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# The isolated cremaster muscle preparation and (external) spermatic nerve-cremaster muscle preparation of the guinea-pig

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The isolated cremaster muscle preparation and spermatic nerve-cremaster muscle preparation of the guinea-pig were studied *in vitro* to determine their suitability as pharmacological test models. The preparation was contracted by acetylcholine, carbachol, succinylcholine and decamethonium ($pD_2$ values, 4·2, 5·3, 7·3 and 7·4, respectively) through an action on a curare-sensitive cholinoceptor. Lobeline and DMPP were ineffective. Nicotine contracted the muscle, but there was tachyphylaxis. Tubocurarine and hexamethonium presumably competitively antagonized acetylcholine ($pA_2$ values, 7·3 and 5·8); lobeline was a non-competitive antagonist ($pD'_2$ value, 6·4). Atropine and mecamylamine exerted a dualistic action against acetylcholine (final $pD'_2$ values, 5·3 and 6·7, respectively). Tubocurarine, succinylcholine and decamethonium exhibited their typical action when tested with spermatic nerve-cremaster muscle preparation; the latter two drugs also produced muscle spasm. Hexamethonium was a weak blocker of neuromuscular transmission. Atropine, mecamylamine, lobeline and DMPP exhibited neuromuscular blocking activity; however, directly evoked muscle twitches were also notably affected. The cremaster muscle preparations seem to add usefully to the list of currently used *in vitro* tests, with the added advantage that a mammalian skeletal muscle model is used for simultaneous quantitative studies.

The cremaster muscle is generally considered to be an extension of the internal oblique muscle of abdomen, although the anatomical features and degree of development of the muscle vary in different species (Sisson, 1962). The cremaster is concerned with position of testes and is responsive to reflex stimulation and to stimuli like temperature changes and general excitation.

In adult guinea-pigs and rabbits the muscle was found to be well developed and in an organ bath, it responded satisfactorily to drugs. Preliminary results obtained with the guinea-pig isolated cremaster and the external spermatic nerve-cremaster muscle preparation constitute the basis of this report.

## MATERIALS AND METHODS

Young male guinea-pigs (750 g or more) were stunned by a blow on head and exsanguinated. After displacing the testes into the abdominal cavity the cremaster can be exposed as a reddish, cone-shaped muscle with apex attached to epididymal pole of testes, and the base fanning out towards the external abdominal ring. A fold of mesentery extends between the muscle and vas deferens.

### *The isolated cremaster muscle preparation*

The muscle was separated from testes and the mesenteric attachment to the vas. Approximately 1½ to 2 cm of the cone was dissected out cutting across the muscle at the level of external abdominal ring. The cone was slit open on two sides starting at the base and cutting upwards to points about 2 mm away from the apex; this converted the cone into a thin strip of muscle of double length. After removal of adherent tissue from the surfaces, the muscle was set up in an organ bath (20 ml) containing oxygenated Tyrode solution (composition, g litre$^{-1}$, NaCl 8·0, KCl, 0·2, $CaCl_2$ 0·2, $MgCl_2$ 0·1, $NaH_2PO_4$ 0·1, $NaHCO_3$, 1·0 and glucose 1·0) maintained at 37° ± 1°. Muscle tone was recorded with an isotonic, frontal writing lever (magnification × 10) with the tissue under a resting tension of about 1 g. Drug testing was started after 2 h and the tissue was washed every 10 min during this period.

Concentration-effect curves for spasmogens were obtained by adding increasing doses of a spasmogen to the bath at intervals of 5 min (acetylcholine, carbachol and succinylcholine) or 15 min (decamethonium). In studies involving antagonists, the curves were obtained before and after the inhibitory

effect of the antagonist added to the bath was maximal (usually 10–15 min). In each instance, the contractile effect (% maximal) was plotted against concentration of agonist (M). The intrinsic activity of agonist ($\alpha$), the negative log of molar concentration of agonist producing 50% of maximal response ($pD_2$ value), the negative log of molar concentration of antagonist that requires doubling the dose of agonist to compensate the inhibition ($pA_2$ value), and the negative log of molar concentration of antagonist inhibiting the maxima by 50% ($pD'_2$ value) were determined (Ariëns, Simonis & van Rossum, 1964a).

*The spermatic nerve-cremaster muscle preparation*

The cremaster muscle was separated from testes and mesenteric attachment to the vas, and was gently pulled down to visualize its continuity with inguinal part of the muscle. The muscular branch of the external spermatic nerve was identified on the posterior abdominal wall as it emerges from the psoas major muscle and courses downwards in front of the circumflex iliac vessels to enter the upper border of cremaster (inguinal part). The muscle was cut across in the inguinal part 1 cm above the point the nerve enters the muscle. The muscle, along with 2½ to 3 cm of the nerve attached to it was separated downwards till the terminal conical part of the muscle came out with the inguinal part.

The inguinal end of the muscle was fixed to the platinum hook of a tissue holder and the muscle was set up in the organ bath (33 ml) in oxygenated Tyrode solution at 37° ± 1°. The conical end of the muscle was attached to a recording lever (spring-loaded, or isotonic frontal writing) through a thin, coated platinum wire. The hook and the wire served as two poles for direct muscle stimulation. The spermatic nerve was threaded through a pair of platinum-ring electrodes for direct stimulation. Electrical stimulation was carried out with submaximal single shocks (0·2 to 2 ms) applied every 20 s. The preparation was rested for 1 h before the experiments and was washed every 10 min during the rest period.

*Drugs*

Concentration of drugs are expressed as molarity in the bath medium. All drugs were dissolved in 0·9% w/v saline just before use. The drugs were: acetylcholine chloride (E. Merck AG, Darmstadt), decamethonium iodide (Fluka AG, Buchs SG), succinylcholine chloride (Midaire, Wellcome, India), carbachol chloride (E. Merck AG, Darmstadt), (+)-tubocurarine chloride and physostigmine salicylate (Burroughs Wellcome & Co., London), atropine sulphate (BDH, London), hexamethonium chloride (Merck, Sharp & Dohme, Ltd., Bombay), lobeline hydrochloride (Sandoz Ltd., Basle) and dimethylphenylpiperazinium iodide (DMPP, Aldrich Chemical Company, Milwaukee). Nicotine, (±)-adrenaline (Sigma Chemical Company, St. Louis) and theophylline (N.U. Societeit Voor Chemische Industrie, Katwijk) were used as bases.

## RESULTS

*The isolated cremaster muscle preparation*

Sensitivity of the muscle to spasmogens increased during the first 2 h; thereafter, the muscle responded reproducibly (Fig. 1) for 4–6 h. In most instances, an initial quicker and a subsequent slower phase could be identified during a contractile response. The spasmogens produced a maximal response in 5 to 10 s, which was followed by a tendency to relax, the 'decay' being most obvious in case of acetylcholine. The preparation, therefore, was not used for cumulative administration of spasmogens.

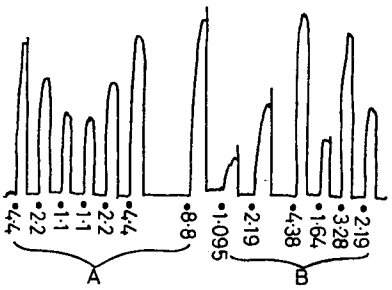


FIG. 1. The guinea-pig cremaster muscle preparation. Responses, at dots, to A-acetylcholine ($\times 10^{-4}$ M) and B-carbachol ($\times 10^{-5}$ M) (added at 5 min intervals).

Table 1 shows the intrinsic activity and $pD_2$ values obtained for 4 acetylcholine-like drugs. The muscle differed from rectus abdominis of frog in its sensitivity to acetylcholine, succinylcholine, and decamethonium. Nicotine ($1{\cdot}17 \times 10^{-5}$ M, n = 5) also contracted the muscle showing sensitivity comparable to that of rectus (Ariëns, Simonis & van Rossum, 1964b). However, the drug was not further studied since tachyphylaxis was evident. Lobeline (up to $1{\cdot}4 \times 10^{-5}$ M, n = 4) or DMPP ($1{\cdot}27 \times 10^{-5}$ M, n = 5) did not contract the muscle.

Physostigmine ($2{\cdot}418 \times 10^{-5}$M, n = 5, Fig. 2) and theophylline ($1{\cdot}795 \times 10^{-4}$M, n = 3) augmented responses to acetylcholine; consequently, threshold

doses were 7–10 times and 2–3 times less, respectively.

*Antagonists.* Tubocurarine and hexamethonium produced a parallel, concentration dependant shift of dose-effect curves for acetylcholine to the right, providing preliminary evidence for a competitive antagonism; lobeline produced only inhibition of maxima (Fig. 2). Atropine (Fig. 2) and mecamylamine produced a dual effect, first a shift to the right (lower concentrations) and then, inhibition of maxima (higher concentrations). Table 1 gives potency of antagonists (as $pA_2$ value if a drug produced a parallel shift of the curves to the right and as $pD'_2$ value if maxima was inhibited). DMPP ($1{\cdot}19$ to $4{\cdot}76 \times 10^{-6}$M, n = 5) exerted a dual antiacetylcholine action of the type of atropine or mecamylamine. It was not further characterized since the degree of inhibition was variable.

Table 1. *Effect of some cholinomimetic and cholinolytic drugs on the isolated cremaster muscle of the guinea-pig.*

| Compound | α | $pD_2$ | $pA_2$ | $pD'_2$ |
|---|---|---|---|---|
| Acetylcholine | — | 4·268 | — | — |
| Carbachol | 1 | 5·358 | — | — |
| Succinylcholine | 1 | 7·225 (5·7)[1] | — | — |
| Decamethonium | 0·8 | 7·4 (5·4)[1] | — | — |
| Tubocurarine | — | — | 7·309 (6·8)[1] | — |
| Hexamethonium | — | — | 5·768 (4·4)[1] | — |
| Atropine | — | — | (5·2)*[2] | 5·30 |
| Mecamylamine | — | — | — | 6·761 |
| Lobeline | — | — | — | 6·446 (4·65)*[3] |

All values are means of 4 to 10 experiments.
α: intrinsic activity, in terms of acetylcholine ( = 1).
In parentheses are corresponding values obtained using frog rectus abdominis: (1) Ariëns, & others, (1964a). (2) Wilson & Schild (1968). (3) Mansuri & others (1974).
* calculated value.

Tubocurarine ($2{\cdot}3 \times 10^{-7}$M) produced a parallel shift of dose effect curves for succinylcholine (n = 4) and for decamethonium (Fig. 2) to right. The shift was of the same magnitude as for acetylcholine.

*The spermatic nerve-cremaster muscle preparation*

Once stabilized, cremasteric responses to electrical stimulation were reproducible for 4–6 h. Table 2 shows the effective concentrations of various drugs (producing about 40 to 60% inhibition of indirectly evoked responses). The preparation exhibited a marked sensitivity to various drugs; also noteworthy was the initial spasm following administration of depolarizing blockers. Hexamethonium was only moderately effective as a blocker (Table 2) since concentrations higher than shown did not produce any better effect. While DMPP was generally inhibitory (Table 2), in 3 other experiments it only augmented the directly and indirectly evoked responses by 30–40% of control. This augmentation could be blocked by hexamethonium ($2{\cdot}9 \times 10^{-5}$M) or mecamylamine ($1{\cdot}2 \times 10^{-5}$M).

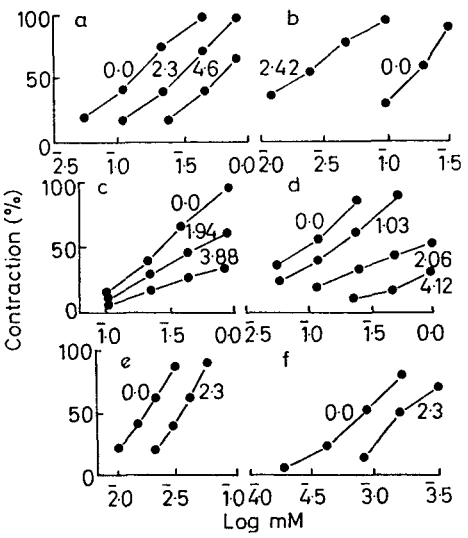


FIG. 2. The guinea-pig cremaster muscle preparation. Effect of various anatagonists (a—Tubocurarine $\times 10^{-4}$ mM, b—physostigmine $\times 10^{-3}$M, c—lobeline $\times 10^{-3}$ mM, d—atropine $\times 10^{-2}$mM, e—tubocurarine $\times 10^{-4}$ mM, f—tubocurarine $\times 10^{-4}$ mM) on responses to cholinergic agents (a–d—acetylcholine, e—carbachol, f—decamethonium). Each curve is based on points which are means of 4 to 8 independent observations.

Physostigmine ($2{\cdot}418 \times 10^{-5}$M, n = 4) markedly augmented the indirectly evoked responses, while adrenaline ($3{\cdot}4 \times 10^{-6}$M, n = 5) or theophylline ($1{\cdot}795 \times 10^{-4}$M, n = 4) produced augmentation of both indirectly and directly evoked responses.

## DISCUSSION

Experiments *in vitro* to study the pharmacology of neuromuscular junction employ classical preparations of frog, leech or chick muscle, and guinea-pig or rat diaphragm (Burgen, Dickens & Zatman, 1949; Quilliam, 1955; Weiss, 1968; also see, Edinburgh University Staff, 1968) or other mammalian test models like guinea-pig or rabbit lumbricals and cat tenuissimus (Jenden, Kamijo & Taylor, 1954; Blaber & Christ, 1967; Waud, Cheng & Waud, 1973). The present report describes a mammalian nerve-muscle preparation which is sensitive and reliable, with an additional, outstanding advantage that a mammalian muscle is used for a simultaneous quantitative study. Also, spasm of the muscle occurring at the outset may give an early indication of a

Table 2. *The guinea-pig spermatic nerve-cremaster muscle preparation.* Effect of drugs on the response to indirect and direct electricalstimulation.

| Drug | Concentration (M) | n | Mean % inhibition: Indirect responses | Mean % inhibition: Direct responses |
|---|---|---|---|---|
| Tubocurarine | $2{\cdot}12 \times 10^{-7}$ | 4 | 44 | 5 |
| | $3{\cdot}54 \times 10^{-7}$ | 5 | 76 | 7 |
| Succinylcholine | $1{\cdot}4 \times 10^{-6}$ | 4 | 38 | 7 |
| | $2{\cdot}8 \times 10^{-6}$ | 4 | 72* | 10 |
| Decamethonium | $1{\cdot}17 \times 10^{-6}$ | 3 | 54 | 10 |
| | $2{\cdot}34 \times 10^{-6}$ | 3 | 75* | 14 |
| Hexamethonium | $5{\cdot}86 \times 10^{-5}$ | 3 | 26 | 10 |
| Atropine | $4{\cdot}32 \times 10^{-5}$ | 3 | 35 | 12 |
| Mecamylamine | $2{\cdot}48 \times 10^{-5}$ | 4 | 40 | 12 |
| Lobeline | $8{\cdot}4 \times 10^{-6}$ | 3 | 64 | 21 |
| DMPP | $2{\cdot}39 \times 10^{-6}$ | 7 | 56 | 25 |

* The muscle responded initially with a spasm.

depolarizing type of blocker. On these grounds, the guinea-pig cremaster is a useful test preparation.

On the strength of the evidence that a certain concentration of tubocurarine caused a parallel shift of their dose-response curves over an identical dose range, we assume that a common receptor is involved in the action of acetylcholine, carbachol, succinylcholine and decamethonium. In view of $pD_2$ values obtained, either carbachol or succinylcholine should be a more suitable agonist if higher $pA_x$ values for antagonists are to be determined. While the difference noted in $pD_2$ values for cremaster and frog rectus is indicative of different sensitivity, a marked difference in $pA_2$ values of tubocurarine and hexamethonium was not anticipated and is intriguing.

A qualitative difference in the blocking activity of hexamethonium and mecamylamine, which recalls a similar difference in their antinicotine activity on jejunum (Ariëns & others, 1964b) could be due to the well known difference in their penetration. Thus, the latter may have an additional, intracellular locus of action. Mecamylamine can produce an intense neuromuscular blockade (Payne & Rowe, 1957) under suitable experimental conditions.

Lobeline and DMPP do not seem to exert a typical 'nicotinic' action, since neither contracted the cremaster muscle. Results with lobeline generally accord with the view (Mansuri, Kelkar & Jindal, 1974) that this drug exerts a procaine-like effect on the neuromuscular junction. While DMPP generally resembled lobeline, its antiacetylcholine activity, reported also with smooth muscle (Barlow & Franks, 1971; Sethi & Gulati, 1974), was not studied further. That DMPP-induced augmentation of muscle contraction was blocked by ganglion blockers could well be due to their antiacetylcholine action at the motor end plate. Atropine exerted a dual type of antiacetylcholine action on the cremaster, which is reminiscent of the finding (Gharpure, 1973) that on smooth muscle atropine may exert a pseudo-irreversible or non-competitive antiacetylcholine action.

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