duced (i) a small tachycardia, independent of nerve stimulation (see Docherty & McGrath, 1978), (ii) a dose-dependent potentiation of nerve-induced responses with equivalent effects from pancuronium (0.3 mg/kg) and NC 45 (10 mg/kg). A similar effect was found on the adrenergically-mediated contraction of the vas deferens in the pithed rat (see McGrath, 1978) which was potentiated to a greater extent by pancuronium than by NC 45.

(3) Cardiac parasympathetic: 50% inhibition of nerve-induced fall in heart rate; pancuronium (0.008 mg/kg), NC 45 (4.3 mg/kg).

NC 45 was, therefore, less potent than pancuronium as a relaxant by $\times 2.1$, as a potentiator of cardiac sympathetic transmission by approximately $\times 33$ and as a blocker of parasympathetic transmission by $\times 538$, when compared at doses producing equivalent effects. The dose/response curves were, however, much steeper for blockade of somatic than of parasympathetic transmission; consequently 'ID 50' values should be used with caution in comparing effects at these two sites.

The blockade of neuronal re-uptake of noradrenaline, by raising the concentration of noradrenaline outside the varicosity, not only potentiates the post-junctional effect but increases the degree of pre-junctional, α-adrenoceptor mediated inhibition (Docherty & McGrath, 1979). Hence, in the presence of pancuronium (1 mg/kg) the α-adrenoceptor antagonists, yohimbine and phentolamine, potentiated the tachycardia produced by low frequency cardioaccelerator stimulation by blocking the inhibitory, pre-junctional effect of transmitter noradrenaline. Pancuronium and pre-junctional α-adrenoceptor antagonists in combination may, therefore, produce tachycardia.

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Three types of muscarinic receptors?

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Although receptors are often classified by the relative activity of agonists it is probably preferable to classify then by comparing the affinities of antagonists. In such a comparison Barlow, Franks & Pearson (1972) found that the muscarine-sensitive acetylcholine receptors of the guinea-pig ileum, bronchial muscle and iris were indistinguishable. Barlow, Berry, Glenton, Nikolaou & Soh (1976), however, subsequently found compounds with lower affinity for muscarine-sensitive acetylcholine receptors in guinea-pig atrial pacemaker cells than for the receptors in the ileum. Muscarine-sensitive acetylcholine receptors can therefore be classified into m_1, in the ileum and m_2 in the atria.

Marshall & Ojewole (1979) have reported that certain neuromuscular blocking agents have greater affinity for receptors associated with the inotropic effects of acetylcholine on the electrically driven atria from reserpinized guinea-pigs than for receptors in the ileum. We have therefore measured the affinity of pancuronium as an antagonist of carbachol and calculated the dose-ratio from the change in the size of the contraction as well as from the change in the rate with guinea-pig atria in Ringer-Locke solution at 30°C. We also made similar experiments with 4-diphenylacetoxy-*N*-methylpiperidine methiodide, the most selective of the compounds previously studied by Barlow *et al.* (1976).

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.