Table 1

	Atria		*Ileum*
	rate	*size*	
A Dose-ratios			
4-diphenylacetoxy-*N*-methylpiperidine methiodide (0.1 μM)	7.1 ± 0.4 (4)	7.2 ± 0.6 (3)	92 ± 12 (2)
Pancuronium bromide (1.0 μM)	4.2 ± 0.8 (4)	3.5 ± 0.5 (4)	3.5 ± 0.9 (5)
B Equipotent molar ratios relative to carbachol			
4-acetoxy-N-methyl-piperidine methiodide	56 ± 12 (2)	68 ± 24 (2)	19 ± 0.6 (5)
3-acetoxy-N-methyl-piperidine methiodide	nd	846 ± 65 (2)	286 ± 10 (4)
Acetyltropine methiodide	62	49	116 ± 7 (4)
(±)-methacholine	nd	1.5	0.78 ± 0.04 (2)

Numbers are means ± s.e. and number of results: *nd* indicates that the ratio could not be determined because the compound blocked the size of the contractions in concentrations which had no effect on the rate. All experiments were made in the presence of hexamethonium (0.28 mM).

The results (Table 1A) confirmed our previous findings with this compound but we observed no selectivity with pancuronium.

We have also measured the equipotent molar ratios for some agonists relative to carbachol (Table 1B) and again found some selectivity in a derivative of piperidine-4-ol. The 3-acetoxy analogue was much weaker but reduced the size of the contractions in concentrations which had no effect on the rate. This occurred also with (±)-methacholine but not with acetyltropine methiodide. The different relative activities of agonists suggest that there may be two types of receptor in the atria, m_2 and m_3, associated with effects on rate and contraction respectively.

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Effects of *p*-chloromercuribenzoate on muscarinic receptor binding in rat brain

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The action of *p*-chloromercuribenzoate (PCMB) on muscarinic receptors has been studied in membrane preparations from rat cerebral cortex and other areas. Membrane preparation and binding measurements were carried out as described previously (Hulme, Birdsall, Burgen & Mehta, 1978) at a membrane protein concentration of 1 mg/ml in 100 mM NaCl buffered at pH 7.0 with HEPES (20 mM).

Reaction between PCMB (1 mM) and cerebral cortical membranes for 15 min at 30°C results in little loss (<20%) of the total binding capacity for antagonists but has a dramatic effect on the binding parameters for carbachol. In the untreated membranes, the carbachol binding curve is flat (Hill Coefficient 0.33) due to the presence of three types of binding site, termed superhigh, high and low (fractional abundance and affinities respectively: 0.1, 2×10^7 M^{-1}; 0.3, 4×10^5 M^{-1}; 0.6, 1×10^4 M^{-1}). After treatment with PCMB, carbachol binding corresponds to a single binding site with affinity 2.5×10^2 M^{-1} (Hill Coefficient 1.0). The new binding state is therefore different from any of the pre-existing states and in the case of the superhigh site the affinity has been reduced by five orders of magnitude. With other full agonists the transformation to a single binding state of lower affinity also occurs. With pilocarpine the Hill Coefficient remains less than 1.0 and apparent heterogeneity of binding persists. The effects of PCMB are not reversed by repeated washing of the membranes.

In the case of most antagonists much smaller reductions in affinity are seen after PCMB treatment.

For example, the affinity of atropine, methylatropine, 3-quinuclidinylbenzilate and dodecyltrimethylammonium are reduced by 3–6 fold, but that of scopolamine and N-methylscopolamine by 35-fold. On the other hand the affinity of propylbenzilylcholine is increased 3-fold.

At higher concentration, PCMB reduces the binding capacity of the receptors. It appears very likely that the effects of PCMB on affinity and on capacity are due to reaction with independent SH groups as suggested by Aronstam, Abood & Hoss (1978).

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Kinetic effects of tubocurarine on skeletal muscle at high agonist concentrations

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Experiments have been performed on voltage-clamped end-plates of *cutaneus pectoris* muscles from *Rana temporaria*, at 7–8°C. The concentrations of agonist that are conventionally employed are low in the sense that they cause a small fraction of ion channels to open at equilibrium, i.e., $\alpha \gg \beta'$ where α is the rate constant for channel shutting and β', which increases with agonist concentration, is the effective rate constant for channel opening. Under these conditions the rate constant, $\tau^{-1} = \alpha + \beta'$, for the exponential relaxation of the agonist-induced membrane current following a step change in membrane potential (a voltage jump), is approximately α, the reciprocal of the mean channel lifetime.

We have examined the effects of high carbachol concentrations (up to 500 μM) after treatment of the muscle with α-bungarotoxin (100 nM) for long enough to reduce the agonist response sufficiently to allow adequate voltage clamp. Desensitization is rapid under these conditions and the peak response appeared to occur before the agonist concentration at the end-plate membrane had reached its maximum value, unless rapid application of the agonist was achieved (confirming observations of B. Sakmann, personal communication). Fast local superfusion of the end-plate area gave responses to low (e.g. 10 μM) carbachol concentrations that reached 90% of maximum in about 6 seconds. Under these conditions the time constant for current relaxation during 64 ms voltage jumps to −150 mV was essentially independent of the degree of desensitization even with high agonist concentrations. All relaxations were fitted with an exponential curve plus a sloping baseline, thus slow relaxations could not be resolved.

The rate constant at −150 mV, τ^{-1}, changed only slightly with carbachol concentration up to 100 μM ($\tau^{-1} \simeq \alpha \simeq 240$ sec^{-1}) but thereafter increased (presumably due to an increase in β') with agonist concentration, as found by Sakmann & Adams (1976), Adams & Sakmann (1978) and, in electroplaque, by Sheridan & Lester (1977). The rate constant was doubled (i.e. 480 s^{-1}) at roughly 380 μM carbachol at which point the rate of increase was about 1.2×10^6 M^{-1}s^{-1}.

The addition of a low concentration, 0.4 μM, of tubocurarine (close to the equilibrium constant for competitive block) might be expected to increase the rate constant by about 11 s^{-1} as a result of 'channel block' (Colquhoun, Dreyer & Sheridan, 1979). In fact, the rate constant was reduced towards α by tubocurarine (e.g.) from 2α to 1.2α with 380 μM carbachol), and could be restored by a further increase in agonist concentration. This observation is similar to that made in electroplaque by Sheridan & Lester (1977). One explanation of it is that tubocurarine equilibrates rather rapidly with the acetylcholine receptor, thus reducing β'. If the dissociation rate constant was much slower than 1 ms^{-1} (implying an astonishingly fast association rate of over 10^9 M^{-1} s^{-1}) two component relaxations would be expected, which we have not been able to detect with any certainty under the difficult conditions of these experiments. The interpretation of the observations is, however, greatly complicated by the rates of diffusion of agonist and antagonist in the synaptic cleft, so the apparently fast rate of competitive block by tubocurarine requires further investigation.

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