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In Vitro and *in Vivo* Binding Characteristics of a New Long-Acting Histamine H_1 Antagonist, Astemizole

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SUMMARY

Binding characteristics of astemizole were studied *in vitro* in various receptor binding models and *in vivo* by determining the occupancy of histamine H_1 receptors in guinea pig lung and cerebellum. *In vitro*, astemizole was found to have a high affinity for histamine H_1 receptors, but great difficulties were encountered in proving this because of its high affinity for nonspecific binding sites. Since the equilibrium conditions were not reached *in vitro*, the real affinity of astemizole remains unclear and its receptor profile must be interpreted with caution. Nevertheless, the drug is certainly much more potent on histamine H_1 receptors than on serotonin S_2 and adrenergic *alpha*$_1$-receptors. Moreover, it was found to be devoid of antimuscarinic and antidopaminergic properties. The most striking property of this drug is its extremely slow dissociation rate from H_1 receptors when assayed *in vitro* using [^{3}H]-pyrilamine. *Ex vivo* experiments were performed in guinea pigs; astemizole was given orally to the animals, and the occupancy of H_1 receptors in the lung and the cerebellum was determined *in vitro* by the [^{3}H]-pyrilamine binding assay. Astemizole was found to occupy H_1 receptors in lung at very low doses. Here again the most striking receptor binding property was its very long duration. The occupancy of H_1 receptors in lung began to decline only 4–6 days after administration of the drug. However, there was a marked difference between the occupancy of peripheral and central receptors; indeed, in contrast to pyrilamine, astemizole at pharmacological doses did not reach the H_1 receptors in the cerebellum, presumably because the drug does not readily cross the blood-brain barrier.

INTRODUCTION

During the last decade, receptors for several neurotransmitters have been identified and characterized in the brain by means of *in vitro* binding studies. In contrast to this, histamine H_1 receptor binding was first identified in homogenates of a peripheral organ, guinea pig ileum (1). As [^{3}H]pyrilamine appeared to bind selectively to H_1 receptors, rapid progress was made in investigating histamine receptors in the central nervous system (2, 3). Although the precise role of such receptors in the brain is still unclear (4), one of the most prominent pharmacological effects of histamine H_1 antagonists is sedation. For instance, there is a reasonable correlation between the sedative properties of the tricyclic antidepressants and their potency as antagonists of the H_1 receptor in the central nervous system (5).

As a rule, the H_1 receptors from different organs in various animal species revealed similar binding characteristics, but the regional distribution differed markedly in various species. In the guinea pig brain, the highest amount of binding was found in the cerebellum, but in man the cortex contains the highest number of receptors (6). In the periphery, the lung of guinea pig contains a higher number of binding sites than that of rat (7), a fact which parallels the higher sensitivity of the guinea pig to bronchoconstricting agents. Recently a new compound, astemizole, 1-[(4-fluorophenyl)methyl]-N-[1-[2-(4-methoxyphenyl)ethyl]-4-piperidinyl]-1H-benzimidazol-2-amine (Fig. 1), was found to be a particularly long-acting H_1 antagonist but devoid of central effects (8).

In the present study, we report on the binding characteristics of astemizole determined under both *in vitro* and *in vivo* conditions. For the latter, the occupancy of H_1 receptors was examined in lung and brain of guinea pigs previously treated with the drug.

MATERIALS AND METHODS

In vitro binding. Wistar rats (150–200 g) and albino guinea pigs (250–350 g) were decapitated and their brains were rapidly removed and dissected.

For determination of the H_1 receptor, membranes were prepared from guinea pig cerebellum as described by Chang *et al.* (2). The binding assay was carried out with

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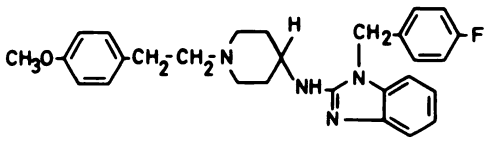

FIG. 1. *Chemical structure of astemizole*

4 nM [^{3}H]pyrilamine in a total volume of 1.1 or 10 ml containing 50 mM phosphate buffer (pH 7.5) and a membrane aliquot corresponding to 20 mg (wet weight) of tissue. In some experiments, Tris-salt buffer [Tris-HCl, 50 mM (pH 7.5); NaCl, 120 mM; KCl, 5 mM; $CaCl_2$, 2 mM; $MgCl_2$; 1 mM, ascorbic acid, 0.1%; and pargyline, 10 μM] was used. After incubation at 25° for 30 min the labeled membranes were harvested and washed (three times with 5 ml of cold buffer each time) by rapid vacuum filtration through Whatman GF/B glass-fiber filters using a 40-well filtration manifold (S.I.D. Janssen Pharmaceutica, Beerse, Belgium). Specific binding was taken as the difference between the total binding and the binding measured in the presence of 10^{-6} M promethazine or astemizole.

5-Hydroxytryptamine (S_2) receptors were measured in rat frontal cortex, using 2 nM [^{3}H]spiperone (9).

Dopamine and muscarinic receptors were determined in rat striata using 2 nM [^{3}H]haloperidol and 2 nM [^{3}H] dexetimide, respectively (10, 11). Binding assay for the *alpha*$_1$-adrenergic receptor was performed in rat forebrain using 0.5 nM [^{3}H]WB-4101 (12). More details of the incubation conditions and the criteria of specific binding have been described elsewhere (13).

Ex vivo binding. After an overnight fast, guinea pigs weighing approximately 250 g were treated orally with the test compound or the vehicle. At different times after drug administration, the animals were decapitated and the lungs and the cerebellum were rapidly removed. The external portion of the lung was dissected and homogenized in 10 volumes of 0.25 M sucrose by means of an Ultra-Turrax homogenizer. After centrifugation at 2000 rpm for 10 min in a refrigerated Sorvall centrifuge, the pellet was again homogenized in 10 volumes and again centrifuged. To both pooled supernatants, phosphate buffer (pH 7.5) was added in order to reach a final concentration of 50 mM. After centrifugation at 18,000 rpm for 10 min, the pellet was suspended in 50 volumes of 50 mM phosphate buffer (pH 7.4), and 3-ml aliquots were used for the binding assay. The incubation conditions were as described above except that the total volume was 3.3 ml.

The cerebellum was prepared and assayed for H_1 receptors, as described for the *in vitro* binding.

In vivo binding. Male Wistar rats (250 g) were given [^{3}H]spiperone (5 μg/kg) by intravenous injection. At variance with earlier work (14), the tested drug was administrated 4 hr before the labeled spiperone. The animals were killed by decapitation 2 hr after labeling. Brain areas were dissected and homogenized in 10 volumes of distilled water. Aliquots (400-μl) were counted for radioactivity in a liquid scintillation counter. *In vivo* binding of [^{3}H]astemizole was carried out in guinea pigs. At different time intervals after oral administration of the labeled drug, the radioactivity was measured in lung and cerebellum.

Materials. [^{3}H]Haloperidol (specific activity 12.5 Ci/mmole), [^{3}H]astemizole (specific activity 28.7 Ci/mmole) and [^{3}H]dexetimide (specific activity 16 Ci/mmole) were obtained from IRE (Fleurus, Belgium). [^{3}H]Spiperone (specific activity 25.7 Ci/mmole), [^{3}H]pyrilamine (specific activity 27.3 Ci/mmole), and [^{3}H]WB-4101 (specific activity 24.4 Ci/mmole) were purchased from New England Nuclear Corporation (Boston, Mass.). Azatadine maleate (Schering), chlorpheniramine HCl (Schering), clemastine fumarate (Sandoz), pyrilamine maleate, and promethazine HCl (Rhône-Poulenc) were used as reference compounds.

For the *in vitro* binding assays, millimolar stock solutions of compounds were made in 100% ethanol and diluted in 10% ethanol; the final ethanol concentration in the incubation mixture was kept below 1%.

RESULTS

Astemizole was tested *in vitro* in the [^{3}H]pyrilamine binding assay and in four different receptor models and then compared with other antihistaminic drugs. The IC_{50} values obtained in the binding *in vitro* are given in Table 1. When [^{3}H]pyrilamine binding was performed under standard conditions (1.1 ml and 30-min incubation), the affinity of astemizole was not very high (IC_{50} 40 nM); indeed, among all of the substances listed in Table 1, only oxatomide was apparently less active than astemizole. However, when the incubation time was increased to 120 min and the volume of the incubation was increased to 10 ml, thus keeping the same total amount of tissue, the affinity of astemizole markedly increased to reach an IC_{50} value of about 4 nM. Figure 2 shows that the inhibition curves for astemizole markedly differed under both experimental conditions but were identical with pyrilamine. Hence, the IC_{50} values of astemizole in the H_1 binding assay became 10 times lower and were thus comparable to the IC_{50} values of most antihistaminic drugs listed in Table 1.

Astemizole is more potent at histamine H_1 receptors than at S_2 receptors. Interestingly, the IC_{50} values for the latter as well as for the *alpha*$_1$-adrenergic receptor remained practically unaffected by changing the incubation conditions. Table 1 also shows that astemizole did not bind to dopamine and muscarinic receptors. In contrast, antihistaminics such as promethazine, clemastine, but especially azatadine were found to compete on muscarinic receptors at relatively low concentrations. Astemizole, (like promethazine, clemastine, and, to a lesser extent, oxatomide) also possesses a lower affinity for *alpha*$_1$-adrenergic receptors than for H_1 receptors. In order to elucidate the problem arising from the different IC_{50} values obtained with astemizole, two different buffers were tested in the [^{3}H]pyrilamine binding assay at increasing tissue concentrations. Table 2 shows that, when astemizole was tested in phosphate buffer, the IC_{50} value was the lowest at low tissue concentrations. In contrast, the result of increasing tissue concentration was less apparent when Tris-salt buffer was used.

Further experimental data were obtained by measuring

TABLE 1

Inhibitory potencies of astemizole and other antihistaminic compounds in various receptor models

Receptor binding was measured under various conditions but using the same amount of membrane.

	IC_{50} (nM)				
	Histamine H_1	Serotonin S_2	Dopamine	Muscarinic acetylcholine	*Alpha*$_1$-adrenergic
Astemizole	40[a]	80[a]	>1,000	>1,000	14[a]
	19[b]	130[b]			25[b]
	4.7[c]	70[c]			10[c]
Pyrilamine	5[a]	>1,000	>1,000	>1,000	>1,000
	7[b]				
	5[c]				
Oxatomide	81	28	100	>1,000	200
Chlorpheniramine	7.7	>1,000	>1,000	>1,000	>1,000
Azatadine	11	280	1,200	36	>1,000
Promethazine	6.8	160	>1,000	100	70
Clemastine	2.3	400	180	100	40

[a] Incubation for 30 min, 1.1 ml.
[b] Incubation for 120 min, 1.1 ml.
[c] Incubation for 120 min, 10 ml.

the amount of labeled ligand remaining bound to the membranes. Table 3 shows the amount of radioactivity associated with the membranes after incubation of membrane preparations with increasing concentrations of [^{3}H]pyrilamine and [^{3}H]astemizole. With [^{3}H]pyrilamine, only a small fraction of the added radioactivity remained associated with the membranes, thus a large amount of [^{3}H]pyrilamine was present as free drug in the incubation mixture even when high tissue concentrations were used (Table 3). On the contrary, for [^{3}H]astemizole—especially at nanomolar concentrations—the largest amount of labeling was associated with the membranes. In addition to this, great difficulties were encountered in preparing standard solutions of the radioactive drug. When [^{3}H]astemizole was diluted in the normal concentrations, i.e., in 10% ethanol, counted radioactivity did not decrease linearly with the dilution, whereas with [^{3}H]pyrilamine the decrease in radioactivity was exactly proportional to the dilution factor (Table 3). This indicates that a non-negligible amount of [^{3}H]astemizole remained, presumably adsorbed to the glassware. This also may explain the apparent contradiction of finding an amount of membrane-bound astemizole higher than 100% (expressed here as the total radioactivity measured in the standard solutions); this must be due to a higher binding capacity of the membranes than of the glass wall, so that the radioactivity counted as standard was lower than that actually added. More recently, a linear relationship between counted radioactivity and dilution factors was found in diluting [^{3}H]astemizole in 100% ethanol (results not shown).

The dissociation rate of astemizole from the H_1 receptors was studied *in vitro* by a centrifugation technique. Figure 3 shows that astemizole and pyrilamine at the concentration of 10^{-6} M totally occupied H_1 receptors. When these membranes were centrifuged the occupancy was completely different for both drugs. With astemizole, H_1 receptors remained completely occupied, whereas after pyrilamine treatment, the receptor immediately became free after washing. After centrifugation, the membranes were further incubated at 37° for different periods of time. After 3 hr, a minute dissociation appeared in the preparations treated with astemizole, indicating that drug remained firmly bound to H_1 receptors.

Ex vivo binding. Guinea pigs were treated orally with different doses of astemizole, and 2 days later the occupancy of H_1 receptors was studied in lung and cerebellum.

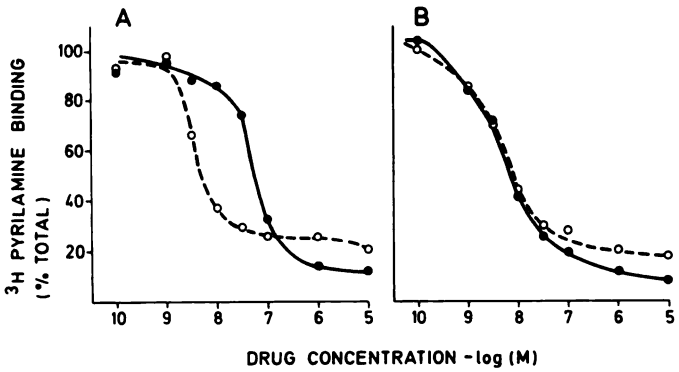

FIG. 2. *Dose-response inhibition of astemizole (A) and pyrilamine (B) in the [^{3}H]pyrilamine binding assay performed under standard conditions (1.1 ml and 30-min incubation) (●—●) or under conditions where the volume was brought to 10 ml and incubation period was increased to 120 min (○- - -○)*

Note that the amount of membrane preparation from guinea pig cerebellum was the same under both conditions.

TABLE 2

Influence of buffer and tissue concentration on the IC_{50} value of astemizole in the [^{3}H]pyrilamine binding assay

Amount of tissue[a]	IC_{50} (nM)	
	Phosphate buffer	Tris-salt buffer
40 mg	10	8
20 mg	8	4
5 mg	2.5	4.5

[a] Corresponding to the amount of tissue (wet weight). Incubation conditions were as follows: volume, 1.1 ml, and 120-min incubation.

TABLE 3

Labeled ligand bound on membranes after incubation at increasing concentrations of [^{3}H]pyrilamine and [^{3}H]astemizole

Membrane fractions from guinea pig cerebellum corresponding to 40 and 5 mg tissue were incubated in phosphate buffer (1.1 ml) for 30 min at 25° in the presence of different concentrations of [^{3}H]pyrilamine or of [^{3}H]astemizole. After filtration the radioactivity was measured on the filter and expressed in percentage of the amount added in the incubation mixtures.

Drug concentration (M)	[^{3}H]Pyrilamine			[^{3}H]Astemizole		
	Standard dpm	Bound % of total		Standard dpm	Bound % of total	
		40 mg tissue	5 mg tissue		40 mg tissue	5 mg tissue
10^{-8}	628,334	0.7	0.3	447,346	63	30
3×10^{-9}	203,118	18	5	123,667	68	38
10^{-9}	67,427	36	7	9,274	97	64
3×10^{-10}	19,346	48	14	3,180	128	108
10^{-10}	6,373	58	23	635	141	111

Figure 4 shows that, even at a low dose of 0.02 mg/kg, H_1 receptors in lung were already occupied. The occupancy by astemizole was found to be dose-dependent. However, when more than 70% of receptors were occupied in lung, the occupancy in cerebellum was still very limited. Only at high doses (2.5 and 5 mg/kg) was astemizole apparently able to occupy H_1 receptors in cerebellum.

Pyrilamine was also tested. However, 2 hr after administration, much higher doses of pyrilamine than of astemizole were required to produce identical occupancy of H_1 receptors in lung (Fig. 5). For instance, to obtain 40% occupancy, 250 times more pyrilamine was needed, so that a 0.08 mg/kg dose of astemizole was approximately equivalent to a 20 mg/kg dose of pyrilamine without taking into consideration the different time schedules for both drugs, 2 days and 2 hr. Moreover, the receptors in brain were also occupied by pyrilamine, even at low doses. Therefore in both organs, lung and brain the occupancy after pyrilamine was dose-dependent.

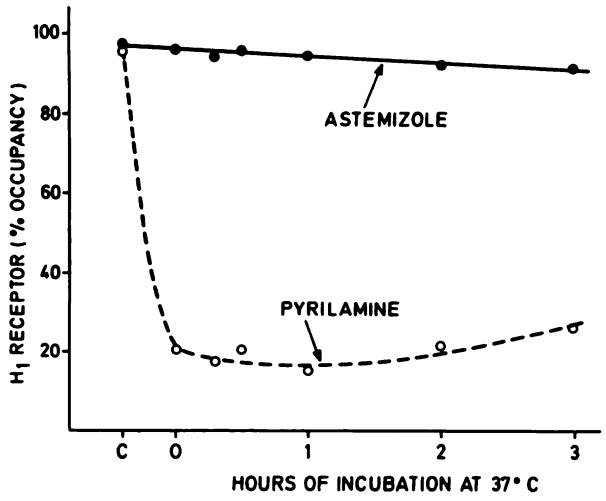

FIG. 3. *Dissociation rate of astemizole and pyrilamine from H_1 receptors in vitro*

Astemizole and pyrilamine were added at the final concentration of 10^{-6} M to membranes prepared from guinea pig cerebellum. Aliquots were directly assayed in the [^{3}H]pyrilamine binding assay (*C*). The remainder was centrifuged at 16,000 rpm for 10 min; the pellet was then suspended in 0.5 mM phosphate buffer (pH 7.5) and aliquots were incubated at 37° for different time periods. After recentrifugation the samples were assayed in the [^{3}H]pyrilamine binding assay and the percentage of occupancy was calculated from the specific binding of untreated membranes.

The time course of occupancy was studied in lung and cerebellum after oral administration of astemizole and pyrilamine. Figure 6 shows that the blockade of H_1 receptors in lung by astemizole, 0.63 and 0.31 mg/kg, remained maximal for approximately 3 days. Thereafter, a net decline occurred and the occupancy disappeared between 6 and 10 days after oral administration. In the cerebellum, the occupancy was quite low and remained practically constant for many days. In contrast, pyrilamine behaved differently, since at the dose of 160 mg/kg, the receptor occupation in lung as well as in brain declined very rapidly so that the duration of occupancy was less than 1 day.

In order to confirm such long-lasting effect of astemizole, the receptor occupancy was determined in two groups of animals treated either with one dose of astemizole for 1 day or once daily for 4 days. The histamine receptors were measured in lung 2 days after the last administration. Figure 7 shows clearly that repeated treatments markedly enhance the receptor occupation. For instance, when 0.31 mg/kg was given once daily for 4 days, 90% of H_1 receptors became occupied by the drug.

In vivo binding. Different doses of astemizole were given orally to rats 4 hr before [^{3}H]spiperone. As shown

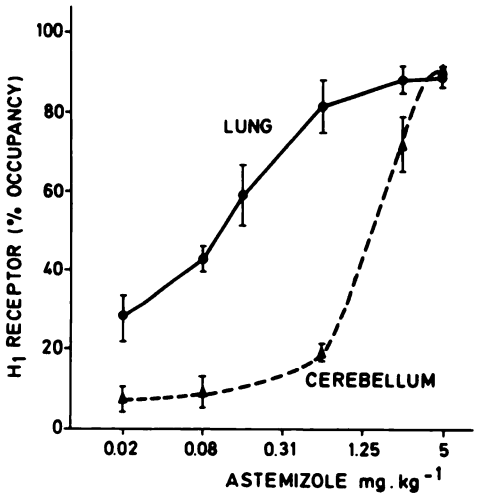

FIG. 4. *Occupancy of histamine H_1 receptors in lung and cerebellum of guinea pigs 2 days after oral administration of different doses of astemizole (see Materials and Methods, ex vivo binding)*

Results are expressed as means (± standard error of the mean) of results obtained from tissues of 6–10 animals measured in triplicate.

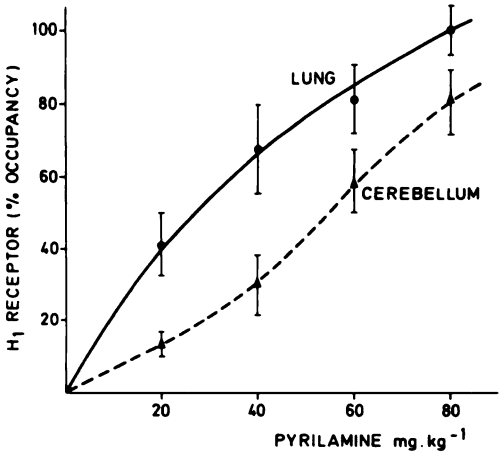

FIG. 5. *Occupancy of histamine H_1 receptors in lung and cerebellum of guinea pigs 2 hr after oral administration of pyrilamine (ex vivo binding)*

Results are expressed as means (± standard error of the mean) of results obtained from tissues of six animals.

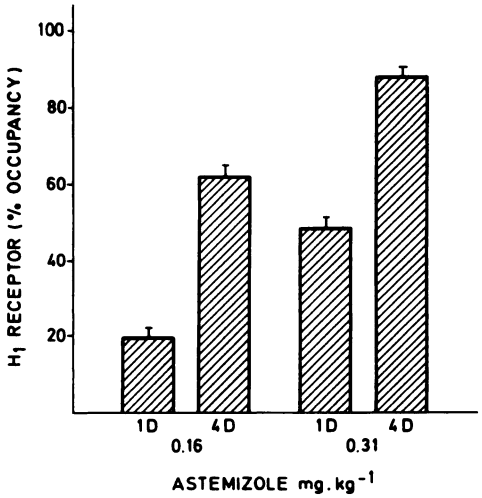

FIG. 7. *Occupancy of histamine H_1 receptors 2 days after oral administration of astemizole*

The drug was given once daily for 1 day (*1D*) or 4 days (*4D*) ($n = 6$, ± SEM)

in Table 4, even at 10 mg/kg, astemizole did not prevent the labeling by [^{3}H]spiperone of brain S_2 receptors in the frontal cortex and of dopamine receptors in the striatum. In contrast, low doses of pipamperone prevented the labeling by [^{3}H]spiperone in the frontal cortex as well as in the striatum but here to a lesser extent.

Finally, guinea pigs were treated orally with [^{3}H]astemizole, 0.31 mg/kg, in order to evaluate the distribution of the radioactivity for up to 2 days in lung and brain. Figure 8 shows that the radioactivity was considerably higher in lung than in brain. Moreover, the decline of labeling in lung was the highest between 2 and 4 days after oral administration of the drug.

DISCUSSION

These results indicate that the very long duration of action and the high potency of astemizole as an antihistamine are directly related to its binding properties. *In vitro*, the drug dissociates very slowly (or perhaps not at all) from histamine H_1 receptors, a fact which may readily explain the long duration of receptor occupation *in vivo*. However, great difficulties were encountered in demonstrating the high potency of this drug toward histamine H_1 receptors, since in the *in vitro* binding profile of astemizole under standard conditions (small volume of incubation mixture, high tissue concentration, and short incubation time) the drug revealed only a weak apparent affinity for histamine H_1 receptors. This is unlikely, since pharmacological studies have shown that astemizole is more potent than other antihistamines against histamine skin reaction and histamine lethality (8). This led us to test astemizole in [^{3}H]pyrilamine binding using different incubation conditions. A 10-fold increase in affinity was obtained by increasing the volume of the incubation mixture from 1 to 10 ml, which suggested anomalous binding properties. The use of [^{3}H]astemizole as ligand allowed us to demonstrate that the drug also possesses a high affinity for nonspecific binding sites. At nanomolar concentrations, practically all [^{3}H]astemizole appeared membrane-bound, whereas in the same concentration

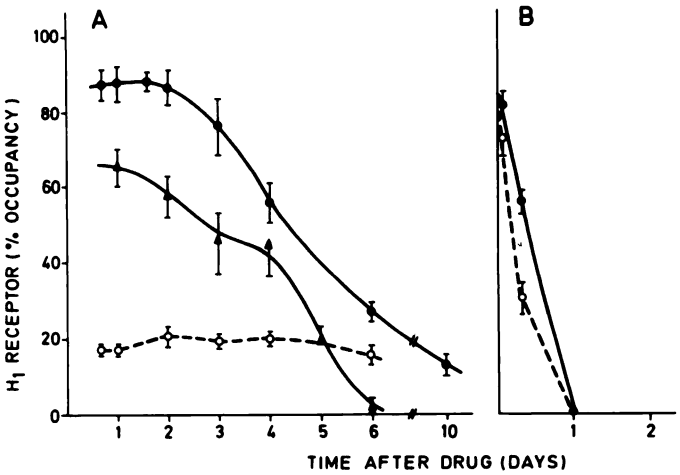

FIG. 6. *Time course of histamine H_1 occupancy in lung (——) and cerebellum (- - -) of guinea pig after oral administration of astemizole (A) and pyrilamine (B)*

Ex vivo binding after astemizole, 0.31 mg/kg (▲——▲) and 0.63 mg/kg (●——●, ○- - -○) (n = 6–10, ± SEM) and after pyrilamine, 160 mg/kg (●——●, ○- - -○) ($n = 6$).

TABLE 4

Effect of astemizole and pipamperone on in vivo [^{3}H]spiperone binding in rat brain

Drug (mg/kg)	Labeled spiperone (ng/mg)		
	Frontal cortex[a]	Striatum[a]	Cerebellum[a]
Astemizole			
Control	2.16 ± 0.06	3.34 ± 0.13	0.52 ± 0.02
0.63	2.23 ± 0.04	3.15 ± 0.04	0.52 ± 0.02
2.5	2.25 ± 0.12	3.59 ± 0.29	0.62 ± 0.02
10	2.36 ± 0.13	3.71 ± 0.29	0.64 ± 0.02
Pipamperone			
Control	2.04 ± 0.13	3.36 ± 0.24	0.48 ± 0.02
0.16	2.14 ± 0.04	3.69 ± 0.16	0.57 ± 0.03
0.63	1.74 ± 0.03	2.88 ± 0.08	0.52 ± 0.01
2.5	1.30 ± 0.09	2.73 ± 0.15	0.52 ± 0.02

[a] Values are means ± standard error of the mean, $n = 6$.

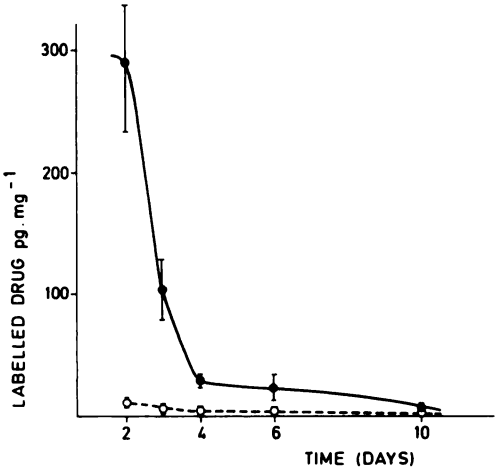

FIG. 8. *Time course of [^{3}H]astemizole accumulation in lung and brain of guinea pig*

Animals were given [^{3}H]astemizole, 0.31 mg/kg^{-1}, orally. Data are presented as means ± standard error of the mean (n = 6).

ranges, [^{3}H]pyrilamine remained largely present as free drug. Since a large amount of [^{3}H]astemizole binding was not displaceable by cold drug, one may conclude that the binding of astemizole at these low concentrations was largely non specific. Finally, the real concentration of [^{3}H]astemizole in the test tube obtained after dilution of the drug in 10% ethanol never corresponded to that normally expected but was always much lower, presumably because of the adsorption of the drug to the walls of the test tubes or to the pipettes. For all of these reasons, the exact determination of the affinity of astemizole toward H_1 receptors remains problematic, and the IC_{50} values obtained thus far (2–4 nM) must be considered as an underestimated value. Therefore, astemizole cannot be considered a reliable ligand for labeling H_1 receptors *in vitro*.

Astemizole demonstrates that certain types of drugs need to be tested under various conditions in the *in vitro* binding assays and the results must be interpreted with great caution. One must remember that, when a drug is tested *in vitro* for its ability to inhibit a specific ligand binding, one assumes that the drug affinity for nonspecific binding sites, including the glass wall, is not so high as to influence the specific binding on the receptor sites. As the rule, the nonspecific binding of a cold drug remains an unknown point.

Such difficulties were not encountered with the other receptor sites, in part because lower tissue concentrations and Tris-salt buffer were used for those sites but also because the affinity values for those receptor sites were much lower than for H_1 receptor, more astemizole (remaining as free drug) being available for competition with the binding sites. When tested in 1 or 10 ml, astemizole displayed identical IC_{50} values in the serotonin S_2 and adrenergic *alpha*$_1$-receptor binding assays. In contrast to certain antihistaminic drugs, astemizole was found to be devoid of antimuscarinic and antidopaminergic properties.

From the foregoing results, one may conclude that astemizole is a very potent histamine H_1 antagonist. The exact nature of the binding between the drug and the receptor is unclear, but covalent binding may be excluded by the chemical structure of the drug.

Ex vivo experiments enabled us to determine to what extent astemizole occupied H_1 receptors after oral administration of the drug. Here again, astemizole revealed its most striking features, a very high potency and a long duration of receptor occupation. For instance, about 40% of H_1 receptors were occupied in lung after administration of astemizole, 0.08 mg/kg, as compared with pyrilamine, 20 mg/kg. Moreover, the occupation remained maximal for 3 days for astemizole but disappeared completely within a few hours for pyrilamine. This is quite compatible with the very slow dissociation rate observed for astemizole *in vitro*. Interestingly, when a dose of astemizole yielding 20% of receptor occupation was given once daily for 4 days, a maximal receptor occupation could be attained in the lung; this might have clinical implications in patients with allergic lung diseases (extrinsic asthma).

The *ex vivo* studies also show a marked difference between lung and brain receptor occupation. At pharmacological doses, astemizole was unable to reach histamine H_1 receptors in brain, whereas they were completely occupied in lung. That 15 or 20% of H_1 receptors were labeled by the drug in the cerebellum does not necessarily mean that the drug penetrates into brain; indeed cerebral microvessels are known to contain a large amount of histamine H_1 receptors (15). Supporting this view is the fact that, when [^{3}H]astemizole was given orally to guinea pigs, only a minute amount of labeling was recovered in brain.

In rats, even at high doses, astemizole did not displace [^{3}H]spiperone in the frontal cortex, thus confirming in another animal species that the drug does not readily cross the blood-brain barrier. Pharmacological studies have also shown that astemizole lacks central effects (8) and that it does not alter sleep patterns in dogs (16). The sedative side effects of conventional antihistamine drugs are generally believed to be the consequence of an interaction with brain histamine H_1 receptors (17).

The remarkably long duration of the action of astemizole and the apparent inability of the compound to cross the blood-brain barrier suggest that it will be a useful antihistamine lacking the sedative properties that represent the major drawback of all classical compounds of this type.

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REFERENCES

1. Hill, S. J., J. M. Young, and D. H. Marrian. Specific binding of [^{3}H]-mepyramine to histamine H_1 receptors in intestinal smooth muscle. *Nature (Lond.)* **270**:361–363 (1977).
2. Chang, R. S. L., V. T. Tran, and S. H. Snyder. Histamine H_1 receptors in brain labelled with ^{3}H-mepyramine. *Eur. J. Pharmacol.* **48**:463–464 (1978).
3. Hill, S. J., P. C. Emson, and J. M. Young. The binding of [^{3}H]-mepyramine to histamine H_1 receptors in guinea pig brain. *J. Neurochem.* **31**:997–1004 (1978).
4. Schwartz, J. C., G. Barbin, A. M. Duchemin, M. Garbarg, J. M. Palacios, T. T. Quach, and J. M. Rose. Histamine receptors in brain: characterization by binding studies and biochemical effects, in *Receptors, Neurotransmitters and Peptide Hormones* (G. Pepen, M. J. Kubar, and S. J. Enna, eds.). Raven Press, New York, 169–182 (1980).
5. Taylor, J. E., and E. Richelson. High affinity binding of tricyclic antidepressants to histamine H_1 receptors: fact and artefact. *Eur. J. Pharmacol.* **67**:41–46 (1980).

6. Chang, R. S. L., V. T. Tran, and S. H. Snyder. Heterogeneity of histamine H_1 receptors: species variations in [^{3}H]-mepyramine binding of brain membranes. *J. Neurochem.* **32**:1653–1663 (1979).
7. Chang, R. S. L., V. T. Tran, and S. H. Snyder. Characteristics of histamine H_1 receptors in peripheral tissues labelled with [^{3}H]mepyramine. *J. Pharmacol. Exp. Ther.* **209**:437–442 (1979).
8. Van Wauwe, J., F. Janssens, C. J. E. Niemegeers, F. Awouters, J. M. Van Nueten, and P. A. J. Janssen. *In vitro* pharmacology of astemizole, a new type of H_1 antihistaminic compound. *Arch. Int. Pharmacodyn. Ther.* **251**:39–51 (1981).
9. Leysen, J. E., C. J. E. Niemegeers, J. P. Tollenaere, and P. M. Laduron. Serotonergic component of neuroleptic receptors. *Nature* (*Lond.*) **272**:168–171 (1978).
10. Leysen, J., J. P. Tollenaere, M. H. J. Koch, and P. Laduron. Differentiation of opiate and neuroleptic receptor binding in rat brain. *Eur. J. Pharmacol.* **43**:253–267 (1977).
11. Laduron, P. M., M. Verwimp, and J. E. Leysen. Stereospecific *in vitro* binding of [^{3}H]-dexetimide to brain muscarinic receptors. *J. Neurochem.* **32**:421–427 (1979).
12. Greenberg, D. A., D. C. U'Prichard, and S. H. Snyder. Alpha noradrenergic receptor binding in mammalian brain: differential labeling of agonist and antagonist. *Life Sci.* **19**:69–76 (1976).
13. Leysen, J. E., F. Awouters, L. Kennis, P. M. Laduron, J. Vandenberk, and P. A. J. Janssen. Receptor binding profile of R 41 468, a novel antagonist at 5-HT_2 receptors. *Life Sci.* **28**:1015–1022 (1981).
14. Laduron, P. M., P. F. M. Janssen, and J. E. Leysen. Spiperone: a ligand of choice for neuroleptic receptors. 2. Regional distribution and *in vivo* displacement of neuroleptic drugs. *Biochem. Pharmacol.* **27**:317–321 (1978).
15. Peroutka, S. J., M. A. Moskowitz, J. F. Reinhard, and S. H. Snyder. Neurotransmitter receptor binding in bovine cerebral microvessels. *Science* (Wash. D. C.) **208**:610–612 (1980).
16. Wauquier, A., W. A. E. Van den Broeck, F. Awouters, and P. A. J. Janssen. A comparison between astemizole and other antihistamines on sleep-wakefulness cycles in dogs. *Neuropharmacology*, **20**:853–859 (1981).
17. Uzan, A., G. Le Fur, and C. Malgouris. Are histamines sedative via a blockade of brain H_1 receptors. *J. Pharm. Pharmacol.* **31**:701–702 (1979).

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