Negligible affinity of histamine H_2-receptor antagonists for central α_1-adrenoceptors

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The interaction between clonidine and histamine H_2-receptor antagonists has indicated that histamine H_2-receptors are involved in the central hypotensive response to clonidine (Finch et al 1978; Karppanen et al 1976, 1977). A recent study by Pilc et al (1979) demonstrated that in very high concentrations only histamine H_2-receptor agonists and antagonists displaced [^{3}H]-clonidine from its specific (α_2) binding sites in membranes from rat cerebral cortex. This finding makes its unlikely that central α_2- as well as histamine H_2-receptors are the common sites of interaction.

[^{3}H]Prazosin labels α_1-adrenoceptors in the rat brain with extreme selectivity (Greengrass & Bremner 1979). Since the drug also interferes with the central hypotensive effect of clonidine (Timmermans et al 1979) we studied the displacement of [^{3}H]prazosin from α_1-adrenoceptor sites in rat brain membranes by the histamine H_2-receptor antagonists metiamide and cimetidine.

Fresh brains (minus cerebella) of male Wistar rats (190–220 g) were homogenized in 20 vol (w/v) ice-cold 50 mM Tris/HCl buffer (pH 7·7 at 25 °C). The homogenate was centrifuged twice (50 000 g; 10 min; 4 °C) with resuspension of the pellet in fresh buffer between spins. The final pellet was homogenized in Tris/HCl buffer (1 mg protein per 1 ml). Aliquots (500 μl) were incubated at 25 °C for 60 min with [^{3}H]prazosin (spec. act. 33 Ci mmol^{-1}; 0·05–10 nM) and various concentrations of drugs in a final volume of 1 ml and terminated by rapid filtration through Whatman GF/B filters followed by three 5 ml washes of ice-cold Tris/HCl buffer. Filters were counted in Instagel at 35–40% efficiency. Specific binding was defined as the excess over blanks containing 2·0 μM phentolamine.

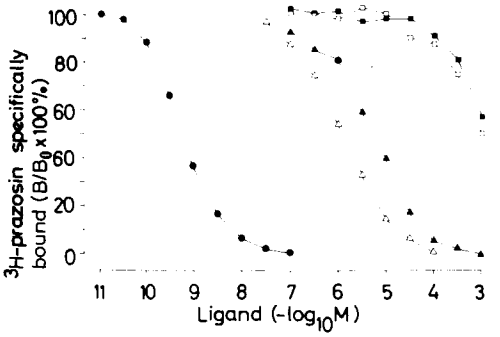

FIG. 1. Displacement of [^{3}H]prazosin (0·2 nM) from its specific binding sites in rat cerebral membranes by increasing concentrations of unlabelled prazosin (●), clonidine (△), diphenhydramine (▲), metiamide (□) and cimetidine (■). Symbols represent mean values out of four separate experiments (s.e.m. < 10%).

In accordance with the results reported by Greengrass & Bremner (1979) the specific binding of [^{3}H]prazosin was saturable and of high affinity. Scatchard analysis indicated a single population of binding sites (K_D = 0·2 nM; B_{max} = 140 fmol mg^{-1} protein). [^{3}H]Prazosin (0·2 nM) was specifically displaced by non-radioactive prazosin (IC50 = 0·6 nM) (Fig. 1).

Clonidine itself also proved a reasonably potent inhibitor of [^{3}H]prazosin binding (IC50 = 1·2 μM). This finding lends support to the pharmacological interaction established between these two drugs (Timmermans et al 1979). The histamine H_2-receptor antagonists metiamide and cimetidine were very weak in competing for [^{3}H]prazosin binding. Displacement occurred at high concentrations only. In contrast, the histamine H_1-receptor antagonist diphenhydramine, which does not reduce the central hypotensive effect of clonidine (Karppanen et al 1976), was more active in inhibiting [^{3}H]prazosin binding (IC50 = 5·2 μM). This may be explained by the moderate α-adrenoceptor antagonist activity of this drug. The present results show the absence of a significant affinity of histamine H_2-receptor antagonists for specific binding sites in rat cerebral membranes labelled by [^{3}H]prazosin (α_1-adrenoceptors). This finding and those of Pilc et al (1979) indicate that neither central α_1- nor α_2-adrenoceptors represent the targets for histamine H_2-receptor antagonists in their antagonism of the central hypotensive effect of clonidine. Moreover, they also reject the hypothesis that clonidine initiates its central hypotensive effect via histamine H_2-receptors.

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