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Actions of some cholinergic antagonists on fast-twitch and slow-twitch skeletal muscle of the cat

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Summary

1. The anticholinergic drug N-ethyl-2-pyrrolidylmethylcyclopentylphenyl glycollate (PMCG) has been studied for its effects on the contraction of fast-twitch (flexor hallucis longus, FHL) and slow-twitch (soleus) muscles in the cat.

2. In both muscles lower doses (0·25 to 10 mg intra-arterially) potentiated and higher doses (10 mg and above) depressed twitches produced by indirect stimulation. Similar effects were obtained in directly stimulated muscles.

3. The effects of the drug on muscles stimulated repetitively were dependent on both dose and frequency of stimulation. Doses which potentiated twitches also potentiated low frequency tetani (5–30 Hz for soleus ; 10–50 Hz for FHL). There was a depression and non-maintenance of higher frequency tetani (80 Hz and above for soleus ; 150 Hz and above for FHL).

4. Both atropine and caramiphen had similar dose dependent potentiating and depressant actions. Hyoscine acted similarly in some cats ; in others it had only a depressant action.

5. It is suggested that the potentiating actions of PMCG, caramiphen, atropine and hyoscine are due to a direct musculotropic action. The depressant actions resulting from higher doses are due, in part, to a curare-like action and in part to a direct action on the muscle fibres.

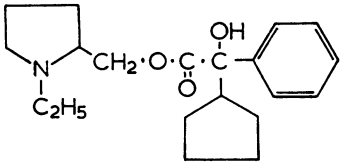
Introduction

In addition to their well known muscarinic receptor blocking activity, anticholinergic drugs are capable of exerting discrete actions at other sites, for example the neuromuscular junction and the skeletal muscle fibre. Thus, dose-dependent potentiation and depression by atropine of the twitches of mammalian and amphibian skeletal muscle have been known for some time (Guttman, Horton & Wilber, 1937 ; Abdon, 1940 ; Bülbring, 1946 ; Dutta, 1949).

Another potent and psychotomimetic anticholinergic drug found to modify skeletal muscle activity is the synthetic compound N-ethyl-2-pyrrolidylmethylcyclopentylphenyl glycollate, the major component (70%) of Ditran. Abood & Biel (1962) reported numerous actions of this compound, which they called PMCG, in both nervous tissue and smooth and skeletal muscle. Isometric twitches of the isolated indirectly stimulated frog sartorius muscle were increased by up to 50% by PMCG (10^{-6}M) in Ringer solution, but spontaneous twitching in calcium-free solution was decreased. Unfortunately, in further publications, Abood and co-

workers are inconsistent in their use of the term PMCG and it is difficult to know which compound was used in studies on membrane phenomena because cross-references within the papers lead one to believe they are all the same. Thus, although the compounds cited would probably have similar properties, the term PMCG has been used for the N-ethyl-2-pyrrolidylmethyl derivative (Abood & Biel, 1962), the N-ethyl-3-pyrrolidylmethyl derivative (Abood, 1968); the N-methyl-2-pyrrolidylmethyl derivative (Abood, Koyama & Kimizuka, 1963a; Abood, Smith, Koyama & Koketsu, 1963b) and the N-methyl-3-piperidinol derivative (Rogeness, Krugman & Abood, 1966) of cyclopentylphenyl glycollate.

In this paper the term PMCG is used for N-ethyl-2-pyrrolidylmethylcyclopentylphenyl glycollate (I).



(I)

Some of the results were included in a communication to the British Pharmacological Society (Brimblecombe & Everett, 1969) in which it was shown that PMCG possesses marked dose-dependent potentiating and depressant actions on the flexor hallucis longus (FHL) and soleus muscles of the cat *in vivo*. Similar effects on the isolated rat diaphragm have been reported by Brimblecombe, Green, Stratton & Thompson (1970).

Methods

The muscles studied were the soleus, a typical slow-twitch muscle, and flexor hallucis longus (FHL), a typical fast-twitch muscle of cats. The animals weighed between 1·5 and 3·5 kg and were prepared according to the methods of Buller & Lewis (1965a, b). The method of recording was essentially that described by Buller & Lewis (1965a).

In most cases the muscles were stimulated indirectly via the cut proximal ends of the motor nerves every 10 or 20 s with pulses of 0·1 ms duration and supramaximal strength (4 × maximal).

In some cats the muscles were stimulated directly with pulses of 0·1 ms and suitable strength between an electrode attached to a drill inserted in the head of the tibia and a silver electrode surrounding a cut tendon. In these experiments the muscles were maintained fully curarized, the absence of any response to indirect stimulation being constantly checked.

Muscle action potentials were recorded using surface electrodes in contact with the belly of the muscle through small pads of saline-impregnated cotton wool.

Drugs were dissolved in saline solution (0·9% w/v) and in most cases injected into the femoral artery via a small branch, but in early experiments they were injected into the jugular vein.

The following drugs were used: atropine sulphate (B.D.H.), hyoscine hydrobromide (B.D.H.), N-ethyl-2-pyrrolidylmethylcyclopentylphenyl glycollate hydro-

chloride (PMCG, synthesized at C.D.E. Porton), (+)-tubocurarine chloride (B.D.H.) and neostigmine (Sigma). Doses expressed in the text refer to the bases or cations.

Results

Effect of PMCG on twitches

Twitches of both FHL and soleus muscles were potentiated markedly by intra-arterial doses of PMCG (0·25 to 10 mg). Intravenous injections of PMCG (10 to 20 mg/kg) caused some increase in twitch tension (maximum 20%), but this route was not satisfactory because doses of this order caused adverse effects on the respiratory and cardiovascular systems. Intra-arterial injections were well localized in the limb concerned and did not affect systemic blood pressure or respiration.

Potentiation of twitches of FHL was more marked than of soleus with increases of up to 200% and 70% respectively, the return to control levels taking up to 2 hours. These increases in twitch tension were characterized by increases in the time to peak (up to 50%) and in the maximum rate of rise (up to 85%). With increasing dosage, the twitches of both muscles were increasingly potentiated until a transition to depression occurred (10 mg and over) as shown in Fig. 1. Increasing the dose from 8 to 16 mg intra-arterially changed the response from one of maximal potentiation to one of pronounced depression. Typical log dose-response curves for both muscles, stimulated alternately every 20 s, are shown in Fig. 2. The upper curves show concurrent changes in tension for each muscle and the lower ones accompanying changes in time to peak and maximum rate of rise of tension. Figure 3 illustrates several consistent findings. First, in any one cat, maximum potentiation of FHL always exceeded that of soleus. Second, the tension increases are accom-

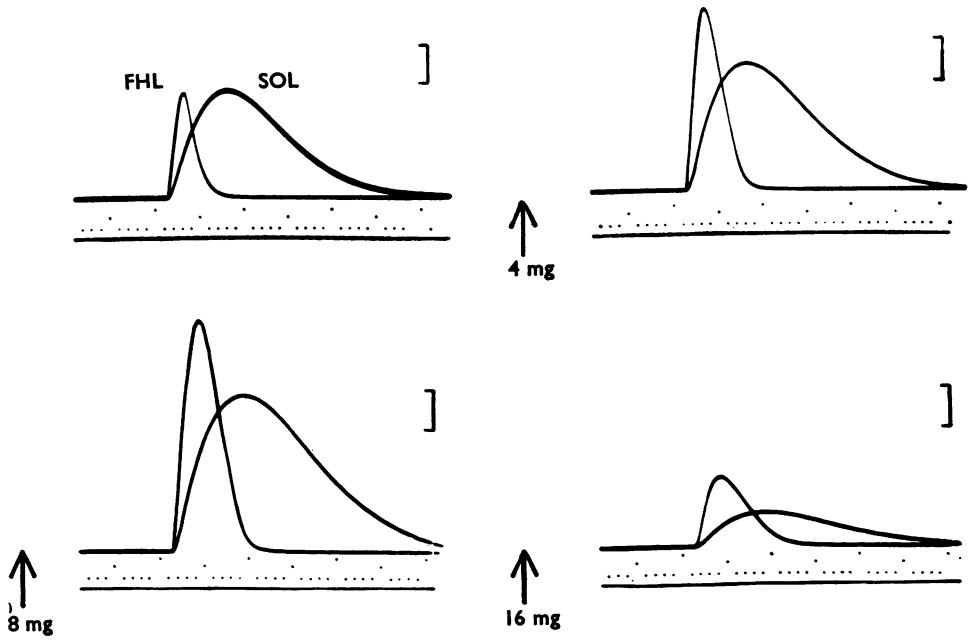


FIG. 1. Dose dependent effects of PMCG. Alternate stimulation of soleus (SOL) and FHL. At the arrows PMCG injected intra-arterially at the doses indicated. Calibration: twitch tension 100 g; time base 10 ms.

panied by increase in both the maximum rate of rise and the time to peak. Third, the transition from potentiation to depression occurs over a narrow dose range. Finally, depression of soleus by PMCG is more marked in extent and duration than that of FHL. In the experiment illustrated in Fig. 2, depression of FHL at a dose of 16 mg was transient only, reverting to a long-lasting potentiation, while depression of soleus was more prolonged. These facts are reflected in the maximum rate of rise and time to peak curves.

PMCG possesses only very weak anticholinesterase activity (pI_{50} against acetylcholinesterase = 1·5 and against cholinesterase = 4·0) and, in addition, surface recordings of muscle action potentials did not show any repetitive firing of the muscle fibres in the presence of PMCG (Fig. 3) although in the presence of anticholinesterases, for example neostigmine (30 μg intra-arterially), it can be readily detected (Fig. 3c). Furthermore, all the effects of PMCG observed in indirectly stimulated muscles can be demonstrated in directly stimulated, fully curarized muscles (Fig. 4). Low doses of PMCG (5 mg and below) potentiated the responses of directly stimulated muscles, sometimes to an even greater extent than those of muscles stimulated through the motor nerve. Although depression also occurred, considerably greater

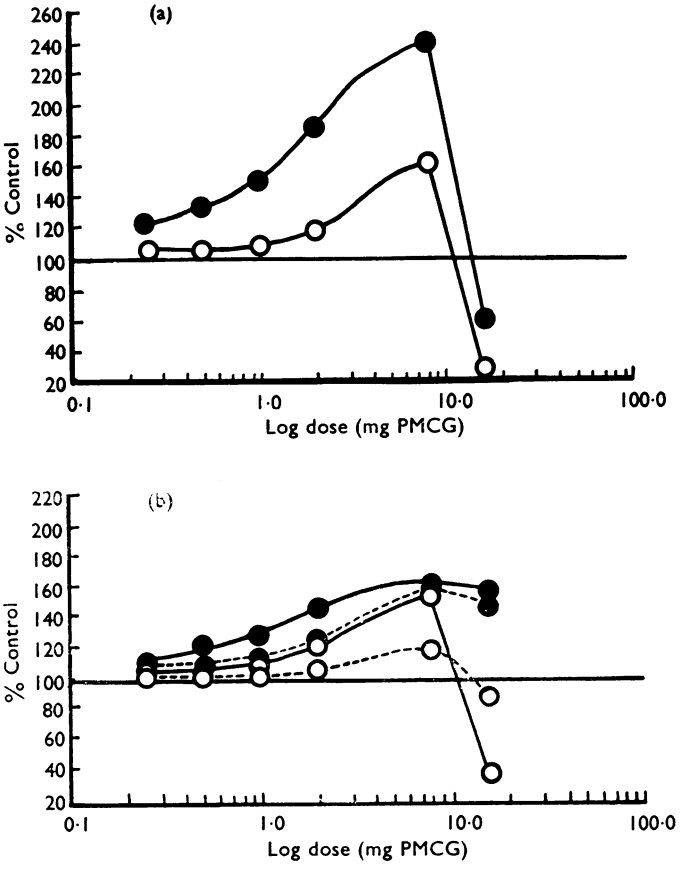


FIG. 2. Log dose-response curves for PMCG. Alternate stimulation of soleus (○) and FHL (●). (a), Changes in twitch tension expressed as % of control; (b), concurrent changes in maximum rate of rise (– – – –) and time to peak (——).

increases in dosage were necessary to give this depression, which again for FHL was transient, even with doses of the order of 50 mg (Fig. 4).

Effects of other anticholinergic drugs on twitches

Other anticholinergic drugs examined for these properties in fast and slow muscle were atropine, hyoscine and caramiphen. Low doses of both atropine and caramiphen intra-arterially (<8 mg) potentiated twitches of the fast muscle (maximum increases observed 45% and 85% respectively) and to a small extent those of the slow muscle (Fig. 5a and b). Higher doses depressed both muscles. In some cats these drugs produced only a depression of soleus but the muscle could still be potentiated by PMCG after recovery from the depressant effects (Fig. 5d). Hyoscine consistently depressed twitches of soleus but its effects on FHL were variable. In some cats (Fig. 5c) it produced effects similar to those of atropine and caramiphen, in others depression only was observed.

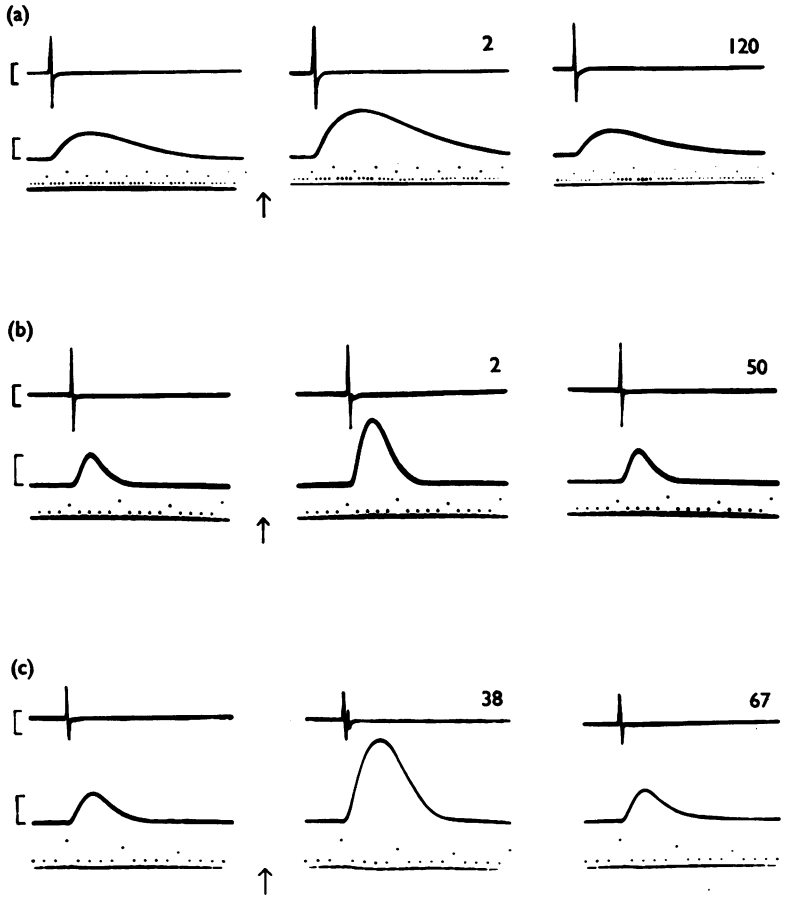


FIG. 3. Potentiation of twitch tension by PMCG in the absence of repetitive firing as determined by muscle action potentials: (a), Soleus; (b) and (c), FHL. At the arrows, PMCG intra-arterially, 5 mg in (a) and 2·5 mg in (b); at the arrow in (c), neostigmine 30 μg/kg intravenously. Note repetitive firing (not maximal) with potentiation equivalent to that in FHL after PMCG (see 3b). Numerals indicate time in minutes after drug injection. Calibration: twitch tension 200 g; muscle action potentials 2·5 mV in (a), 5 mV in (b) and (c); time base 10 ms.

Effects of drugs on tetani

The responses of both fast and slow muscles to repetitive stimulation are also modified by PMCG. The changes are dependent on dose and frequency of stimulation. For a valid comparison between the two muscles their difference in response

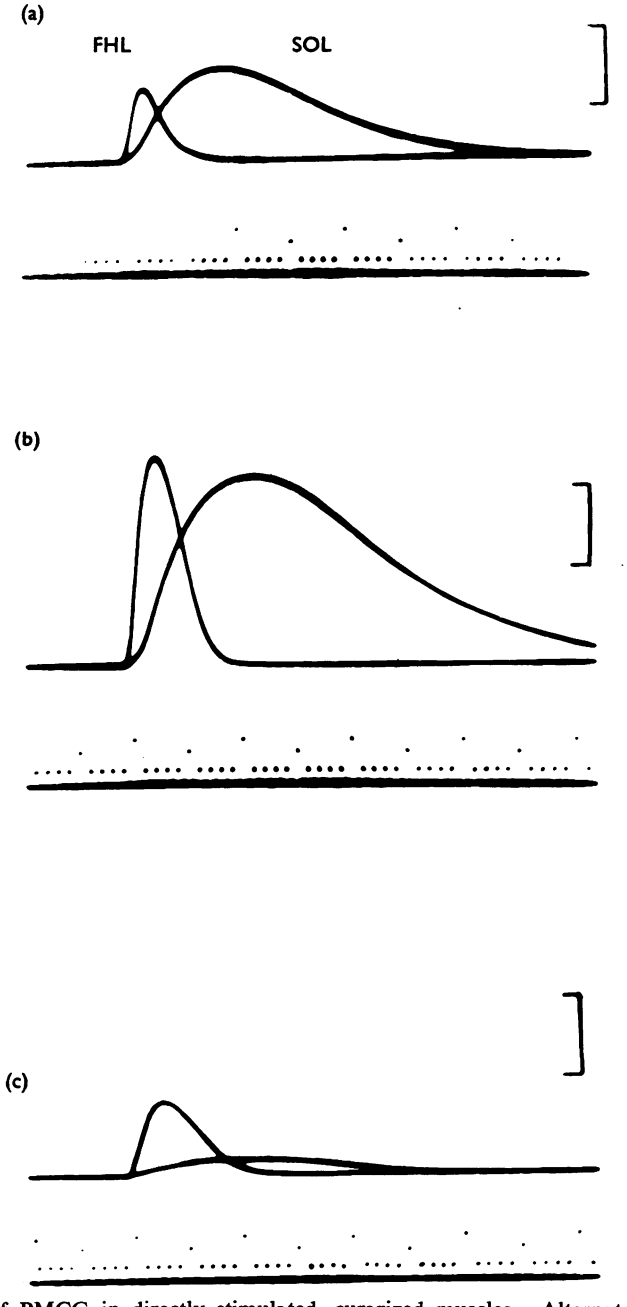


FIG. 4. Effect of PMCG in directly stimulated, curarized muscles. Alternate stimulation of soleus (SOL) and FHL. (a), Control twitches ; (b) after PMCG 5 mg intra-arterially and (c) after 50 mg intra-arterially. Calibration: twitch tension 200 g ; time base 10 ms.

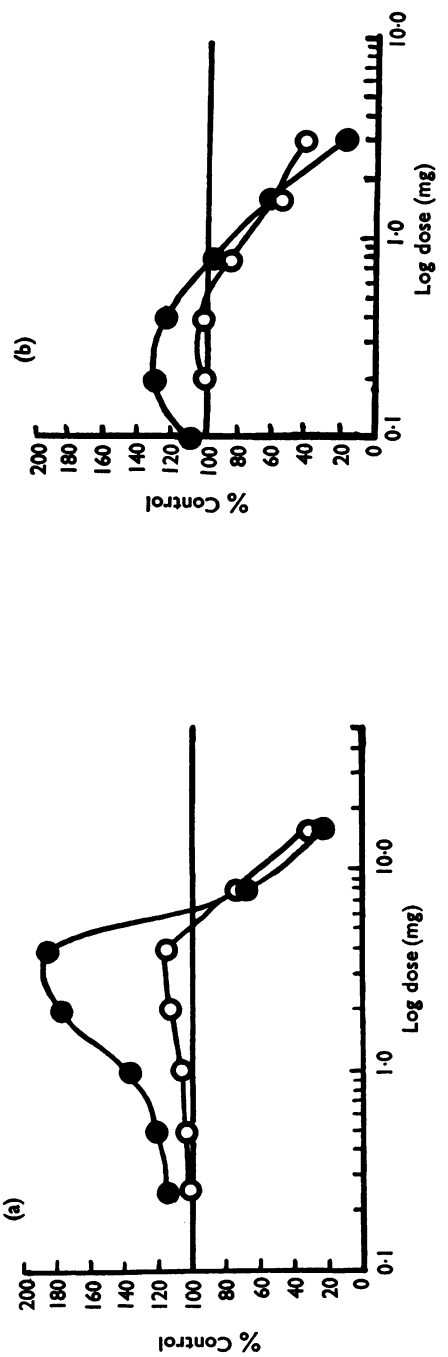


FIG. 5. Log dose-response curves for other anticholinergic drugs. Alternate stimulation of soleus (○) and FHL (●). (a), Caramiphen; (b), atropine; (c), hyoscine (compare with Fig. 2a for PMCG); (d), soleus, PMCG (○), atropine (▲), caramiphen (×). All curves in (d) were obtained on the same muscle.

to stimulation at a given frequency must be taken into account. The degree of fusion of the tension record is related to twitch speed, maximum tetanic tension occurring for soleus at about 50 Hz and for FHL at about 125 Hz, although maximum rate of tension change (as determined by the differentiated record) occurs at considerably greater frequencies of stimulation. Therefore, for the purpose of this study equivalent frequencies were chosen to suit each muscle.

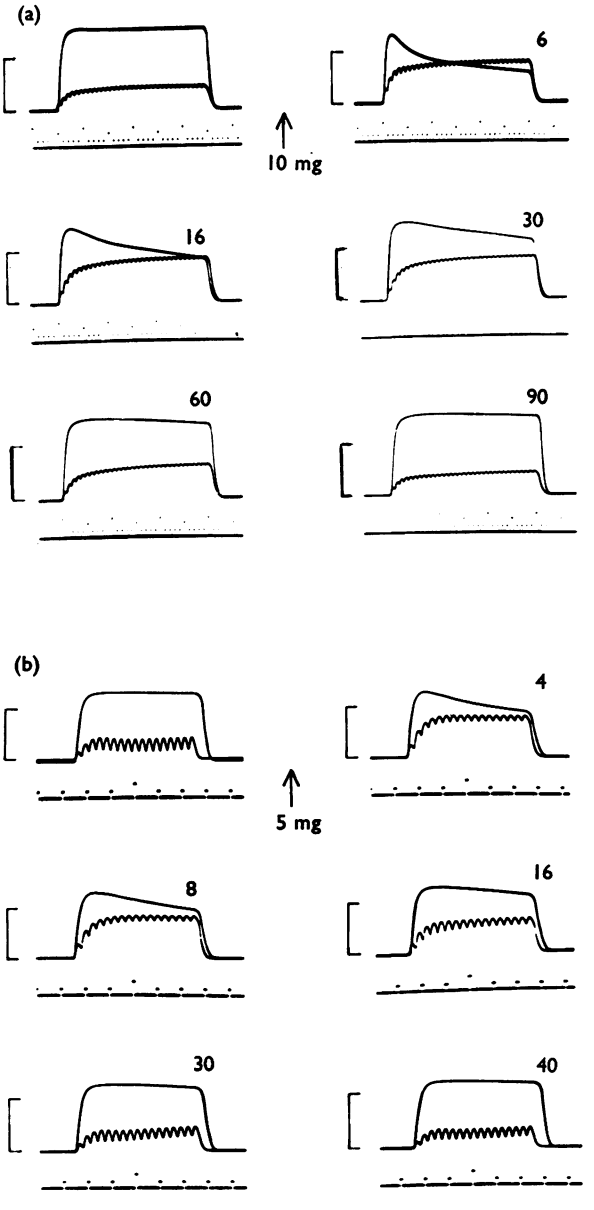


FIG. 6. Effect of PMCG on tetani at low and high frequencies. (a), Soleus stimulated at 10 and 100 Hz for 3 s; (b), FHL stimulated at 35 and 200 Hz for 0·5 s. At the arrows, PMCG intra-arterially in the doses indicated. Numerals indicate minutes after PMCG. Calibration: tetanic tension 1 kg; time base 100 ms.

Doses of PMCG which potentiated twitches also potentiated low frequency tetani (5–30 Hz for soleus, 10–50 Hz for FHL) both by augmenting the units of contraction and by increasing the degree of fusion at a given frequency. In contrast, higher frequency tetani (80 Hz and above for soleus, 150 Hz and above for FHL) were depressed by PMCG and waned during the stimulation period. These effects of

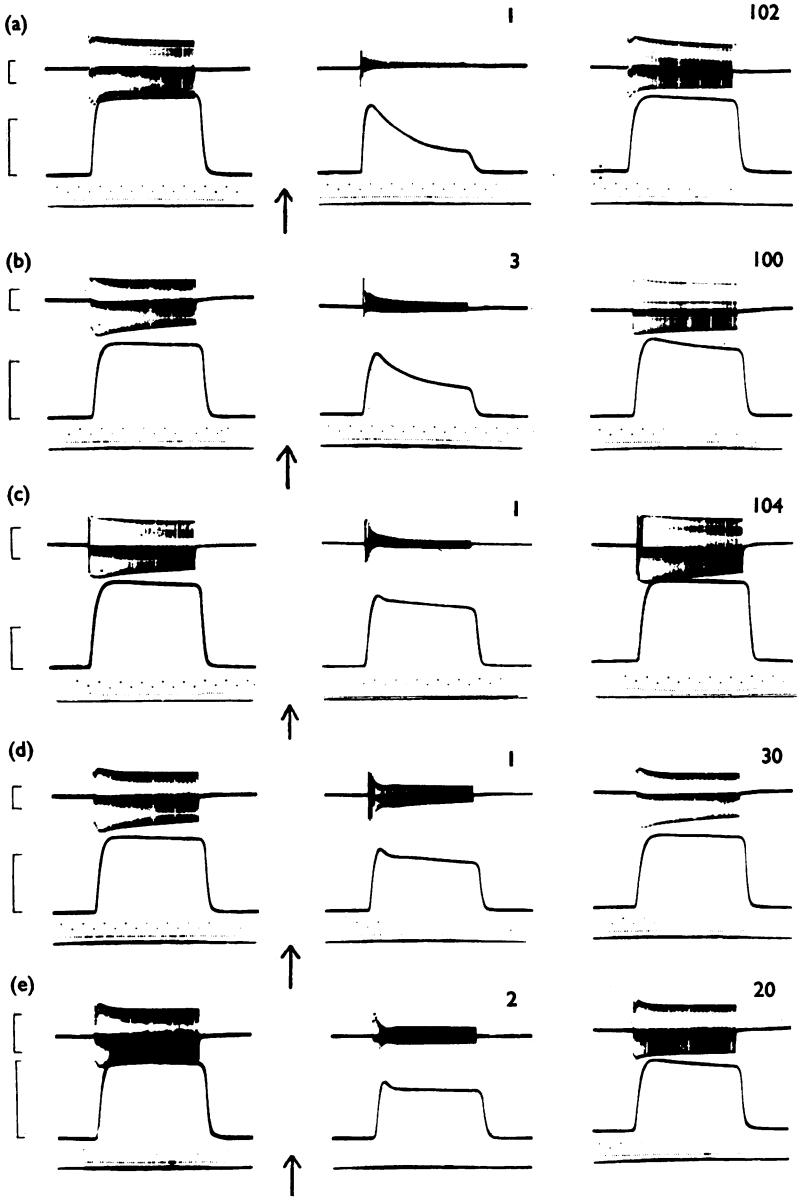


FIG. 7. Effect of various drugs on tetanic stimulation of FHL. 150 Hz for 0·5 s. At the arrows, the following drugs were injected: (a), PMCG, total 15 mg intra-arterially; (b), caramiphen, 5 mg intra-arterially; (c), atropine, total 7 mg intra-arterially; (d), hyoscine, total 20 mg intra-arterially; (e) curare, 75 μg intra-arterially. Numerals indicate time in minutes after last dose of drug. Calibration: tetanic tension 1 kg; muscle action potentials 10 mV; time base 10 ms.

PMCG are shown for the cat muscles in Fig. 6. FHL fatigues more easily than soleus with high frequency tetani of several seconds' duration. Hence, in the experiments illustrated in Fig. 6, tetanic stimulation of FHL was reduced to 0·5 s in order to facilitate recovery while that of soleus was continued for 3 s. However, the similarity between the two muscles is evident in spite of the difference in duration of tetani. Over a range of intermediate frequencies (40–60 Hz for soleus, 80–100 Hz for FHL), these opposing influences of PMCG resulted in little or no change in the appearance of tetani. Doses of PMCG sufficient to block twitches also reduced or abolished tetani at all frequencies. Simultaneous recordings of muscle action potentials show an accompanying decrease in size during the waning of tetanic tension in the presence of 5 mg PMCG (Fig. 7). Similar inhibitory effects on tetani are shown for atropine (7 mg), caramiphen (5 mg) and hyoscine (25 mg) in the same Figure. For purposes of comparison typical "Wedensky" inhibition by tubocurarine (0·5 mg intra-arterially) is also included.

Discussion

Several workers have reported that low concentrations of atropine potentiate the indirectly stimulated twitches of the rat phrenic nerve-diaphragm preparation *in vitro* (Bülbring, 1946; Dutta, 1949; Segawa, Kojima & Takagi, 1967). Large concentrations of atropine, however, depress the twitches of the diaphragm (Bülbring, 1946; Dutta, 1949) and also depress tetanic contractions and the degree of post-tetanic potentiation of the frog gastrocnemius-sciatic nerve preparation (Guttman *et al.*, 1937). Following these reports it was not surprising that similar, dose-dependent actions of atropine administered intra-arterially were observed in cat fast-twitch muscle *in vivo*. Of greater interest is the finding that the ability to potentiate supramaximal twitches and submaximal tetani is not unique to atropine among anticholinergic drugs. Particularly interesting is the extent of potentiation by the potent centrally acting drug PMCG, especially in the cat fast-twitch muscle, FHL. Caramiphen was somewhat less active in this respect, while hyoscine produced variable results, being inactive in some preparations and only weakly active in others. Only caramiphen and PMCG appreciably increased the twitches of the slow-twitch cat muscle.

It is improbable that potentiation by these anticholinergic agents is the result of any weak anticholinesterase activity they might possess. This view is supported by the lack of repetition in the action potentials and by the observation of identical effects in directly stimulated curarized muscles. Dutta (1949) similarly reported potentiation by atropine of the directly stimulated curarized rat diaphragm preparation. It can be concluded, therefore, that the site of action is beyond the neuromuscular junction—that is, within the skeletal muscle fibre itself. As far as PMCG is concerned, this accords with the work of Abood & Biel (1962) on the isolated frog sartorius muscle. These workers found that PMCG-induced increases in the isometric contractions of the muscle in Ringer solution were accompanied by prolongation of the negative after-potential. In calcium-free solution PMCG decreased the spontaneous twitching and restored the intracellular resting potential of the muscle after its partial depolarization by this treatment (Abood *et al.*, 1963b). The relationship between several compounds, all termed PMCG, and calcium, with muscle glycolysis and membrane phenomena have been studied by Abood and co-workers (see Abood, 1968). They conclude that the drugs are capable of inter-

acting with ATP, Ca^{2+} and lipophilic components in the excitatory membrane and that they interfere with the calcium-dependent excitation-contraction coupling of the sartorius muscle and can substitute for Ca^{2+} in restoring the resting potential. Abood *et al.* (1963b) found that atropine (10^{-4}M) increased twitch tension by 20% but differed from PMCG (10^{-5}M) in that it did not inhibit lactate production and that higher concentrations were required to inhibit spontaneous twitchings of frog sartorius muscles in calcium-free EDTA Ringer solution.

In the presence of PMCG the magnitude of the action potentials of individual frog muscle fibres was essentially unchanged although the negative after-potential was prolonged (Abood & Biel, 1962). Although gross muscle action potentials are considerably less accurate than those from individual fibres, our results are in general agreement with this finding. Some slight increase in the height of the action potential occasionally occurred after PMCG ; a similar observation has been made in the rat diaphragm after atropine (Kojima & Takagi, 1969).

The mechanism of the " curare-like " action of atropine has been the subject of many reports, most workers finding both similarities and discrepancies between the actions of atropine and curare at the neuromuscular junction. Adbon (1940) concluded that acetylcholine antagonism and " curare-like " action are not of the same nature in the frog gastrocnemius muscle. Doses of atropine which blocked the quick contraction in response to intra-arterially injected acetylcholine did not block indirectly elicited twitches, but high doses blocked both direct and indirect excitation. Atropine, unlike tubocurarine, does not prevent the depression of twitches of the rat diaphragm brought about by acetylcholine in the presence of an anticholinesterase (Bülbring, 1946). Atropine, in concentrations 2,000 times greater than that of tubocurarine, resembles the latter in reducing the magnitude of the end plate potentials of the rat diaphragm and in lacking presynaptic actions at these concentrations (Beranek & Vyskočil, 1967). In the frog sartorius neuromuscular junction, however, the concentration of atropine required to mimic tubocurarine is only 100 times greater (Beranek & Vyskočil, 1968), but differences in the responses of the e.p.p.s. and m.e.p.p.s. to the two drugs were noted.

It seems likely that PMCG contains some " curare-like " action since depression of indirectly stimulated muscles occurs more readily and at lower doses than that of directly stimulated muscles. However, tetanic depression in the presence of PMCG and curare differ somewhat. Thus, after PMCG, tetanic tension falls relatively smoothly during the stimulation period, while after curare, a rapid initial decline is followed by a plateau at a lower level. These differences are reflected in the amplitude of the accompanying action potentials. It is interesting to note that hyoscine and atropine both show greater curare-like characteristics than does caramiphen, which more closely resembles PMCG, both from the twitch potentiating and tetanus depressing points of view. Although the effects of caramiphen may be compared to some extent with those of both PMCG and curare, generally the initial fall in tension and action potential amplitude is less abrupt and longer-lasting than in the presence of curare and is followed by a further decline at a slower but steady rate.

As a final note, it may be significant that local anaesthetics have been reported as producing effects somewhat similar to those reported here. Thus, procaine, quinine and quinidine all increase twitch tension, at the same time prolonging the

negative after-potential (Lammers & Ritchie, 1955; Falk, 1961). Both Bülbring (1946) and Dutta (1949) found similarities between the effects of atropine and procaine on the rat diaphragm. Preliminary studies have revealed that PMCG is more potent as a local anaesthetic than atropine while hyoscine is devoid of such activity. It is planned to examine the actions of some local anaesthetics on fast-twitch and slow-twitch muscles in the cat.

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