Br. J. Pharmac. (1976), **56**, 457–467

ISOTONIC AND ISOMETRIC RESPONSES OF DIFFERENT TONIC MUSCLES TO AGONISTS AND ANTAGONISTS

M.J. MICHELSON & S.A. SHELKOVNIKOV

Pharmacological Laboratory, Sechenov Institute of Evolutionary Physiology and Biochemistry, Leningrad, K-223, Academy of Sciences, USSR

1 With isotonic recording the percentage of muscle shortening as compared with the maximal possible shortening, and with isometric recording the percentage of developed tension were determined. In relatively 'thick' muscles, such as dorsal leech muscle, frog rectus abdominis or protractor pharynx of holothuria (0.3–0.8 mm thick), the concentrations of a full agonist (carbachol) producing a given percentage of tension, (e.g. 50%) are about 5 times greater than the concentrations, producing the same percentage of shortening. In 'thin' muscles the difference between the percentage of shortening and tension is either small (retractor dentis of the sea urchin, 0.1 mm thick, response to carbachol) or absent (guinea-pig ileum, 0.06 mm thick, responses to methylfurmethide). The possible mechanism underlying this difference is discussed.

2 With partial agonists (dodecamethonium and heptamethonium) the fractional tension of the frog rectus abdominis is always less than the fractional shortening and the correlation between shortening and tension is the same as in the case of full agonists.

3 The blocking activity of (+)-tubocurarine on the frog rectus abdominis is the same in isotonic and in isometric conditions.

4 On the frog rectus abdominis the alkylating agent, decamethonium mustard, does not produce any 'parallel shift' of the dose-response curve for carbachol, the only result of alkylation being a decrease in maximal response, which is more pronounced in isometric than in isotonic conditions. The degree of decrease is in accordance with the correlation between percentage of shortening and percentage of tension in the absence of alkylating agent. Probably this muscle does not possess any 'spare receptors'.

5 On the frog muscle the dose-isometric response curve for acetylcholine (ACh) is shifted toward greater concentration about 33-fold as compared with the dose-isotonic response curve but after the inhibition of cholinesterases the shift is only about 6-fold. The same shift (5-fold) is observed for carbachol, which is not hydrolysed by cholinesterases. The results with ACh are due to the fact, that after cholinesterase inhibition the sensitivity to ACh increases in isotonic conditions only 13-fold, but in isometric conditions it increases 71-fold. Probably under isometric conditions, when the muscle remains in the extended state, the rate of hydrolysis of ACh is much greater than under isotonic conditions when the muscle is shortened during contraction.

Introduction

The isotonic response (the shortening of the muscle during its contraction) and the isometric response (the tension developed during the contraction) coincide in some muscles, but differ greatly in other muscles. Clark (1926) compared the percentage of shortening of the frog rectus abdominis muscle produced by different concentrations of acetylcholine (ACh) under isotonic conditions and the percentage of tension developed under isometric conditions, taking as 100% the maximal shortening and the maximal tension of which the tissue was capable. He found that with shortening by 50% the tension developed was negligible (Figure 1). On the other hand Colquhoun & Tattersall (1970) observed no difference between the percentage of shortening and tension of the guinea-pig ileum longitudinal muscle in response to histamine.

Some physiological observations have also been published. For example indirect stimulation of tonic fibres of frog gastrocnemius muscle in isotonic conditions produced a strong tonic shortening but the tension was less than 50 g, whereas the maximal tension under isometric conditions was about 300 g (Vereshchagin, Zhukov & Leushina, 1950). Csapo (1954; 1960) studied the contractions of rabbit uterus segments elicited by electrical stimulation and showed that with practically maximal (100%) shortening the tension was only 5%.

In the present work we tried to determine why the

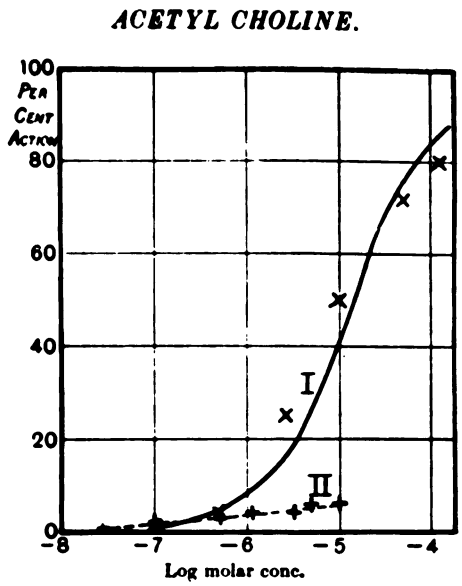


Figure 1 Action of acetylcholine on rectus abdominis as a function of concentration. I-isotonic; II-isometric. Ordinate scale: percentage of maximal possible action. Abscissa scale: logarithm of molecular concentration. From A.J. Clark (1926), *J. Physiol.*, **61**, 530–546. By permission of *The Journal of Physiology*.

correlation between shortening and tension is so different in different muscles. We also considered the importance of isotonic and isometric conditions when determining: (1) the EC_{50} values; (2) the capacity of drugs to produce a maximal contraction, showing whether it is a full or a partial agonist; (3) the blocking effect of antagonists; (4) the capacity of alkylating agents to bind covalently to the receptors; (5) the effect of anticholinesterases.

Methods

Frog rectus abdominis muscle (Rana temporaria)

A preparation of two unseparated muscles (left and right) was suspended in a bath, containing 25 ml of Ringer solution bubbled with air. For isometric recording the upper end of the muscle was connected to a tensoregister and the muscle was extended to its normal length. Even with maximal tension the muscle shortened only by 0.1 mm, that is no more than 0.2% of its length. With isotonic recording the load was 5–10% of maximal tension, that is about 1.5 g, the maximal tension being about 20 grams. In some experiments isometric responses were obtained first, and then isotonic responses, in other experiments the sequence was reversed. The response to isotonic KCl solution both in isotonic and isometric conditions was taken as 100%. The drugs were added to the bath in a volume not exceeding 0.5 ml. The interval between complete relaxation and the next contraction was 20 minutes.

Leech dorsal muscle (Hirudo medicinalis)

A piece of muscle containing 14 segments was used and the muscle was thoroughly cleaned of any traces of internal organs or nerve elements. Recordings were made as with frog muscle. In isotonic conditions the load was 1 g, the maximal tension being about 15 grams.

The pharynx protractor muscle of Holothuria (Cucumaria japonica)

This muscle was studied at the Putiatin Island Biostation in the Sea of Japan near Vladivostok. The animals were obtained by trawling and stored in a fish-pond in the sea. After excision the muscle was allowed to relax for 2 h in sea water with a load of 0.5 g and extended to its normal length of about 25 mm; this length was taken as 100%. The maximal tension developed by the muscle was 10–13 grams.

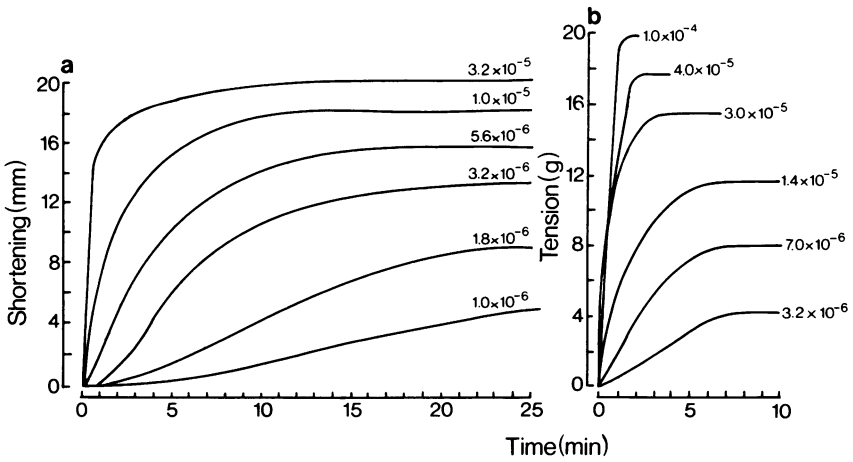
The retractor dentis muscle of the sea urchin (Strongylocentrotus droebachiensis)

This muscle was also studied at the Putiatin Biostation. With isotonic recording the load was 0.5 g; the maximal tension developed by the muscle was about 10 grams.

Guinea-pig ileum

A piece of guinea-pig ileum 5 cm long was suspended in a bath containing 5 ml Tyrode solution (bubbled with air) at pH 7.4 and 37°C. Hexamethonium (50 μg/ml) was used to block the nicotinic cholinoceptors of intestinal ganglia. The load in isotonic conditions was 400 mg, the maximal tension being about 6 grams. The drugs were added to the bath in a volume of 0.1 ml.

The full agonist used for all muscles was carbaminoylcholine (carbachol); on the frog muscle ACh was also used; on the guinea-pig ileum methylfurmethide was used. Dose-response curves were plotted from the results and the values for EC_{50} were calculated, that is, the concentration producing shortening or tension which was half of the maximum of which the tissue was capable. Heptamethylene-bis-trimethylammonium (BTM-7) and dodecamethylene-bis-trimethylammonium (BTM-12) were used as partial agonists on the frog rectus muscle. (+)-



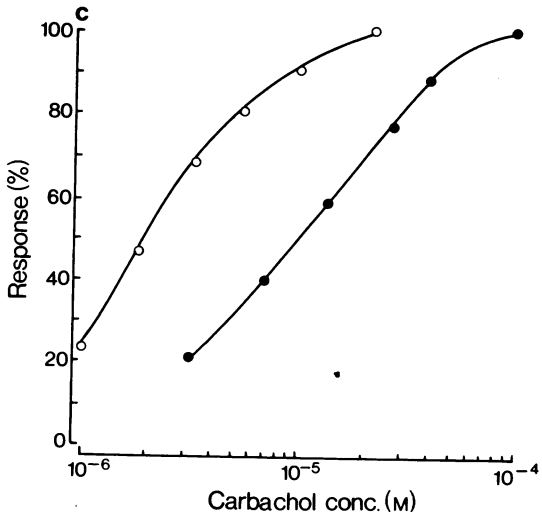
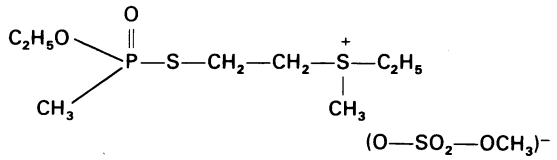


Figure 2 Frog rectus abdominis. Isotonic shortening (a) and isometric tension (b) of the same muscle, produced by different concentrations of carbachol. (c) Isotonic and isometric dose-response curves obtained in the same experiment: (O) isotonic shortening; (●) isometric tension. Both shortening and tension as % of maximum.

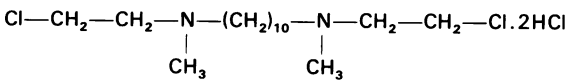
Tubocurarine was added to the bath for an hour and then the dose-response curve for carbachol was obtained and compared with the similar curve obtained without tubocurarine, both in isotonic and isometric conditions.

The irreversible organophosphorus cholinesterase inhibitor Gd-42 3×10^{-7} M was added to the bath containing the frog muscle for 30 min and then washed out for an hour. Isotonic and isometric dose-response curves were obtained before and after cholinesterase inhibition.



Gd-42

An alkylating agent A7 (decamethonium mustard) synthesized by D.V. Ioffe in S.G. Kuznetsov's laboratory was used for the irreversible block of frog rectus nicotinic cholinoceptors. This compound when



Decamethonium-mustard

dissolved in water forms an ethyleniminium ion, which alkylates the cholinoceptors. The formation of covalent bonds makes the blocking action very prolonged in spite of repeated washing. A7 was dissolved in Ringer solution at pH 7.4 and left for an hour at room temperature for formation of ethyleniminium ion. This agent was added to the bath in a concentration of 1×10^{-5} M for 1 h, after which the alkylating agent was washed out for 15 min with Ringer solution containing sodium thiosulphate 5×10^{-2}M, and then with fresh Ringer solution for 45 minutes. Sodium thiosulphate reacts with the free ethyleniminium ion and binds those molecules of the alkylating agent which have not bound covalently to the receptor. The action of A7 can be prevented by adding sodium thiosulphate (see Gill & Rang, 1966). The dose-response curves were obtained before and after alkylation.

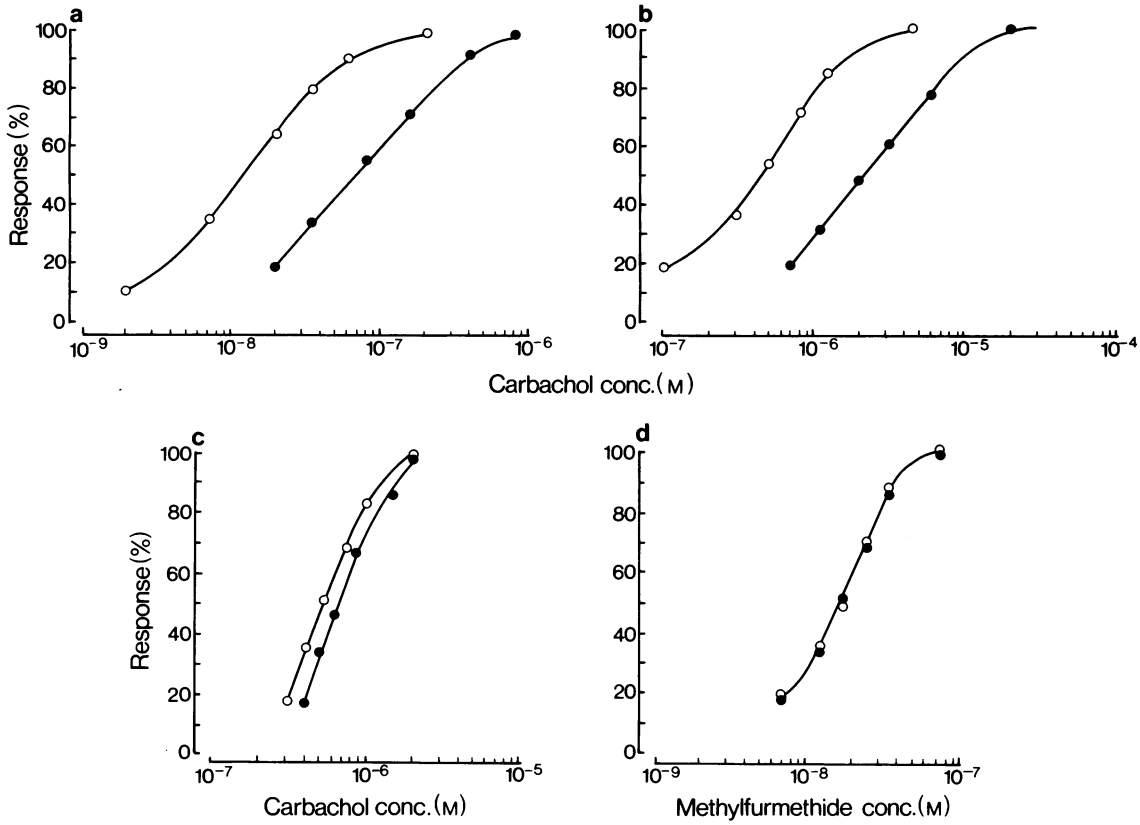


Figure 3 Isotonic and isometric dose-response curves, obtained on the same muscle: (O) isotonic shortening, (●) isometric tension, both as % of maximum. (a) Protractor pharynx of holothuria, responses to carbachol; (b) leech dorsal muscle, responses to carbachol; (c) retractor dentis of the sea urchin, responses to carbachol; (d) guinea-pig ileum, responses to methylfurmethide.

Results and Discussion

1. *The responses of different muscles to full agonists*

Frog rectus abdominus Figure 2 illustrates the isotonic (Figure 2a) and isometric (Figure 2b) responses of the frog rectus abdominis to different concentrations of carbachol. Comparison of the dose-response curves (Figure 2c) shows that a strong shortening corresponds to much less tension. For example, with a carbachol concentration producing 70% of maximal shortening only 20% of maximal tension is developed. On the other hand the carbachol concentration producing 50% tension can produce a shortening of about 90%. Table 1 shows that for this muscle the isometric EC_{50} is 5 times as great as the isotonic EC_{50}.

Holothurian pharynx protractor muscle The isotonic and isometric responses of this muscle (Figure 3a) are similar to those of frog muscle. For a given shortening in length the tension developed is much less; so with shortening by 80% the tension is only 30%. The isometric EC_{50} value is about 4.4 times as great as the isotonic value (Table 1).

Leech dorsal muscle With carbachol concentrations producing shortening of leech dorsal muscle by 80% the tension is 30% (Figure 3b). The isometric EC_{50} value is 4.3-times as great as the isotonic value (Table 1).

Sea urchin retractor dentis Both isotonic and isometric responses of the sea urchin retractor dentis develop much more rapidly than the responses of holothurian, leech or frog muscles. There is only a small discrepancy between the isotonic and the isometric dose-response curves (Figure 3c), but nevertheless the isometric curve is shifted towards greater concentrations. With shortening by 50% the

tension is 40%. The isometric EC_{50} value is only 1.5 times as great as the isotonic value (Table 1).

Guinea-pig ileum In the guinea-pig ileum both isotonic and isometric responses develop very quickly, within a few seconds. The isotonic and isometric dose-response curves are practically superimposed (Figure 3d). A given methylfurmethide concentration produces the same percentage of shortening and of tension, hence the isotonic and isometric EC_{50} coincide (Table 1).

Thus on the frog, leech and holothurian muscles a given agonist concentration, that is the same stimulus, always produces less tension than shortening. The graphic relationship between shortening and tension has the form of a hyperbola (Figure 4). On the sea urchin muscle the difference is far less pronounced, but still the tension is less than the shortening. On the guinea-pig ileum there is no difference: any given concentration of the agonist produces the same percentage of shortening and of tension.

The different properties of the muscles studied can be compared with the differences in their thickness. Great differences between shortening and tension are observed in relatively thick muscles. The muscles of frog, holothuria and leech are 0.5, 0.8 and 0.3 mm thick respectively (Table 2). With the thin sea urchin muscle (0.1 mm) the discrepancy between shortening and tension is negligible. The guinea-pig ileum muscle is even thinner (0.06 mm) and the percentage of shortening coincides with the percentage of developed tension.

It is evident that a strong shortening of 'thick' muscles may be achieved when only a part of the muscle fibres is excited. With great shortening not all the fibres are shortened actively. If the fibres are parallel the active shortening of one part of the muscle

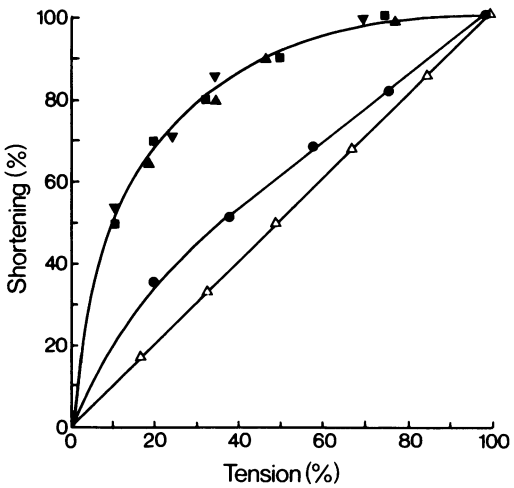


Figure 4 Relationships between shortening and tension: (■) frog rectus abdominis; (▲) protractor pharynx of holothuria; (▼) leech dorsal muscle; (●) retractor dentis of the sea urchin; (△) guinea-pig ileum. In all cases shortening and tension are expressed as % of maximum.

fibres must be accompanied by passive folding of another part. But the passively folded fibres do not contribute to the muscle tension. Therefore a given agonist concentration may produce a considerable shortening and only a small tension.

One supposition that explains why only some fibres contract actively in the thick muscles is that in low concentrations the agonist cannot diffuse quickly into the deep internal layers of the muscle and only the superficial fibres are actively contracted, whereas the

Table 1 EC_{50} values for different muscles with isotonic and isometric recording

Muscle	Agonist	EC_{50} (M) Isotonic recording (mean ± s.e.)	EC_{50} (M) Isometric recording (mean ± s.e.)	Ratio: EC_{50} isometric / EC_{50} isotonic
Frog rectus abdominis	Carbachol	$5.0 \times 10^{-6} \pm 0.7 \times 10^{-6}$ (10)	$2.5 \times 10^{-5} \pm 0.3 \times 10^{-5}$ (10)	5.0
Protractor pharynx of holothuria	Carbachol	$1.4 \times 10^{-8} \pm 0.2 \times 10^{-8}$ (15)	$6.1 \times 10^{-8} \pm 0.8 \times 10^{-8}$ (15)	4.4
Leech dorsal muscle	Carbachol	$4.0 \times 10^{-7} \pm 0.7 \times 10^{-7}$ (5)	$1.7 \times 10^{-6} \pm 0.14 \times 10^{-6}$ (5)	4.3
Retractor dentis of the sea urchin	Carbachol	$5.0 \times 10^{-7} \pm 0.6 \times 10^{-7}$ (4)	$7.0 \times 10^{-7} \pm 0.8 \times 10^{-7}$ (4)	1.4
Guinea-pig ileum	Methylfurmethide	$2.0 \times 10^{-8} \pm 0.3 \times 10^{-8}$ (10)	$2.0 \times 10^{-8} \pm 0.2 \times 10^{-8}$ (10)	1.0

The number of estimations is shown in parentheses.

deep fibres are passively folded (Figure 5a). This theory easily explains why in the quinea-pig ileum shortening coincides with tension: in a very thin muscle the agonist can act simultaneously on all fibres.

But this supposition may be correct only if the time of development and maintenance of the contracture is less than the time of diffusion of the agonist to the deep muscle fibres.

In Table 2 the time of development and maintenance of 50% shortening is compared with the time of diffusion of drugs into the deep layers of the muscles. To calculate the time of diffusion, that is the time of equalization of concentrations in the solution and in the deepest layers of muscle fibres, we used the diffusion equation $t=L^2/2D$, where t is the time in s, L=thickness of the muscle in cm and D=the diffusion coefficient (Setlow & Pollard, 1962). As we did not know the diffusion coefficient for carbachol, we used the diffusion coefficient for ACh determined on the rat diaphragm after inhibition of muscle cholinesterases, which is equal to 2.1×10^{-6} cm^2/s (Krnjević & Mitchell, 1960). The diffusion coefficients for the different drugs, ACh, decamethonium, gallamine, tubocurarine, are similar (Brookes & Mackay, 1971a, b).

One can see from the equation that the time of equalization of concentrations does not depend on the drug concentration, but only on the muscle thickness and diffusion coefficient. Hence the diffusion time will be the same with any agonist concentration in the bath. In the frog rectus, the holothurian and sea urchin muscles, and probably the leech muscle, the agonist can diffuse from both sides; therefore we used half the muscle thickness as the value of L. The thickness of the longitudinal muscle of guinea-pig ileum is not more than 60 µm (Paton & Rang, 1965). Probably the agonist can diffuse into this muscle only from one side.

Table 2 shows that the diffusion time is comparatively short in all muscles. The time of development and maintenance of 50% shortening is much greater, especially for the thick muscles. For instance the frog rectus muscle develops 50% shortening during 20 min and maintains it for hours whereas the diffusion into the muscle takes about 3.5 minutes. During the long time taken for the development and maintenance of the contraction the agonist has time to reach the deep fibres and contract them: in this case there should be no difference between the isotonic and isometric responses. These considerations therefore do not support the suggestion that the superficial fibres alone account for the isotonic response of thick muscles.

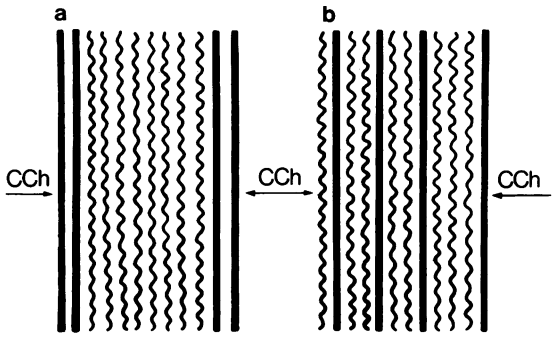


Figure 5 Two schemes of isotonic contraction of a thick muscle. (a) Only superficial fibres are actively contracted (thick straight lines) and the deep fibres are passively folded (thin twisted lines). (b) Only the most sensitive fibres are actively contracted independently of their position in the muscle (thick straight lines) and the less sensitive fibres are passively folded (thin twisted lines).

Another suggestion is that in a thick muscle the most sensitive fibres contract first, independently of their position in the muscle. The shortening of these sensitive fibres is accompanied by a passive folding of less sensitive fibres and therefore the percentage of

Table 2 Muscle thickness, the time of diffusion and the time of development and maintenance of isotonic shortening

Muscle	*Thickness (cm)*	*Half thickness (cm)*	$t=L^2/2D$	*Time of development and maintenance of 50% shortening*
Frog rectus abdominis	0.05	0.025	approx. 200 s	> 1 h
Protractor pharynx of holothuria	0.08	0.04	approx 400 s	approx. 1 h
Leech dorsal muscle	0.03	0.015	approx. 60 s	> 40 min
Retractor dentis of sea urchin	0.01	0.005	approx. 10 s	> 5 min
Guinea-pig ileum	0.006	—	approx. 10 s	approx. 1 min

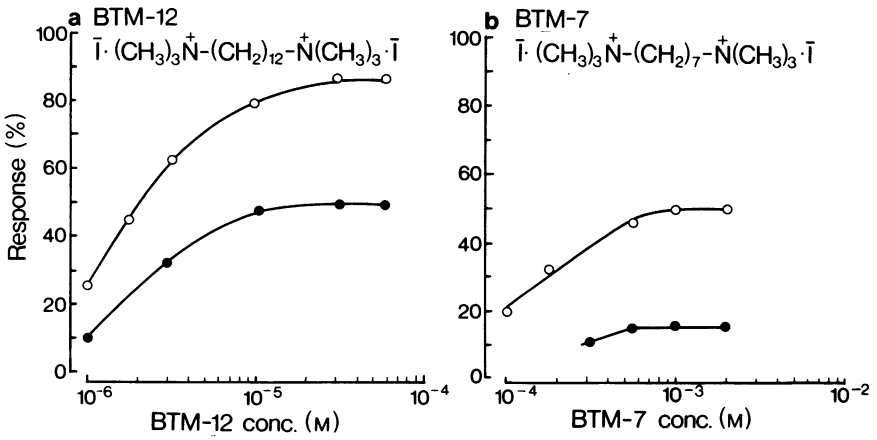


Figure 6 Frog rectus abdominis. Isotonic (O) and isometric (●) dose-response curves for partial agonists: (a) dodecamethylene-bis-trimethylammonium (BTM 12); (b) heptamethylene-bis-trimethylammonium (BTM 7). In both cases the isotonic and the isometric curves are obtained on the same muscle.

tension is always less than the percentage of shortening (Figure 5b). This suggestion seems more valid. It explains better the difference between the isotonic and isometric response of 'thick' muscles. It is more difficult to explain on this basis why isotonic and isometric responses in thin muscles are equal, because the fibres of a thin muscle may also differ in their sensitivity to an agonist. Possibly the conditions for folding of less sensitive fibres are better in a 'thick' than in a 'thin' muscle. The problem is still more complicated because the muscles studied have quite different structures.

2. *The action of partial agonists*

Each partial agonist (BTM-12 and BTM-7) was studied on five frog muscles. Figure 6a shows that the isometric dose-response curve is not only shifted towards greater concentrations as compared with the isotonic curve, but also that there is a more pronounced decrease in the maximal response. Thus the isotonic maximal response reaches 83% of shortening induced by KCl, whereas the isometric maximal response reaches 47% of the KCl-induced tension. In 5 muscles the mean isotonic maximal response was 85% ± 2%, and the mean isometric maximal response was 45% ± 2%. BTM-7 can produce only 50% shortening and the maximal tension developed is only 15% (Figure 6b). In 5 muscles the mean maximal isotonic response was 50% ± 3% and the mean maximal isometric response was 15% ± 1%.

These results are in accordance with the relationship between the isotonic shortening and isometric tension of frog rectus in response to a full agonist, carbachol. Thus if the relationship between isotonic and isometric responses to full agonists and the maximal response to a partial agonist with one mode of recording is known, it is possible to predict the maximal response with the other mode of recording.

3. *The blocking effect of (+)-tubocurarine*

On the frog rectus abdominis the isotonic and isometric responses to carbachol were obtained in the absence and in the presence of (+)-tubocurarine (2×10^{-6} M. In one series of experiments (4 muscles) the shift of isotonic responses towards greater concentrations of carbachol in the presence of tubocurarine was determined; in another series (5 muscles) the shift of isometric responses was studied. Figure 7 shows that the shift is equal in isotonic and in isometric conditions (Table 3). Such an equal shift could be predicted because the dose-response curves for carbachol are parallel with both methods of recording. Tubocurarine does not decrease either the isotonic or the isometric maximal responses; in both cases a maximal response can be obtained in the presence of tubocurarine provided the concentration of carbachol is sufficiently increased.

4. *The action of an alkylating agent A7*

On the frog rectus muscle the alkylating derivative of decamethonium, decamethonium-mustard A7, in a concentration of 1×10^{-5} M acts as a potent cholinolytic agent. A7 reduces the action of ACh, carbachol, suberyldicholine and tetramethylammonium, but does not influence the contracture produced by KCl. The action of A7 is very slowly reversible: the maximal response diminished by 50% is restored to 100% in 10 hours. Tubocurarine

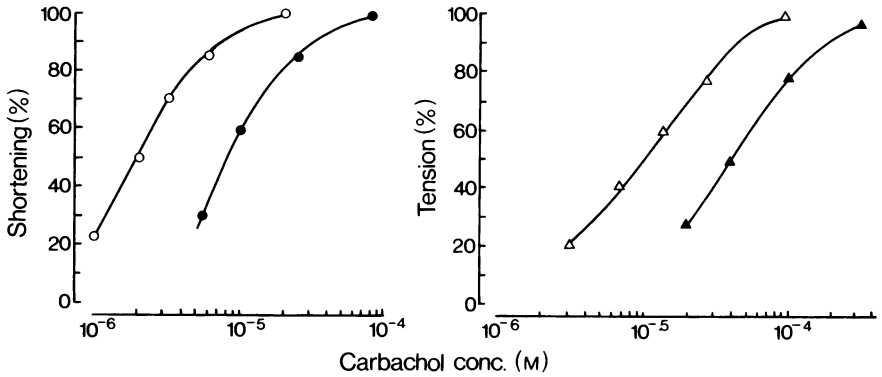


Figure 7 Frog rectus abdominis. The parallel shift of dose-response curves for carbachol towards greater concentrations in presence of (+)-tubocurarine 2×10^{-6}M: (a) isotonic responses; (b) isometric responses. In both cases the shift is about 4-fold. (● and ▲) In the presence; (○ and △) in the absence of (+)-tubocurarine.

3×10^{-5}M (the concentration blocking 99% of cholinoceptors) provides full protection from A7. A7 produces a similar block of contractions induced by ACh, carbachol and tetramethylammonium and the change of pH from 6 to 11 does not influence the alkylating activity. It may be thought therefore that A7 is blocking the anionic site of the cholinoceptor, probably a carboxylic group the ionization of which hardly changes with changes of pH within these limits.

The action of A7 on isotonic responses was studied on 5 rectus muscles and on the isometric responses of another 5 muscles. In both cases A7 1×10^{-5} M was added for 60 minutes. Figure 8 shows that A7 produced a shift in the dose-response curves both for isotonic (Figure 8a) and isometric (Figure 8b) recording toward greater concentrations but the decrease of maximal tension was more pronounced than the decrease of maximal shortening. In 5 muscles the mean decrease of the isotonic response was to $82\% \pm 2\%$ of maximal and the mean decrease of the isometric response was to $47\% \pm 1\%$ of maximal. Figure 3 shows that without alkylating agents the shortening by 82% corresponds to a tension of 40%. Thus in muscles with a great difference between shortening and tension the effect of alkylating agents will also be different and, probably, the effect in isometric conditions is more informative.

Nickerson (1956) observed that after the action of an alkylating agent on the guinea-pig ileum the dose-response curve for histamine was shifted towards greater concentrations by two orders of magnitude. Nickerson concluded that only a small fraction of the receptor pool was necessary for production of maximal response, all others being 'spare' (or 'reserve') receptors which can be blocked without preventing the muscle from developing a maximal response provided the agonist concentration is sufficiently high. Waud (1968) doubted this interpretation because Nickerson recorded isotonic responses and administered the alkylating agent for a short time. Waud supposed that only superficial cells were blocked, but not the deep ones, and concluded that Nickerson was examining not spare receptors but spare cells. But on the guinea-pig ileum the isotonic

Table 3 Isotonic and isometric EC_{50} values for carbachol on the frog rectus abdominis in the presence and in the absence of (+)-tubocurarine (Tbc)

Mode of recording	EC_{50} (M) before tubocurarine	EC_{50} (M) in presence of tubocurarine 2×10^{-6} M	Dose-ratio: EC_{50} after Tbc / EC_{50} before Tbc
Isotonic	$5.0 \times 10^{-6} \pm 0.8 \times 10^{-6}$ (4)	$2.1 \times 10^{-5} \pm 0.3 \times 10^{-5}$ (4)	4.2
Isometric	$2.5 \times 10^{-5} \pm 0.4 \times 10^{-5}$ (4)	$1.0 \times 10^{-4} \pm 0.1 \times 10^{-4}$ (4)	4.0

The number of experiments is shown in parentheses.

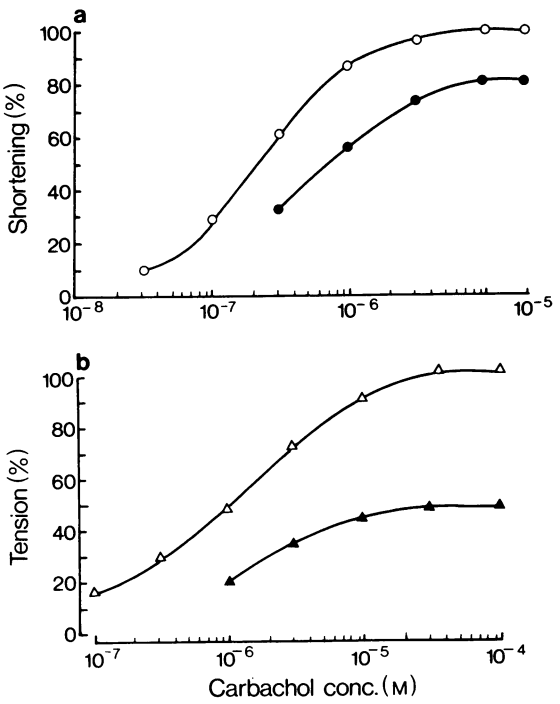


Figure 8 Frog rectus abdominis. The blockade of responses to carbachol after the action of the alkylating agent decamethonium-mustard (A7) 1×10^{-5}M during one hour: (a) dose-isotonic response curves before (○) and after (●) the action of the alkylating agent; (b) dose-isometric response curves before (△) and after (▲) the action of the alkylating agent.

and isometric responses coincide, indicating that there are no spare cells. Indeed, we observed a similar action of alkylating agents (benzilylcholine-mustard and others) on isotonic and isometric responses of guinea-pig ileum to methylfurmethide.

On the frog rectus 60% of tension corresponds to nearly 100% of shortening, hence there is no more than 40% of spare cells in this muscle. Thus after the action of an alkylating agent the isometric dose-response curve can be shifted toward greater concentrations (due to spare fibres) only 1.5-fold more than the isotonic curve. In our experiments on this muscle with A7 (1×10^{-5} M for 1 h) both isotonic and isometric responses to carbachol diminished at once and no parallel shift of dose-response curves was obtained. This invites the conclusion that there are no spare fibres and no spare receptors in this muscle.

5. *Isotonic and isometric responses of the frog rectus abdominis to acetylcholine: the effect of anticholinesterases*

The dose-isometric response curve for ACh is markedly shifted toward greater concentrations as compared with isotonic responses (Figure 9a), the mean dose-ratio being about 33 (Table 4) whereas for carbachol it is about 5. It seems probable that the greater dose-ratio in the case of ACh is due to the difference in the rate of its hydrolysis by the muscle in isotonic and in isometric conditions. To check this supposition we determined the dose-ratio for ACh after inhibition of muscle cholinesterases. The irreversible organophosphorus cholinesterase inhibitor Gd-42 in a concentration of 3×10^{-7} M was added to the bath for 30 min (this produces complete inhibition

Table 4 Isotonic and isometric responses of the frog rectus abdominis to acetylcholine before and after the inhibition of muscle cholinesterases by an organophosphorus inhibitor Gd-42

	EC_{50} (M)		Dose-ratio: $\frac{EC_{50} \text{ before blockade}}{EC_{50} \text{ after blockade}}$
	before the inhibition of cholinesterases	*after the inhibition of cholinesterases*	
Isotonic responses	$4.5 \times 10^{-6} \pm 0.5 \times 10^{-6}$ (10)	$3.5 \times 10^{-7} \pm 0.4 \times 10^{-7}$ (10)	13
Isometric responses	$1.5 \times 10^{-4} \pm 0.3 \times 10^{-4}$ (5)	$2.1 \times 10^{-6} \pm 0.2 \times 10^{-6}$ (5)	71
Dose-ratio: Isometric EC_{50} / Isotonic EC_{50}	33	6	

The number of experiments is shown in parentheses.

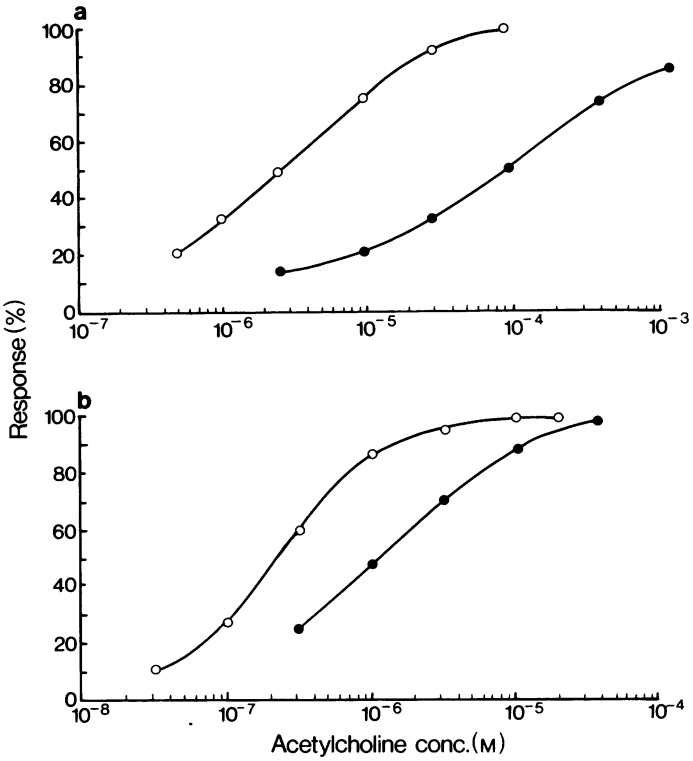


Figure 9 Frog rectus abdominis. Isotonic (O) and isometric (●) dose-response curves; (a) before exposure to the cholinesterase inhibitor; (b) after exposure to an irreversible cholinesterase inhibitor, Gd-42 3×10^{-7} M, for 30 minutes.

of muscle cholinesterases), and then washed out for one hour. After this the mean dose-ratio of EC_{50} tension and EC_{50} shortening was not 33 but only about 6 (Table 4). After the inhibition of cholinesterases the isotonic dose-response curve was shifted 13-fold toward lesser concentrations, but the isometric dose-response curve was shifted 71-fold in the same direction (Table 4). Probably the rate of ACh hydrolysis by an extended muscle (isometric recording) is much greater than by a contracted (shortened) muscle (isotonic recording). Possibly the negligible increase of tension on the frog rectus muscle in response to ACh observed by Clark (1926) (see Figure 1) was due to the fact that in his experiments the muscle cholinesterases were not inhibited. Mittag, Ehrenpreis & Patrick (1971) have shown that the rate of ACh hydrolysis by an extended piece of guinea-pig ileum is much greater than by a contracted piece of ileum.

On the holothurian pharynx protractor without anticholinesterases we obtained similar results: the dose-ratio between isometric EC_{50} and isotonic EC_{50} was about 100. Unfortunately it was not possible to check this ratio after the inhibition of cholinesterases because the anticholinesterases induce spontaneous contractions of the muscle, but for carbachol the mean ratio was about 4.4 (Table 1).

Hence when studying agonists which are hydrolysed by cholinesterases it is important to take into account the fact that the rate of hydrolysis may be much greater with isometric recording than with recording under isotonic conditions.

We thank Dr Edith Bülbring for valuable criticism and help in the preparation of this manuscript.

References

BROOKES, N. & MACKAY, D. (1971a). Rate of onset and offset of neuromuscular block in the isolated rat diaphragm. *Br. J. Pharmac.*, **41**, 339–343.

BROOKES, N. & MACKAY, D. (1971b). Diffusion of labelled substances through isolated rat diaphragm. *Br. J. Pharmac.*, **41**, 367–378.

CLARK, A.J. (1926). The reaction between acetylcholine and muscle cells. *J. Physiol.*, **61**, 530–546.

COLQUHOUN, D. & TATTERSALL, M.L. (1970). Rapid histamine assays: a method and some theoretical considerations. *Br. J. Pharmac.*, **38**, 241–252.

CSAPO, A. (1954). Dependence of isometric tension and isotonic shortening of uterine muscle on temperature and on strength of stimulation. *Am. J. Physiol.*, **177**, 348–354.

CSAPO, A. (1960). Molecular structure and function of smooth muscle. In *The Structure and function of smooth muscle*, ed. Bourne, G.H., Vol. I, pp. 229–264. New York: Academic Press.

GILL, E.W. & RANG, H.P. (1966). An alkylating derivative of benzilylcholine with specific and long-lasting parasympatholytic activity. *Mol. Pharmac.*, **2**, 284–297.

KRNJEVIĆ, K. & MITCHELL, J.F. (1960). Diffusion of acetylcholine in agar cells and in the isolated rat diaphragm. *J. Physiol.*, **153**, 562–572.

MITTAG, T.W., EHRENPREIS, S. & PATRICK, P. (1971). Some properties of cholinesterases in intact guinea-pig ileum in vitro. *Arch. int. pharmacodyn.*, **191**, 270–278.

NICKERSON, M. (1956). On receptor occupancy and tissue response. *Nature, Lond.*, **178**, 697–698.

PATON, W.D.M. & RANG, H.P. (1965). The uptake of atropine and related drugs by intestinal smooth muscle of guinea-pig in relation to acetylcholine receptors. *Proc. Roy. Soc. B.*, **163**, 1–44.

SETLOW, R.B. & POLLARD, E.C. (1962). *Molecular Biophysics*. Massachusets, USA, London, England: Addison-Wesley Inc.

VERESHCHAGIN, S.M., ZHUKOV, E.K. & LEUSHINA, L.I. (1950). The problem of the role of parabiotic excitation in the tonic contraction of striated muscle. *Physiolog. J. USSR*, **34**, 673–678. (In Russian.)

WAUD, D.R. (1968). Pharmacological receptors. *Pharmac. Rev.*, **20**, 49–88.

(Received October 1, 1975.)