history, allergic state, and drug exposure on the role of the muscarinic-receptor system in peripheral lung responses in health and disease.

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Inhibition by H_1-antihistamines of the uptake of noradrenaline and 5-HT into rat brain synaptosomes

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There are a number of reports that a range of structurally diverse H_1-receptor antagonists can interfere with the reuptake of the two biogenic amine neurotransmitters, noradrenaline and 5-hydroxytryptamine (5-HT), into presynaptic nerve endings in both the peripheral and central nervous system [1–4]. Isaac and Goth [1] first showed that diphenhyramine and chlorpheniramine potentiated the cardiovascular responses of noradrenaline by reducing the neuronal uptake of this neurotransmitter. Similar *in vitro* [2–6] and *in vivo* [2] studies have shown that mepyramine, diphenhyramine and chlorpheniramine can inhibit the neuronal uptake of noradrenaline and 5-HT in the mammalian central nervous system. Few studies, however, have attempted to quantify the effectiveness of H_1-receptor antagonists as inhibitors of the different neurotransmitter reuptake processes. In those studies that have been made, there is an indication that mepyramine [4, 5] and diphenhyramine [4, 6] can discriminate between the high affinity transport systems for noradrenaline and 5-HT. The present study was undertaken to quantify and compare the specificity of seven representative H_1-receptor antagonists as inhibitors of noradrenaline and 5-HT uptake.

Materials and methods

Measurement of uptake. A crude synaptosomal preparation of rat (Wistar, males, 250 g) cerebral cortex was prepared as described by Kellar *et al.* [7]. Aliquots (100 μl) of the synaptosomal suspension were added to incubation tubes containing H_1-receptor-antagonist in 660 μl of Krebs–Tris–Ringer medium (mM concentrations: NaCl, 118; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; Tris base, 50.4; glucose, 0.07; pargyline, 0.05, pH 7.4), gassed with O_2/CO_2 (95:5) and incubated at 37° for 10 min. [^{3}H]noradrenaline or [^{3}H]5-HT was then added in 40 μl of Krebs medium, to give a final concentration of 10 nM [^{3}H]noradrenaline or 2 nM [^{3}H]5-HT, and the incubation continued for a further 5 min. Incubations were terminated by addition of 4 ml of ice-cold 0.9% saline and filtered immediately through Whatman GF/B glass fibre filters under vacuum. The tubes were rinsed and the filters washed twice with 4 ml 0.9% saline and tritium was determined by liquid scintillation counting. Triplicate measurements were made at each incubation condition. The extent of the non-specific transport of [^{3}H]noradrenaline and [^{3}H]5-HT into rat brain synaptosomes was determined to be the uptake measured in the presence of 10 μM imipramine. Inhibitor constants (K_Is) for blockade of monoamine uptake were calculated from the concentration of drug (IC_{50}) required for 50% inhibition of the imipramine-sensitive transport of noradrenaline or 5-HT using the relationship $K_I = IC_{50}/(A/K_t + 1)$ where A is the concentration of [^{3}H]monoamine and K_t is its transport constant (i.e. the concentration at one half maximal imipramine-sensitive transport). K_t values were calculated from curves of inhibition of [^{3}H]monoamine uptake by the corresponding nonradioactive monoamine (added simultaneously with the radioactive ligand) according to the expression $K_t = IC_{50} - A$.

Materials. [^{3}H]5-HT creatinine sulphate (20 Ci/mmol) and [^{3}H]noradrenaline hydrochloride (13.5 and 15 Ci/mmol) were purchased from Amersham International. Mepyramine maleate, 5-HT creatinine sulphate, noradrenaline hydrochloride and methapyrilene hydrochloride were obtained from Sigma. Gifts of (+)-chlorpheniramine maleate (Schering), imipramine hydrochloride (Courtin & Warner), diphenhydramine (Parke Davis), promethazine hydrochloride (May & Baker), triprolidine hydrochloride and chlorcyclizine hydrochloride (Wellcome) are gratefully acknowledged.

Results and discussion

The transport constants (K_t) for [^{3}H]noradrenaline and [^{3}H]5-HT (0.17 and 0.09 μM respectively, Table 1) in the present study agreed well with those reported previously [6]. The antidepressant drug imipramine inhibited the uptake of both monoamines in a dose related fashion yielding similar inhibitor constants (K_I) of 0.04 and 0.11 μM for

Table 1. Effect of H_1-receptor antagonists on the high affinity uptake of [^{3}H]noradrenaline and [^{3}H]5-HT into rat brain synaptosomes

Inhibitor	Inhibitor constant (K_I, μM) Noradrenaline (A)	(N)	5-HT (B)	(N)	Ratio B/A
(+)-Chlorpheniramine	0.51 ± 0.04	(3)	0.06 ± 0.01	(4)	0.12
Mepyramine	1.20 ± 0.10	(3)	0.83 ± 0.39	(3)	0.69
Methapyrilene	0.80 ± 0.35	(3)	0.65 ± 0.07	(3)	0.81
Chlorcyclizine	3.43 ± 0.86	(3)	>10	(3)	>2.78
Promethazine	1.42 ± 0.29	(4)	>10	(3)	>6.67
Diphenhydramine	0.91 ± 0.36	(4)	9.20 ± 4.40	(4)	10.20
Triprolidine	0.33 ± 0.14	(4)	3.60 ± 0.70	(3)	10.90
Imipramine	0.04 ± 0.01	(6)	0.11 ± 0.02	(4)	2.75
Noradrenaline	0.17 ± 0.04	(4)	N.D.		
5-HT	N.D.		0.09 ± 0.02	(3)	

Values represent mean ± SE of the K_I values obtained as described under Materials and Methods. The number of individual experiments conducted with each inhibitor are given in parentheses. ND = not determined.

noradrenaline and 5-HT uptake respectively (Fig. 1 and Table 1). The extent of the non-specific transport (i.e. that insensitive to inhibition by 10 μM imipramine), however, differed markedly for noradrenaline (46.3 ± 2.0%, N = 27) and 5-HT (9.9 ± 1.0%, N = 24) uptake.

All of the H_1-antagonists tested inhibited noradrenaline uptake over the concentration range (10^{-9} M–10^{-5} M) employed. Triprolidine and (+)-chlorpheniramine were the most potent inhibitors of noradrenaline uptake, although with the exception of chlorcyclizine the K_I values for all of the H_1-antihistamines fell within a fairly narrow range (0.3–1.4 μM; Table 1). These values were all, however, an order of magnitude higher than the value obtained for the antidepressant imipramine (Fig. 1, Table 1).

The inhibition by H_1-antihistamines of the reuptake of 5-HT into rat brain synaptosomes varied to a greater extent between drugs. In agreement with earlier qualitative studies [2, 3], (+)-chlorpheniramine was found to be a potent inhibitor of 5-HT transport (Fig. 2) with a K_I of 0.06 μM. It was notable that this value was similar to that obtained for imipramine (0.11 μM, Table 1) and the values reported for the 5-HT selective agents fluoxetine and zimelidine [6]. Interestingly, (+)-chlorpheniramine also showed a degree of selectivity (8-fold) towards inhibition of 5-HT uptake (Table 1). Mepyramine and its structural analogue methapyrilene were an order of magnitude weaker than chlorpheniramine and, in contrast to the data obtained by Tuomisto and Tuomisto [5] showed little discrimination between the two uptake systems (Table 1). The remaining H_1-antagonists were weak inhibitors of 5-HT uptake and showed a preference for the noradrenaline carrier (Table 1). The data obtained with diphenhydramine (Table 1) confirms the selectivity towards inhibition of noradrenaline uptake reported previously [4, 6].

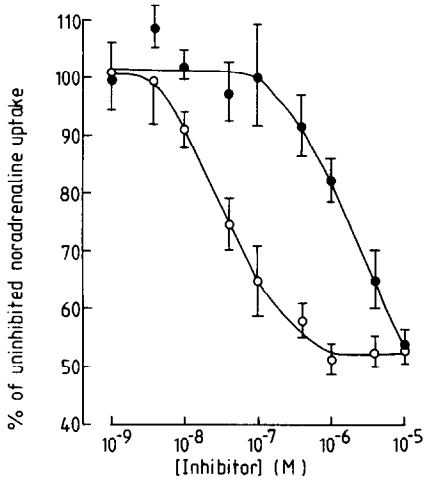

Fig. 1. Inhibition by imipramine (○) and promethazine (●) of the uptake of 10 nM [^{3}H]noradrenaline into rat brain synaptosomes. Values represent the mean ± SE of triplicate measurements in a single experiment. Uptake is expressed as a percentage of the uninhibited uptake of noradrenaline. Similar results were obtained in five (imipramine) and three (promethazine) other experiments (Table 1).

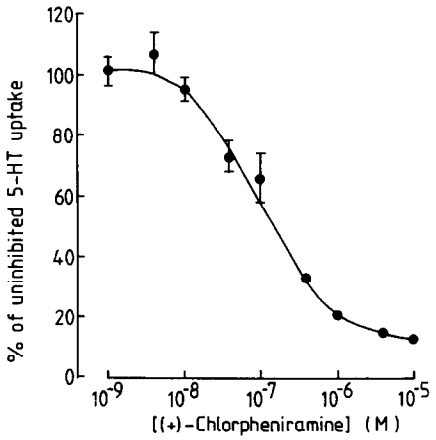

Fig. 2. Inhibition by (+)-chlorpheniramine of the uptake of 2 nM [^{3}H]5-HT into rat brain synaptosomes. Values represent the mean ± SE of triplicate measurements in a single experiment. Uptake is expressed as a percentage of the uninhibited uptake of 5-HT. Similar results were obtained in three other experiments (Table 1).

The marked difference in the selectivity of these H_1-receptor antagonists for the two monoamine uptake systems suggests that these effects are not secondary to the well known membrane-stabilising properties of the H_1-antihistamines [8]. Indeed it was notable that the H_1-antagonists inhibited amine uptake to the same maximal extent as imipramine and did not affect the imipramine-insensitive uptake over the concentration range employed. However, in general the concentrations of these drugs required to inhibit monoamine uptake systems are much higher than those required for occupancy of histamine H_1-receptors [9]. Nevertheless, the uptake inhibiting properties of these drugs may well contribute to their pharmacological activity *in vivo* where the concentration of these H_1-receptor antagonists may reach the range at which noradrenaline and 5-HT uptake is inhibited. In this respect it is important to note that histamine can induce a release of noradrenaline and 5-HT from certain central and peripheral tissues via a non-receptor tyramine-like action [5, 10–12]. These effects can be prevented by inhibitors of monoamine uptake [10, 11]. Consequently, if the *in vivo* concentrations of H_1-antihistamines reach the micromolar range then many of the H_1-receptor antagonists tested here will inhibit this non-receptor action of exogenous histamine. This has important implications for the interpretation of *in vivo* studies of the action of histamine in the CNS.

In summary, the present study shows that a range of structurally diverse H_1-receptor antagonists can inhibit the uptake of both 5-HT and noradrenaline in the CNS. Many of these agents show selectivity towards one particular monoamine transport system. The finding that at low concentrations (+)-chlorpheniramine is a potent and relatively selective inhibitor of 5-HT transport suggests that this agent may inhibit the neuronal uptake of 5-HT at the therapeutic doses required to achieve H_1-receptor antagonism. These data therefore raise questions concerning the pharmacological consequences of chronic administration of this popular antihistamine and the therapeutic potential of these effects. For example, (+)-chlorpheniramine may have an additional therapeutic application in the treatment of depression if selective 5-HT uptake blockade underlies the clinical efficacy of antidepressants such as zimelidine [13] and fluoxetine [14].

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Cyclopiazonic acid inhibition of the Ca^{2+}-transport ATPase in rat skeletal muscle sarcoplasmic reticulum vesicles*

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Cyclopiazonic acid (Fig. 1), a mycotoxin produced by certain fungi of the *Aspergillus* and *Penicillium* genera, may be found as a natural contaminant of corn, peanuts and certain mold-fermented cheeses and meats [1]. Although the clinical signs of toxicity are species dependent, the skeletal muscle is frequently involved and symptoms include muscular incoordination and altered motor activity [2, 3]. It has been suggested that cyclopiazonic acid toxicity may be due to a direct effect on the muscle [4]. The following studies were conducted to investigate the effect of cyclopiazonic acid on the Ca^{2+}-transport ATPase found

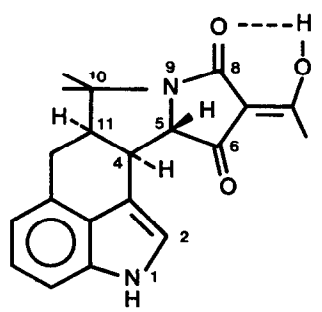

Fig. 1. Structure of cyclopiazonic acid.

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