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The profiles of human and primate [^{3}H]N^{α}-methylhistamine binding differ from that of rodents

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Abstract

Characterization of the histamine H_3 receptor in rodent species has been extensive but limited characterization has been done with primate or human tissue. We have characterized the binding of [^{3}H]N^{α}-methylhistamine to cynomolgus monkey and human brain membranes to determine whether there are any significant differences among species' pharmacology. In monkey, [^{3}H]N^{α}-methylhistamine bound, in a guanine nucleotide-sensitive fashion, to an apparently homogeneous class of sites at equilibrium ($K_D = 1.4$ nM, $B_{max} = 34$ fmol/mg protein). The profile of binding was broadly similar to that of rodents, with a couple of significant differences. Most notably, the potency of the histamine H_3-receptor-specific antagonist thioperamide ($K_i = 240$ nM) was substantially less than reported for rodents and under assay conditions that yield a two-site curve fit in rodents only a single class of thioperamide binding sites was detected in monkey. Burimamide, however, yielded a two-site curve fit ($K_{iH} = 6.7$ nM, $K_{iL} = 1100$ nM) independent of the presence of sodium in the assay, as it does in rodents. Characterization of the human brain histamine H_3 receptor showed that it was similar to the monkey and not rodent receptor. Our findings indicate that differences between primate and rodent histamine H_3 receptors of potentially serious importance for the discovery of antagonists active in humans do exist. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Histamine H_3 receptor; [^{3}H]N^{α}-methylhistamine binding, human, primate; Thioperamide; Burimamide

1. Introduction

The histamine H_3 receptor was originally recognized as a high-affinity [^{3}H]histamine binding site in rodent brain (Barbin et al., 1980). Subsequently, it was shown to modulate the synthesis and release of histamine and later the release of other neurotransmitters as well, and an antagonist, thioperamide, specific for this receptor was discovered (Arrang et al., 1987).

Radioligand binding has been done with the agonists [^{3}H](*R*)-α-methylhistamine (Arrang et al., 1987; West et al., 1990a) and [^{3}H]N^{α}-methylhistamine (West et al., 1990b) or antagonists (Jansen et al., 1992, 1994; Ligneau et al., 1994; Yanai et al., 1994; Alves-Rodrigues et al., 1996; Brown et al., 1996) in studies of rodent tissues. Four functional studies have employed human tissue, in addition to the number of rodent functional assays that have been reported, but the data from these studies provide only a limited pharmacological profile of the human receptor (Arrang et al., 1988; Cherifi et al., 1992; Oike et al., 1992; Imamura et al., 1995). For this reason, we have characterized primate brain binding of [^{3}H]N^{α}-methylhistamine.

2. Materials and methods

2.1. Materials

[^{3}H]N^{α}-methylhistamine (81 Ci/mmol) was from Dupont NEN. Drugs were from Research Biochemicals, except for impromidine, which was from Smith, Kline and French Laboratories.

2.2. Tissue preparation

Brains were obtained from cynomolgus monkeys at necropsy, kept briefly on ice until processing, then added to 10 volumes (w/v) of ice-cold 50 mM Tris–HCl buffer, pH 7.5, and disrupted with a Polytron (PTA 35/2 tip, about 1 min at setting 7). After low-speed centrifugation (10 min, $1000 \times g$), the supernatant was centrifuged for

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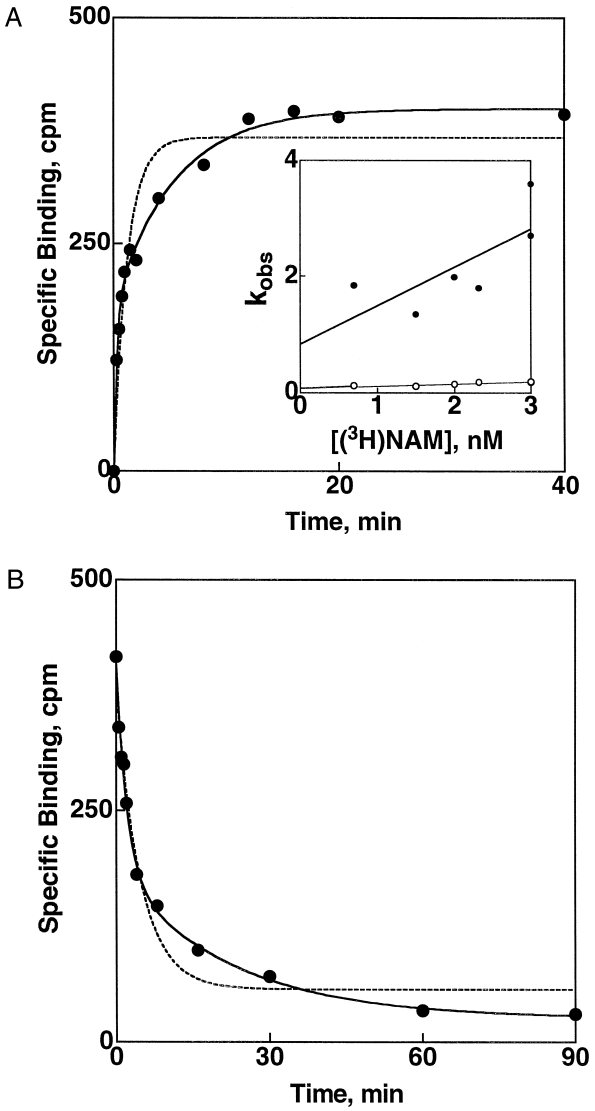

Fig. 1. Kinetics of [^{3}H]N^{α}-methylhistamine binding to monkey brain membranes. (A) Association time course. Values for k_{obs} obtained from curve fits were plotted vs. [^{3}H]N^{α}-methylhistamine concentrations as shown in the inset [(•) rapid component; (◦) slow component]; k_{-1} values were determined from the *y*-intercepts and k_1 values from the slopes of these lines. (B) Dissociation time course. (dotted line) Monoexponential fit; (solid line) biexponential fit.

10 min at 50,000 × *g*. The high-speed pellet was resuspended in half the original volume of buffer, a sample was taken for protein assay (bicinchoninic acid, Pierce) and the suspension was centrifuged again at 50,000 × *g*. After final resuspension and aliquoting, the material was recentrifuged and the pellets were frozen at −80°C until use.

Human cerebral cortex was obtained from autopsy specimens and frozen at −80°C until use. Four specimens from different donors were processed together. These were thawed at room temperature then added to 10 volumes (w/v) of ice-cold 50 mM Tris–HCl buffer, pH 7.5, and disrupted with a Polytron (PTA 35/2 tip, about 1 min at setting 6). After low-speed centrifugation (10 min, 1000 × *g*), the supernatant was centrifuged for 10 min at 50,000 × *g*. The high-speed pellet was resuspended in the original volume of buffer, a sample was taken for protein assay, and the suspension was centrifuged again at 50,000 × *g*. Tissue was resuspended at 3 mg of protein/ml and frozen at −80°C until use.

2.3. Binding assays

For equilibrium assays, membrane (250–300 μg of protein) was incubated with [^{3}H]N^{α}-methylhistamine (0.7 nM for monkey, 1.5 nM for human competition binding

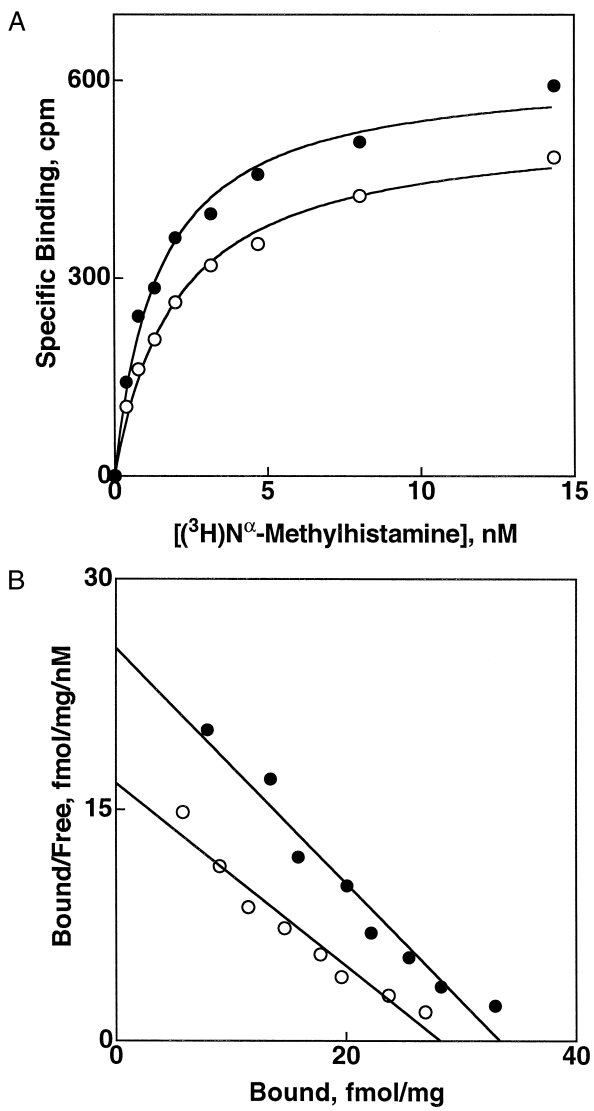

Fig. 2. [^{3}H]N^{α}-methylhistamine saturation binding to monkey brain membranes. (A) Saturation isotherm. Various concentrations of [^{3}H]N^{α}-methylhistamine were incubated with membranes as described in Section 2, without or with 100 μM GTPγS, which had been determined to be a maximally efficacious concentration in a dose–response experiment. (B) Scatchard plot of the data; (•) control, (◦) 100 μM GTPγS.

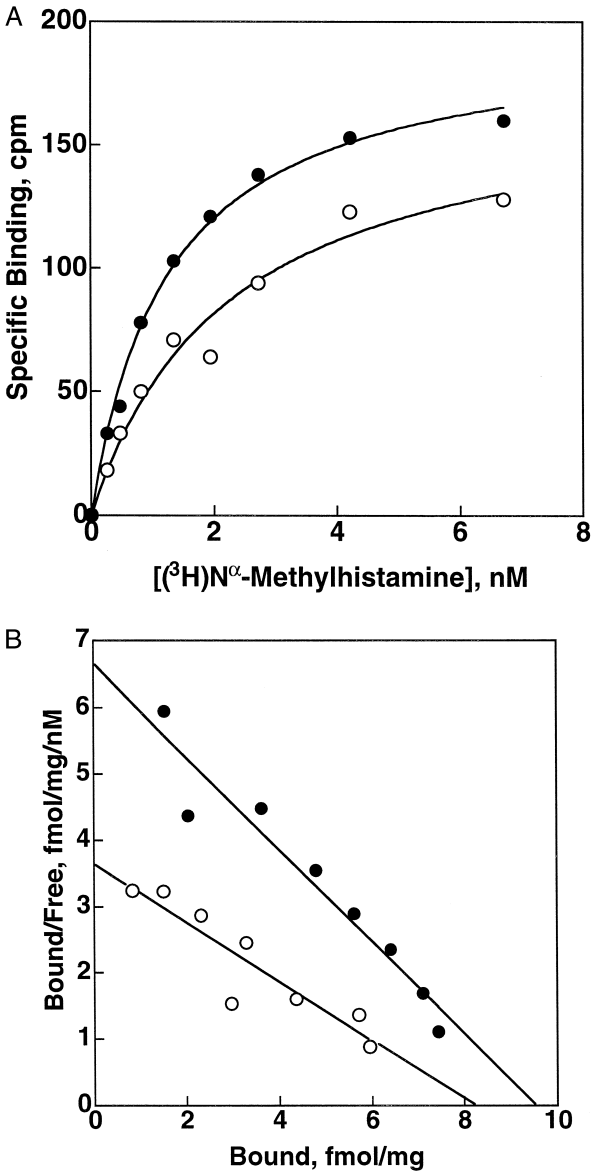

Fig. 3. [^{3}H]N^{α}-methylhistamine saturation binding to human brain membranes. (A) Saturation isotherm. Various concentrations of [^{3}H]N^{α}-methylhistamine were incubated with membranes as described in Section 2, without or with 100 μM GTPγS, which had been determined to be a maximally efficacious concentration in a dose–response experiment. (B) Scatchard plot of the data; (•) control, (◦) 100 μM GTPγS.

assays, concentrations as indicated for other assays) without or with inhibitor compounds in a total volume of 200 μl 50 mM Tris, pH 7.5. A concentration of thioperamide, 10^{-5} M, that gave maximum inhibition, at least equal to that of any compound tested, was used to determine nonspecific binding. Assays mixtures were incubated for 30 min at 30°C in polypropylene, 96-well, deep-well plates then filtered on a Mach II harvester (Tomtec) through 0.3% polyethylenimine-soaked GF/B filters. These were washed three times with 1.2 ml of Tris buffer, dried in a microwave oven, impregnated with Meltilex wax scintillant and counted at 40% efficiency in a Betaplate scintillation counter (Wallac).

Conditions were modified for kinetic assays. For association time courses, tissue (180 μl) was incubated at 30°C in quadruplicate without (total binding) and with 1 μM N^{α}-methylhistamine (nonspecific binding) for 45 min at which time it was filtered. At the appropriate time prior to filtration, binding was initiated with the addition of 20 μl of [^{3}H]N^{α}-methylhistamine. For dissociation time courses, tissue was incubated 30 min at 30°C with radioligand (3 nM). N^{α}-methylhistamine (10 μM final concentration) was added at various times thereafter and the incubation allowed to proceed. Samples were filtered simultaneously. Total and nonspecific binding were determined from samples incubated for 2 h.

2.4. Dissection

For gross localization of binding, 10 areas were dissected from the brains of two monkeys. Samples from the two monkeys were combined and prepared as above, except with a PT10 Polytron tip at setting 5 for 30 s. For the binding assay, quadruplicate determinations of total and nonspecific binding of 1.5 nM [^{3}H]N^{α}-methylhistamine were made in each experiment.

2.5. Data analysis

Curves were fit to the data with Prism nonlinear least squares curve-fitting program (GraphPad Software, San Diego, CA). One- and two-site or mono- and biexponential fits were tested. For all experiments, a two-site fit was considered the better when $p < 0.05$. Values shown are the means ± the standard error of the means from at least three experiments. Figures are one from among three or more of the same experiment.

Table 1
Inhibitor potency vs. [^{3}H]N^{α}-methylhistamine binding
[^{3}H]N^{α}-methylhistamine was incubated with membranes and 11 concentrations of inhibitor compounds and a curve was fit to the data. One- and two-site fits were tested. For all these compounds, a two-site fit offered no statistically significant ($p < 0.05$) improvement over a one-site fit. K_i values were calculated from IC_{50} values according to the equation of Cheng and Prusoff (1973).

Compound	Monkey K_i (nM)	Human K_i (nM)
Imetit	0.97 ± 0.52	0.31 ± 0.08
N^{α}-methylhistamine	1.7 ± 0.8	0.83 ± 0.15
Clobenpropit	2.2 ± 0.3	5.7 ± 1.1
(*R*)-α-methylhistamine	3.1 ± 2.0	1.5 ± 0.2
Histamine	13 ± 3	8.3 ± 2.6
(*S*)-α-methylhistamine	20 ± 1	32 ± 5
Thioperamide	240 ± 60	200 ± 30
Impromidine	250 ± 20	290 ± 100
Dimaprit	2100 ± 1000	850 ± 140

3. Results

3.1. Kinetic assays

Binding parameters were determined in kinetic experiments for monkey brain. [^{3}H]N^{α}-methylhistamine binding reached steady-state by 20 min (Fig. 1A). Association kinetics were biexponential as determined from curves fit to the data. A plot of k_{obs} vs. [^{3}H]N^{α}-methylhistamine concentration for a series of assays run at various [^{3}H]N^{α}-methylhistamine concentrations (inset) yielded two lines with slopes equivalent to association rate constants of 0.034 and 0.66 nM^{-1} min^{-1}; *y*-intercepts, equivalent to dissociation rate constants, were 0.082 and 0.83 min^{-1} for each line, respectively. The dissociation constants for [^{3}H]N^{α}-methylhistamine calculated as $K_D = k_{-1}/k_1$ from these values are 2.4 and 1.3 nM. The dissociation time course was also characterized by biexponential kinetics (Fig. 1B) with 60% of the sites rapidly and 40% slowly dissociating; dissociation rate constants were 0.036 and 0.5 min^{-1}. The dissociation constants calculated from these values are 1.1 and 0.8 nM.

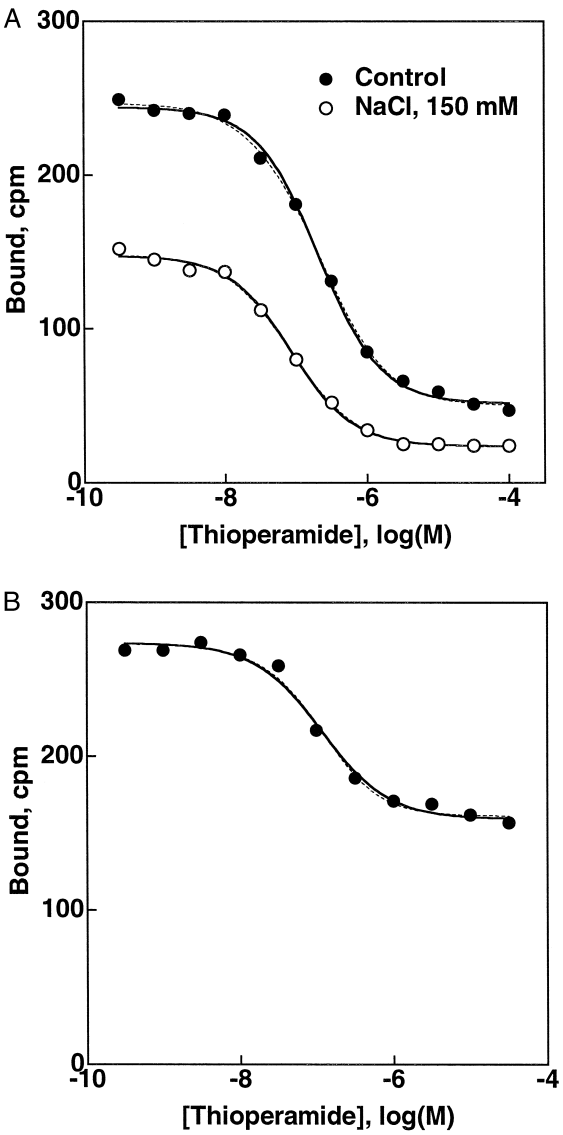

Fig. 4. Thioperamide competition vs. [^{3}H]N^{α}-methylhistamine binding. (A) Monkey, (B) human. Curves were fit for one and two sites. (solid line) One-site fit, (dotted line) two-site fit.

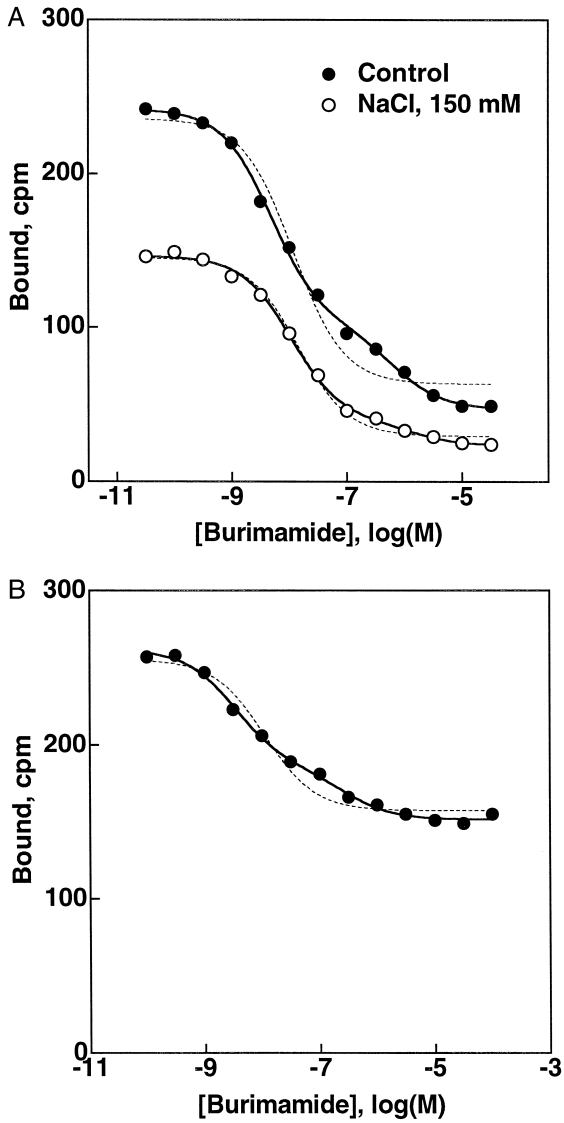

Fig. 5. Burimamide competition vs. [^{3}H]N^{α}-methylhistamine binding. (A) Monkey, (B) human. (solid line) Two-site fit, (dotted line) one-site fit.

3.2. Saturation binding

Equilibrium saturation binding data for monkey (Fig. 2A,B) show that [^{3}H]N^{α}-methylhistamine bound to an apparently single class of sites ($K_D = 1.4 \pm 0.1$ nM, $B_{max} = 34 \pm 4$ fmol/mg of protein) and binding was sensitive to the guanine nucleotide GTPγS ($K_D = 1.9 \pm 0.3$ nM, $B_{max} = 27 \pm 1$ fmol/mg of protein). Saturation binding data for human (Fig. 3A,B) also show that [^{3}H]N^{α}-

methylhistamine bound to an apparently single class of sites ($K_D = 1.3 \pm 0.2$ nM, $B_{max} = 10 \pm 1$ fmol/mg of protein). In the presence of 100 μM GTPγS, the K_D value was 2.2 ± 0.1 nM and B_{max} value was 10 ± 2 fmol/mg of protein.

3.3. Competition binding

For both human and monkey, the profile of inhibitor potencies in Table 1 is generally consistent with a histamine H_3 receptor. Thioperamide inhibition was better characterized by a one- than a two-site curve in monkey (Fig. 4A; Table 1) unlike rat, but was similar to rat in being salt-sensitive ($K_i = 73 \pm 18$ nM). A one-site curve-fit was similarly appropriate to thioperamide inhibition of binding to human brain (Fig. 4B). Burimamide inhibition of [^{3}H]N^{α}-methylhistamine binding was, however, better characterized by a two- than one-site curve-fit for monkey ($K_{iH} = 4.4 \pm 0.6$ nM, $K_{iL} = 770 \pm 230$ nM) (Fig. 5A) as we and others have seen in rat brain, and the two sites persisted in the presence of sodium chloride ($K_{iH} = 5.7 \pm 1.1$ nM, $K_{iL} = 370 \pm 160$ nM). Two sites were also indicated in human brain ($K_{iH} = 2.7 \pm 1.2$ nM, $K_{iL} = 120 \pm 25$ nM) (Fig. 5B). Dimaprit was equipotent in human or monkey to rat brain and impromidine was only slightly less potent. Binding was stereoselective for (*R*)-α-methylhistamine over (*S*)-α-methylhistamine, although it appears to a lesser degree than has been reported in rodent (Arrang et al., 1987). There were otherwise no substantial differences in the potencies of agonists among human, monkey and rodent.

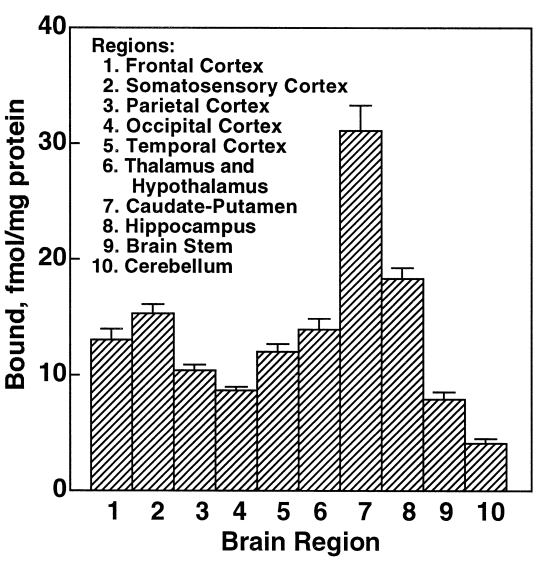

Fig. 6. Localization of [^{3}H]N^{α}-methylhistamine binding in monkey brain. Membrane (300 μg of protein) from each region was incubated with 1.5 nM [^{3}H]N^{α}-methylhistamine in quadruplicate determinations of total and nonspecific binding. Shown are the averages ± the standard errors of the means of three experiments.

3.4. Distribution of binding within brain

The distribution of binding in monkey brain is summarized in Fig. 6. The density of binding varied seven-fold among the various regions. Highest levels were seen in the caudate–putamen, followed by the hippocampus, thalamus and hypothalamus, and the entire cortex. Lowest levels were seen in the cerebellum and brainstem.

4. Discussion

The histamine H_3 receptor has been characterized by high affinity for histamine and the related agonists (*R*)-α-methylhistamine, N^{α}-methylhistamine and imetit and for the antagonist thioperamide (Barbin et al., 1980; Arrang et al., 1987; Garbarg et al., 1992; Howson et al., 1992; Van der Goot et al., 1992). We see similarly high affinity for histamine, (*R*)-α-methylhistamine, N^{α}-methylhistamine, and imetit in human and monkey brain membranes. As we saw with [^{3}H](*R*)-α-methylhistamine binding to rat brain membranes (West et al., 1990a), the kinetics of [^{3}H]N^{α}-methylhistamine binding to monkey brain membranes are biexponential while equilibrium binding data, from both saturation and homologous competition assays, disclose only a single class of sites. The K_D values calculated from the kinetic data are so close to one another that it is reasonable they appear as a single class of sites with high affinity for the radioligand.

Where our results with the primate brain receptor differ significantly from those with rodent is primarily in the behavior of thioperamide. We have previously reported for the antagonists thioperamide and burimamide that inhibition of [^{3}H]N^{α}-methylhistamine binding to rat brain is better modeled by a two- than a one-site curve-fit. We had remarked upon the salt-sensitivity of thioperamide potency (West et al., 1990a) and others have shown that thioperamide conforms to a one-site fit in the presence of sodium and interpreted this as evidence of thioperamide's being a negative antagonist at the rat brain receptor (Clark and Hill, 1995), whereas we had concluded that thioperamide and burimamide behavior was consistent with two histamine H_3 receptor subtypes. Our findings in competition studies vs. [^{3}H]N^{α}-methylhistamine binding to primate brain membranes are that thioperamide inhibition, while it is sensitive to salt, is monophasic — and burimamide inhibition in the presence or absence of sodium is still biphasic.

A reasonable conclusion is that the biological basis for the two-site fit for thioperamide in rodent may be different from the basis for the burimamide two-site fit in rodent. The occurrence of a single population of sites being detected in monkey or human brain with thioperamide as inhibitor is consistent with only one histamine H_3 receptor

subtype being detected and/or thioperamide's not being a negative antagonist in the monkey brain. In any event, the biphasic burimamide inhibition curve persists in the presence of sodium, inconsistent with negative antagonism as an explanation for its behavior. The existence of two populations of burimamide binding sites in primate tissue is consistent with receptor subtypes. This and the substantially lower potency of thioperamide in the primate than in the rodent binding assays indicate that discovery of efficacious antagonists for histamine H_3 receptors may be more complex than is apparent from rodent models alone.

A limited number of other histamine H_3 receptor assays have been performed with human tissue. Thioperamide shows varying degrees of potency in these. For example, in the human brain histamine release assay, the K_i value for thioperamide was 16 nM (Arrang et al., 1988), higher than what had been reported in rat (Arrang et al., 1987), but substantially lower than we find in the human binding assay. As a blocker of histamine H_3 receptor-mediated inhibition of human saphenous vein contraction, thioperamide had a K_i value we calculate from published data (Oike et al., 1992) to be 33 nM. The one other binding assay that has been reported in human tissue, the gastric tumor cell line HGT-1, clone 6, yielded a K_i value for thioperamide of 80 nM, with a purified receptor (Cherifi et al., 1992). These values are all between those reported in the rat and what we find in human, this last value, from the binding assay of the gastric tumor cell line, being closest to what we find in the brain binding assay. As more detailed pharmacology from these human assays becomes available, we will have a better profile of the receptors and an idea, perhaps, of whether they reflect subtypes of histamine H_3 receptor.

One might suppose, the tissue being obtained at autopsy, that the human differences could be ascribed to human tissue being less fresh than what is obtained from rodents — and this might be true with regard to the binding site density — but human and monkey histamine H_3 receptor binding are similar. Thioperamide is also less potent an inhibitor in the monkey than the rodent brain. In the monkey, like the human, thioperamide inhibition conforms to a one-site curve-fit, this in a tissue that yields a receptor density equivalent to that of guinea-pig brain (Korte et al., 1990), and about half that of rat (West et al., 1990b). Again, the human tissue may not be as fresh as the animal specimens and this is reflected in the reduced site density, but in all other respects, the human data is consistent with the monkey. Worth noting, too, the potency of the agonists tested in the various species is very similar among species. Whatever the biochemical basis for it, there are real differences between rodent and primate brain histamine H_3 receptors in the pharmacology of thioperamide.

In the context of primate histamine H_3 receptors, the only other report about the histamine H_3 receptor in nonhuman primates is an autoradiographic localization with [^{3}H](*R*)-α-methylhistamine (Martinez-Mir et al., 1990). Results from our gross dissection are consistent with that study and with autoradiography in rodents (Schwartz et al., 1990), showing highest levels of [^{3}H]*N*$^\alpha$-methylhistamine binding to basal ganglia followed by hippocampus, thalamus and hypothalamus, and cortex, with an almost two-fold range of site-density among different regions of cortex. Levels of binding in cerebellum and brainstem are very low in contrast. In summary, there are no clear, gross differences in the distribution of the histamine H_3 receptor between primate and rodent but there are fine differences in the pharmacology between species.

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