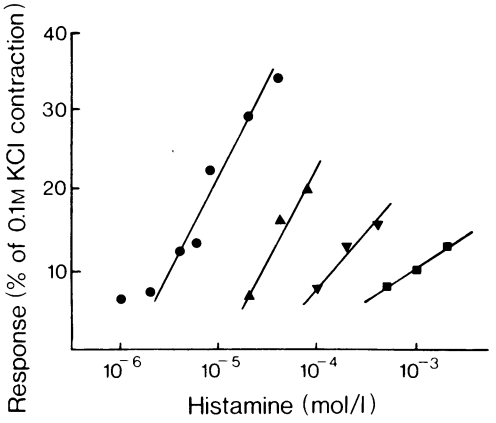

Figure 8 Effect of mepyramine on histamine-induced contractions of denervated cremaster muscle: (●) histamine alone, (▲) histamine + mepyramine 2×10^{-9} M, (▲) histamine + mepyramine 4×10^{-9} M, (■) histamine + mepyramine 8×10^{-9} M.

immediately after the drug was given and reached its peak in 20 s and on washing the drug out there was a rapid relaxation. With histamine there was a latency of 20–30 s before the response began, the contraction reached maximum 20–45 s later and recovery was very much slower. For experiments with histamine a drug contact time of 1 min was used, with a 15 min interval between doses. With higher concentrations full relaxation did not occur even after 15 minutes. Desensitization was a problem in most experiments with histamine. After the first fairly large contraction in response to a small dose, the sensitivity would drop and then remain steady. Then after about 10 responses it would decrease again unless a long rest period of about an hour was allowed. An example of the response to histamine is given in Figure 6.

The responses were small in magnitude compared with those to ACh. With isotonic recording the maximum histamine contractions were about 35% of the contracture produced by 0.1 M KCl.

Whereas the optimum time for eliciting responses to ACh was 2 to 4 weeks after denervation, the optimum time for histamine responses was 4 to 6 weeks after denervation. Earlier than this the muscle was relatively insensitive to histamine and later than this the muscle was usually too atrophied to give good responses.

The effect of histamine antagonists

Metiamide This H_2-receptor antagonist appeared to have little or no effect on the response to histamine. The dose-response curves for histamine alone and in the presence of metiamide 10^{-6} M were indistinguishable. The dose-response curve in the presence of 10^{-5} M and 10^{-4} M metiamide showed a slight shift to the right but this probably represented loss of sensitivity since no recovery occurred when the antagonist was washed out of the bath (see Figure 7).

Mepyramine This H_1-receptor antagonist produced a dose-related shift in the histamine dose-response curve. Mepyramine 10^{-9} M produced a parallel shift while higher doses produced non-parallel shifts (Figure 8). One possible interpretation is that mepyramine is a non-competitive antagonist in this system, but it is much more likely that the effect is due to non-specific loss of sensitivity of the tissue. Certainly there was poor recovery of the histamine response after washing the high doses of antagonist out of the bath.

If the dose-ratio given by the lowest concentration of mepyramine was used to calculate the affinity constant, the values obtained in 2 experiments were 6×10^9 M^{-1} ($pA_2 = 9.7$) and 4×10^9 M^{-1} ($pA_2 = 9.6$). These fall within the range reported for mepyramine in other tissues with H_1-receptors, which vary from $pA_2 = 9.1$ in the guinea-pig tracheal chain preparation (Arunlakshana & Schild, 1959) to $pA_2 = 9.8$ for guinea-pig ileum (Ash & Schild, 1966).

The response to catecholamines

Adrenaline at a dose of 5×10^{-6} M produced a slowly developing contracture over the course of 2 min or so.

It was not possible to obtain a dose-response curve because not only was the effect slow in wearing off, but in some cases the muscle continued to contract after the adrenaline was washed out of the bath. With isotonic recording, the peak response was usually about 30% of the 0.1 M KCl contracture (in one case it was 50%) and was sometimes little reduced after 20 minutes. Atropine and (+)-tubocurarine did not affect this contraction. Noradrenaline 5×10^{-6} M also produced a slow contracture in two preparations in which it was tested. This reached 30% of the KCl maximum and also continued to increase after the drug was washed out.

Isoprenaline at 5×10^{-6} M produced no effect and at 5×10^{-5} M produced only a very tiny response, less than 5% of the KCl maximum.

Responses to other pharmacological agents

Some substances thought to be involved in anaphylactic or other immunological responses which were tested on the denervated muscle but which had no effect were, 5-hydroxytryptamine (up to 10^{-3} M), bradykinin (up to 50 μg/ml) and slow-reacting substance of anaphylaxis.

Response of sensitized muscle to antigen

The denervated cremaster muscle manifested Schultz-Dale responses. In five preparations from guinea-pigs sensitized to ovalbumin, ovalbumin 100 μg/ml elicited a contraction about 25% of the 0.1 M KCl maximum. The contraction had a long latency and a slow rate of shortening and desentitization to subsequent doses was observed. As with histamine the best results were obtained 4 to 6 weeks after denervation. Mepyramine, 5×10^{-9} M, prevented the response and no response to antigen was seen in innervated preparations or in denervated preparations from non-sensitized animals.

Discussion

The usual preparation used in studies of denervated skeletal muscle is the rat diaphragm, which is denervated by cutting the phrenic nerve in the neck. The denervated diaphragm is not an ideal preparation, particularly in the guinea-pig. Not only is the actual operation technically more difficult in the guinea-pig than in the rat, but having a diaphragm quiescent on one side increases the possibility of respiratory infections in an animal which is already very susceptible to such infections. Another important factor is that the setting up of a diaphragm preparation necessitates cutting through muscle fibres, which decreases the viability of the preparation. The cremaster muscle on the other hand forms a neat sac which can be dissected out with little damage to the muscle. Grant (1966) in a study on the effect of denervation on skeletal muscle blood vessels, used the cremaster muscle of the rat and reported that section of the genito-femoral nerve caused most of the myelinated nerve fibres to the muscle to degenerate, though many of the perivascular nerve fibres were left intact. We found that section of the genito-femoral nerve generally produced gradual atrophy of the cremaster muscle indicating that this nerve provides the main innervation for the muscle. The genito-femoral nerve can easily be isolated and divided and as it innervates little else besides the cremaster muscle and a small area of skin, its section produces little interference with the animal in comparison with the cutting of the phrenic nerve.

It should be stated that when using isotonic recording with the cremaster preparation the actual change of length of the muscle is much less than the change seen in a smooth muscle preparation such as guinea-pig ileum or uterus. The maximum contraction produced by KCl 0.1 M represented about 50% change in actual muscle length and the maximum contraction to ACh in denervated preparations was between 25% and 35% of the muscle length. It was nevertheless possible to obtain surprisingly consistent graded responses to drugs within quite a narrow range of change of muscle length. With isometric recording on the other hand quite substantial increases of tension could be obtained not only with KCl but also with ACh.

The response of the innervated muscle to acetylcholine

One unexpected finding was that the innervated preparations exposed to ACh gave slow dose-related contractile responses. These responses were competitively antagonized by tubocurarine but not atropine, and values obtained for the affinity constants of the antagonists indicated that the receptors were mainly if not entirely nicotinic. This implies that the muscle involved is almost certainly skeletal muscle. Ninomiya (1975) had suggested that some of the slow pharmacological responses which he reported were due to the presence of smooth muscle, possibly that associated with blood vessels. We feel that the ACh responses obtained in the present study (involving contractures of up to 5 g, competitively antagonized by (+)-tubocurarine) could not be attributed to the presence of smooth muscle.

Slow contractile responses with ACh are not usually seen with mammalian skeletal muscle preparations. One could postulate that the cremaster muscle gave sustained contractions with ACh, instead of twitches, because the distribution of receptors is different in cremaster muscle from that in most other mammalian skeletal muscle and more like that in the classical frog rectus preparation and some avian

muscles (Hess, 1970). There is evidence that there are some skeletal muscles in mammals, for example, the extraocular muscles in many species, which contain muscle fibres that have multiple nerve terminals and which, after a nerve impulse or the application of ACh undergo a slow contracture rather than a twitch (see Hess, 1970). It is possible that the guinea-pig cremaster contains a component of this slow tonic skeletal muscle. Another explanation is that the thinness of the muscle and the open 'basket-work'-like nature of the arrangement of the muscle fibres allows more rapid diffusion of the ACh so that it is able to reach a high concentration at the end plates of individual muscle fibres before there is marked electrical accommodation, or hydrolysis. Preliminary microscopic observations did not support the view that a substantial part of the tissue was smooth muscle. This is in accord with the observations of Grant (1966) and Majno, Palade & Schoefl (1961) who did detailed histological studies on rat cremaster muscle and described the muscle as predominantly striated in nature. However, it is still possible that some smooth muscle could be present, though it is unlikely that it is implicated in the responses to cholinomimetic agents described in this study.

The response of the denervated muscle to acetylcholine

On the basis of the results obtained in the present study the ACh receptors in the denervated preparations were also nicotinic. In this respect our results with this muscle differ from those obtained with the denervated anterior gracilis muscle of the rat *in situ*. Turkanis (1969) using this latter preparation found that equal doses of atropine and (+)-tubocurarine produced equivalent effects in that they necessitated the same increase in the amount of ACh required to produce a given amount of depolarization of the muscle fibre membrane. However, Dale & Gaddum (1930) and Beránek & Vyskočil (1967) using *in vitro* preparations found that the concentration of atropine had to be 300 times greater than that of tubocurarine to produce the same effect. Our results with the denervated cremaster muscle are in accord with these latter observations.

The effect of anticholinesterases

One other perhaps rather unexpected finding with this muscle was that the ACh response in denervated muscle showed some potentiation in the presence of anticholinesterases. In fact the potentiation was similar to that seen in innervated muscle. One explanation is that the muscle had not been completely denervated and that some cholinesterase was still present in association with a few remaining nerve fibres (Grant, 1966). It would be of interest to examine the distribution of cholinesterase in this muscle before and after denervation.

The response to histamine

It was reported by Alonso-de Florida *et al.* (1965) that denervated guinea-pig diaphragm responded to histamine. Ninomiya (1975) claimed that histamine increased basal tension and generated spontaneous contractions in a cut section of innervated cremaster muscle—the tip. In the present study we were unable to obtain contractions to histamine in whole innervated cremaster muscle, but a reasonable dose-response curve to histamine could be obtained with the denervated preparation. The histamine responses could be antagonized by the H_1-receptor antagonist, mepyramine, but not by the H_2-receptor antagonist metiamide. The affinity constant obtained for mepyramine fits in with those obtained from other tissues with H_1-receptors. Similarly selective effects with these two types of antagonists were also obtained in denervated guinea-pig diaphragm by Dale & Evinç (unpublished results). If the histamine response of denervated guinea-pig skeletal muscle is due to the appearance or uncovering of receptors for histamine, it would seem that these receptors are H_1-receptors.

Spontaneous contractions

Ninomiya (1975) reported spontaneous contractions in cremaster preparations. We did not obtain spontaneous contractions in any preparations in which isotonic recording methods were employed. In a few preparations in which isometric-recording was used, spontaneous contractions of 100–250 mg were seen towards the end of a long experiment. These small fluctuations in basal tension could well have been due to smooth muscle, but we did not find that either histamine or ACh increased them.

The difference between our results and Ninomiya's could possibly be due to strain differences in guinea-pigs or to the fact that he used a cut section of the muscle, the tip, whereas all our experiments were performed on whole intact muscles.

The response of the denervated preparation to catecholamines

Many studies have reported that denervated skeletal muscle develops sensitivity to catecholamines (Ellis, 1959; Bhoola & Schachter, 1961; Bowman & Raper, 1965) though Turkanis (1969) reported that topically applied catecholamines had no effect on the denervated anterior gracilis muscle of the rat. In the present study adrenaline and noradrenaline both produced sustained contractures. These responses also occurred in the presence of atropine and (+)-tubocurarine, so, as was concluded by Bowman & Raper (1965), cholinergic mechanisms are not involved. Isoprenaline produced virtually no response except in very high concentration. These findings suggest that if catecholamine receptors are present they are possibly α-receptors. Paterson (1963) using rat hemi-

diaphragm concluded that α-receptors were present in denervated skeletal muscle, whereas Bowman & Raper (1965) using the denervated tibialis anterior of the cat concluded that β-receptors were present.

The adrenaline response reported by Ninomiya (1975) in innervated muscle could be due either to the smooth muscle which he postulates is present, or to slow tonic skeletal muscle, which has also been reported to give contractures with adrenaline (Bowman & Nott, 1969).

The anaphylactic response of denervated muscle

The denervated cremaster muscle from guinea-pigs sensitized with ovalbumin gave typical Schultz-Dale responses when exposed to the antigen *in vitro*. The peak time for the reaction coincided with the peak time for the histamine response, 6 weeks after denervation, and like the histamine response it could be antagonized by mepyramine. A similar anaphylactic response has been reported by Alonso-de Florida *et al.* (1965) and Alonso-de Florida, del Castillo, García & Gijón (1968) and attributed by them to direct antigen-antibody stimulation of muscle contraction. It was pointed out by Dale & Ziletti (1970) that most evidence supports the view that the anaphylactic contraction of smooth muscle is mainly due to mediators released from mast cells and that this could well be the case for the denervated skeletal muscle preparation used by Alonso-de Florida *et al.* (1965), because the tendinous part of guinea-pig diaphragm is certainly very rich in mast cells. One would have to postulate that if, in the cremaster muscle, mediator-release was indeed the basis of the anaphylactic reaction, the main mediator involved was probably histamine, because other substances which are believed to be implicated in the anaphylactic contraction of smooth muscle are not active in denervated cremaster muscle. It would be of interest to examine the histamine content and mast cell distribution of the denervated cremaster muscle.

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