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Interaction of the Neuromuscular Blocking Drug Atracurium with Muscarinic Acetylcholine Receptors

J. MUSÍLKOVÁ[1], L.A. STARSHINOVA[2], S.A. SHELKOVNIKOV[2], S. TUČEK[1]

[1]*Institute of Physiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia, and* [2]*Sechenov Institute of Evolutionary Physiology and Biochemistry, USSR Academy of Sciences, Leningrad, USSR*

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Summary

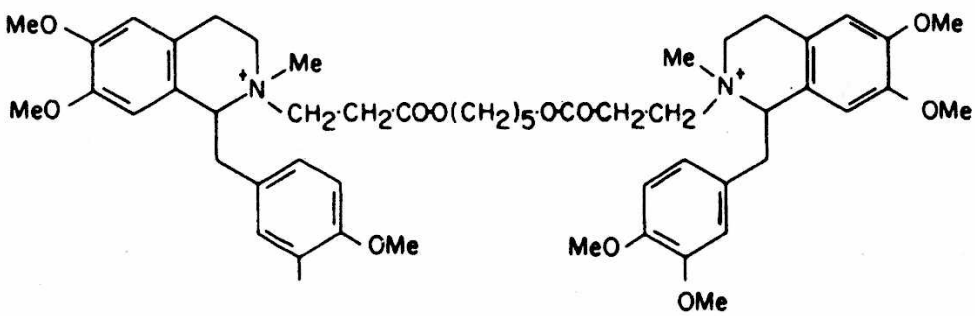
On isolated rat heart atria, atracurium competitively antagonized the negative chronotropic effect of methylfurmethide, shifting the concentration-response curve to the right without diminishing the agonist's maximal effect; K_d calculated from dose ratios was 3.0 μmol/l. On the longitudinal muscle of rat ileum, atracurium antagonized the effect of methylfurmethide in a non-competitive manner; at 50 μmol/l atracurium, the maximum response to methylfurmethide was diminished by about 50 %. Atracurium antagonized the binding of (^{3}H)quinuclidinyl benzilate ((^{3}H)QNB) to muscarinic binding sites in the atria, ileal longitudinal muscle and cerebellum with IC_{50} values of 5 – 8 μmol/l, and in brain cortex of 25 μmol/l. Atracurium was little efficient, however, in antagonizing the binding of N-(^{3}H-methyl) scopolamine ((^{3}H)NMS) to muscarinic binding sites. Complete blockade was not achieved at concentrations up to 1 mmol/l. Concentrations required to diminish the binding by 50 % were 10 – 1000 times higher for (^{3}H)NMS than for (^{3}H)QNB. Atracurium brought about the dissociation of (^{3}H)QNB-receptor complexes, but its effect was considerably stronger at a concentration of 30 μmol/l than at 1 mmol/l. Atracurium slowed down the dissociation of (^{3}H)QNB-receptor complexes observed after the addition of atropine. The effects of atracurium on the dissociation of (^{3}H)NMS-receptor complexes were similar to those on (^{3}H)QNB-receptor complexes, but a high concentration of atracurium (1 mmol/l) produced a transient increase in (^{3}H)NMS binding preceding its subsequent dissociation. Although the observations of the antagonism by atracurium of the effect of methylfurmethide on the heart atria, and of the inhibition of the specific binding of (^{3}H)QNB to the atria, ileal smooth muscle, cerebellum and brain cortex are compatible with the assumption of a competitive interaction, the discrepancy between the effects of atracurium on the binding of (^{3}H)QNB and (^{3}H)NMS indicates that atracurium does not bind to the same binding site as (^{3}H)QNB and (^{3}H)NMS. It appears that most effects of atracurium on muscarinic receptors are allosteric and that both negative and positive cooperativities play a role in interactions between atracurium and muscarinic ligands.

Key words:
Muscarinic receptors – Atracurium

Introduction

Several of the drugs that are being used as neuromuscular blocking agents are known to have antimuscarinic effects (reviews Kharkevich and Shorr 1983, Mitchelson 1987, 1988). The antimuscarinic action of neuromuscular blockers has attracted considerable attention not only because of the risk that it might produce unwanted side effects in patients, but also because its investigation revealed new features of muscarinic receptors and of the regulation of their binding properties.

The most widely studied neuromuscular blocker with antimuscarinic effects is gallamine, noted to be cardioselective in its autonomic action (Riker and Wescoe 1951, Nedoma *et al.* 1985). The analysis of the effects of gallamine on the binding of radiolabelled muscarinic antagonists led to the conclusion that gallamine acts not only on the classical muscarinic binding site, but also (or exclusively, in the view of some investigators) on an allosteric binding site, from which it alters the binding properties of the classical binding site of muscarinic receptors (Stockton *et al.* 1983, Dunlap and Brown 1983, Ellis and Lenox 1985b, Nedoma *et al.* 1986, Narayanan and Aronstam 1986, Burke 1986, Lee and El-Fakahany 1988). The allosteric effects of gallamine include both a decrease in the binding of muscarinic antagonists and a stabilization of the receptor-antagonist complexes that had already been formed. Alcuronium, another neuromuscular blocking agent, has been found to exert positive cooperativity *vis-a-vis* the binding of N-(^{3}H-methyl)scopolamine((^{3}H)NMS) but not of (^{3}H)quinuclidinyl benzilate ((^{3}H)QNB) (Nedoma *et al.* 1987, Tuček *et al.* 1990).



ATRACURIUM

Fig. 1
Chemical formula of atracurium.

Recently, a new synthetic neuromuscular blocking agent has been introduced into clinical practice under the name of atracurium (Payne and Utting 1983, for formula see Fig. 1). As there are no published data available with regard to its possible interaction with muscarinic receptors, we decided to investigate its effects on rat heart atria and ileal longitudinal muscle (muscarinically stimulated with methylfurmethide) and on the binding of labelled muscarinic antagonists (^{3}H)QNB and (^{3}H)NMS to homogenates of the heart atria, ileal smooth muscle, brain cortex and cerebellum. We have found that atracurium interacts with muscarinic receptors in a more complex way than other neuromuscular blockers, but we refrained from

investigating the complex interaction in full detail and report its basic features revealed with elementary approaches.

Methods

Materials

Atracurium besylate was provided by Wellcome Foundation, Berkhamsted, England. L-(-)-quinuclidinyl benzilate (benzil-4,4'-^{3}H) was from Amersham International, Amersham, England, and N-(^{3}H)methyl-scopolamine was from Du Pont de Nemours, NEN Research Products, Dreieich, F.R.G.

Experiments on isolated atria and ileum

Wistar albino rats of both sexes and of 160 – 190 g body weight were used. Isolated heart atria and the longitudinal smooth muscle of the ileum (prepared according to Rang 1964) were bathed in Krebs solution of the following composition (mmol/l): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11.1. The solution was bubbled through with 95 % O_2 + 5 % CO_2 and kept at 37 °C . On the atria, contractions were recorded isotonically and the chronotropic effect of muscarinic agents was evaluated. On the ileal smooth muscle, contractions were registered isometrically. The evaluation of the antagonistic action of atracurium was performed (Edinburgh Staff 1970) with methylfurmethide as the agonist .

Radioligand binding experiments

Wistar female rats of 180 – 220 g body weight were used. Tissues were homogenized in an all-glass homogenizer and an ice-cold solution of the following composition (mmol/l): NaCl 137, KCl 5, $CaCl_2$ 2, $MgSO_4$ 1, Na_2HPO_4 1, Na-HEPES (N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonate)) 10 (pH 7.4). The homogenization medium was slightly modified in experiments with (^{3}H)NMS binding to ileal longitudinal muscles; in these experiments, the Ca^{2+} and Mg^{2+} ions were replaced by Na^+ ions and the medium was supplemented with 0.5 mmol/l EDTA and 1 mmol/l phenylmethylsulfonyl fluoride. Atrial and ileal homogenates were centrifuged (10 min, 700 x g) and the sediment was discarded; the supernatants were stored at −20 °C and used within less than 4 weeks. Cerebrocortical and cerebellar homogenates were used immediately after preparation.

Incubations for radioligand binding and dissociation were at 38 °C and the composition of the incubation medium corresponded to that of the homogenization medium with added radioligand and atracurium. In experiments with (^{3}H)NMS binding to the ileum, 0.2 ml of the homogenate (prepared in the modified medium) was diluted by 0.4 ml of the unmodified medium and used for incubation. Final incubation volume was 2.0 ml in experiments with (^{3}H)QNB and 0.6 ml in those with (^{3}H)NMS; the incubation tubes contained 20 – 40 fmol of (^{3}H)QNB binding sites in experiments with (^{3}H)QNB) and 30 – 60 fmol in those with (^{3}H)NMS. (^{3}H)QNB was used at a concentration of 100 pmol/l both in experiments with the inhibition of radioligand binding and in experiments with radioligand dissociation. The duration of incubation is indicated in the Results section at each series of experiments. Incubations were stopped by filtration on Whatman GF/C glass fibre filters with immediate washing the tubes and filters with 4 x 5 ml of ice-cold Na phosphate buffer (10 mmol/l, pH 7.4). Radioactivity retained on the filters was measured using Bray (1960) scintillation solution and Beckman LS 7800 scintillation spectrometer. Parallel incubations with 1 μmol/l atropine sulphate were performed to determine non-specific binding.

Results

Pharmacological experiments

Atracurium was found to antagonize the negative chronotropic effect of methylfurmethide on the heart atria (Fig. 2). Increasing concentrations of atracurium brought about strictly parallel rightward shifts of the concentration-response curves for methylfurmethide, without diminishing its maximal effect, suggesting that atracurium acted as a competitive antagonist. Apparent K_d value for the binding of atracurium to the heart atria, calculated from dose ratios obtained in five experiments illustrated by Fig. 2 (Gaddum 1957), was 3.0 ± 0.5 μmol/l (mean ± S.E.M., Tab. 1).

On the longitudinal muscle of the ileum, atracurium produced non-parallel rightward shifts of the concentration-response curve for methylfurmethide; the curve became flattened and, at 50 μmol/l atracurium, maximum response to methylfurmethide diminished by approximately 50 % (Fig. 2). The effect of atracurium on the ileum developed slowly; it took about 3 h to reach equilibrium.

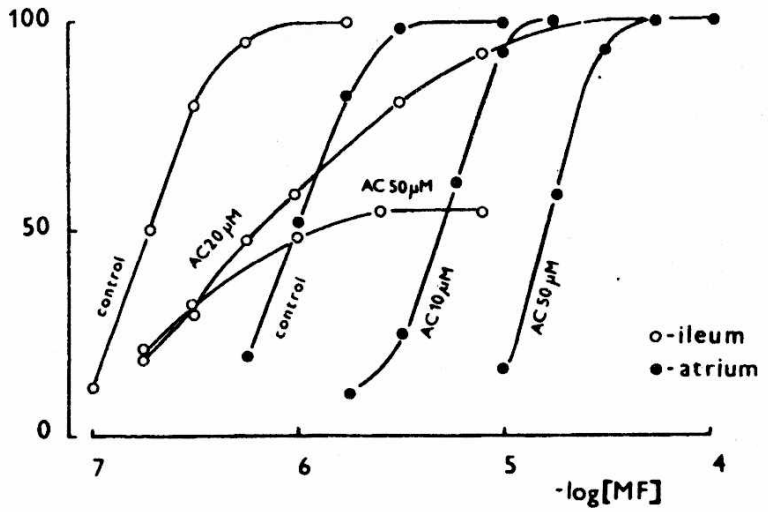


Fig. 2
Concentration-response curves for the action of methylfurmethide on the frequency of atrial contractions (closed circles) and on the tension (isometric contraction) of the ileal longitudinal smooth muscle (open circles) in the absence or presence of atracurium (AC) at concentrations indicated in the Figure. Abscissa: negative logarithm of the concentration of methylfurmethide. Ordinate: per cent decrease in the rate of spontaneous contractions (for the atria) or per cent of maximum contraction (for the ileum). Data from a single experiment which have been confirmed in four other experiments.

Inhibition of (^{3}H)QNB binding

Low-speed supernatants obtained from the homogenates of heart atria and ileal longitudinal muscles and complete homogenates of the cerebellum and brain cortex were incubated 120 min in the presence of 100 pmol/l (^{3}H)QNB and increasing concentrations of atracurium (Fig. 3). Atracurium inhibited the specific

(atropine displaceable) binding of (^{3}H)QNB with IC_{50} values of 7.7, 5.6, 6.1, and 25.5 μmol/l in the atria, ileal smooth muscle, cerebellum and brain cortex, respectively (Tab. 1). The binding of (^{3}H)QNB was completely prevented at high (> 1 mmol/l) concentrations of atracurium. The displacement curve for the cortex was shifted to the right. Hill slope factors n_H were 0.86, 0.95, 0.60 and 0.77 in the atria, ileum, cerebellum and brain cortex, respectively (Tab. 1).

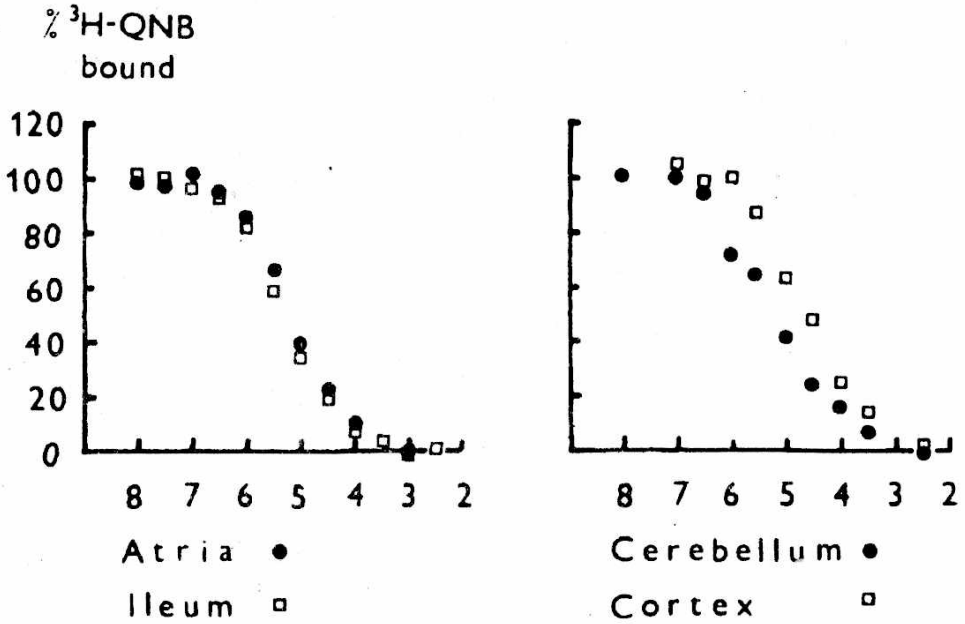


Fig. 3
Displacement of specific (^{3}H)QNB binding by atracurium. Abscissa: negative logarithm of the concentration of atracurium (mol/l). Ordinate: per cent of the binding in the absence of atracurium. Points are means of three experiments in the atria and ileum and of two experiments in the cerebellum and brain cortex. Each experiment was performed with duplicate incubations for total and duplicate incubations for non-specific binding.

Inhibition of (^{3}H)NMS binding

During 60 min incubations of low-speed supernatants or homogenates with 350 pmol/l (^{3}H)NMS, the inhibition of the binding by atracurium was much smaller than that observed with (^{3}H)QNB (Fig. 4). The displacement curves obtained in the atria, cerebellum and cortex were flat and a complete inhibition of (^{3}H)NMS binding could be demonstrated in none of the tissues examined, not even with 3 mmol/l atracurium. Concentrations of atracurium needed to diminish the binding of (^{3}H)NMS by 50 % (IC_{50}) extrapolated from Fig. 4 were 1.0, 0.5 and 0.2 mmol/l in the atria, cerebellum and brain cortex, respectively (Tab. 1). In the ileum, the binding of (^{3}H)NMS remained higher than 50 % at atracurium concentrations of up to 3 mmol/l.

In experiments with ileal smooth muscle, atracurium at concentrations of 10 and 30 μmol/l produced a significant increase ($p < 0.05$ and $p < 0.01$, respectively) in the binding of (^{3}H)NMS, which was followed by an inhibition of binding at higher concentrations. It was a characteristic feature of results obtained with (^{3}H)NMS that its binding also appeared slightly (insignificantly, but consistently) increased at certain concentrations of atracurium in tissues other than the ileum (0.3 μmol/l in the atria and cortex and 0.1 μmol/l in the cerebellum).

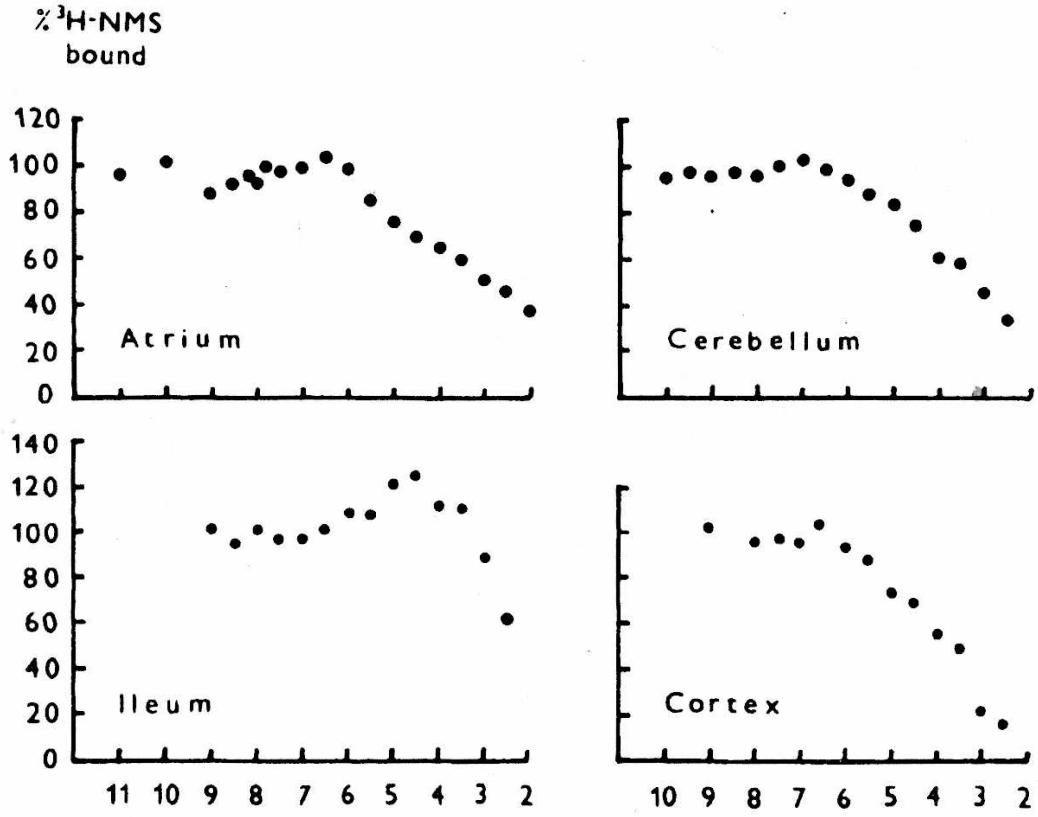


Fig. 4

Displacement of specific (^{3}H)NMS binding by atracurium. Abscissa: Negative logarithm of the concentration of atracurium (mol/l). Ordinate: per cent of the binding in the absence of atracurium. In the heart atria, data in the range of $10^{-7} - 10^{-3}$ mol/l atracurium are means of 5 experiments, while the other points are means of 1 – 4 experiments. In the ileal longitudinal muscle, data in the range of $10^{-6.5} - 10^{-3}$ mol/l atracurium are means of 3 experiments end the other points are means of 2 experiments. In the cerebellum, the data are means of 2 – 3 experiments, and in the brain cortex of 2 experiments. Each experiment was performed with duplicate incubations for total and duplicate incubations for non-specific binding.

Dissociation of (^{3}H)QNB-receptor complexes

Low speed supernatants of heart atria were incubated with 100 pmol/l (^{3}H)QNB for 80 min to reach equilibrium binding; atropine and/or atracurium was then added and the time course the disappearance of (^{3}H)QNB-receptor complexes

was followed (Fig. 5). Within 60 min, the amount of bound (^{3}H)QNB diminished by 74 % in the presence of 1 μmol/l atropine, by 49 % in the presence of 30 μmol/l atracurium and 11 % in the presence of 1 mmol/l atracurium. Atracurium diminished the effect of atropine: in the presence of 1 μmol/l atropine plus 30 μmol/l atracurium, the amount of bound (^{3}H)QNB diminished by 62 %, and in the presence of 1 μmol/l atropine plus 1 mmol/l atracurium by only 16 % within 60 min (Fig. 5).

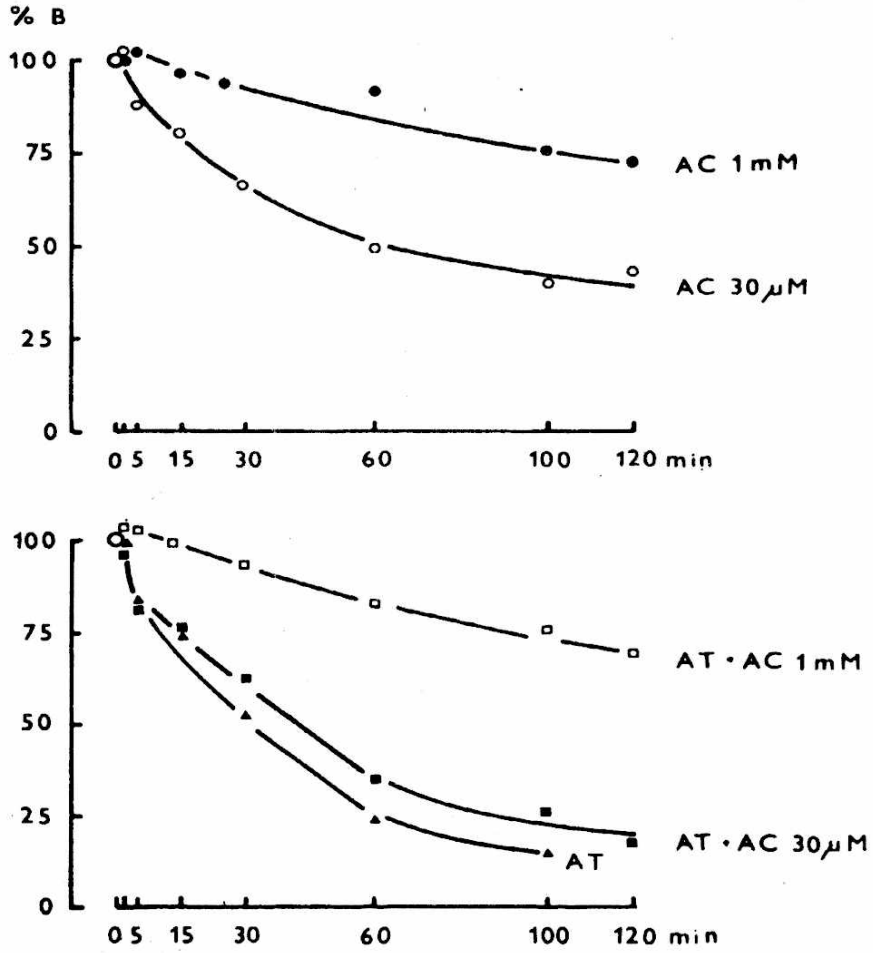


Fig. 5
Dissociation of (^{3}H)QNB-receptor complexes in atrial membranes after the addition of (top) atracurium (AC) 1 mmol/l (full circles), atracurium 30 μmol/l (open circles), or (bottom) of atropine (AT) 1 μmol/l (closed triangles), atropine 1 μmol/l plus atracurium 30 μmol/l (closed squares), or of atropine 1 μmol/l plus atracurium 1 mmol/l (open squares). Atropine and atracurium were added to the incubation tubes after 80 min preincubation of the homogenate with (^{3}H)QNB (100 pmol/l) and the amounts of bound (^{3}H)QNB recovered after 2 – 120 min were compared with (and expressed as per cent of) the amount of bound (^{3}H)QNB recovered at the end of the preincubation. Data for 15, 30 and 60 min are means of at least 4 incubations, while those for the other time points are means of 2 incubations. The lines were drawn by eye.

Dissociation of (^{3}H)NMS-receptor complexes

The addition of 1 μmol/l atropine to (^{3}H)NMS-receptor complexes preformed during 80 min preincubation was followed by rapid dissociation of (^{3}H)NMS; within 2 min the amount of bound (^{3}H)NMS diminished to 13 % of the initial (equilibrium) value (Fig. 6). The addition of 30 μmol/l atracurium was followed by a slow dissociation of (^{3}H)NMS (with 71 % of (^{3}H)NMS remaining bound after 60 min), while the addition of 1 mmol/l atracurium was followed by a transient increase in the binding of (^{3}H)NMS (to 115 % of the initial value after 2 min ($p < 0.05$), with subsequent extremely slow dissociation (79 % of (^{3}H)NMS still remaining bound after 140 min). When 30 μmol/l atracurium was added simultaneously with atropine, the dissociation was slightly slower than with atropine alone, and the rate of atropine-induced dissociation was markedly slowed down by 1 mmol/l atracurium.

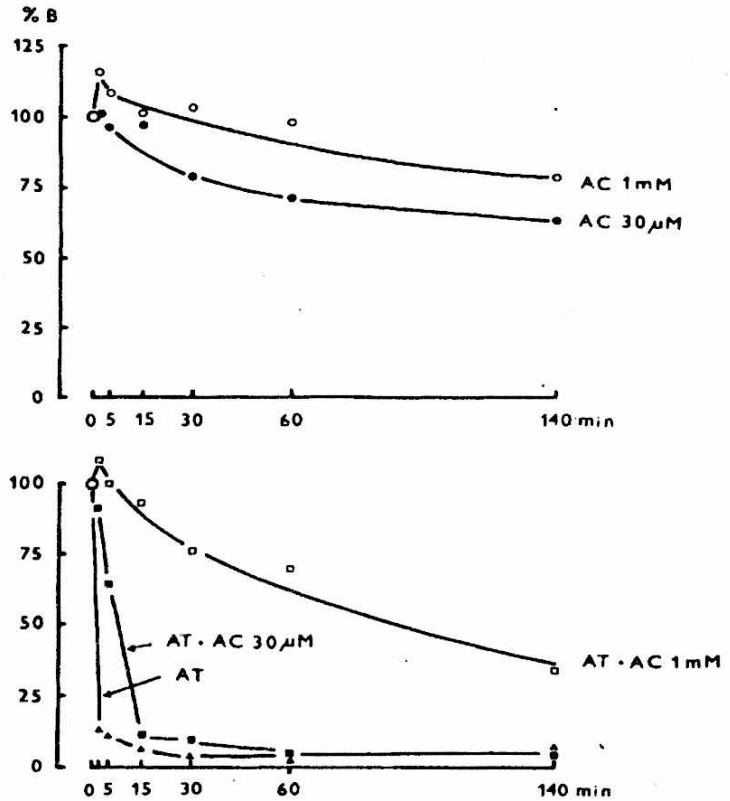


Fig. 6

Dissociation of (^{3}H)NMS-receptor complexes in atrial membranes after the addition of (top) atracurium (AC) 1 mmol/l (open circles), atracurium 30 μmol/l (closed circles), or (bottom) of atropine (AT) 1 μmol/l (closed triangles) atropine 1 μmol/l plus atracurium 30 μmol/l (closed squares), or of atropine 1 μmol/l plus atracurium 1 mmol/l (open squares). Atropine and atracurium were added to the incubation tubes after 80 min preincubation of the homogenate with (^{3}H)NMS (350 pmol/l) and the amounts of bound (^{3}H)NMS recovered after 2 – 120 min were compared with (and expressed as per cent of) the amount of bound (^{3}H)NMS recovered at the end of the preincubation. All points are means of at least 6 incubations. The lines were drawn by eye.

Stability of atracurium

Although we did not investigate chemical stability of atracurium, it has been checked that its effects were not altered by prolonged incubations. The binding of 100 pmol/l (^{3}H)QNB to ileal smooth muscle membranes was inhibited by 64 % at the end of 2 h incubations during which 10 μmol/l atracurium was present simultaneously; when the incubation medium containing atracurium had been preincubated for 1 or 2 h at 38 °C before the addition of homogenate, the binding of (^{3}H)QNB at the end of subsequent 2 h incubations was again inhibited by 64 or 60 %, respectively. The inhibitions of (^{3}H)QNB binding by 10 μmol/l atracurium observed at the end of incubations lasting 1 h, 2 h or 3 h (with no preincubations) were by 66 %, 64 %, or 58 %, respectively. These data (means of 2 – 3 observations) suggest that atracurium was reasonably stable during prolonged incubations.

Table 1

K_d, IC_{50} and n_H values for the inhibition by atracurium of the chronotropic effects of methylfurmethide on the heart atria and of the specific binding of (^{3}H)QNB and (^{3}H)NMS to homogenates of the atria, ileal smooth muscle, cerebellum and brain cortex.

	Atria	Ileum	Cerebellum	Brain cortex
K_d (μmol/l, from dose ratios)	3.0±0.5 (5)			
IC_{50} (μmol/l) for (^{3}H)QNB displacement	7.7±0.6 (3)	5.6±0.2 (3)	6.1 (2)	25.5 (2)
IC_{50} (μmol/l) for (^{3}H)NMS displacement	1000	>3000	500	200
n_H for (^{3}H)QNB displacemen	0.86±0.03 (3)	0.95±0.05 (2)	0.60 (2)	0.77 (2)

Apparent K_d for binding of atracurium to the heart atria was calculated from dose ratios (Gaddum 1957) obtained in pharmacological experiments illustrated in Fig. 2. IC_{50} values for the inhibition of (^{3}H)QNB binding were determined from Hill plots of data from experiments in Fig. 3; values of Hill slope factors n_H are from the same experiments. IC_{50} values for the inhibition of the binding of (^{3}H)NMS were extrapolated visually from data plotted in Fig. 4. The results are given as means (or means ± S.E.M., where n > 2); the number of experiments is given in parentheses. The IC_{50} value for (^{3}H)QNB displacement by atracurium was significantly ($p < 0.05$) higher in the brain cortex than in the atria, ileal smooth muscle and cerebellum (one way analysis of variance with Newman-Keuls multiple comparison method global test).

Discussion

In pharmacological experiments on the heart atria, atracurium antagonized the effects of methylfurmethide in a manner which appeared to be competitive, with a K_d of 3 μmol/l. The affinity of atracurium for cardiac muscarinic receptors thus resembles that of several other neuromuscular blockers; under comparable conditions, Nedoma *et al.* (1985) have found the K_d for gallamine to be 0.75 μmol/l

and for alcuronium 6.0 μmol/l, while d-tubocurarine did not antagonize muscarinic stimulation up to a concentration of 100 μmol/l.

The way in which atracurium antagonized the effect of methylfurmethide on the smooth muscle of the ileum was, however, clearly non-competitive (Fig. 2): the shift of the concentration-response curve was not parallel, and the agonist failed to produce its full effect in the presence of 50 μmol/l atracurium. We do not know the mechanism of the non-competitive effect of atracurium on the ileum; direct action on ionic channels in smooth muscle cells is one of the possibilities.

Increasing concentrations of atracurium diminished the binding of (^{3}H)QNB to cardiac membranes with an IC_{50} value of 7.7 μmol/l, which agrees well with the K_d of 3 μmol/l obtained on isolated heart atria. The action of atracurium on the binding of (^{3}H)QNB to membranes from the ileal smooth muscle, cerebellum and brain cortex was similar to its action on cardiac membranes, except for two features: (1) Hill coefficients (Tab. 1) for the binding of atracurium were low in the cerebellum and brain cortex, suggesting that the pool of atracurium binding sites in these organs was not homogeneous. (2) The affinity of atracurium for the binding sites was lower in the brain cortex than in other tissues (Fig. 3, Tab. 1), suggesting that atracurium prefers M_2 to M_1 receptors. Similar data have been reported for gallamine (Burke 1986, Price *et al.* 1986).

Quite surprisingly, atracurium has been found to have much smaller effects on the binding of (^{3}H)NMS than on the binding of (^{3}H)QNB (Tab. 1). In none of the tissues examined did atracurium completely prevent the binding of (^{3}H)NMS. In comparison with control incubations, the amount of (^{3}H)NMS bound in the presence 1 mmol/l atracurium still corresponded to 50 % in the atria, 90 % in the ileum, 46 % in the cerebellum and 22 % in the brain cortex. The concentrations of atracurium needed to diminish the binding of (^{3}H)NMS by 50 % were about 1000 times (atria), 100 times (cerebellum) or 10 times (cortex) higher than the corresponding IC_{50} values with regard to the binding of (^{3}H)QNB (Tab. 1). This discrepancy has serious implications which will be discussed later. The displacement curves obtained with (^{3}H)NMS and increasing concentrations of atracurium (Fig. 4) were flat, with bumps suggesting multiplicity of negative and perhaps also positive interactions with subpopulations of binding sites. Particularly conspicuous was the increase in the binding of (^{3}H)NMS observed with 10 – 30 μmol/l atracurium in the ileal smooth muscle The most likely explanation of the increase seems to be positive cooperativity, similar to that revealed in experiments with (^{3}H)NMS and alcuronium (Nedoma *et al.* 1987, Tuček *et al.* 1991).

In earlier work with gallamine and some other neuromuscular blockers, their ability to bind to muscarinic receptors simultaneously with (^{3}H)QNB or (^{3}H)NMS (i.e., to bind to a different site than the labelled antagonists) has been demonstrated by showing that the blockers slowed down the dissociation of preformed (^{3}H)QNB-receptor or (^{3}H)NMS-receptor complexes (Stockton *et al.* 1983, Ellis and Lenox 1985b, Nedoma *et al.* 1986, Narayanan and Aronstam 1986, Gillard *et al.* 1986, Ellis and Seidenberg 1987).

The same has now been found for atracurium with both (^{3}H)QNB- and (^{3}H)NMS-receptor complexes (Figs. 5 and 6). In experiments with the dissociation of (^{3}H)NMS-receptor complexes, an increase in the binding of (^{3}H)NMS was noted at short intervals after the addition of 1 mmol/l atracurium, followed by dissociation, which was considerably slower in the presence of atropine plus

atracurium than in the presence of atropine alone (Fig. 6). The short-lived increase in the binding after the addition of a high concentration of atracurium is unusual and difficult to interpret in terms of known properties of muscarinic receptors. Perhaps it reflects a transient positively cooperative effect of atracurium which becomes desensitized within a few minutes.

It is generally assumed that (^{3}H)NMS and (^{3}H)QNB bind to the same sites on muscarinic receptors, except that some sites accessible to (^{3}H)QNB are not accessible to (^{3}H)NMS (Ellis and Lenox 1985a, El-Fakahany *et al.* 1986, Brown and Goldstein 1986, Lenox 1985a, Lee and El-Fakahany 1988). The discrepancy between the effects of atracurium on the binding of (^{3}H)QNB and of (^{3}H)NMS indicates that atracurium does not bind to the same sites as (^{3}H)NMS and (^{3}H)QNB do. If, e.g., 1 μmol/l atracurium completely inhibited the binding of (^{3}H)QNB to cardiac membranes (Fig. 3), it must have been bound to all muscarinic receptors available; yet under the same conditions 50 % of (^{3}H)NMS still remained bound. The quantitative discrepancy was even larger in the case of smooth muscle membranes. By implication, atracurium must bind to another site than (^{3}H)NMS and (^{3}H)QNB. This points to a strong possibility that the observed antimuscarinic effects of atracurium were all due to negative cooperativity rather than to competition for muscarinic binding sites. The difference between the effects on the binding of (^{3}H)QNB and of (^{3}H)NMS is not surprising because cooperative effects are strongly dependent on the chemical nature of respective ligands; e.g., alcuronium was observed to exert positive cooperativity with regard to the binding of (^{3}H)NMS, but not of (^{3}H)QNB (Nedoma *et al.* 1987, Tuček *et al.* 1990). The difficulties of distinguishing between competition and strong negative cooperativity have been pointed out by Stockton *et al.* (1983), Tomlinson (1988) and others. The antagonism between gallamine and acetylcholine or carbamoylcholine on the smooth muscle could also be best explained in terms of an allosteric action of gallamine (Mitchelson and Ziegler 1984).

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Reprint Requests:
Dr. J. Musílková, Institute of Physiology, Czechoslovak Academy of Sciences, CS–142 20 Prague 4, Vídeňská 1083.