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RESEARCH PAPER

Peripheral and central H_1 histamine receptor occupancy by levocetirizine, a non-sedating antihistamine; a time course study in the guinea pig

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Background and purpose: The H_1 receptor occupancy (H1RO) in brain is an indicator of central side effects of antihistamines. Here, we determined the kinetics of central and peripheral H1RO by levocetirizine in relation to its brain and plasma concentration, and investigated the role of the blood-brain barrier in any delay in brain H1RO.
Experimental approach: Concentration-time profiles in plasma and brain were obtained after 0.1 and 1 mg kg^{-1} oral doses of levocetirizine in guinea pigs. H1RO in brain was measured *ex vivo* using [^{3}H]-mepyramine and, in the periphery, by measuring the degree of inhibition of histamine-induced contractions of isolated guinea pig ileum.
Key results: The concentration-time profile of levocetirizine indicated lower levels (partition coefficient, $K_p = 0.06$-0.08), higher t_{max} (2-4 h vs 1-1.5 h) and longer terminal half-life (4-5.6 h vs 2.1-2.8 h) in brain than plasma. The H1RO at 0.1 and 1 mg kg^{-1} were 75% and 97%, respectively, at 1 hr in the periphery and, in the brain, were <20% and 28-67% respectively, at all time points studied. Brain H1RO vs plasma concentrations profile showed a delay, but not when compared to brain concentrations.
Conclusions and Implications: This study demonstrates an effective peripheral antihistamine effect of levocetirizine without central adverse effects at the dose close to human therapeutic dose. The slow increase in H1RO in the brain with time was caused by slow blood-brain barrier transport of levocetirizine. This demonstrates the importance of measuring time course of brain H1RO in relation to brain concentrations of drugs.
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Keywords: levocetirizine; brain pharmacokinetics; H_1 receptor occupancy; pharmacokinetic–pharmacodynamic relationship; antihistamines

Abbreviations: BBB, blood-brain barrier; IS, internal standard; LC-MS/MS, liquid chromatography tandem mass spectrometry; LLOQ, lower limit of quantification

Introduction

In the central nervous system (CNS), histamine is associated with a wide range of physiological functions such as arousal, cognition, regulation of the sleep wake cycle, learning and memory that are mediated mainly through H_1 receptors (Watanabe and Yanai, 2001). In consequence, the antagonism of H_1 receptors in the brain can lead to side effects like sedation and impairment of cognitive functions as experienced with first generation antihistamine in the treatment of allergic disorders (Kay, 2000).

The central side effects caused by antihistamines can be estimated in humans by objective psychometric tests and subjective rating scales (Hindmarch and Shamsi, 1999). Using various antihistamines at therapeutic doses in humans, Yanai *et al*. (1999) demonstrated the existence of a correlation between impairment of cognitive performance and the central histamine H_1 receptor occupancy and proposed that an occupation of approximately 30% of histamine H_1 receptors in the human cerebral cortex seems to be tolerated before the emergence of central side effects (Yanai *et al*., 1999). If the sedative effects or cognitive impairment observed in humans are difficult to assess in animal models, receptor occupancy determination could be easily assessed in animal species using *ex vivo* binding assays or functional isolated organ assays at central and peripheral sites, respectively. Levocetirizine is a non-sedative (Hindmarch *et al*., 2001; Verster *et al*., 2003a, b) antihistamine effective against allergic conditions (Devalia *et al*., 2001). To determine H_1 receptor occupancy of levocetirizine *ex vivo* in

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brain and peripheral tissues, we have taken advantage of the fact that levocetirizine has a long residence time on the H_1 histamine receptor owing to very slow dissociation kinetics (dissociation half-life = 142 min, Gillard *et al*., 2002). In this respect, therefore, we studied central H_1 receptor occupancy using displacement of [^{3}H]-mepyramine binding to the cerebellum and peripheral occupancy using the shift of the histamine concentration–response curve in segments of ileum obtained from the drug-treated guinea pigs in comparison to vehicle-treated guinea pigs.

The central H_1 receptor occupancy is commonly measured at the time of maximal plasma concentration (Wiech and Martin, 1982; Snowman and Snyder, 1990; Kreutner *et al*., 2000; Yakuo *et al*., 2001). However, for any drug, the pharmacokinetics in brain could differ from plasma pharmacokinetics depending on the involvement of passive and active processes at the blood-brain barrier (BBB) and binding within the brain (Hammarlund-Udenaes *et al*., 1997; Syvanen *et al*., 2006). Our previous study on the pharmacokinetics of cetirizine enantiomers in guinea pigs showed that the terminal half-life of cetirizine enantiomers in brain was longer than in blood (3.94 vs 1.90 h for levocetirizine) indicating pharmacokinetic differences between the brain and blood (Gupta *et al*., 2006). Therefore, the present study in the guinea pig was designed in order to determine the time course of central histamine H_1 receptor occupancy and brain concentration of levocetirizine. The time course of peripheral histamine H_1 receptor occupancy and plasma concentration of levocetirizine were also determined in order to investigate the influence of the BBB. Two dose levels of levocetirizine were selected, one close to the human therapeutic dose of cetirizine, which also reduces the histamine-induced wheal area by 50% in guinea pig (De Vos *et al*., 1987) and the other 10-fold higher. This study demonstrated levocetirizine to be an effective antihistamine at doses with very low brain H_1 receptor occupancy and the BBB to play an important role in delay in its brain H_1 receptor occupancy.

Materials and methods

Animals

Male Dunkin Hartley guinea pigs weighing 350–450 g (Charles River, France) were acclimatized for at least 5 days at 22°C and controlled humidity in an alternating 12 h light/dark cycle before the experiment. Standard diet and water were provided *ad libitum*. The protocol was approved by the Animal Ethics Committee of Uppsala University (C218/1) and UCB (orgisol-GP).

Study design

Levocetirizine was administered orally at doses of 0.1 and 1 mg kg^{-1} to conscious guinea pigs at time zero. The guinea pigs were killed 1, 2, 4, 8 and 16 h after administration and blood, cerebellum and ileum were sampled. The plasma was separated from blood by centrifugation for 5 min at 7200 *g*. Four animals were included for each time point. One saline control group was included to obtain estimates of absence of H_1 receptor occupancy (two animals for each time point). One chlorpheniramine group ($n = 4$) was included as a positive control to define the maximal H_1 receptor occupancy. Chlorpheniramine (2 mg kg^{-1}) was administered intravenously and blood, cerebellum and ileum were sampled at 1 h after dosing. One-half of the cerebellum was used to study the *ex vivo* H_1 receptor occupancy and the other half was used to measure concentrations of levocetirizine. The ileum was removed and used to measure *ex vivo* peripheral H_1 receptor occupancy.

Another group of animals was included to characterize the full plasma concentration–time profile after oral administration of levocetirizine. The guinea pigs were anaesthetized by inhalation of Isoflurane (2.5% balanced with 1.5 l min^{-1} oxygen and 1.5 l min^{-1} nitrous oxide) and 0.25 ml of midazolam (5 mg ml^{-1}) intraperitoneally (i.p.). To maintain the body temperature at 38°C during surgery, the guinea pigs were placed on a heating pad and the body temperature was controlled with a thermometer. Fluoro ethylene propylene (FEP) tubings were inserted into the left common carotid artery for blood sampling. In order to avoid clotting, the catheters were filled with heparinized saline solution (100 IU ml^{-1}). The ends of the catheters were passed subcutaneously to a plastic cup on the surface of the neck, out of reach of the guinea pigs. The animals were placed in a CMA/120 system for freely moving animals (CMA Microdialysis, Sweden) with free access to water and food, and the experiment started approximately 24 h later in the morning. The oral doses of 0.1 ($n = 3$) and 1 mg kg^{-1} levocetirizine ($n = 4$) were then administered and 250 μl of blood was collected at 0, 15, 30, 45, 60, 90, 120, 180, 240, 480 and 600 min post-dosing and the plasma was separated. All samples were stored at −20°C until analysis.

Ex vivo *H_1 receptor occupancy in the brain*

Half cerebellum, from saline, chlorpheniramine or levocetirizine-treated guinea pigs, were homogenized in 250 μl of a 20 mM Tris-HCl buffer (pH 7.4) containing 250 mM sucrose and stored in liquid nitrogen. Subsequently, prewarmed homogenates (10 μl) were added to 490 μl of a 50 mM Tris-HCl buffer (pH 7.4) containing 2 mM $MgCl_2$ and 3 nM [^{3}H]-mepyramine. Samples were incubated for 1 min at 25°C. After the incubation period, the receptor bound radioligand was separated from the free ligand by vacuum filtration of samples over GF/C glass fibre filters (Whatman, VEL, Belgium). Filters were presoaked in 0.1% polyethylenimine to reduce the nonspecific binding (NSB) of the radioligand. Adsorbed samples were washed four times with 1 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4) and radioactivity trapped onto the filter was determined in a liquid scintillation counter (30–40% efficiency). NSB of [^{3}H]-mepyramine to the homogenate was measured in the presence of 10 μM cetirizine. Receptor-specific binding (RSB) was obtained by subtracting the NSB from the total binding observed in the absence of cetirizine (B0). For each sample, two B0 and two NSB were obtained. The specific

binding was also corrected for the amount of proteins in each sample. The percentage of H_1 receptor occupied was expressed as

$$\%\,\mathrm{H_1\ Re ceptor\ Occupancy} = \frac{\mathrm{RSB_{saline}} - \mathrm{RSB_{treatment}}}{\mathrm{RSB_{saline}}} \times 100$$

Ex vivo *H_1 receptor occupancy in ileum*

Sections of ileum, prepared from saline, chlorpheniramine- or levocetirizine-treated guinea pigs, were mounted in 20 ml organ baths filled with Tyrode solution. The bathing solution was maintained at 37°C and gassed with 95% O_2–5% CO_2. Tissues were allowed to equilibrate for a period of 20 min under a resting tension of 1 g. Isometric contractions were measured by force-displacement transducers coupled to an IOX computer system (EMKA Technologies, Paris, France). At the end of the 20 min period of stabilization, a cumulative concentration–response curve to histamine was elicited.

Agonist activity of histamine was determined by the calculation of pD_2 values (Van Rossum *et al*., 1963). In the presence of levocetirizine, a higher concentration of histamine will be required to produce the same effect and the histamine concentration–response curve on the guinea pig ileum will be shifted to the right. The shift between the saline- and levocetirizine-treated ileum curves was estimated from the ratio of histamine concentrations required to produce a half-maximal response in saline- or levocetirizine-treated animals. Since levocetirizine is a competitive histamine H_1 antagonist as described previously (Christophe *et al*., 2003), a theoretical calculation based on the shift between these curves allows an estimation of the peripheral receptor occupancy, for example, a twofold shift would correspond to 50% of the receptors being occupied by the antagonist.

Chemical analysis

Plasma and cerebellum samples were analysed using a liquid chromatography tandem mass spectrometry (LC-MS/MS) method. The chromatographic column used was a Zorbax SB-CN (150 × 4.6 mm, Agilent Technologies, Wilmington, DE, USA) tandem with a triple quadropole mass spectrometer (Quattro Ultima; Micromass, Manchester, UK). The mass spectrometer was in positive ion mode with following settings: multiple reaction monitoring (389.1 > 200.9), cone voltage 40 V, dwell time 0.5 s, interchannel delay 0.1 s, source temperature 130°C, desolvation temperature 400°C, desolvation gas flow 700 l h^{-1} and cone gas flow 250 l h^{-1}. The mobile phase consisted of 10 mM ammonium acetate buffer at pH 7.0 and 35% acetonitrile.

For extraction of levocetirizine from brain samples, the brain was homogenized in four volumes of saline. A portion of the homogenate (100 μl) was precipitated using 200 μl acetonitrile containing the internal standard (IS) ucb 20028 (5 ng ml^{-1}). The contents were mixed and centrifuged. Supernatant (200 μl) was transferred to an Eppendorf tube and evaporated at 40°C under N_2. The dried sample was dissolved in 200 μl mobile phase and 40 μl was injected on to the LC-MS/MS system. The column was maintained at room temperature and the flow-rate of the mobile phase was 0.9 ml min^{-1}. The peak area was used for the quantification of levocetirizine. The assay was linear over the range of 0.1–100 ng g^{-1} brain. Precision (expressed as coefficient of variation) and accuracy for the lower limit of quantification (LLOQ) (0.1 ng g^{-1} brain) was 7.3 and 109%, respectively.

For plasma samples, 50 μl of plasma was mixed with 100 μl acetonitrile containing IS ucb 20028 (100 ng ml^{-1}) and centrifuged. The supernatant (50 μl) was evaporated at 40°C under N_2. The dried sample was dissolved in 250 μl mobile phase and 40 μl was injected on to the LC-MS/MS system. For plasma samples, the curve was split into two parts, a low (0.5–250 ng ml^{-1}) and a high (250–2500 ng ml^{-1}) concentration curve. The precision and accuracy for the LLOQ (0.5 ng ml^{-1}) was 10 and 100%, respectively.

Data analysis

Owing to the destructive nature of brain sample collection, only one sample was collected per animal. Therefore, the data were analysed using a naïve pooling approach. All calculations were based on the mean concentration–time data; each data point was the mean of four animals. Therefore, the pharmacokinetic parameter estimates are expressed only as mean values. The amount of levocetirizine in the brain was corrected for the amount of the drug present in the blood vessels in the brain. The reported value in the literature for plasma content in the brain of the guinea pig (11.5 μl g^{-1} brain) was used for this purpose (Bosse and Wassermann, 1970). The terminal half-life in plasma and brain was expressed as $\ln 2/\lambda_{pl}$ and $\ln 2/\lambda_{br}$. The λ_{pl} and λ_{br} are the terminal rate constants obtained by log-linear regression of the terminal phase of the concentration-time profile in the plasma and the brain, respectively. To measure the extent of brain transport, the partition coefficient K_p was determined, which is the ratio of brain to plasma $AUC_{0-\infty}$. The whole areas under the concentration–time curves $AUC_{0-\infty}$ were expressed as the sum of the area under the corresponding concentration vs time curve until the last observation (AUC_{0-t}) and the residual area ($AUC_{t-\infty}$). The AUC_{0-t} was calculated by the trapezoidal method. The $AUC_{t-\infty}$ was determined as the ratio of the concentration at the last time point to respective terminal rate constants.

For the ileum contraction data, the concentration of histamine inducing half the maximal response and the maximal response of each concentration–response curve (E_{max}) were calculated using an iterative computer software (XLfit, ID Business Solutions, UK or Prism, GraphPad software, San Diego, CA, USA) fitting the experimental data to the four parameter logistic equation:

$$Y = A + \frac{B - A}{1 + \left(\frac{10^C}{10^X}\right)^D}$$

where Y is the observed effect, A is minimum Y, B is maximum Y, C is logarithm of the molar concentration of the histamine inducing 50% of B ($-pD_2$), D is the slope factor and X is the log of the molar concentration of histamine.

Chemicals

Levocetirizine (XYZAL) [2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid chlorhydrate and an IS (ucb 20028) {2-[2-(4-benzhydrylidene-piperidin-1-yl)-ethoxy]-ethoxy}-acetic acid chlorhydrate were synthesized at UCB SA (Braine l'Alleud, Belgium). Histamine and (+) chlorpheniramine were from Sigma Aldrich (Bornem, Belgium) and low molecular weight heparin was purchased from Sigma (Sigma Chemical Co., St Louis, MO, USA). [^{3}H]mepyramine (27 Ci mmol^{-1}) was purchased from Amersham Biosciences (Rosendaal, the Netherlands). All other chemicals were of analytical grade and solvents were of HPLC grade. The water was purified by a Milli-Q Academic system (Millipore, Bedford, MA, USA).

Results

The concentration–time profiles of levocetirizine in plasma and brain after the 0.1 and 1 mg kg^{-1} oral doses are shown in Figure 1. The maximum concentrations in plasma were 112 ± 43 and 950 ± 206 ng ml^{-1}, respectively. The time of maximum concentration (t_{max}) in plasma was 1–1.5 h after dosing. This was observed from the plasma concentration–time profile in the group of guinea pigs with more frequent sampling (data not shown). In the brain, the concentrations were much lower than in plasma. After the low dose, a maximum concentration of 2.7 ± 0.3 ng g^{-1} brain was attained at 2 h, and remained at the same level for up to 8 h after the dose. After the high dose, the maximum concentration of levocetirizine in the brain was 23.6 ± 2.7 ng g^{-1} brain, attained 4 h after dosing. The K_p values were 0.08 and 0.06 for the low and high dose, respectively. The half-life of levocetirizine in the brain (5.6 and 4.0 h for the 0.1 and 1 mg kg^{-1} doses, respectively) was longer than in the plasma (2.8 and 2.1 h, respectively).

The binding of [^{3}H]-mepyramine incubated with brain homogenates is shown in Figure 2. In the control (saline) group, the binding of [^{3}H]-mepyramine was high and represented 0% occupancy. The binding of [^{3}H]-mepyramine in chlorpheniramine-treated guinea pigs was totally prevented and was used as a positive control to determine 100% H_1 receptor occupancy. In the presence of 1 mg kg^{-1} levocetirizine, [^{3}H]-mepyramine binding decreased to about 40% of the level observed in the saline group, indicating a receptor occupancy of about 60%, while in the 0.1 mg kg^{-1} levocetirizine treated group, [^{3}H]-mepyramine binding was close to 80% of its value in the saline group indicating a 20% receptor occupancy. The actual H_1 receptor occupancy of levocetirizine in the brain as a function of time is shown in Figure 3. For the 1 mg kg^{-1} dose, the H_1 receptor occupancy was maximal at 2 h after dosing and remained on the same level until 8 h. It ranged from 30 to 70% occupancy during the 16 h study period. At the dose of 0.1 mg kg^{-1}, the H_1 receptor occupancy was $<20\%$ at all time points.

Histamine induced a concentration-dependent contraction of the isolated guinea pig ileum (Figure 4). For the saline group, the pD_2 value was 6.22 ± 0.25 ($n = 10$) and the maximal contraction induced by histamine was 4.09 ± 1.48 g ($n = 10$). The concentration–response curve to

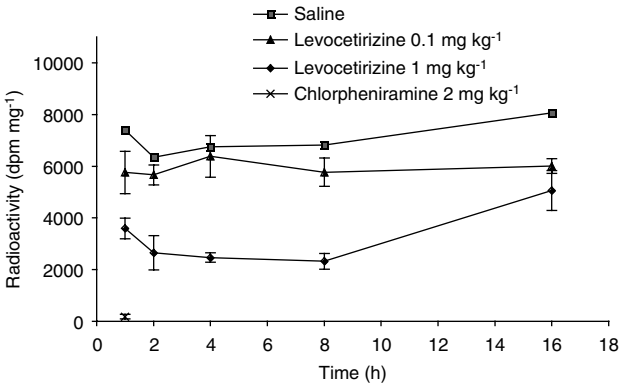

Figure 2 Plot of receptor-specific binding of [^{3}H]-mepyramine vs time in the cerebellum isolated from guinea pigs pretreated with saline, 0.1 mg kg^{-1} levocetirizine, 1 mg kg^{-1} levocetirizine and 2 mg kg^{-1} chlorpheniramine. The binding was corrected for the amount of proteins in each sample. Points represent mean value ($n = 2$–4). Vertical bars represent s.d.

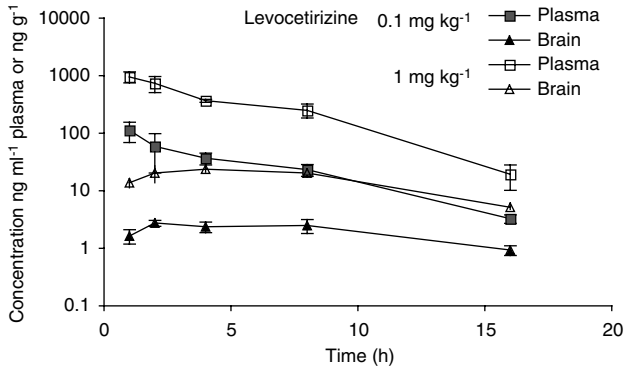

Figure 1 Concentration–time profiles in plasma and brain obtained after the oral administration of 0.1 and 1 mg kg^{-1} levocetirizine in guinea pigs. Each time point is presented as mean ± s.d. from four animals.

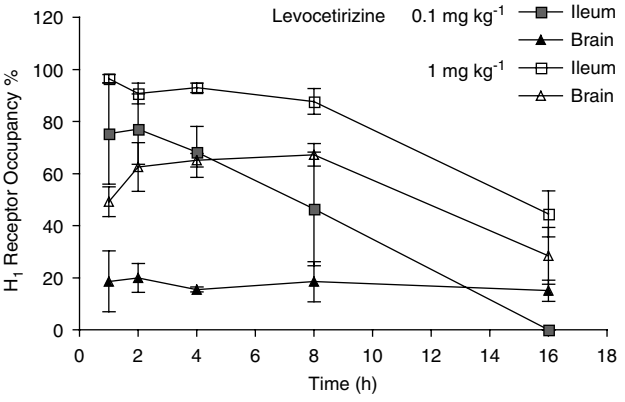

Figure 3 The H_1 receptor occupancy vs time profiles in ileum and brain following oral administration of 0.1 and 1 mg kg^{-1} levocetirizine in guinea pigs. Each point represents mean ± s.d. from four animals.

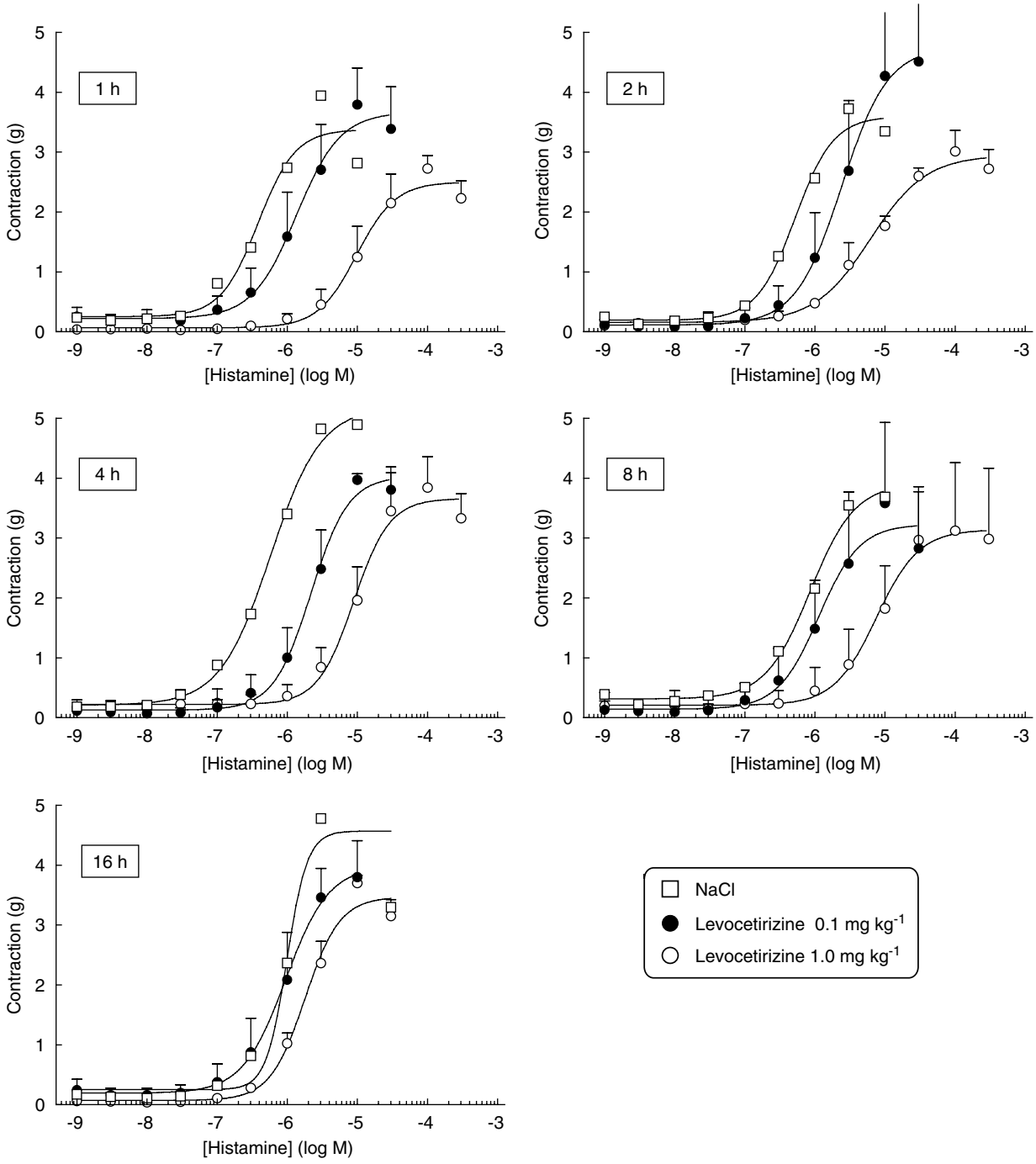

Figure 4 Concentration–response curves to histamine elicited on the ileum isolated from guinea pigs pretreated with saline, 0.1 mg kg^{-1} levocetirizine and 1 mg kg^{-1} levocetirizine. Different panels represent time of death of the animal after dosing. Points represent mean values ($n = 2$–4). Vertical bars represent s.d.

histamine was displaced to the right in the presence of levocetirizine. The maximum shift was observed 1 h after dosing and this progressively decreased. The maximal amplitude of the concentration–response curve to histamine was also reduced in the presence of levocetirizine. The H_1 receptor occupancy time profiles in the ileum after the 0.1 and 1.0 mg kg^{-1} dose of levocetirizine are shown in Figure 3. The H_1 receptors were maximally occupied 1 h after both levocetirizine doses. At the dose of 0.1 mg kg^{-1}, the H_1 receptor occupancy ranged from 75% at 1 h to 0% at 16 h. At the dose of 1 mg kg^{-1}, the H_1 receptor occupancy was 97% at 1 h and 46% at 16 h with more than 90% of receptors remaining occupied for up to 8 h after dosing.

The concentration–H_1 receptor occupancy relationship of levocetirizine in the periphery and the CNS are shown in Figures 5 and 6. The plasma concentration vs ileum H_1 receptor occupancy curve showed that there was no equilibration delay between plasma concentration and effect

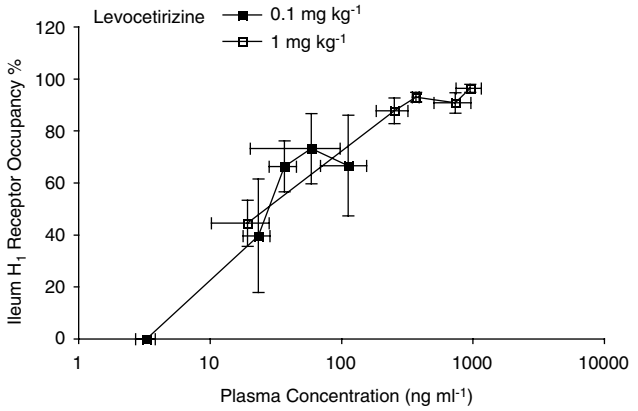

Figure 5 The plasma concentrations vs ileum H_1 receptor occupancy curve of levocetirizine after oral administration of 0.1 and 1 mg kg^{-1} in guinea pigs.

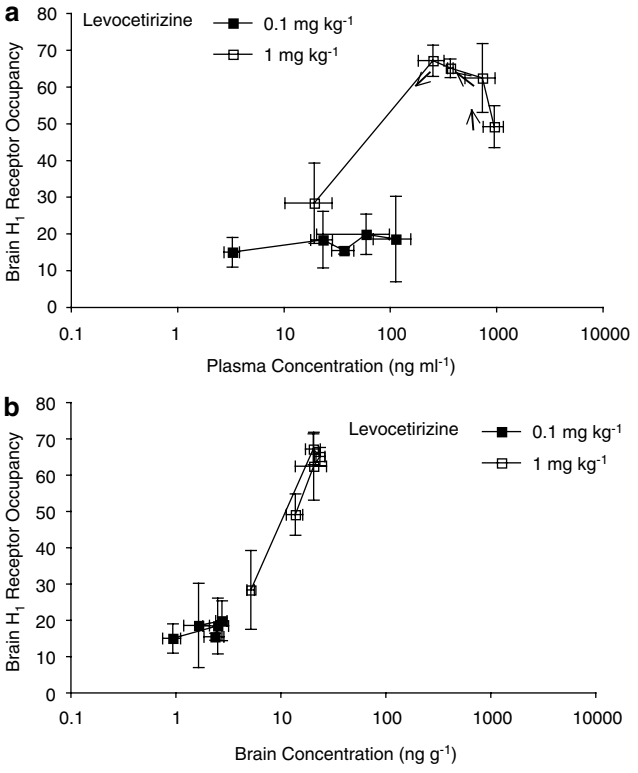

Figure 6 (**a**) Plasma concentrations vs brain H_1 receptor occupancy curve (**b**) Brain concentrations vs brain H_1 receptor occupancy curve of levocetirizine, after the oral administration of 0.1 and 1 mg kg^{-1} doses in guinea pigs.

site (Figure 5). A plot of the plasma concentration vs H_1 receptor occupancy in the brain, however, indicated that the central H_1 receptor occupancy lags behind the plasma concentration resulting in counterclockwise hysteresis (Figure 6a). However, in the plot of the brain concentrations vs H_1 receptor occupancy in the brain, this hysteresis collapsed and brain H_1 receptor occupancy-concentrations appeared to follow a sigmoidal E_{max} model (Figure 6b).

Discussion

The aim of this work was to study the time course of H_1 receptor occupancy of levocetirizine in the periphery and the brain in relation to the plasma and brain concentrations of the drug. Comparison of H_1 receptor occupancy in the brain and the ileum showed that brain H_1 receptor occupancy is lower and increases slowly with time compared to ileum occupancy. At the low dose of 0.1 mg kg^{-1} (a dose close to the recommended therapeutic dose in man; 10 mg day^{-1}), levocetirizine occupied $<20\%$ of central H_1 receptors at all time points, while nearly 80% of peripheral receptors were blocked 1 h following oral dosing. These results are in accordance with clinical data showing effective peripheral effect of levocetirizine with no central side effects (Hindmarch *et al*., 2001; Verster *et al*., 2003a, b). With the high dose (1 mg kg^{-1}) of levocetirizine, 90–96% of H_1 receptor was occupied in the periphery up to 8 h post-dosing, whereas in the brain, the H_1 receptor occupancy ranged from 50 to 70% during those 8 h. Low brain H_1 receptor occupancy of racemic cetirizine has been reported previously in rats and humans. In rats, the brain H_1 receptor occupancy was 22.5 and 34.2%, 1 h after doses of 10 and 30 mg kg^{-1} i.p., respectively (Snowman and Snyder, 1990). Tashiro *et al*. (2002) measured H_1 receptor occupancy in eight healthy volunteers by positron emission tomography using [^{11}C]-doxepin 90 min after a 20 mg oral cetirizine dose (the recommended daily dose of cetirizine is 10 mg orally) and showed that cetirizine occupied 30% of the H_1 receptors. Since an occupation of approximately 30% of histamine H_1 receptors in the human cerebral cortex seems to be tolerated before the emergence of central side effects (Yanai *et al*., 1999), the present results confirmed in an animal model that levocetirizine at the 0.1 mg kg^{-1} dose showed significant peripheral antihistamine activity while occupying marginal central H_1 receptors. The 10 times higher dose studied resulted in increased central H_1 receptor occupancy, which would lead to central side effects.

The concentration–time profile of levocetirizine in the brain showed that levocetirizine entered into the brain slowly and to a low extent (Figure 1). The K_p of 0.06 is close to values reported previously (Chen *et al*., 2003; Polli *et al*., 2003; Gupta *et al*., 2006). Lower levels of levocetirizine in brain compared to plasma could be due to cetirizine being a P-glycoprotein substrate (Chen *et al*., 2003; Polli *et al*., 2003). A slow transport of levocetirizine into the brain could be due to low permeability across the BBB. It has been reported that cetirizine has low/moderate passive permeability (P_{app} $B \rightarrow A = 56$ nm s^{-1}) determined *in vitro* in Madin Darby canine kidney type II cells transfected with the human multidrug resistance 1 gene (Polli *et al*., 2003). Levocetirizine has a longer half-life in brain than in plasma (4.8 vs 2.5 h). Thus, the pharmacokinetics of this drug in brain is slower than in plasma.

The differences in the pharmacokinetics of levocetirizine in the plasma and the brain affect brain pharmacodynamics in relation to the plasma concentrations. This was evident when H_1 receptor occupancy in the brain was compared to both plasma and brain concentrations (Figure 6). A plot of the levocetirizine plasma concentrations against H_1 receptor

occupancy in the brain indicated that the occupancy lags behind the plasma concentration. Plotting the H_1 receptor occupancy in brain against brain concentration resulted in collapse of the hysteresis loop. This suggests that the delay in the brain H_1 receptor occupancy compared to plasma concentrations is due to a slow equilibration of levocetirizine across the BBB.

As with levocetirizine, for carebastine, the active metabolite of ebastine, the brain H_1 receptor occupancy was not correlated to its plasma concentrations (Tagawa *et al*., 2001). However, for (+) chlorpheniramine a linear relationship was observed between its brain H_1 receptor occupancy and plasma concentrations. Again these differences in (+) chlorpheniramine and carebastine could be due to different pharmacokinetics in the blood and the brain caused by different BBB transport properties of the two compounds. Chlorpheniramine has high passive permeability and is not effluxed by P-glycoprotein (Mahar Doan *et al*., 2002), whereas carebastine has been shown to be a substrate for P-glycoprotein-mediated efflux (Tamai *et al*., 2000).

In the present study, the peripheral receptor occupancy of levocetirizine was measured indirectly using the cumulative concentration–response curve to histamine of ileum sections taken from guinea pigs pretreated with levocetirizine. Using this method, it was possible to get measurement of peripheral H_1 receptor occupancy of levocetirizine and compare its occupancy in brain in the same animal. Levocetirizine has a dissociation half-life from H_1 receptors of 142 min (Gillard *et al*., 2002), which makes it reasonable to assume that the H_1 receptors remained occupied by levocetirizine in these *ex vivo* studies thus permitting measurement of the shift in the concentration–response curve to histamine in comparison with the saline-treated group. However, it should be noted that this shift could be underestimated due to the time elapsed between the death of the animal and the *in vitro* experiment, which included a 20 min of stabilization period in the organ bath. The concentration at which 50% of H_1 receptors were occupied was approximately the same in ileum (Figure 5) and brain (Figure 6b) validating the assumption that levocetirizine remained associated with H_1 receptors under these *ex vivo* conditions. A reduction of the maximum amplitude of the concentration–response curve to histamine observed in the presence of levocetirizine could be explained by the slow dissociation rate of levocetirizine from H_1 receptors. It has been demonstrated previously that, in functional studies, high levocetirizine concentrations depressed the maximum tissue response to histamine. However, at the receptor level, levocetirizine interacts competitively with histamine (Christophe *et al*., 2003).

In conclusion, the present results demonstrate that it is important to characterize the time course of central histamine H_1 receptor occupancy in relation to the brain concentrations of drugs. For levocetirizine, a slow increase in H_1 receptor occupancy in the brain could be fully accounted for by slow transport across the BBB. A dose of levocetirizine ($0.1\,mg\,kg^{-1}$) producing effective blockade of histamine H_1 receptors in the periphery had no effect on such receptors in the brain. These results are in agreement with clinical data demonstrating potent peripheral antihistamine effects of levocetirizine without adverse effects in the CNS.

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Conflict of interest

The authors state no conflict of interest.

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