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Characterisation of the specific binding of the histamine H_3 receptor antagonist radioligand [^{3}H]GR168320

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Abstract

We have examined the specific binding of the tritiated derivative of the potent histamine H_3 receptor antagonist, [3,4-$^3H_2$]-cyclohexyl-{[4-(3*H*-imidazol-4-yl)-piperidin-1-yl]iminomethyl}-amine ([^{3}H]GR168320), to homogenates of rat cerebral cortex. Specific binding of [^{3}H]GR168320 at 37°C associated and dissociated rapidly. Binding was saturable (B_{max} 412 ± 89 fmol/mg protein) and of high affinity (K_d 0.12 ± 0.11 nM). Saturation studies suggested the involvement of a single site. Histamine H_3 receptor agonists and antagonists inhibited [^{3}H]GR168320 binding with high affinity. Agonist and antagonist affinities correlated when compared with affinities obtained using the tritiated histamine H_3 agonist radioligand N^{α}-methylhistamine.

Keywords: Histamine H_3 receptor; Radioligand binding; [^{3}H]GR168320; Cerebral cortex, rat; [^{3}H]N^{α}-Methylhistamine

1. Introduction

It is now well established that histamine is able to exert it's effects in both the central nervous system (CNS) and the periphery via activation of at least three histamine receptor subtypes, H_1, H_2 and H_3 (see Schwartz and Haas, 1992, for review). The most recently discovered receptor, the histamine H_3 receptor, is characterised by its ability to subserve an autoregulatory function on the release and synthesis of histamine (Arrang et al., 1983). Moreover the H_3 receptor has also been shown to act as a heteroreceptor to influence the release of other neurotransmitters (Fink et al., 1990; Taylor and Kilpatrick, 1992; Molderings et al., 1992; Clapham and Kilpatrick, 1992).

The highly potent agonist [^{3}H](*R*)-α-methylhistamine (Arrang et al., 1987) constitutes a suitable probe for the selective labelling of the H_3 receptor. Affinity values for H_3 receptor antagonists and agonists from these binding studies were highly correlated with the corresponding values obtained at functional H_3 autoreceptors regulating histamine release or synthesis (Schwartz et al., 1990; Timmerman, 1990). However using another highly potent and selective agonist [^{3}H]N^{α}-methylhistamine, West et al. (1990) showed that two H_3 receptor antagonists, thioperamide and burimamide could identify two affinity sites termed H_{3A} and H_{3B}. Although these data implicate possible subtypes of the H_3 receptor, interpretation of these data is confounded by the use of an agonist radioligand. G-protein coupled receptors can exist in low and high affinity states for agonists. This makes interpretation of agonist binding difficult for two reasons. Firstly, when two sites are identified it is difficult to determine whether these represent two affinity 'states' or two distinct receptor sites. Secondly, agonist radioligands will often not occupy the low affinity site or will occupy only a small proportion of receptors in this state. This makes estimation of total number of receptors (B_{max}) and affinity value (K_i) difficult. Antagonists do not usually differentiate high and low affinity states or G-protein coupled receptors and hence an antagonist radioligand will have significant advantages over an agonist radioligand.

Until now, few radiolabelled H_3 receptor antagonists have been synthesised. Jansen et al. (1992) described the first iodinated histamine receptor antagonist [^{125}I]-iodophenpropit which appeared to fulfill the criteria for radiolabelling of the H_3 receptor, although the proportion

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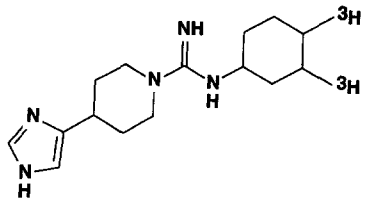

Fig. 1. Structure of GR168320.

of specific binding was only 50–60% of the total binding. This was followed by another novel iodinated histamine receptor antagonist [^{125}I]iodoproxyfan (Ligneau et al., 1994). This radioligand bound to rat striatum giving 65% specific binding. More recently Yanai et al. (1994) have reported on the characterisation of [^{3}H]S-methylthioperamide, although limited pharmacology of the binding sites labelled by this antagonist was performed.

We now report on the characteristics of the binding of another tritiated derivative of thioperamide, [3,4-$^{3}H_2$]-cyclohexyl-{[4-(3*H*-imidazol-4-yl)-piperidin-1-yl]imino-methyl}-amine ([^{3}H]GR168320) (Fig. 1), to homogenates of rat cortex and compare the affinity values obtained for this binding assay with those obtained from an assay using [^{3}H]N^{α}-methylhistamine in rat cortex for a range of histamine receptor compounds.

2. Materials and methods

2.1. Membrane preparation

Cerebral cortices were obtained from male Lister hooded rats (200–250 g). Tissue samples were homogenised using an Ultra Turrax at maximum setting for three 5-s intervals in 20 volumes of Hepes buffer (50 mM, pH 7.4, 4°C) and centrifuged for 12 min at 48 000 × *g* (4°C). The supernatant was discarded and the homogenisation and centrifugation process repeated twice for the pellet. The final pellet was resuspended in Hepes buffer at a concentration of 400 mg/ml (wet weight of tissue) and kept at −80°C until required.

2.2. [^{3}H]GR168320 binding assay

The binding assay was performed in Hepes buffer (50 mM, pH 7.4) at a final volume of 5 ml containing between 0.8–0.9 mg tissue protein. [^{3}H]GR168320 (4.8 Ci/mmol) was routinely used at a final concentration of 0.1 nM. In saturation studies this was varied between 0.02 nM and 10 nM. All assays were performed in duplicate. Non-specific binding was defined by the inclusion of thioperamide (10 μM). Tubes were incubated at 37°C for 30 min. The incubation was terminated by filtration through polyethyleneimine (0.1%) pretreated Whatman GF/B filters using a Brandel cell harvester. Filters were washed with 3 × 5 ml of Hepes buffer (4°C) and subjected to liquid scintillation counting (5 min at 40% efficiency).

2.3. [^{3}H]N^{α}-Methylhistamine binding assay

The binding assay of [^{3}H]N^{α}-methylhistamine to rat cortex was essentially as that described by Kilpatrick and Michel (1991) using the histamine H_3 agonist radioligand [^{3}H](*R*)-α-methylhistamine except for the following changes. The radioligand used was [^{3}H]N^{α}-methylhistamine (78.9 Ci/mmol) at a concentration of 0.2 nM and the assay was performed in a Hepes buffer (50 mM, pH 7.4) in a final assay volume of 0.5 ml.

2.4. Functional preparation.

For the assessment of GR168320 antagonist potency at histamine H_3 receptors, the methods described by Taylor and Kilpatrick (1992) were used. In brief, longitudinal smooth muscle strips were prepared and mounted in organ baths in Krebs-Henseleit solution, maintained at 37°C and aerated with 5% CO_2 in oxygen. Tissues were subjected to electrical field stimulation (1 stimulation/min, 20 pulses of 100 Hz frequency, 0.5 ms pulse duration and supramaximal intensity of 30 V). All experiments were conducted in the presence of atropine (0.1 μM), mepyramine (1 μM) and ranitidine (10 μM). At the start of each experiment, the inhibition of twitch responses to electrical field stimulation by (*R*)-α-methylhistamine was established and control agonist cumulative concentration-response curves were repeated every hour. Tissues were then incubated with antagonist and 30 min later, the agonist curves were reconstructed. 'Dose-ratio' shifts were measured graphically and used in Schild analysis. Where the gradient of the Schild slope was not significantly different from unity, estimates of potency (pK_i) were measured from the Schild plot, with the gradient constrained to 1.

2.5. Data analysis

Saturation studies were analysed using the program LIGAND (Munson and Rodard, 1980). Competition curves were analysed using iterative curve fitting techniques (Michel and Whiting, 1984). Protein was measured using the method of Bradford (1976) using bovine serum albumin as the standard.

2.6. Materials

[^{3}H]N^{α}-Methylhistamine (specific activity 78.9 Ci/mmol) was purchased from New England Nuclear. [^{3}H]GR168320 was radiolabelled with tritium by the Radiochemical group, Glaxo Research & Development. Its specific activity was determined to be 4.8 Ci/mmol by gravimetric analysis and liquid scintillation counting. Impromidine and burimamide were gifts from Dr M.E. Par-

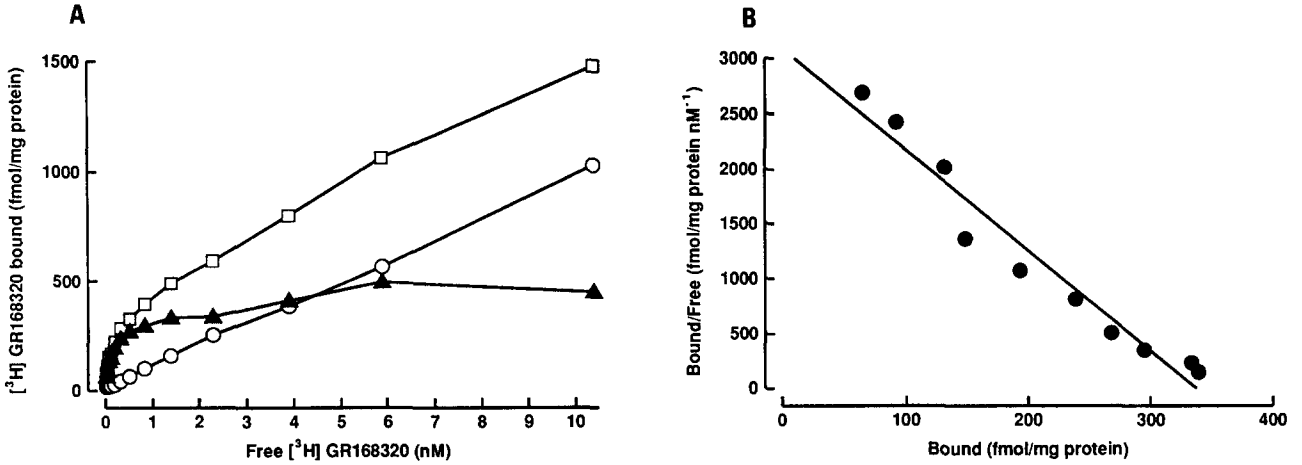

Fig. 2. (a) Equilibrium saturation analysis of specific [^{3}H]GR168320 binding to homogenates of rat cerebral cortex. (□) Total binding, (○) non-specific binding, (▲) specific binding. Results are from a single representative experiment. [^{3}H]GR168320 binding was examined over the concentration range 0.02–10 nM. (b) The Scatchard transformation of these data. Analysis using the computer program LIGAND revealed a K_d of 0.11 nM and a B_{max} of 334.0 fmol/mg protein.

sons, Smith Kline and Beecham. Mepyramine maleate was obtained from Rhone Poulenc. VUF 9153 (*S*-[3-(4(5)-imidazolyl)]propyl-*N*-(4-chlorobenzyl)isothiourea) (as the dihydrochloride salt) and other compounds were synthesised by the Chemistry Research Department, Glaxo Research and Development.

3. Results

Using rat cortex homogenates, total binding of [^{3}H]GR168320 (0.1 nM) and [^{3}H]N^{α}-methylhistamine (0.2 nM) was routinely 600–900 dpm and 3500–4000 dpm, respectively. Non-specific binding, measured in the presence of thioperamide (10 μM), was 90–150 dpm for [^{3}H]GR168320 and 250–300 dpm for [^{3}H]N^{α}-methylhistamine. For both radioligands at these concentrations, 5–7% of added radioactivity was bound at equilibrium.

Specific binding of both [^{3}H]GR168320 (0.02–10 nM) and [^{3}H]N^{α}-methylhistamine (0.02–10 nM) to rat cerebral cortex membranes was saturable and of high affinity (Fig. 2 and Fig. 3). For both ligands, Scatchard transformation of the specific binding from saturation binding experiments revealed a single class of high affinity binding sites. Analysis with the computer program LIGAND gave K_d values of 0.11 ± 0.01 nM and 0.20 ± 0.04 nM for [^{3}H]GR168320 and [^{3}H]N^{α}-methylhistamine, respectively (Fig. 2 and Fig. 3). The density of histamine H_3 receptors in this tissue were 412 ± 89 fmol/mg protein and 150 ± 7.4 fmol/mg protein for [^{3}H]GR168320 and [^{3}H]N^{α}-methylhistamine respectively. The time course of association and dissociation of [^{3}H]GR168320 and [^{3}H]N^{α}-

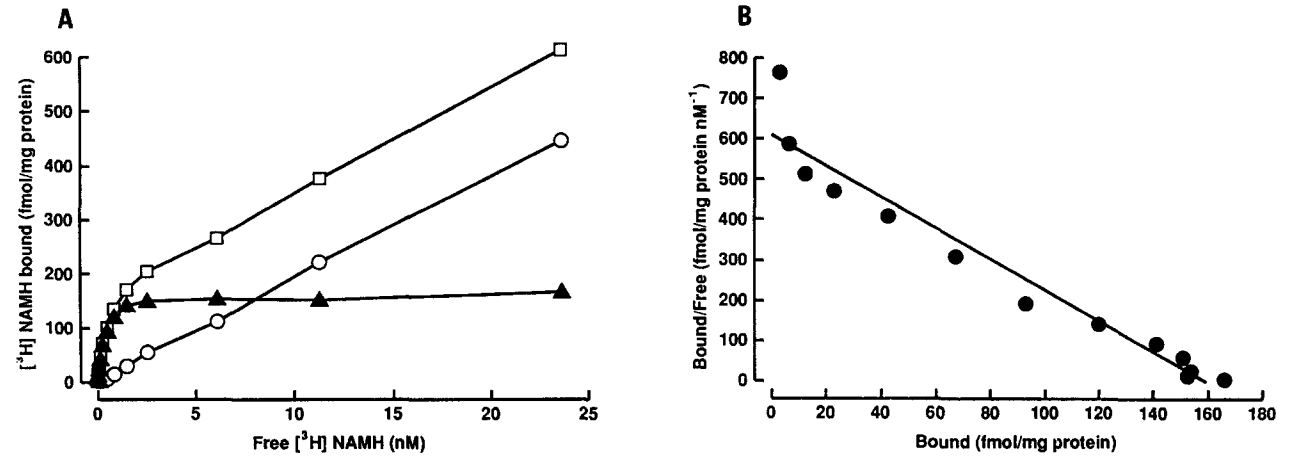

Fig. 3. (a) Equilibrium saturation analysis of specific [^{3}H] N^{α}-methylhistamine binding to homogenates of rat cerebral cortex. (□) Total binding, (○) non-specific binding, (▲) specific binding. Results are from a single representative experiment. [^{3}H]N^{α}-Methylhistamine binding was examined over the concentration range 0.02–10 nM. (b) shows the Scatchard transformation of these data. Analysis using the computer program LIGAND revealed a K_d of 0.13 nM and a B_{max} of 142 fmol/mg protein.

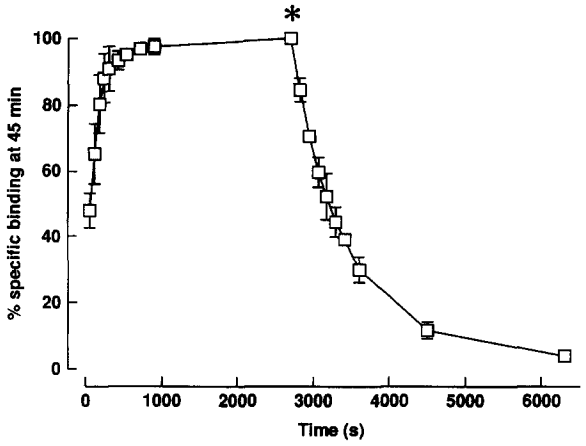

Fig. 4. The association and dissociation of specific [^{3}H]GR168320 (0.1 nM) binding to homogenates of rat cerebral cortex. Data points are the means (±S.E.M.) of 3 separate experiments. Dissociation, denoted by *, was initiated by the inclusion of thioperamide (10 μM).

methylhistamine in rat cerebral cortex are shown in Fig. 4 and Fig. 5, respectively. The association of specific [^{3}H]GR168320 binding to rat cortex at 37°C was complete within 30 min. The derived association rate (K_{+1}) was $3.00 \pm 0.61 \times 10^7$ M^{-1} s^{-1}. Specific [^{3}H]GR168320 binding was reversible. The half life for dissociation, initiated by the addition of 10 μM thioperamide, was approximately 7.5 min. Transformation of the dissociation data by plotting ln (bound at time t/bound at time 0) versus time revealed a linear plot. The derived dissociation rate (K_{-1}) was $1.25 \pm 0.31 \times 10^{-2}$ s^{-1}. The association of specific [^{3}H]N^{α}-methylhistamine binding to rat cortex at 25°C was complete within 45 min. The derived association rate (K_{+1}) was $1.92 \pm 0.24 \times 10^6$ M^{-1} s^{-1}. Specific [^{3}H]N^{α}-methylhistamine binding was reversible. The half life for dissociation initiated by the addition of 10 μM thioperamide, was approximately 10 min. Transformation of the dissociation data by plotting ln(bound at time

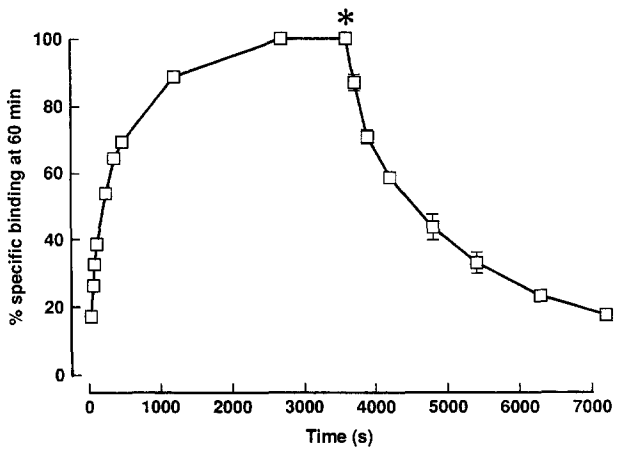

Fig. 5. The association and dissociation of specific [^{3}H]N^{α}-methylhistamine (0.1 nM) binding to homogenates of rat cerebral cortex. Data points are the means (±S.E.M.) of 3 separate experiments. Dissociation, denoted by *, was initiated by the inclusion of thioperamide (10 μM).

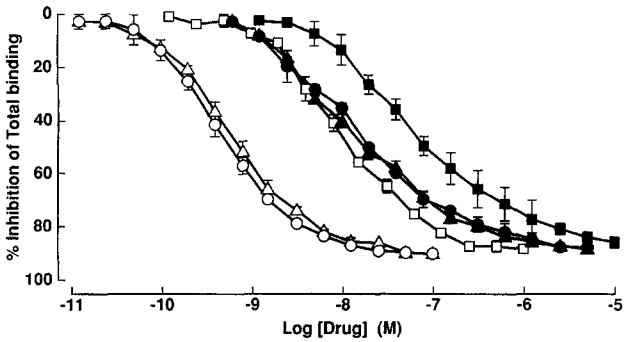

Fig. 6. The inhibition of [^{3}H]GR168320 (0.1 nM) binding to homogenates of rat cerebral cortex by H_3 agonists and antagonists. (○) (*R*)-α-methylhistamine (△) N^{α}-methylhistamine, (□) histamine, (■) thioperamide, (▲) GR168320, (●) VUF 9153. Results are the means (±S.E.M.) of 3 separate experiments.

t/bound at time 0) versus time revealed a linear plot. The derived dissociation rate (K^{-1}) was $6.72 \pm 0.62 \times 10^{-4}$ s^{-1}. Determination of the equilibrium dissociation constants from the kinetic data for [^{3}H]GR168320 and [^{3}H]N^{α}-methylhistamine were 0.42 nM and 0.35 nM, respectively. These values were similar to the K_d values obtained from the saturation experiments.

Histamine H_3 receptor agonists and antagonists competed potently for both [^{3}H]GR168320 and [^{3}H]N^{α}-methylhistamine binding to rat cortex (Fig. 6 and Fig. 7 and Table 1). All compounds were able to totally inhibit specific binding of both radioligands. In the [^{3}H]GR168320 binding assay, Hill slopes for the antagonist competition curves were close to unity in all cases while those for the agonists were significantly less than one in all cases ($P < 0.01$ two sided one sample t-test). Agonist inhibition curves in these instances were fitted to a two site model (Table 2). In the [^{3}H]N^{α}-methylhistamine binding assay, with the exception of thioperamide and burimamide Hill slopes for the antagonist competition curves were close to unity. All agonist Hill slopes were close to unity. The H_1 receptor antagonist and the H_2 receptor antagonist raniti-

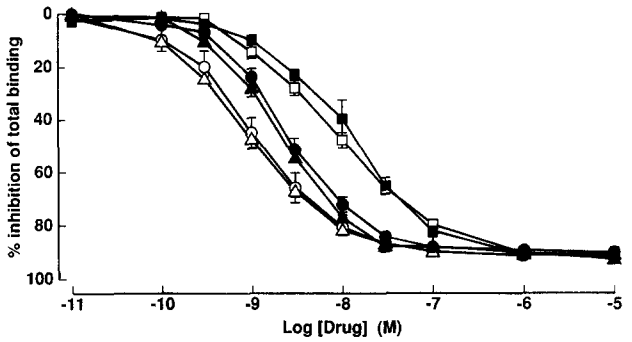

Fig. 7. The inhibition of [^{3}H]N^{α}-methylhistamine (0.1 nM) binding to homogenates of rat cerebral cortex by H_3 agonists and antagonists. (○) (*R*)-α-Methylhistamine, (△) N^{α}-methylhistamine, (□) histamine, (■) thioperamide, (▲) GR168320, (●) VUF 9153. Results are the means (±S.E.M.) of 3 separate experiments.

Table 1
Comparison of agonist and antagonist affinity values in the [^{3}H]GR168320 and [^{3}H]N^α-methylhistamine binding assays

Compound	[^{3}H]-GR168320		[^{3}H]-NAMH	
	pK_i	Hill slope	pK_i	Hill slope
Thioperamide	8.4±0.03	1.11±0.05	8.7 ±0.04	0.82±0.01
VUF9153 *	9.6±0.03	1.05±0.06	9.8 ±0.06	0.89±0.03
GR168320	9.8±0.03	1.07±0.02	9.7 ±0.11	0.88±0.05
Burimamide	6.9±0.17	1.02±0.07	7.20±0.08	0.81±0.04
Impromidine	7.7±0.03	1.16±0.06	7.9 ±0.03	0.86±0.09
NAMH	8.3±0.12	0.72±0.01	9.4 ±0.08	1.04±0.02
RAMH	8.4±0.06	0.75±0.04	9.3 ±0.10	1.05±0.07
Histamine	7.6±0.09	0.67±0.07	8.6 ±0.10	0.91±0.04
Mepyramine	5.1±0.01	1.08±0.02	< 5.0	–
Ranitidine	5.3±0.01	1.26±0.08	< 5.0	–

Results are the means (±S.E.M.) of 3 separate experiments. * Van der Goot et al. (1992).

Table 2
Two site analysis of agonist curves against [^{3}H]GR168320 in rat cortex

Agonist	pK_H	pK_L
NAMH	8.7±0.06	7.1±0.26
RAMH	8.9±0.12	7.6±0.05
Histamine	8.0±0.10	6.8±0.41

Results are the means (±S.E.M.) of three separate experiments.

dine were weak inhibitors of both [^{3}H]GR168320 and [^{3}H]N^α-methylhistamine binding. The comparison of compound affinities for both radioligands is shown in Table 1.

In the isolated longitudinal muscle preparation of the guinea pig ileum, GR168320 antagonised the (*R*)-α-methylhistamine induced inhibition of electrically induced contractions. GR168320 (0.3, 1, 3 and 10 nM) produced a rightward and parallel displacement of the concentration-response curve to (*R*)-α-methylhistamine. At these concentrations, GR168320 produced no suppression of the maximum agonist response. Schild analysis of antagonist data up to concentrations of 10 nM revealed a slope of the linear regression of 1.25 ± 0.15 (not significantly different from 1) and the pK_B value estimated from the slope constrained to unity was 9.68 ± 0.06.

The specificity of GR168320 was checked at $5HT_3$ and $5HT_4$ receptors where its binding affinity pK_i < 5 and functionally, using guinea pig ileum, at the muscarinic M_3 receptor (pK_B < 5, data not shown).

4. Discussion

When characterising the binding of a novel ligand, because functional responses are not measured in a binding assay, it is important to establish that the specific binding meets the criteria for identification of a receptor (see Burt, 1978). From functional studies in the guinea pig ileum, GR168320 elicits surmountable antagonism. Consistent with this, specific [^{3}H]GR168320 binding was also reversible, as well as being of high affinity. Receptors exist in infinite numbers and therefore, one would expect specific binding to be saturable. This was the case for [^{3}H]GR168320 although the number of receptors labelled by [^{3}H]GR168320 (B_{max} of 412 ± 89 fmol/mg protein), was significantly higher than that for [^{3}H]N^α-methylhistamine (B_{max} of 150.0 ± 7.4 fmol/mg protein). This is likely to be because the agonist radioligand was only labelling the high affinity state of the H_3 receptor whereas [^{3}H]GR168320 was labelling all the receptors.

The most important criteria for identification of the H_3 receptor is that drug affinities derived from the binding assay should correlate with those derived from the functional models of the H_3 receptor. Antagonist and agonist affinities that we report correlate closely with those derived from functional (histamine release) models of the H_3 receptors (see Schwartz et al., 1990; Timmerman, 1990). Moreover histamine H_1 and H_2 receptor antagonists showed very weak affinity for [^{3}H]GR168320 binding.

Further evidence to suggest that [^{3}H]GR168320 is specifically binding H_3 to receptors is the close correlation between antagonist and agonist affinities with those determined in the already well characterised [^{3}H]N^α-methylhistamine binding assay. However, agonist affinities in the [^{3}H]GR168320 binding assay are ten fold less than those obtained in the [^{3}H]*N*-methylhistamine binding assay and the slopes for the agonists are much shallower. These shallow slopes can be attributed to high and low affinity states of the H_3 receptor. In the [^{3}H]GR168320 binding assay the antagonist radioligand is able to label both the high and low affinity state of the receptor which can be discriminated by the cold agonists, as shown by their shallow slopes. Two site analysis of these slopes (Table 2) revealed that the K_H values obtained for the agonists when using [^{3}H]GR168320 are slightly lower than their pK_i values obtained when using [^{3}H]N^α-methylhistamine. This can be atributed to the fact that high affinity states for agonists may be underestimated in the buffer used (Hepes) in which divalent cations are omitted. In the [^{3}H]N^α-methylhistamine binding assay the agonist radioligand at the concentration used is only able to bind to the high affinity state and therefore the cold agonists only give a monophasic competition curve of high affinity.

Hill slopes for all the antagonist competition curves in the [^{3}H]GR168320 binding assay were close to unity. This included both the competition curves for thioperamide and burimamide which were shallow in the [^{3}H]N^α-methylhistamine binding assay. These data obtained with the agonist radioligand have also been reported by Arrang et al. (1987) and West et al. (1990). An explanation for these shallow curves with agonist radioligands was provided by Clark and Hill (1994) who have shown that in the [^{3}H]N^α-methylhistamine binding assay, the sites labelled by thioperamide can be altered by changing the ionic composition of the assay buffer. By increasing the sodium

concentration, there is a reduction in binding and thioperamide is only able to label the high affinity site of the receptor. These data together suggest that the shallow Hill slope to thioperamide and burimamide is unlikely to reflect binding to distinct subtypes of the receptor, but more likely reflects conformational states of the same receptor dependent on agonist binding or ionic composition of the assay buffer. However subtypes for the histamine receptor cannot be ruled out because recently Jansen et al. (1994), using the iodinated H_3 receptor antagonist [^{125}I]-iodophenpropit, showed that burimamide and dimaprit gave biphasic displacement curves. Clearly there is a need for further investigation into the above phenomena which will be helped by the introduction of more selective ligands.

In conclusion [^{3}H]GR168320 specifically labels histamine H_3 receptors in homogenates of rat cortex and therefore this radioligand provides a useful tool for the study of histamine H_3 receptors.

References

Arrang, J.M., M. Garbarg and J.C.Schwartz, 1983, Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor, Nature 302, 832.

Arrang, J.M., M. Garbarg, J.C. Lancelot, J.M. Lecomte, H. Pollard, M. Robba, W. Schunack and J.C. Schwartz, 1987, Highly potent and selective ligands for histamine H_3-receptors, Nature 327, 117.

Bradford, M., 1976, A rapid and sensitive method for quantitation of microgram quantities of proteins utilizing the principles of protein-dye binding, Anal. Biochem. 72, 248.

Burt, D.R, 1978, Criteria for receptor identification, in: Neurotransmitter Receptor Binding, eds. H.I. Yammamura, S.J. Enna and M.J. Kuhar (Raven Press, New York) p. 41.

Clapham, J. and G.J. Kilpatrick, 1992, Histamine receptors modulate the release of [^{3}H]acetylcholine from slices of rat entorhinal cortex: evidence for the possible existence of H_3 receptor subtypes, Br. J. Pharmacol. 107, 919.

Clark, E.A. and S.J. Hill, 1994, Influence of sodium ions on the binding of thioperamide to histamine H_3 receptors in rat cerebral cortex, Br. J. Pharmacol. 190P.

Fink, K., E. Schlicker, A. Neise and M. Göther, 1990, Involvement of presynaptic H_3 receptors in the inhibitory effect of histamine on serotonin release in the rat brain cortex, Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 513.

Jansen, F.P., B. Rademaker, A. Bast and H. Timmerman, 1992, The first radiolabeled histamine H_3 receptor antagonist, [^{125}I]iodophenpropit: saturable and reversible binding to rat cortex membranes, Eur. J. Pharmacol. 217, 203.

Jansen, F.P., T.S. Wu, H.-P. Voss, H.W.M. Steinbusch, R.C. Vollinga, B. Rademaker, A. Bast and H. Timmerman, 1994, Characterization of the binding of the first selective radiolabelled histamine H_3 receptor antagonist, [^{125}I]iodophenpropit, to rat brain, Br. J. Pharmacol. 113, 355.

Kilpatrick, G.J. and A.D. Michel, 1991, Characterisation of the binding of the histamine receptor agonist [^{3}H](R)-α-methylhistamine to homogenates of rat and guinea pig cortex, in: New Perspectives in Histamine Research, eds. H. Timmerman and H. Van der Goot (Birkhäuser Verlag, Basle, Boston, Berlin) p. 69.

Ligneau, X., M. Garbarg, M.L. Vizuete, J. Diaz, K. Purand, H. Stark, W. Schunack, and J.C. Schwartz, 1994, [^{125}I]Iodoproxyfan, a new antagonist to label and visualize cerebral histamine H_3 receptors, J. Pharmacol. Exp. Ther. 271, 452.

Michel, A.D. and R.L. Whiting,1984, Analysis of ligand binding data using a microcomputer, Br. J. Pharmacol. 83, 460P.

Molderings, G.J., G. Weibenborn, E. Schlicker, J. Likungu, and M. Göther, 1992, Inhibition of noradrenaline release from the sympathetic nerves of the human saphenous vein by presynaptic histamine receptors, Naunyn-Schmiedeberg's Arch. Pharmacol. 346, 46.

Munson, P.J. and D. Rodard, 1980, LIGAND: a versatile computerised approach for characterisation of ligand binding system, Anal. Biochem. 107, 220.

Schwartz, J.C., J.M. Arrang, M. Garbarg and H. Pollard, 1990, A third histamine receptor subtype: characterisation, localisation and functions of the H_3 receptor, Agents Actions 30, 13.

Schwartz, J.C. and H. Haas, 1992, The histamine receptor, in: Receptor Biochemistry and Methodology, Vol. 16 (Wiley-Liss, New York).

Taylor, S.J. and G.J. Kilpatrick, 1992, Characterisation of histamine H_3 receptors controlling non-adrenergic non-cholinergic contractions of the guinea-pig isolated ileum, Br. J. Pharmacol. 105, 667.

Timmerman, H., 1990, Histamine H_3 ligands: just pharmacological tools or potential therapeutic agents?, J. Med. Chem. 33, 4.

Van der Goot, H., M.J.P. Schepers, G.J. Sterk and H. Timmerman, 1992, Isothiourea analogies of histamine as potent agonists or antagonists of the histamine H_3 receptor, Eur. J. Med. Chem. 27, 511.

West, R.E., A. Zweig, N.-Y. Shih, M.I. Siegel, R.W. Egan, and M.A. Clark, 1990, Identification of two H_3 histamine receptor subtypes, Mol. Pharmacol. 38, 610.

Yanai, K., J.H. Ryu, N. Sakai, T. Takahashi, R. Iwata, T. Ido, K. Murakami and T. Watanabe, 1994, Binding characteristics of a histamine H_3 receptor antagonist, [^{3}H]S-methylthioperamide: comparison with [^{3}H](R)α-methylhistamine binding to rat tissues, Jpn. J. Pharmacol. 65, 107.