Abstract We have characterized the binding of the histamine H3 receptor antagonist \([^{125}\text{I}]\text{iodophenpropit}\) to mouse brain. \([^{125}\text{I}]\text{iodophenpropit}\) saturably bound to mouse brain membranes with a \(pK_d\)-value of 9.31±0.04 nM and a receptor binding density of 290±8 fmol per mg protein. Saturation binding analysis revealed binding of \([^{125}\text{I}]\text{iodophenpropit}\) to a single class of sites, showing linear Scatchard plots and Hill coefficients not different from unity (\(n_H=0.98±0.02\)). At a concentration of 0.25 nM \([^{125}\text{I}]\text{iodophenpropit}\), specific binding represented about 75% of the total binding. Competition binding curves for H3 receptor antagonists were fitted best to a one-site model, showing \(pK_i\)-values in general accordance with the \(pA_2\)-values obtained in mouse cerebral cortex. Displacement of \([^{125}\text{I}]\text{iodophenpropit}\) by the H3 receptor agonists (R)-\(\alpha\)-methylhistamine, immepip, imetit and histamine were fitted best to a two-site model. Competition binding curves of (R)-\(\alpha\)-methylhistamine showed a rightward shift upon incubation with GTP\(_{\text{gS}}\) (10 \(\mu\)M), indicating the involvement of G-proteins in H3 agonist binding. In contrast, competition binding curves of the antagonists iodophenpropit, thioperamide and burimamide were not affected by GTP\(_{\text{gS}}\) (10 \(\mu\)M). Autoradiographic experiments showed that \([^{125}\text{I}]\text{iodophenpropit}\) binding sites were heterogeneously distributed, similarly to the distribution of histamine H3 receptors reported in rat brain. Highest densities were observed in the cerebral cortex, the striatum, the nucleus accumbens, the globus pallidus and the substantia nigra.

In conclusion, we have demonstrated that in mouse brain, \([^{125}\text{I}]\text{iodophenpropit}\) selectively binds to histamine H3 receptors. We also observed that the mouse brain H3 receptors labelled by \([^{125}\text{I}]\text{iodophenpropit}\) displayed binding characteristics and a distribution similar to rat brain.

Key words Histamine H3 receptors · \([^{125}\text{I}]\text{iodophenpropit}\) · Receptor binding study · Mouse brain · Autoradiographic distribution

Introduction

The histamine H3 receptor has been well documented as a presynaptic receptor modulating neuronal histamine release in the CNS and the release of other neurotransmitters, both in CNS and PNS (Schwartz et al. 1991; Leurs and Timmerman 1992; Schlicker et al. 1994). Histamine H3 receptors have been identified in different mammalian species, from rodents to primates (Martinez-Mir et al. 1990; Schlicker et al. 1994). Activation of H3 receptors in the CNS has been demonstrated to modulate sleep and wakefulness, cognition, locomotion, feeding behaviour and electrically induced convulsions (for review see Schwartz et al. 1991; Onodera et al. 1994; Leurs et al. 1998). The molecular pharmacological mechanisms underlying these effects are likely related to modulation of the release of different neurotransmitters like histamine (H3 autoreceptors; Arrang et al. 1983; Van der Werf et al. 1987), serotonin (Fink et al. 1990; Alves-Rodrigues et al. 1995), noradrenaline (Schlicker et al. 1989), acetylcholine (Clapham and Kilpatrick 1992; Arrang et al. 1995) and neuropeptides (Matsubara et al. 1992). Histamine H3 receptors may also attain their effects via yet unidentified postsynaptic mechanisms (Cumming et al. 1991; Ryu et al. 1994).

Histamine H3 receptors have been characterized by receptor binding studies performed in rat (Arrang et al. ...
1990; West et al. 1990a; Jansen et al. 1994; Ligneau et al. 1994), guinea pig (Korte et al. 1990; Kilpatrick and Michel 1991) and, to a less extent, in bovine (Zweig et al. 1992) and primate brain (Martinez-Mir et al. 1990). Characterization of H3 receptors in mouse brain is based almost exclusively on functional studies (Kathmann et al. 1993; Leurs et al. 1996). The mouse has been frequently used to study the pharmacology of CNS H3 receptors in vivo, using the H3 agonist (R)-α-methylhistamine and the H3 antagonist thioperamide (Schwartz et al. 1991; Onodera et al. 1994). The in vivo potencies of both ligands in the mouse were consistent with their potencies reported in other species. The H3 antagonist clobenpropit, however, was reported to inhibit electrically induced convulsions in mice (Yokoyama et al. 1994) at doses about tenfold lower than the doses required to observe CNS effects in rats (Barnes et al. 1993). At present it is not clear whether this difference is due to a species difference or to H3 receptor heterogeneity.

The distribution of binding sites of the H3 agonist [3H]Nα-methylhistamine in mouse forebrain has been visualized with autoradiography (Cumming et al. 1994). A pharmacological characterization of the [3H]Nα-methylhistamine binding sites using different selective ligands was not performed, however. The present study was conducted to provide a more detailed characterization of cerebral histamine H3 receptors in mouse brain. We have used the H3 antagonist [125I]iodophenpropit as a radioligand (Jansen et al. 1992, 1994). The affinities of different ligands for [125I]iodophenpropit binding sites were determined in mouse brain membrane preparations. Moreover, we examined the distribution of [125I]iodophenpropit binding sites in mouse CNS using receptor autoradiography.

Materials and methods

Preparation of mouse cerebral membranes and receptor binding studies. Balb/c mice (20–25 g; Harlan, Zeist, The Netherlands) were killed by decapitation and the brains were rapidly removed. The brains were homogenized in 15 volumes (v/w) ice-cold Tris-HCl buffer (50 mM Tris-HCl, 5 mM MgCl₂, 145 mM NaCl, pH 7.4 at 4°C) using a glass-teflon homogenizer. Subsequently, membrane fractions were prepared as previously described (Jansen et al. 1994). Receptor binding experiments were performed at 37°C in Tris-HCl buffer (pH 7.4 at 37°C) with a total incubation volume of 0.5 ml. Determinations were performed in triplicate. In saturation experiments membranes were incubated with [123I]iodophenpropit in final concentrations ranging from 0.025 nM to 3 nM. In competition binding experiments a concentration of 0.25 nM [125I]iodophenpropit was used. There was no significant difference between [125I]iodophenpropit binding displaced by (R)-α-methylhistamine (10 μM; 73±2% of total binding, n=4) and by thioperamide (0.3 μM; 77±3% of total binding, n=11, P>0.05; two-tailed unequal Student’s t-test). H3 receptor agonists and H3 receptor antagonists displaced [125I]iodophenpropit to the same level. Membrane suspensions were incubated with [125I]iodophenpropit (Menge et al. 1992) for 60 min to reach equilibrium. Incubations were started by the addition of 100-μl membrane preparations (20–60 μg protein per tube) and were terminated by adding 2 ml ice-cold Tris-HCl buffer (pH 7.4 at 4°C) immediately followed by filtration through Whatman GF/C filters (pretreated with 0.3% polyethyleneimine) using a Brandel filtration apparatus. The radioactivity bound to the filters was measured by an LKB gamma counter. Protein concentrations were determined using the Bio-Rad Protein Assay (Bradford 1976). Bovine serum albumin (BSA) was used as a standard.

Data analysis and statistical evaluation. Dissociation constants for H3 ligands in the absence and presence of GTPγS were compared using a two-tailed unpaired Student’s t-test. Saturation and competition binding experiments were evaluated using the non-linear curve fitting program LIGAND (Munson and Rodbard 1980) on a Macintosh computer. Binding curves were fitted (unweighted) to a one- and two-site model, respectively, and statistically tested on the increasing goodness of the fit for a model with additional parameters (i.e. the two-site model), using a probability level of 5% (Munson and Rodbard 1980). pKᵦ-values and pKᵦᵢ-values are expressed as the average ± SD of the indicated number of experiments (n). The fitting procedure includes a correction to account for receptor occupancy, converting IC₅₀-values into Kᵦ-values (Munson and Rodbard 1980).

Receptor autoradiographic experiments. Balb/c mice (20–25 g; Harlan, Zeist, The Netherlands) were killed by decapitation and the brains were rapidly removed. Brains were frozen in isopentane at −40°C. Cryostate sections (transversal or sagittal, 14 μM) were cut, mounted onto gelatin/chromalum-coated glass slides and were stored at −80°C until use. For the autoradiographic experiments, tissue sections were thawed and were incubated with 0.25 nM [125I]iodophenpropit for 60 min at 37°C in 50 mM Tris-HCl buffer containing 145 mM NaCl, 5 mM MgCl₂ and 0.25% BSA (pH 7.4 at 37°C). Non-specific binding was determined by incubation of adjacent sections in the presence of 1 μM (R)-α-methylhistamine or with 0.3 μM thioperamide. To stop the incubations, the sections were rinsed once with ice-cold Tris-HCl buffer (50 mM Tris-HCl, 5 mM MgCl₂, 145 mM NaCl, pH 7.4 at 4°C) and were subsequently washed twice for 15 min in ice-cold Tris-HCl buffer and 15 s in ice-cold distilled water. Sections were dried by a stream of cold air and were exposed to Hyperfilm (Amersham International, UK) for 20 h.

Chemicals. [125I]Iodophenpropit (Radionuclide Center, Leiden/Amsterdam Center for Drug Research, Vrije Universiteit, Amsterdam) was labelled to a specific activity of 1900 Ci mmol⁻¹ as described by Menge et al. (1992). The following drugs were used: iodophenpropit dihydrobromide (laboratory stock), clobenpropit dihydrobromide (laboratory stock), thioperamide maleate (laboratory stock), idopfoxylan (gift from Prof. Dr. W. Schunack, Berlin, Germany), burimamide (gift from SK&F Laboratories, UK), imipramine trihydrochloride (gift from SK&F Laboratories), dimaprit dihydrochloride (gift from SK&F Laboratories), histamine dihydrochloride (Sigma, St. Louis, Mo., USA), betahistine dihydrochloride (gift from Solvay Pharmaceuticals, Netherlands), ondansetron (gift from Solvay Pharmaceuticals, Netherlands), (R)-α-methylhistamine dihydrochloride (Research Biochemicals International, Natick, Mass., USA), (S)-α-methylhistamine dihydrobromide (Tocris Cookson, St. Louis, Mo., USA), imetit dihydrobromide (VUF8325, laboratory stock), immepp dihydrobromide (VUF4708; 4-(1H-imidazol-4-yl)methylpiperidine dihydrobromide, laboratory stock), mepyramine hydrochloride (Sigma), tiotidine (ICL Macclesfield, UK), yohimbine (Roth, Karlsruhe, Germany), guanoxine 5′-O-(3-thio)triphosphate (GTPγS; Sigma), bovine serum albumin (BSA; Sigma) and polyethyleneimine (Aldrich, Zwijndrecht, Netherlands). Ligands (solutions of 1–10 mM) were dissolved in distilled water or in Tris-HCl buffer and were further diluted in Tris-HCl buffer. Ondansetron (1 mM) and haloperidol (10 mM) were dissolved in DMSO and ethanol, respectively.

Results

Binding of [125I]iodophenpropit to mouse brain membranes was saturable and of high affinity (pKᵦᵢ=9.31±0.04;