dog and cat both receptors mediate depressor responses.

This communication describes further experiments on the role of H_1- and H_2-receptors in the depressor response to histamine in the cat.

Experiments have been made in cats, body weight 1.5-3.1 kg, anaesthetized with pentobarbitone sodium 60 mg/kg i.p. Blood pressure was measured from a cannula inserted into the right carotid artery. Histamine was administered by i.v. injection. Mepyramine was administered i.v. 10 min before repeating the doses of histamine. Metiamide (Black, Duncan, Emmett, Ganellin, Hesselbo, Parsons & Wyllie, 1973) was administered by continuous i.v. infusion for 30 min before and during the injection of histamine.

Histamine, over the dose-range 10^{-10} to 10^{-7} mol/kg (30 ng/kg to 30 μg/kg), caused dose-dependent depressor responses. Administration of mepyramine, 2.5×10^{-5} mol/kg (1 mg/kg), caused a shift, to the right, in the histamine dose-response curve, with a dose-ratio of less than 10. Administration of larger doses of mepyramine, up to 2.5×10^{-5} mol/kg (20 mg/kg), caused no further shift in the dose-response curve. When the maximal blocking effect of mepyramine had been achieved, metiamide, 4×10^{-7} and 2×10^{-6} (mol/kg)/min (100 and 500 (μg/kg)/min), caused further parallel, dose-dependent shifts, to the right, of the histamine dose-response curve.

In contrast, metiamide alone, up to 1×10^{-5} (mol/kg)/min (2.5 (mg/kg)/min), had no significant effect on the histamine dose-response curve. Administration of mepyramine, 2.5×10^{-6} to 8.25×10^{-5} mol/kg (1 to 33 mg/kg), during continued infusion of metiamide caused parallel, dose-dependent shifts, to the right, of the dose-response curve. These were bigger than the maximal shifts caused by mepyramine in untreated animals but comparable to the shifts caused by the same doses of mepyramine and metiamide when the order of antagonist administration was reversed.

The results reported in this communication indicate that histamine can interact with both H_1- and H_2-receptors within the cardiovascular system of the cat. In the cat, like the dog, stimulation of either receptor elicits hypotension. The different effects of the selective antagonists suggest that histamine has different ED_{50} for the two receptors.

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The inhibition of α-bungarotoxin binding to denervated rat muscle by tubocurarine and other drugs

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The experiments reported here were done in order (a) to test the specificity of α-bungarotoxin (BuTX) binding in skeletal muscle in the light of reports (Berg, Kelly, Sargent, Williamson & Hall, 1972; Porter, Chiu, Wieckowski & Barnard, 1973) that much of the BuTX binding to the endplates cannot be inhibited by (+)-tubocurarine (TC), and (b) to provide information about extrajunctional receptors in denervated muscle for comparison with endplate receptors in normal muscle. Because BuTX combines virtually irreversibly with acetylcholine (ACh) receptors, no inhibition of BuTX binding at all would be expected *at equilibrium*. In this situation it is appropriate to measure the effect of inhibitors on the rate of binding.

The simplest model for inhibition of binding of an irreversible ligand by a fast-acting competitive inhibitor predicts that the rate constant, and, *a fortiori*, the initial rate, will be reduced by a factor

of $1 + (x/K)$, where x is the concentration, and K the equilibrium dissociation constant, of the inhibitor.

To avoid diffusion limitation of the rate of BuTX binding homogenates of rat diaphragm (denervated 13-15 days previously) were used. Binding in intact muscle was found to be much slower, and, as expected, the degree of inhibition by TC much less.

The initial rate of BuTX binding (measured after 7 min incubation with [^{125}I]-BuTX) could be depressed to 10% or less of its control value by sufficiently high concentrations of TC, hexamethonium (C6), decamethonium (C10), carbachol (CCh) and suxamethonium (SCh). After subtraction of this small uptake of the BuTX which cannot be inhibited by (+)-tubocurarine, the initial rate was found to be related to inhibitor concentration as predicted above. This method gave equilibrium constants of 0.3 μM for TC, 175 μM for C6, 2.0 μM for C10, and 1.3 μM for SCh. The value for TC is near to the value (0.5 μM) calculated from the inhibition of CCh-induced depolarizations in denervated muscle fibres.

In other experiments the BuTX binding was followed nearly to equilibrium over 3 hours. The rate was measured in the presence of high concentrations of TC (60 μM) and CCh (800 μM) to obtain the uptake of the BuTX which cannot be inhibited. The latter at 3 h, was about 20% of the total uptake. After subtraction of this, the remaining uptake was approximately exponential, the rate constants indicating equilibrium constants of 0.33 μM for TC and 4 μM for CCh.

These experiments suggest that the finding that BuTX binding to the endplates of normal rodent muscle can be reduced to only about half the control value by high concentrations of TC (Berg *et al.*, 1972; Porter *et al.*, 1973) may not mean that only half the BuTX binding sites are ACh receptors. A reduced degree of inhibition of BuTX binding would be expected if measurements were made at a fixed time that was not short (compared with the rate constant for binding), or if the rate of BuTX binding were diffusion controlled.

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Tetrodotoxin binding to innervated and denervated rat diaphragm homogenates

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The binding of tritium labelled tetrodotoxin ([^{3}H]-TTX) to intact innervated rat diaphragm was reported by Colquhoun, Rang & Ritchie (1973). We have now measured the binding of [^{3}H]-TTX to denervated muscle, which is known to become partly resistant to the action of TTX (Redfern & Thesleff, 1971; Harris & Thesleff, 1971). Muscle homogenates were used, rather than intact muscle; this improved the precision of the binding measurements.

The homogenate, washed with 0.6 M KCl and resuspended in buffered saline, was incubated for 1-4 h at 20°C with [^{3}H]-TTX (Colquhoun, Henderson & Ritchie, 1972) followed by centrifugation at 10^5 *g*. The unbound [^{3}H]-TTX trapped in the pellet was measured by means of [^{14}C]-mannitol.

As before, the results could be fitted by the sum of a hyperbolic saturable component and a linear component. Pooling the results from intact and homogenized normal (innervated) diaphragm indicated a saturable component with an equilibrium constant of $K = 6.1$ nM (95% interval 4.7-7.8 nM), and a binding capacity of $M = 2.5$ fmole/mg wet weight (95% interval 2.1-2.8 fmole/mg). The value of M/K, which is more precisely determined than either M or K separately, was 0.40 μl/mg (0.36-0.46 μl/mg). The saturable component was abolished in the presence of 100 nM saxitoxin. In diaphragm muscle which had been denervated 5-14 days previously, the binding of [^{3}H]-TTX was reduced. Measurements on intact denervated muscle gave $M/K = 0.13$ μl/mg, but were not precise enough for M and K to be separately determined. Homogenized denervated muscle showed a saturable component of [^{3}H]-TTX binding with $K = 8.5$ nM (4.3 to 15.3 nM), little different from the value in innervated muscle, and