Binding Characteristics of a Histamine H3-Receptor Antagonist, \([^{3}H]S\)-Methylthioperamide: Comparison with \([^{3}H](R)\alpha\)-Methylhistamine Binding to Rat Tissues

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ABSTRACT—The release and synthesis of neuronal histamine are regulated by histaminergic autoreceptors named as histamine H3 receptors. The development of radiolabeled histamine H3 antagonists is needed to characterize the binding of antagonists to these receptors. Here we describe the binding characteristics of a new histamine H3-receptor antagonist, \([^{3}H]S\)-methylthioperamide (SMT), to rat tissues, and compare its binding with that of \([^{3}H](R)\alpha\)-methylhistamine ((R)\(\alpha\)MH), a selective histamine H3-receptor agonist. The binding of \([^{3}H]SMT\) to the membranes of rat forebrain was found to be stereoselective, saturable, reversible and temperature-dependent. Saturation binding experiments indicated a single class of high affinity sites for \([^{3}H]SMT\) in forebrain membranes (\(K_D=2.1\) nM, \(B_{\text{max}}=24.3\) pmol/g of tissue at 4°C). The \(B_{\text{max}}\) was approximately 3 times that of \([^{3}H](R)\alpha\)MH binding to rat forebrain membranes (\(K_D=2.5\) nM, \(B_{\text{max}}=7.3\) pmol/g of tissue at 25°C). Autoradiographic images of \([^{3}H]SMT\) binding in the brain were essentially the same as those of \([^{3}H](R)\alpha\)MH. \([^{3}H]SMT\) also bound appreciably to peripheral tissues (the liver, adrenal, stomach, ileum, kidney, lung and bladder), whereas the \([^{3}H](R)\alpha\)MH bindings to these peripheral tissues were negligible. These results indicate that \([^{3}H]SMT\) binds to H3 receptors primarily in the central nervous system, and that it also has high affinity toward non-H3 receptors, probably hemoproteins, in peripheral tissues.

Keywords: Histamine H3 receptor, Histamine, \([^{3}H]S\)-Methylthioperamide, \([^{3}H](R)\alpha\)-Methylhistamine, Binding

Histamine receptors have been divided into three major subtypes, named H1, H2 and H3 receptors. The histamine H3 receptor was initially proposed to be an autoreceptor of the histaminergic neuron system, modulating release and synthesis of neuronal histamine in the central nervous system (1–3). However, it is now considered to act as a heteroreceptor in the central and peripheral nervous systems (4, 5). It also regulates the release of other neurotransmitters such as 5-hydroxytryptamine, noradrenaline, acetylcholine and \(\gamma\)-aminobutyric acid (6–9).

\((R)\alpha\)-Methylhistamine ((R)\(\alpha\)MH) and thioperamide are often used to demonstrate the presence of H3 receptors, acting as an agonist and antagonist, respectively (2). The tritium-labeled agonists \([^{3}H]N^2\)-methylhistamine (10, 11) and \([^{3}H](R)\alpha\)MH (12) are currently available for ligand-receptor binding studies. However, no tritium-labeled histamine H3 antagonist has yet been reported. Here we describe the binding of a new H3 antagonist, \([^{3}H]S\)-methylthioperamide (SMT), to rat brain and its comparison with \([^{3}H](R)\alpha\)MH, a selective histamine H3 agonist.

MATERIALS AND METHODS

Chemicals

\([^{3}H](R)\alpha\)MH (39 mCi/\(\mu\)mol) was obtained from Amersham Japan (Tokyo). \([^{3}H]SMT\) (Fig. 1) was synthesized by \(S\)-methylation of the \(S\)-desmethylated precursor thioperamide with \([^{3}H]\)iodomethane (Amersham). The chemically and radiochemically labeled product was purified by preparative HPLC (TSK-Gel ODS-80\(^TM\), 7.8 mm \(\times\) 30 cm). Its specific activity (S.A.) was determined to be 50.3 mCi/\(\mu\)mol based on its UV absorption (254 nm) on analytical HPLC (TSK-Gel ODS-80\(^TM\), 3.4 mm \(\times\) 10 cm). \((R)\alpha\)MH, \((S)\alpha\)MH and thioperamide were
Measurement of histamine content of mouse brain

ICR mice were injected intraperitoneally with S-methylthioperamide dihydrochloride (10 and 20 mg/kg) and thioperamide maleate (20 mg/kg) 1 hr before sacrifice. Their brains were homogenized in 5–10 volumes of 3% perchloric acid containing 5 mM EDTA-2 Na in a Polytron, and the homogenate was centrifuged at 12,000 x g for 20 min. Histamine in the resulting supernatant was then measured by the HPLC-fluorescence method.

Preparation of subcellular fractions from rat brain

Enriched membrane fractions were prepared rapidly by excising rat brain tissue and placing it in ice-cold 50 mM Tris-HCl buffer (pH 7.5). The rat forebrain (cerebral cortex and striatum) was homogenized in a Polytron (setting 8, 30–60 sec). In subcellular distribution studies, the brain homogenate was centrifuged twice at 1,000 x g at 4°C to separate the nuclear fraction and cell debris. The resulting supernatants were combined and then centrifuged twice at 50,000 x g for 20 min to obtain the membrane fraction. Unless otherwise indicated, the brain homogenate was only centrifuged twice at 50,000 x g to obtain the crude membrane fraction. Protein concentration was determined by the method of Bradford with a kit obtained from BioRad (Richmond, CA, USA).

Bindings of [3H]SMT and [3H](R)aMH

Incubation (total volume 0.5 ml) with [3H]SMT was carried out at 4°C for 90 min unless otherwise indicated, and the reaction was terminated by addition of 5 ml of the ice-cold buffer and rapid filtration on a glass fiber filter (GF/B) precoated with 0.3% polyethyleneimine. The filter was washed with three 5-ml volumes of cold buffer, and the radioactivity trapped on the filter was counted in 10 ml of Aquasol 2 (Amersham). Specific binding was defined as the radioactivity bound after subtraction of nonspecific binding, determined in the presence of 10 μM thioperamide. The binding assay of [3H](R)aMH was performed by a modification of the method of Arrang et al. (2, 12).

Table 1. Effects of S-methylthioperamide on brain histamine content of ICR mice

| Drug           | Saline | S-Methylthioperamide 10 mg/kg | S-Methylthioperamide 20 mg/kg | Thioperamide 20 mg/kg |
|----------------|--------|-------------------------------|-------------------------------|----------------------|
| Histamine      | 683 ± 84 | 556 ± 66*                    | 497 ± 65**                   | 467 ± 72**           |

Values are means ± S.D. s for 5–10 determinations. The histamine content of the brain is expressed in pmol/g of wet tissue. The doses of S-methylthioperamide and thioperamide are expressed as those of their dihydrochloride and maleate salts, respectively. *P < 0.05, **P < 0.01.
rium within 5 min and then gradually decreased for 60 min. After adding a large amount of SMT, the total binding rapidly decreased in 2 min. On the other hand, the total binding at 4°C increased gradually in 45 min and then remained constant for at least 60 min. Binding of \([\text{H}]\)SMT to rat brain at 4°C was gradually reversed on addition of excess cold SMT.

Saturation experiments were carried out at 4°C to determine the maximum binding capacity (B_{max}) and dissociation constant (K_D). As shown in Fig. 2, a saturation binding study disclosed an apparent single class of binding sites for \([\text{H}]\)SMT in the rat forebrain (B_{max} = 24.3 ± 4.2 pmol/g of tissue, 551 ± 152 fmol/mg of protein; K_D = 2.1 ± 0.7 nM; n = 3). On the other hand, the results revealed a single class of binding sites for \([\text{H}]\)({\text{R}})\alpha MH with a B_{max} of 7.3 ± 1.2 pmol/g of tissue (189 ± 40 fmol/mg of protein; n = 3) and a K_D of 2.5 ± 0.5 nM. Specific binding of \([\text{H}]\)SMT and \([\text{H}]\)({\text{R}})\alpha MH at a concentration of 1 nM accounted for approximately 70–80% and 85–90%, respectively, of the total binding.

**Fig. 2.** Saturation bindings of \([\text{H}]\)S-methylthioperamide (A) and \([\text{H}]\)({\text{R}})\alpha-methylhistamine (B) to rat forebrain membranes. (●), total binding; (■), nonspecific binding; (○), specific binding. Points are averages for triplicate experiments.
Inhibitions of $[^3H]$SMT binding by (R) and (S)$\alpha$MH

To determine whether the binding of $[^3H]$SMT was stereoselective, we carried out competition binding experiments using stereoselective (R) and (S)$\alpha$MH. The displacement curve for the agonist (R)$\alpha$MH was shallow and revealed two binding sites (Fig. 3). The biphasic displacement of $[^3H]$SMT by (R)$\alpha$MH coincided well with the binding characteristics of the antagonist $[^125I]$iodophenpropit (14). In contrast, the displacement of $[^3H]$SMT by the antagonist thioperamide fitted best to a one-site binding model (15). (S)$\alpha$MH was 3 orders of magnitude less potent than its (R) stereoisomer.

Autoradiographic demonstration of $[^3H]$SMT binding sites in rat brain

The localizations of H$_3$ receptors determined with $[^3H]$-SMT and $[^3H]$(R)$\alpha$MH are shown in Fig. 4. The levels of binding sites of $[^3H]$SMT were high in the cerebral cortex, striatum, nucleus accumbens and substantia nigra. The distribution pattern of $[^3H]$SMT was essentially the same as that of $[^3H]$(R)$\alpha$MH except that in the cerebellum. $[^3H]$SMT bound slightly to the cerebellum, whereas $[^3H]$(R)$\alpha$MH did not bind appreciably.

Tissue distribution of $[^3H]$ligand-binding in rats

$[^3H]$SMT was bound preferably to peripheral tissues, high levels of binding sites being found in the adrenal, liver, stomach, small intestine, bladder, trachea and kidney (Table 2). In contrast, no $[^3H]$(R)$\alpha$MH binding was detectable in most peripheral organs. Specific binding of $[^3H]$(R)$\alpha$MH was mainly observed in the cerebral cortex and midbrain. The specific bindings of the two ligands to various organs, determined by subtraction of nonspecific binding measured with 10 $\mu$M thioperamide, were almost the same as those measured with 10 $\mu$M (R)$\alpha$MH.
Table 2. Tissue distribution of [3H]S-methylthioperamide binding in rats

| Tissue      | Specific binding [3H]S-Methylthioperamide | [3H](R)α-Methylhistamine |
|-------------|-------------------------------------------|--------------------------|
| Forebrain   | 51 ± 14                                   | 36 ± 11                  |
| Midbrain    | 36 ± 5                                    | 21 ± 4                   |
| Cerebellum  | 15 ± 7                                    | 2.9 ± 0.4                |
| Lung        | 45 ± 6                                    | 2.8 ± 1.6                |
| Trachea     | 28 ± 18                                   | 1.9 ± 0.16               |
| Pituitary   | 9.8 ± 3.5                                 | 1.6 ± 0.1                |
| Testis      | 16 ± 2.7                                  | 0.83 ± 0.53              |
| Liver       | 270 ± 177                                 | 0.52 ± 0.07              |
| Adrenal     | 286 ± 34                                  | —                        |
| Stomach     | 206 ± 152                                 | —                        |
| Bladder     | 56 ± 41                                   | —                        |
| Small intestine | 50 ± 44                               | —                        |
| Kidney      | 50 ± 32                                   | —                        |
| Spleen      | 17 ± 4.7                                  | —                        |
| Large intestine | 9.0 ± 2.2                           | —                        |
| Pancreas    | 4.3 ± 1.3                                 | —                        |
| Heart       | 3.6 ± 0.67                                | —                        |
| Muscle      | 1.3 ± 0.5                                 | —                        |

Values are means ± S.D.s for 3 determinations. Binding of [3H]-ligands is expressed in fmol/mg protein. Non-specific binding is defined as that with 10 μM thioperamide. —, less than 0.5 fmol/mg protein.

DISCUSSION

Thioperamide has often been used as an antagonist to characterize histamine H3 receptors in pharmacological and biochemical experiments (2, 12). However, its labeling with tritium has not been successful. As we found that S-methylation of thioperamide does not change its antagonistic activity, we synthesized [3H]SMT using [3H]-CH3I and compared its binding to rat tissues with that of [3H](R)αMH, with which histamine H3 receptors have been characterized.

SMT reduced the content of histamine in the mouse brain dose-dependently as well as thioperamide. The decrease in histamine content by SMT was probably due to facilitated release of neuronal histamine. This release of histamine could be measured using microdialysis (data not shown). As shown in Table 1, the effect of SMT on the brain content of histamine was similar to that of thioperamide described previously (16).

The main difference between the bindings of [3H]SMT and [3H](R)αMH is that the maximum binding capacity of [3H]SMT is approximately 3 times more that of [3H](R)αMH. Autoradiography showed that the distributions of binding sites of [3H]SMT and [3H](R)αMH were similar, except in the cerebellum. Displacement of [3H]SMT by the agonist (R)αMH revealed two distinctive binding sites, high (Kd = 0.048 nM) and low (Kd = 100 nM) affinity binding sites. The existence of high and low affinity binding sites may arise from the presence of acceptor binding sites such as hemoproteins in the brain, although the involvement of G-proteins in agonist binding is not ruled out. The binding characteristics observed here are essentially the same as those of [125I]iodophenpropit, a radioiodinated antagonist (14). The biphasic displacement of H3-receptor antagonist binding by agonists is a common feature in the both antagonist-binding studies. Further studies are needed to clarify the characteristics of the H3-antagonist binding.

The peripheral binding sites labeled with [3H]SMT and [3H](R)αMH differed markedly in distribution and quantity. Large amounts of [3H]SMT binding sites were found in almost all tissues, whereas binding of [3H](R)αMH was observed at a lesser extent only in the lung, trachea and pituitary. The peripheral binding sites of [3H]SMT should be taken into account in studies on not only the functional roles of histamine H3 receptors using thioperamide, but also the purification of the H3 receptors (17, 18). Imidazole derivatives have been observed to bind to diverse cytochrome P-450 isoforms (19–21). Thioperamide interacts with the heme moiety, producing a type-II difference spectrum, suggesting that it has high affinity to non-H3 receptors, probably hemoprotein, in peripheral tissues (22).

In conclusion, [3H]SMT, a new methylated derivative of thioperamide, can be used as a 3H-labeled H3 antagonist to measure the histamine H3 receptors in the brain because it shows the criteria of a ligand for histamine H3 receptors. Use of both [3H]SMT and [3H](R)αMH allows analysis of the difference in characteristics of binding of agonists and antagonists to H3 receptors. It should be remembered that thioperamide and its related derivatives may have considerable levels of non-H3 binding sites in central and peripheral tissues in studies designed to elucidate functional roles of H3 receptors.

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REFERENCES

1. Arrang JM, Garbarg M and Schwartz JC: Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. Nature 302, 832–837 (1983)
2 Arrang JM, Garbarg M, Lancelot JC, Lecomte JM, Pollard H, Robba M, Schunack W and Schwartz JC: Highly potent and selective ligands for histamine H3-receptors. Nature 327, 117–123 (1987)

3 Arrang JM, Garbarg M and Schwartz JC: Autoregulation of histamine release in brain by presynaptic H3-receptors. Neuroscience 15, 553–562 (1985)

4 Cumming P, Shaw C and Vincent SR: High affinity histamine binding sites is the H3 receptor: Characterization and autoradiographic localization in rat brain. Synapse 8, 144–151 (1991)

5 Pollard H, Moreau J, Arrang JM and Schwartz JC: A detailed autoradiographic mapping of histamine H3 receptors in rat brain areas. Neuroscience 52, 169–189 (1993)

6 Schüller E, Fink K, Hinterthaner M and Göthert M: Inhibition of noradrenaline release in the rat brain cortex via presynaptic H3 receptors. Naunyn Schmiedebergs Arch Pharmacol 340, 633–638 (1989)

7 Schüller E, Behling A, Lünmen G, Malinowska B and Göthert M: Mutual interaction of histamine H2-receptors and α2-adrenoceptors on noradrenergic terminals in mouse and rat brain cortex. Naunyn Schmiedebergs Arch Pharmacol 345, 639–646 (1992)

8 Gulat-Marnay C, Lafitte A, Arrang JM and Schwartz JC: Modulation of HA release and synthesis in the brain mediated by α2 receptors. J Neurochem 53, 519–524 (1989)

9 Clapham J and Kilpatrick GJ: Histamine H3 receptors modulate the release of [3H]acetylcholine from slices of rat entorhinal cortex: evidence for the possible existence of H3 receptors subtypes. Br J Pharmacol 107, 919–923 (1992)

10 Korte A, Myers J, Shih N-Y, Egan RW and Clark MA: Characterization and tissue distribution of H3 histamine receptors in guinea pigs by N'-methylhistamine. Biochim Biophys Res Commun 168, 979–986 (1990)

11 West RE Jr, Zweig A, Shin N-Y, Siegel MI, Egan RW and Clark MA: Identification of two H3-histamine receptor subtypes. Mol Pharmacol 38, 610–613 (1990)

12 Arrang JM, Roy J, Morgat JL, Schunack W and Schwartz JC: Histamine H3-receptor binding sites in rat brain membranes: modulation by guanine nucleotides and divalent cations. Eur J Pharmacol 188, 219–227 (1990)

13 Yanai K, Ryu JH, Watanabe T, Iwata R and Ido T: Receptor autoradiography with 11C and [3H]-labelled ligands visualized by imaging plates. Neuro Report 3, 961–964 (1992)

14 Jansen FP, Rademaker B, Bast A and Timmerman H: The first radiolabeled histamine H3 receptor antagonist, [125I]iodophenpropl: saturable and reversible binding to rat cortex membranes. Eur J Pharmacol 217, 203–205 (1992)

15 Munson PJ and Rodbard D: Ligand: A versatile computerized approach for characterization of ligand-binding systems. Anal Biochem 107, 220–239 (1980)

16 Sakai N, Onodera K, Maeyama K, Yanai K and Watanabe T: Effects of thioperamide, a histamine H3 receptor antagonist, on locomotor activity and brain histamine content in mast cell-deficient W/Wv mice. Life Sci 48, 2397–2404 (1991)

17 Cherifi Y, Pigeon C, Romancer ML, Bado A, Rey-Desmars F and Lewin MJM: Purification of a histamine H3 receptor negatively coupled to phosphoinositide turnover in the human gastric cell line HGT1. J Biol Chem 267, 25315–25320 (1992)

18 Zweig A, Siegel MI, Egan KW, Clark MA, Shorr RGL and West RE Jr: Characterization of a digitonin-solubilized bovine brain H3 histamine receptor coupled to a guanine nucleotide-binding protein. J Neurochem 59, 1661–1666 (1992)

19 Swanson RA and Dus KM: Specific covalent labeling of cytochrome P-450(CAM) with 1-(4-azidophenyl) imidazole, an inhibitor-derived photoaffinity probe for P-450 heme proteins. J Biol Chem 254, 7238–7246 (1979)

20 Wolff DJ, Datto GA, Samatovicz RA and Tempwick RA: Calmodulin-dependent nitric-oxide synthase. Mechanism of inhibition by imidazole and phenylimidazoles. J Biol Chem 268, 9425–9429 (1993)

21 Wolff DJ, Datto GA and Samatovicz RA: The dual mode of inhibition of calmodulin-dependent nitric-oxide synthase by antifungal imidazole agents. J Biol Chem 268, 9430–9436 (1993)

22 LaBella FS, Queen G, Glavin G, Durant G, Stein D and Brandes LJ: H3 receptor antagonist, thioperamide, inhibits adrenal steroidogenesis and histamine binding to adrenocortical microsomes and binds to cytochrome p450. Br J Pharmacol 107, 161–164 (1992)