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Histamine H_1 receptor occupancy in human brains after single oral doses of histamine H_1 antagonists measured by positron emission tomography

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1 Histamine H_1 receptor occupancy in the human brain was measured in 20 healthy young men by positron emission tomography (PET) using [^{11}C]-doxepin.

2 (+)-Chlorpheniramine, a selective and classical antihistamine, occupied 76.8±4.2% of the averaged values of available histamine H_1 receptors in the frontal cortex after its administration in a single oral dose of 2 mg. Intravenous administration of 5 mg (+)-chlorpheniramine almost completely abolished the binding of [^{11}C]-doxepin to H_1 receptors (H_1 receptor occupancy: 98.2±1.2%).

3 Terfenadine, a nonsedative antihistamine, occupied 17.2±14.2% of the available H_1 receptors in the human frontal cortex after its administration in a single oral dose of 60 mg.

4 There was no correlation between H_1 receptor occupancy by terfenadine and the plasma concentration of the active acid metabolite of terfenadine in each subject.

5 PET data on human brain were essentially compatible with those on H_1 receptor occupancy in guinea-pig brain determined by *in vivo* binding techniques, although for the same H_1 receptor occupancy the dose was less in human subjects than in guinea-pigs.

6 The PET studies demonstrated the usefulness of measuring H_1 receptor occupancy with classical and second-generation antihistamines in human brain to estimate their unwanted side effects such as sedation and drowsiness quantitatively.

Keywords: Antihistamines; H_1 receptor antagonists: chlorpheniramine; brain; terfenadine; sedation in man: positron emission tomography; receptor occupancy

Introduction

Many antihistamines cause drowsiness and impaired performance (Nicholson, 1983; 1987), which are thought to be due mainly to their blockade of central histamine H_1 receptors (Nicholson *et al.*, 1991). However, there has been a resurgence of interest in histamine H_1 receptor antagonists since the recent discovery and development of antihistamines with low, if any, side effects of sedation (Ganellin, 1992). It has been proposed that the effect of sedation results from occupation of cerebral H_1 receptors, but there is no conclusive evidence for this, particularly in man. Central H_1 receptor occupancy has usually been determined by *ex vivo* binding techniques in animals (Quanch *et al.*, 1979; 1980; Diffley *et al.*, 1980).

By positron emission tomography (PET) neurotransmitter receptors in human subjects can be quantitated non-invasively, permitting *in vivo* measurements of receptor occupancy (Frost & Wagner, 1990; Pike, 1993). We have developed and validated *in vivo* imaging methods to measure histamine H_1 receptors in human subjects using [^{11}C]-mepyramine and [^{11}C]-doxepin (Villemagne *et al.*, 1991; Yanai *et al.*, 1992b,c).

In this study, central H_1 receptor occupancy in human brain after single oral doses of classical and non-sedative antihistamines was measured by positron emission tomography using [^{11}C]-doxepin. Central histamine H_1 receptor occupancy was also determined in guinea-pigs after oral doses of antihistamines by *in vivo* binding techniques because PET measurement of H_1 receptor occupancy in human brain is based on *in vivo* binding.

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Methods

PET measurements

(*E*)-Doxepin labelled with ^{11}carbon was obtained by N-alkylation of the desmethylated (*E*)-doxepin ((*E*)-isomer, 97.7%) with [^{11}C]-iodomethane as described previously (Yanai *et al.*, 1992b,c). The precursor and product were generous gifts from Pfizer Inc., Japan. The radiochemical and chemical purities of the ligand were >99% and >95%, respectively. The means±1 s.d. of the specific activity at the time of administration, injected dose, and injected mass were 65.3± 9.3 GBq μmol^{-1} (1766±252 mCi μmol^{-1}), 518±98 MBq (14.0±2.7 mCi), and 8.6±1.5 nmol (1.5±0.3 μg), respectively.

Young male volunteers of 20-25 years old received an injection of [^{11}C]-doxepin 1 h after the oral administration of (+)-chlorpheniramine or terfenadine. They had no personal history of allergy or of taking antiallergic H_1 antagonists. They ate a light lunch before 12 h 00 min and they did not eat again until the end of the study. They received a single oral dose of an antihistamine at 15 h 30 min, 2 mg of (+)-chlorpheniramine or 60 mg of terfenadine. These doses were chosen on the basis of recommended doses. In another experiment, 5 mg of (+)-chlorpheniramine was injected intravenously 10 min before [^{11}C]-doxepin administration. Written informed consent was obtained from all volunteers before PET studies, and permission for these studies was granted by the Committee on Clinical Investigation, Tohoku University School of Medicine, and the Advisory Committee of Radioactive Substances, Tohoku University School of Medicine.

In all 20 PET studies were carried out. The whole-body positron emission tomograph used (Model 931; CTI Inc., Knoxville, TN, U.S.A.) acquired 7 simultaneous transverse planes. The subject's head was positioned according to the

standard CT atlas so as to obtain planes parallel to the orbitomeatal (OM) line. A Ge-68/Ga-68 transmission scan was performed to correct for auto-attenuation. [^{11}C]-doxepin was injected in 3–5 ml of saline over a period of 60 s, and PET data were acquired with the subject's eyes closed for 90 min from 30 s after [^{11}C]-doxepin administration according to the following protocol of scans: six of 1.5 min, seven of 3 min, six of 5 min, and then three of 10 min. Arterial blood samples were collected for 60 min from 6 volunteers after the injection through a transcutaneous catheter inserted into the left radial artery. The radioactivity of the plasma samples was measured, and the percentage of [^{11}C]-doxepin in the total ^{11}C radioactivity was analysed by high performance liquid chromatography (h.p.l.c.) as described previously (Yanai *et al.*, 1990; 1992b).

The PET scanner and the well scintillation detector for counting blood samples were calibrated using a 10 cm cylindrical phantom and a test tube containing a known concentration of ^{11}C, respectively. Calibration data, together with the measured radioactivity of [^{11}C]-doxepin at the time of injection, were used to determine the brain radioactivity obtained by PET as a percentage of the injected dose per litre of brain volume (%dose l^{-1}) in each subject. Radioligand concentrations in the frontal and cerebellar cortices were determined in bilateral brain regions using several elliptical regions of interest (ROIs) in the PET images of the subjects. These structures were readily identifiable in the early PET images (10–20 min after injection). In the late PET images, in which their visual identification was difficult, the sets of templates of ROIs defined in early PET images were transferred to the late images of each subject. Average values for each structure were calculated from data in several records of both the left and right sides.

The term 'binding potential' is often used as a measure of the combined density and affinity (B_{max}/K_D) of a receptor population *in vivo* because B_{max} and K_D cannot be determined separately in studies of single-tracer kinetics. The specific *in vivo* binding parameter, binding potential, in the cortex was determined by graphical analysis by the method of Logan *et al.* (1990) from the ratio of the slopes for the two ROIs of cerebellum and cortical structures because the cerebellum contains a very low density of H_1 receptors (Chang *et al.*, 1979; Yanai *et al.*, 1992b). The values of binding potential in the frontal cortex were calculated from the following equation: (ratio of slopes of the radioactivity in the frontal cortex and cerebellum)-1. The slopes were determined from last 12 points of linear region by linear regression analysis. H_1 receptor occupancy (as a % of the control) was calculated by the following equation: 100 - (binding potential in each treated subject)/(mean value of binding potential in control subjects) × 100 (%).

Determination of terfenadine and its acid metabolite

Plasma samples (5 ml) were taken 60 min after oral administration of terfenadine. Terfenadine, the carboxylic acid metabolite (M1), and piperidine methanol metabolite (M2) were measured by h.p.l.c. with fluorescence detection by a reported method with a slight modification (Coutant *et al.*, 1991). Briefly, samples and standards were prepared by solid-phase extraction on C18 minicolumns (Bond Elute C18, VARIAN, CA, U.S.A.). Minicolumns were preconditioned with two 2 ml volumes of methanol followed by 2 ml of water and 1.5 ml of 0.2 M acetate buffer (pH 4.0). The internal standard solution (1 ml 150 ng ml^{-1} in acetate buffer) was added to the column. Then 1 ml of plasma sample or calibration standard (0–1000 ng of M1 and 0–100 ng of M2 and terfenadine per 1 ml of drug-free plasma) was added to the solution in the minicolumn, and the solution was passed through the column under reduced pressure. The column was rinsed with 2 ml of water, two 1 ml volumes of methanol-vater (50:50 v/v), and 1 ml of methanol. The sample was eluted from the column into a tube with two 1 ml volumes of 0.05 M triethylamine in methanol. The eluted samples were evaporated under a stream of nitrogen at 50°C, and reconstituted in 200 µl of the mobile phase for h.p.l.c. and 100 µl samples were injected into an h.p.l.c. column (Spherisorb 5CN, Phase Separation, Norwark, CT, U.S.A.) maintained at 40°C. All separations were performed with a vacuum-degassed mobile phase consisting of acetonitrile/methanol/0.012 M ammonium acetate buffer (15/23/62 v/v) at a flow rate of 1.5 ml min^{-1}. The h.p.l.c. eluate was monitored with a fluorometer (821-FP, JASCO, Tokyo) at excitation and emission wavelengths of 230 and 280 nm, respectively.

Animal experiments

The *in vivo* binding of [^{3}H]-mepyramine (Amersham, 1036 MBq μmol^{-1}) was examined in adult male Hartley guinea-pigs weighing 280–310 g. A Teflon catheter was implanted in the jugular vein under pentobarbitone anaesthesia (25 mg kg^{-1}). The animals received an intravenous injection of [^{3}H]-mepyramine (370 kBq, 357 pmol) 1 h after oral administration of various doses of (+)-chlorpheniramine and terfenadine. They were killed by decapitation 60 min after [^{3}H]-mepyramine administration, and their brains were rapidly removed and cut on ice into specific regions (cerebellum, thalamus, striatum, hippocampus, and cortex). The brain samples were weighed and solubilized with Protosol (NEN) at 50°C for 2 days, and then their radioactivity was measured in a liquid scintillation counter (Tri-carb Packard 2050). The results were calculated and plotted as ratios of values to those in the striatum, which was essentially no H_1 receptors (Hill *et al.* 1978; Chang *et al.*, 1979).

Results

PET measurements

Histamine H_1-receptors in human brain were measured with [N-$^{11}CH_3$]-(*E*)-doxepin by positron emission tomography. As shown in Figure 1c and 1f, oral administration of 2 mg of (+)-chlorpheniramine decreased the accumulation of the ^{11}C-ligand in the brain. There was no difference in the radioactivities in the cortex and cerebellum of (+)-chlorpheniramine-treated subjects. On the contrary, terfenadine did not apparently block the accumulation in the cortex (Figure 1b and 1e).

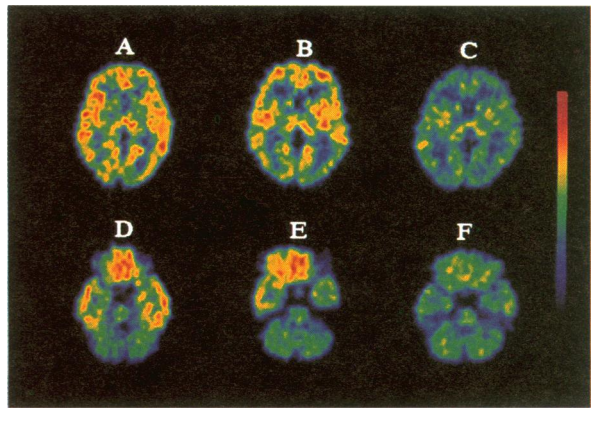

Figure 1 PET images showing radioactivity in horizontal brain sections at the striatal (**A**, **B**, **C**) and cerebellar (**D**, **E**, **F**) levels after i.v. injection of [^{11}C]-doxepin into healthy volunteers. (**A**), (**D**): PET images in a control subject. (**B**), (**E**): PET images in a subject treated with 60 mg of terfenadine. (**C**), (**F**): PET images in a subject treated with 2 mg of (+)-chlorpheniramine.

The method of graphical analysis developed by Logan *et al.* (1990) was applicable to [^{11}C]-doxepin binding *in vivo*. The averaged plasma radioactivity of [^{11}C]-doxepin in 6 cases (3 control, 2 treated studies with 2 mg (+)-chlorpheniramine, and 1 treated studies with 60 mg terfenadine) was used for the calculation instead of the individual plasma concentrations. The treatment with (+)-chlorpheniramine and terfenadine did not apparently change the metabolism of [^{11}C]-doxepin in human subjects as shown in Figure 2d. The differences between the values of binding potential calculated from averaged and individual plasma concentrations were less than 5% in six subjects from whom arterial blood samples were taken. The average concentrations of [^{11}C]-doxepin (corrected for metabolites) in the arterial blood at 0.33, 0.66, 1, 1.3, 1.7, 2, 2.5, 3, 4, 5, 7.5, 10, 15, 20, 25, 30, 40, 50, and 60 min were 2.6, 4.1, 4.5, 4.7, 3.1, 2.0, 1.4, 1.1, 0.91, 0.78, 0.58, 0.54, 0.43, 0.37, 0.32, 0.27, 0.27, and 0.26% dose l^{-1}, respectively.

According to this method, we plotted $\int ROI(\tau)d\tau/ROI(T)$ vs. $\int Cp(\tau)d\tau/ROI(T)$, where $ROI(\tau)$ and $Cp(\tau)$ are functions of time (τ) describing the variation of radioactivity in an ROI of brain and metabolite-corrected radioactivity in the plasma (average data for 6 volunteers), respectively. As shown in Figure 2, a linear relationship was observed from approximately 5 min after the injection. The differences in the slopes in the frontal cortex and cerebellum were greater in control and terfenadine-treated subjects than in (+)-chlorpheniramine-treated subjects.

Histamine H_1 receptor occupancy in human brain

The calculated values of binding potential in the control, terfenadine-treated, and (+)-chlorpheniramine-treated groups are summarized in Figure 3. Administration of (+)-chlorpheniramine at a dose of 2 mg significantly decreased the binding potential. However, the parameters were not significantly changed from control values by treatment with 60 mg of terfenadine. Intravenous treatment with (+)-chlorpheniramine at a dose of 5 mg almost completely abolished the *in vivo* binding of [^{11}C]-doxepin, its blockade of ^{11}C-radio-

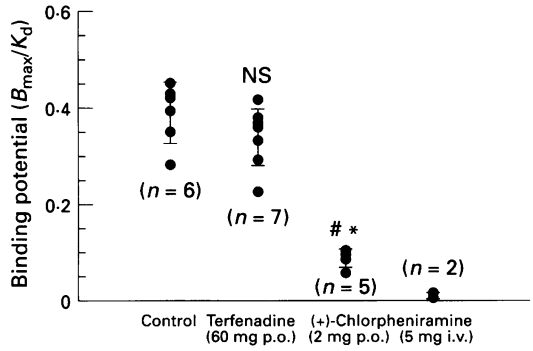

Figure 3 Histamine H_1 receptors measured by PET after administration of sedative and non-sedative antihistamines. The values and means ± 1 s.d. of binding potential are plotted as (●). The significances of differences in binding potentials of control, terfenadine, and (+)-chlorpheniramine (p.o.) groups were determined by ANOVA followed by Duncan's test. $^*P<0.05$ (vs. control group); $^{\#}P<0.05$ (vs. terfenadine group); NS (not significant compared with control group).

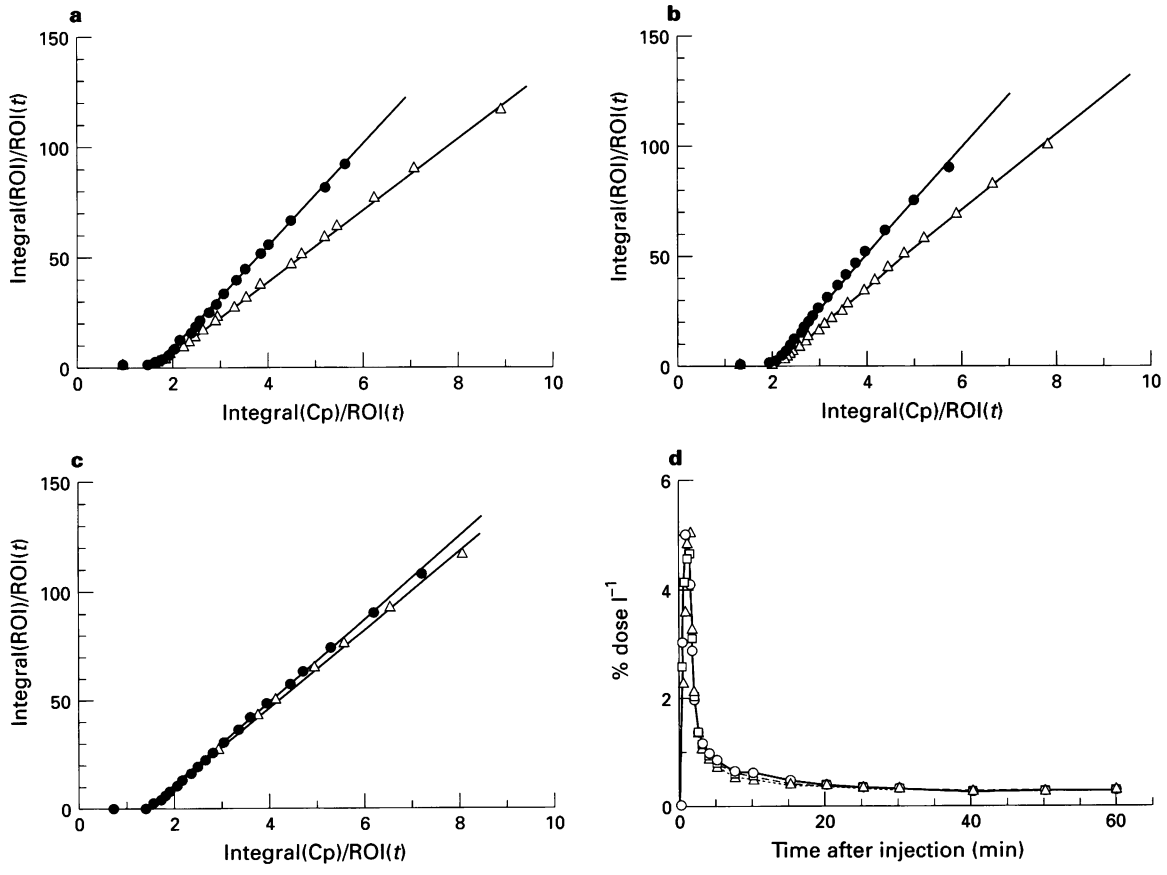

Figure 2 Graphical analysis of the time-activity data from the frontal cortex (●) and cerebellum (△) regions of interest (ROIs), ROI(t) indicates the radioactivity in the region of interest at time t. Cp is the plasma radioactivity corrected for metabolites. Data are representatives for control (**a**), terfenadine-treated (**b**), and (+)-chlorpheniramine-treated (**c**) subjects. (**d**) Radioactivity of [^{11}C]-doxepin in arterial plasma. Three control subjects (○); 2 (+)-chlorpheniramine-treated subjects (△); one terfenadine-treated subject (□).

activity in the brain being dose-dependent. The histamine H_1 receptor occupancies by these antihistamines were calculated from the values of binding potential, and are listed in Table 1.

Table 1 Histamine H_1 receptor occupancy in human brain

Treatment	*% receptor occupancy*	n
None	0 ± 15.0	6
Terfenadine (60 mg p.o.)	17.2 ± 14.2^{NS}	7
(+)-Chlorpheniramine (2 mg p.o.)	76.8 ± 4.2$^{*,\#}$	5
(+)-Chlorpheniramine (5 mg i.v.)	96.5 ± 1.9	2

Values are means ± 1 s.d. for 2–7 determinations. $*P < 0.05$ (*vs.* control group); $\#P < 0.05$ (*vs.* terfenadine group); NS (not significant compared with values of control group). The statistical significance of differences in values in the control, terfenadine, and (+)-chlorpheniramine (p.o.) groups were determined by ANOVA followed by Duncan's test.

Histamine H_1 receptor occupancies were 76.8 ± 4.2% and 96.5 ± 1.9% after a single oral dose of 2 mg and intravenous administration of 5 mg of (+)-chlorpheniramine, respectively. In contrast, the H_1 receptor occupancy was 17.2 ± 14.2% after an oral dose of 60 mg of terfenadine.

Correlation of H_1 receptor occupancy in human brain with the plasma concentration of terfenadine acid metabolite

Terfenadine is subject to extenive first-pass effect (Garteiz *et al.*, 1982; Coutant *et al.*, 1991). On oxidation, its main metabolite is a carboxylic acid (M1) which is a potent H_1 antagonist (Figure 4a) and which penetrates the blood-brain barrier (BBB) with difficulty because of its negative charge (Leeson *et al.*, 1982; Wiech *et al.*, 1982). A very large variation in the concentration of M1 was observed in different subjects in this study. The time-dependent change in concentration of the terfenadine acid metabolite was examined for 2.5 h after oral administration of terfenadine, and the peak concentration was observed during PET scanning (data not shown). The H_1 receptor bindings in these subjects were not correlated with the plasma concentrations of M1, as shown in Figure 4b.

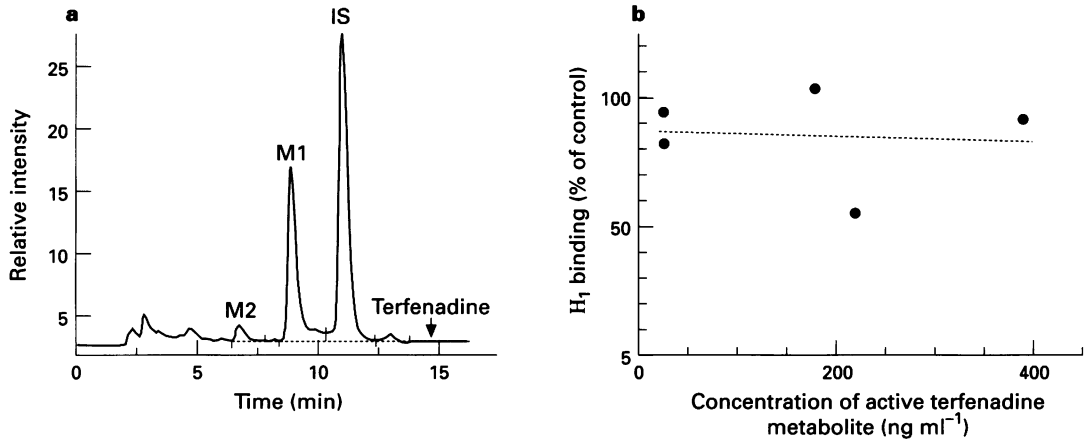

Figure 4 Determination of terfenadine and its metabolites in plasma. (**a**) H.p.l.c. chromatogram of a typical plasma sample from a subject treated with 60 mg of terfenadine. 4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl-1-piperidinyl)butyl]-α-α-dimethylbenzene acetic acid (M1) and α-α-diphenyl-4-piperidinemethanol (M2) were detected in the plasma. However, the level of terfenadine was too low to detect. IS: internal standard (4-[1-hydroxy-4-[4-(hydroxybis(4-methylphenyl)methyl-1-piperidinyl]-α-α-dimethylbenzene acetic acid). (**b**) Absence of correlation between H_1 receptor binding measured by PET and the plasma concentration of active terfenadine acid metabolite (M1) 60 min after oral administration of terfenadine ($r = 0.077$).

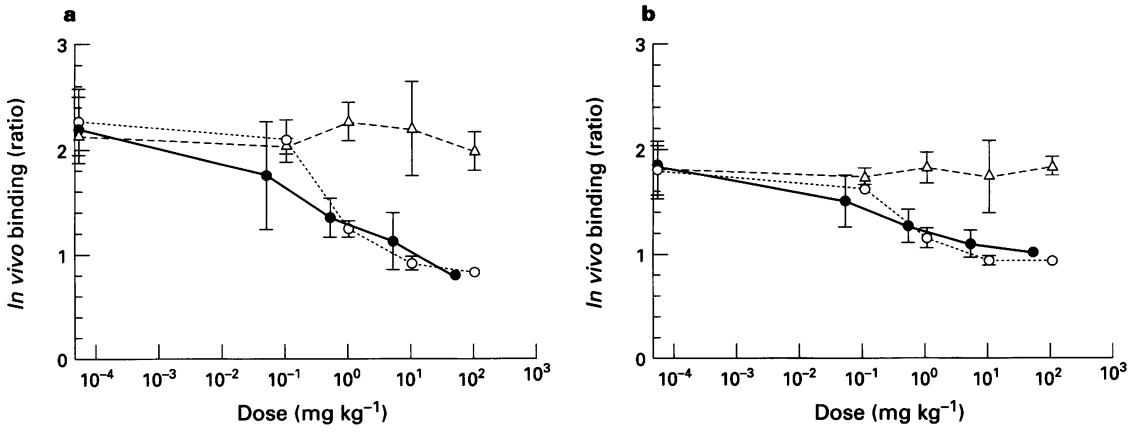

Figure 5 Effects of pretreatment with (+)-chlorpheniramine (oral; ○: intraperitoneal; ●) and terfenadine (oral; △) on specific *in vivo* binding of [^{3}H]-mepyramine in guinea-pig cerebellum (a) and thalamus (b). The data were plotted as ratios of values to those in the striatum because striatum contains the lowest density of H_1 receptors. (+)-Chlorpheniramine blocked the binding dose-dependently, while terfenadine had little effect on *in vivo* binding. Points are means ± 1 s.d. for 2–5 determinations.

Animal experiments

Marked species differences were observed in the distribution of H_1 receptors. In guinea-pigs, the cerebellum and thalamus contain the highest density of H_1 receptors in the brain while the striatum has negligible H_1 receptors (Chang *et al.*, 1979). Single oral doses of (+)-chlorpheniramine resulted in dose-dependent occupation of central H_1 receptors in the guinea-pig cerebellum and thalamus, as shown in Figure 5a and b, respectively. Intraperitoneal administration of (+)-chlorpheniramine similarly decreased the *in vivo* binding of [^{3}H]-mepyramine to H_1 receptors in the brain. In the cerebellum, the ID_{50} values on intraperitoneal and oral administration of (+)-chlorpheniramine were approximately 0.12 and 0.43 mg kg^{-1} body weight, respectively. The oral dose causing 80% receptor occupancy was approximately 2 mg kg^{-1} in guinea-pigs. On the other hand, terfenadine did not occupy central H_1 receptors in guinea-pig brain as determined under the conditions for *in vivo* binding.

Discussion

H_1-receptor antagonists are among the most widely used drugs in the world. But even at recommended doses, first-generation H_1 antagonists such a triprolidine, diphenhydramine, hydroxyzine, and chlorpheniramine, often have adverse effects on the central nervous system, such as induction of somnolence, diminished alertness, slowed reaction times, and impaired cognitive function. Thus in epidemiological studies, first-generation antihistamines have been implicated as causes of fatal traffic accidents (Cimbura *et al.*, 1982). These adverse effects are thought to be due to the occupation of central histamine H_1 receptors, although this has not been proved conclusively in human subjects (Nicholson *et al.*, 1991). Second-generation H_1 antagonists, such as terfenadine, astemizole, loratidine, and cetirizine, have relatively weak side effects as sedatives, as shown indirectly by electroencephalographic monitoring, sleep-latency studies and simple measurements of reaction times in complex sensorimotor tasks such as computer-monitored driving (Bhatti & Hindmarch, 1989; Simons *et al.*, 1994). This difference was originally attributed to their higher affinity for peripheral receptors than for central H_1 receptors. It now appears more likely, however, that their lower sedative effects are associated with their difficulty in crossing BBB, although there is no proof of this in man (Rose *et al.*, 1982; Timmerman, 1992; Zhang *et al.*, 1993).

Central H_1 receptor occupancy has usually been measured by *ex vivo* binding techniques in animals (Quanch *et al.*, 1979; 1980; Diffley *et al.*, 1980). We previously reported that H_1 receptors could be measured by *in vivo* binding techniques in the guinea-pig (Yanai *et al.*, 1990). *In vivo* measurement of binding is useful in determining the potencies of drugs *in vivo*. Neurotransmitter receptors have been measured non-invasively in human subjects by PET with suitable radioligands. An alternative use of PET is for measurement of receptor occupancy after administration of receptor-binding drugs. We have used PET-imaging methods for measuring histamine H_1 receptor occupancy in human brain to demonstrate differences in penetration through the BBB of sedative and non-sedative H_1 antagonists.

In the present study, we measured central H_1 receptor occupancies after single oral doses of a classical antihistamine, (+)-chlorpheniramine, and a second-generation H_1 antagonist, terfenadine, using [^{11}C]-doxepin in male volunteers of 20–25 years of age. We previously reported that the binding of both [^{11}C]-doxepin and [^{11}C]-pyrilamine were age-dependent, and that [^{11}C]-doxepin was a ligand of choice for measuring H_1 receptors by PET because of its higher affinity for H_1 receptors (Yanai *et al.*, 1992a,c). [^{3}H]-doxepin is reported to have two saturable binding sites with higher and lower affinities in the brain. These high and low affinity binding sites were attributed to the H_1 receptor and an undefined receptor, respectively (Tran *et al.*, 1981; Kanba & Richelson, 1984). Doxepin was the most potent drug at histamine H_1 receptors in human brain. Moreover, the affinities of doxepin for muscarinic receptors, α_1-, α_2-adrenoceptors, 5-HT_{1A}, 5-HT_2, and dopamine D_2-receptors were about 200 fold less than that for H_1 receptors (Cusack *et al.*, 1994). Actually, the binding of [^{11}C]-doxepin in the frontal cortex was significantly lowered after administration of a specific H_1 antagonist, (+)-chlorpheniramine and the radioactiivty in the cortex was approximately the same as that in cerebellum (Yanai *et al.*, 1992c). [^{11}C]-doxepin was assumed to occupy only a small fraction of H_1 receptors in the brain because of its high specific activity, suggesting that most of the [^{11}C]-doxepin might be bound to the higher-affinity binding sites, H_1 receptors, in the brain.

PET measurements of receptor occupancy in human brain have been used in studies of opiate receptors (Lee *et al.*, 1988; Jones *et al.*, 1991; Villemagne *et al.*, 1994), dopamine D_1 and D_2 receptors (Farde *et al.*, 1992; Nordström *et al.*, 1993), and benzodiazepine receptors (Shinotoh *et al.*, 1989). Farde *et al.* (1992) demonstrated that D_2 receptor occupancy induced by classical neuroleptics in the basal ganglia of drug-treated schizophrenic patients was 70 to 89% at conventional dosages. The 'atypical' antipsychotic drug, clozapine, occupied 38 to 63% of D_2 receptors in human brain. Our findings of H_1 receptor occupancy by antihistamines are analogous to theirs in some respects. The histamine H_1 receptor occupancy after a single oral dose of 2 mg of (+)-chlorpheniramine was calculated to be approximately 80% of the total H_1 receptors, whereas the occupancy after treatment with terfenadine at a dose of 60 mg was less than 20% of the total H_1 receptors in the frontal cortex, which was not significantly different from the control value. The specific binding of [^{3}H]-mepyramine sometimes disappeared in autopsied human brains of patients who were taking conventional doses of classical antihistamines immediately before their death (Yanai *et al.*, unpublished data). These data suggest that classical antihistamines at the recommended doses occupied almost all H_1 receptors in the brain and that the full occupancy of H_1 receptors was closely related to their unwanted central side effects.

Central receptor occupancy has been investigated with respect to pharmacological effects in both animals and man. A linear relationship between D_2 receptor occupancy and antipsychotic effects was observed in schizophrenic patients (Nordström *et al.*, 1993). In studies on benzodiazepine receptors, the fractional receptor occupancy by a full agonist was perfectly correlated with its graded pharmacological effects. However, partial and partial-inverse agonists required a large fractional receptor occupancy to produce significant anti- and proconvulsant effects, respectively (Brouillet *et al.*, 1991; Chavoix, *et al.*, 1991). In some animal studies, receptor occupancy was not correlated with pharmacological effects (Ishizuka *et al.*, 1989). These relationships probably depend on receptor subtypes, the characteristics of the drugs, and pharmacological effects. There may also be a species difference in the relationship. In our studies, about 80% of the H_1 receptors in human brain were occupied on administration of 2 mg of (+)-chlorpheniramine, but more than 10 fold this dose of (+)-chlorpheniramine was needed for the same receptor occupancy in guinea-pigs. This species difference might be attributed to variation of absorption and metabolism in human subjects and guinea-pig.

The plasma concentrations of terfenadine and its metabolites were determined simultaneously in our PET studies. There was no correlation between H_1 receptor binding and the concentration of terfenadine's active metabolite (M1) in the plasma. This indicated that the variation of binding potential in terfenadine-treated subjects was probably due to individual differences in H_1 receptors among the volunteers. In this study, we did not perform sequential PET studies on one subject because of views from our ethical committee. Nordström *et al.* (1993) demonstrated a hyperbolic relationship between dopamine D_2 receptor occupancy and the plasma concentration of raclopride in schizophrenic patients. The low permeability of

the BBB to terfenadine's active metabolite would result in the large individual variation of binding potential in contrast to highly permeable drugs such as raclopride and (+)-chlorpheniramine. Concomitant determination of the plasma concentration of administered drugs would be helpful for understanding the relationship between receptor occupancy and pharmacological effects in non-paired PET measurements.

Our PET studies demonstated that receptor occupancy by a second-generation H_1 antagonist was less than 20% of the total H_1 receptors and that the low receptor occupancy was closely related to the low incidence of central side effects. Our preliminary studies also demonstrated low occupancies of H_1 receptors by other second generation antihistamines (Yanai *et al.*, unpublished data). PET measurement of H_1 receptor occupancy by H_1 antagonists is the most sensitive and definitive method available to estimate the incidence of central adverse effects induced by newly developed H_1 antagonists.

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References

BHATTI, J.Z. & HINDMARCH, I. (1989). The effects of terfenadine with and without alcohol on an aspect of car driving performance. *Clin. Exp. Allergy*, **19**, 609–611.

BROUILLET, E., CHAVOIX, C., BOTTLAENDER, M., KHALILI-VARASTEH, M., HANTRAYE, P., FOURNIER, D., DODD, R.H. & MAZIERE, M. (1991). In vivo bidirectional modulatory effect of benzodiazepine receptor ligands on GABAergic transmission evaluated by positron emission tomography in nonhuman primates. *Brain Res.*, **557**, 167–176.

CHANG, R.S.L., TRAN, V.T. & SNYDER, S.H. (1979). Heterogeneity of histamine H_1 receptors: species variations in [^{3}H]pyrilamine binding of brain membranes. *J. Neurochem.*, **32**, 1653–1663.

CHAVOIX, C., BROUILLET, E., KUNIMOTO, M., DE LA SAYETTE, V., KHALILI-VARASTEH, M., HANTRAYE, P., DOOD, R.H., GUIBERT, B., PRENANT, C. & NAQUET, R. (1991). Relationships between benzodiazepine receptors, impairment of GABAergic transmission and convulsant activity of beta-CCM: a PET study in the baboon *Papio papio*. *Epilepsy Res.*, **8**, 1–10.

CIMBURA, G., LUCAS, D.M., BENETT, R.C., WARREN, R.A. & SIMPSON, H.M. (1982). Incidence and toxicological aspects of drugs detected in 484 fatally injured drivers and pedestrians in Ontario. *J. Forensic Sci.*, **27**, 855–867.

COUTANT, J.E., WESTMARK, P.A., NARDELLA, P.A., WALTER, S.M. & OKERHOLM, R.A. (1991). Determination of terfenadine and terfenadine acid metabolite in plasma using solid-phase extraction and high-performance liquid chromatography with fluorescence detection. *J. Chromatogr.*, **570**, 139–148.

CUSACK, B., NELSON, A. & RICHELSON, E. (1994). Binding of antidepressants to human brain receptors: focus on newer generation compounds. *Psychopharmacology*, **114**, 559–565.

DIFFLEY, D., TRAN, V.T. & SNYDER, S.H. (1980). Histamine H_1 receptor labeled in vivo: antidepressants and antihistamine interactions. *Eur. J. Pharmacol.*, **64**, 177–181.

FARDE, L., NORDSTRÖM, A.L., WIESEL, F.A., PAULI, S., HALLIDIN, C. & SEDVALL, G. (1992). Positron emission tomographic analysis of central D_1 D_2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. *Arch. Gen. Psychiatry*, **49**, 538–544.

FROST, J.J. & WAGNER, Jr, H.N. ed. (1990). *Quantitative Imaging: Neuroreceptors, Neurotransmitters and Enzymes*. New York: Raven Press.

GANELLIN, C.R. (1992). Pharmacochemistry of H_1 and H_2 receptors. In *The Histamine Receptor*. ed. Schwartz J.C. & Haas, H.L. pp. 1–56. New York: WileyLiss.

GARTEIZ, D.A., HOOK, R.H., WALKER, B.J. & OKERHOLM, R.A. (1982). Pharmacokinetics and biotransformation studies of terfenadine in man. *Arzneim. Forsch. Drug Res.*, **32(II)**, **9a**, 1185–1190.

HILL, S.J., EWSON, P.C. & YOUNG, J.M. (1978). The binding of [^{3}H]mepyramine to histamine H_1-receptors in guinea-pig brain. *J. Neurochem.*, **31**, 997–1004.

ISHIZUKA, H., SAWADA, Y., ITO, K., SUGIYAMA, Y., SUZUKI, H., IGA, T. & HANANO, M. (1989). Nonlinear relationship between benzodiazepine receptor occupancy and glucose metabolic response in the conscious mouse brain in vivo. *J. Pharmacol. Exp. Ther.*, **251**, 362–367.

JONES, A.K.P., FRISTON, K.J., QI, L.Y., HARRIS, M., CUNNINGHAM, V.J., JONES, T., FEINMAN, C. & FRACKOWIAK, R.S.L. (1991). Sites of action of morphine in the brain. *Lancet*, **338**, 825.

KANBA, S. & RICHELSON, E. (1984). Histamine H_1 receptors in human brain labeled with [^{3}H]doxepin. *Brain Res.*, **304**, 1–7.

LEE, M.C., WAGNER, Jr, H.N., TANADA, S., FROST, J.J., BICE, A.N. & DANNALS, R.F. (1988). Duration of occupancy of opiate receptors by naltrexone. *J. Nucl. Med.*, **29**, 1207–1211.

LEESON, G.A., CHAN, K.Y., KNAPP, W.C., BIEDENBACH, S.A., WRIGHT, G.J. & OKERHOLM, R.A. (1982). Metabolic disposition of terfenadine in laboratory animals. *Arzneim. Forsch. Drug Res.*, **32**, 1173–1178.

LOGAN, J., FOWLER, J.S., VOLKOW, N.D., WOLF, A.P., DEWEY, S.L., SCHLYER, D.J., MACGREGOR, R.R., HITZEMANN, R., BENDTIEM, B., GATLEY, S.J. & CHRISTMAN, D.R. (1990). Graphical analysis of reversible radioligand binding from time-activity measurements applied to [N-^{11}C-methyl]-(-)-cocaine PET studies in human subjects. *J. Cereb. Blood Flow Metab.*, **10**, 740–747.

NICHOLSON, A.N. (1983). Antihistamines and sedation. *Lancet*, **ii**, 211–212.

NICHOLSON, A.N. (1987). New antihistamines free of sedative side effects. *Trends Pharmacol. Sci.*, **8**, 247–249.

NICHOLSON, A.N., PASCOE, P.A., TURNER, C., GANELLIN, C.R., GREENGRASS, CASY, A.F. & MERCER, A.D. (1991). Sedation and histamine H_1 receptor antagonism: studies in man with the enantiomers of chlorpheniramine and dimethindene. *Br. J. Pharmacol.*, **104**, 270–276.

NORDSTRÖM, A.L., FARDE, L., WIESEL, F.A., FORSLUND, K., PAULI, S., HALLIDIN, C. & UPPFELDT, G. (1993). Central D_2 dopamine receptor occupancy in relation to antipsychotic drug effects: a double-blind PET study of schizophrenic patients. *Biol. Psychiatry*, **33**, 227–235.

PIKE, V.W. (1993). Positron-emitting radioligands for studies in vivo - probes for human psychopharmacology. *J. Psychopharmacol.*, **7**, 139–158.

QUANCH, T.T., DUCHEMIN, A.M., ROSE, C. & SCHWARTZ, J.C. (1979). In vivo occupation of cerebral histamine H_1-receptors evaluated with [^{3}H]-mepyramine may predict sedative properties of psychoactive drugs. *Eur. J. Pharmacol.*, **60**, 391–392.

QUANCH, T.T., DUCHEMIN, A.M., ROSE, C. & SCHWARTZ, J.C. (1980). Labeling of histamine H_1 receptors in the brain of living mouse. *Neurosci. Lett.*, **17**, 49–54.

ROSE, C., QUANCH, T.T., LLORENS, C. & SCHWARTZ, J.C. (1982). Relationship between occupation of cerebral H_1-receptors and sedative properties of antihistamines. Assessment in the case of terfenadine. *Arzneim. Forsch. Drug Res.*, **32**, 1171–1173.

SHINOTOH, H., IYO, M., YAMADA, T., INOUE, O., SUZUKI, K., ITOH, T., FUKUDA, H., YAMAZAKI, T., TATENO, Y. & HIRAYAMA, K. (1989). Detection of benzodiazepine receptor occupancy in the human brain by positron emission tomography. *Psychopharmacology*, **99**, 202–227.

SIMONS, F.E.R. & SIMONS, K.J. (1994). The pharmacology and use of H_1-receptor antagonist drugs. *N. Engl. J. Med.*, **330**, 1663–1670.

TIMMERMAN, H. (1992). Factors involved in the incidence of central nervous system effects of H_1-blockers. In *Therapeutic Index of Antihistamines*. ed. Church, M.L. & Rihoux, J.P., pp. 19–31. New York: Hogrefe & Huber.

TRAN, V.T., LEBOVITZ, R., TOLL, L. & SNYDER, S.H. (1981). [^{3}H]Doxepin interactions with histamine H_1 receptors and other sites in guinea pig and rat brain homogenates. *Eur. J. Pharmacol.*, **70**, 501–509.

VILLEMAGNE, V.L., DANNALS, R.F., SANCHEZ-ROA, P.M., RAVERT, H.T., VAQUEZ, S., WILSON, A.A., NATARAJAN, T.K., WONG, D.F., YANAI, K. & WAGNER, Jr, H.N. (1991). Imaging histamine H_1 receptors in the living human brain with ^{11}C-pyrilamine. *J. Nucl. Med.*, **32,** 308–311.

VILLEMAGNE, V.L., FROST, J.J., DANNALS, R.F., LEVER, J.R., TANADA, S., NATARAJAN, T.K., WILSON, A.A., RAVERT, H.T. & WAGNER, Jr, H.N. (1994). Comparison of [^{11}C]diprenorphine and [^{11}C]carfentanil in vivo binding to opiate receptors in man using a dual detector system. *Eur. J. Pharmacol.*, **257,** 195–197.

WIECH, N.L. & MARTIN, J.S. (1982). Absence of an effect of terfenadine on guinea pig brain histamine H_1-receptors *in vivo* determined by receptor binding techniques. *Arzneim. Forsch. Drug Res.*, **32,** 1167–1170.

YANAI, K., WATANABE, T., MEGURO, K., YOKOYAMA, H., SATO, I., SASANO, H., ITOH, M., IWATA, R., TAKAHASHI, T. & IDO, T. (1992a). Age-dependent decrease in histamine H_1 receptor in human brains revealed by PET. *Neuroreport*, **3,** 433–436.

YANAI, K., WATANABE, T., YOKOYAMA, H., HATAZAWA, J., IWATA, R., ISHIWATA, K., MEGURO, K., ITOH, M., TAKAHASHI, T., IDO, T. & MATSUZAWA, T. (1992b). Mapping of histamine H_1 receptors in the human brain using [^{11}C]pyrilamine and positron emission tomography. *J. Neurochem.*, **59,** 128–136.

YANAI, K., WATANABE, T., YOKOYAMA, H., MEGURO, K., HATAZAWA, J., ITOH, M., IWATA, R., ISHIWATA, K., TAKAHASHI, T. & IDO, T. (1992c). Histamine H_1 receptors in human brain visualized in vivo by [^{11}C]doxepin and positron emission tomography. *Neurosci. Lett.*, **59,** 145–148.

YANAI, K., YAGI, N., WATANABE, T., ITOH, M., ISHIWATA, K., IDO, T. & MATSUZAWA, T. (1990). Specific binding of [^{3}H]pyrilamine to histamine H_1 receptors in guinea pig brain in vivo: determination of binding parameters by a kinetic four-compartment model. *J. Neurochem.*, **55,** 409–420.

ZHANG, M.Q., TER LAAK, A.M. & TIMMERMAN, H. (1993). Structure-activity relationships within a series of analogues of the histamine H_1 antagonist terfenadine. *Eur. J. Med. Chem.*, **28,** 165–173.

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