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Differential effect of sodium ions and guanine nucleotides on the binding of thioperamide and clobenpropit to histamine H_3-receptors in rat cerebral cortical membranes

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1 Conflicting reports in the literature over heterogeneity (West *et al.*, 1990) or homogeneity (Arrang *et al.*, 1990) of histamine H_3-receptor binding sites may be attributed to the use of different incubation conditions. In the present study we have investigated the extent to which the binding of H_3-receptor ligands to rat cerebral cortical membranes can be modified by both sodium ions and guanine nucleotides.

2 The H_3-selective antagonist, thioperamide, discriminated between two specific binding sites for [^{3}H]-N$^\alpha$-methylhistamine ($IC_{50\,1}$ = 2.75 ± 0.87 nM, $IC_{50\,2}$ = 101.6 ± 12.0 nM, % site 1 = 24 ± 2%) in 50 mM Tris HCl buffer, but showed homogeneity of binding in 50 mM Na/K phosphate buffer.

3 Sodium ions markedly altered the binding characteristics of thioperamide (i.e. heterogeneity was lost and IC_{50} value shifted towards the high affinity site). The competition curves for a second H_3-antagonist, clobenpropit and the H_3-agonist N$^\alpha$-methylhistamine however, were unaltered in the presence of sodium ions.

4 Guanylnucleotides displaced only 60% of specific [^{3}H]-N$^\alpha$-methylhistamine binding and modulated thioperamide binding in the same way as sodium ions.

5 These data suggest that the H_3-receptor can exist in different conformations for which thioperamide, but not N$^\alpha$-methylhistamine and clobenpropit, show differential affinity.

6 The potential nature of these sites, and the implications of this apparent receptor heterogeneity for H_3-receptor antagonism by thioperamide, are discussed.

Keywords: Histamine H_3-receptors; [^{3}H]-N$^\alpha$-methylhistamine; sodium ions; guanylnucleotides

Introduction

Histamine receptors have been divided into three classes on the basis of pharmacological studies with receptor-selective agonists and antagonists (H_1, H_2 and H_3, Hill, 1990). Arrang *et al.* (1983; 1987b) have shown that histamine H_3-receptors can act presynaptically to inhibit the neuronal release and synthesis of histamine in rat cerebral cortical slices. In addition to their role in histaminergic neurotransmission (Arrang *et al.*, 1983; 1985; 1987b; Van der Werf *et al.*, 1987; Van der Vliet *et al.*, 1988), H_3-receptors have also been shown to interact with a variety of other neurotransmitter systems, e.g. cholinergic, peptidergic, 5-hydroxytryptaminergic and adrenergic (Schlicker *et al.*, 1988; 1989; Hew *et al.*, 1990; Clapham & Kilpatrick, 1992; Taylor & Kilpatrick, 1992).

Radioligand binding studies with the high affinity H_3-agonists [^{3}H]-R$^\alpha$-methylhistamine (Arrang *et al.*, 1990; Kilpatrick & Michel, 1991) and [^{3}H]-N$^\alpha$-methylhistamine (West *et al.*, 1990; Korte *et al.*, 1990) have successfully identified binding sites with the characteristics of H_3-receptors in both rat and guinea-pig tissues. The binding of ^{3}H-agonists to these sites has been shown to be regulated by guanine nucleotides (implying a G-protein linkage) and several cations (Arrang *et al.*, 1987a; 1990; Zweig *et al.*, 1992). Magnesium and sodium have been shown to inhibit [^{3}H]-R$^\alpha$-methylhistamine binding (Kilpatrick & Michel, 1991), and the presence of calcium ions was found to produce some heterogeneity of agonist binding (Arrang *et al.*, 1990).

Heterogeneity of H_3-receptor binding has also been reported by West *et al.* (1990) who identified two components in the binding of [^{3}H]-N$^\alpha$-methylhistamine to rat brain membranes in 50 mM Tris buffer that could be discriminated by the selective H_3-antagonist thioperamide. These data have been interpreted as evidence for multiple H_3-receptor subtypes (West *et al.*, 1990); however, an alternative possibility is that this heterogeneity of thioperamide binding reflects a differential affinity of this antagonist for different conformations of a single class of H_3-receptor. For example, in NG108-15 cells, heterogeneity of binding to opiate receptors has been observed for certain antagonists which display 'negative efficacy' i.e. they disrupt the spontaneous tendency of receptors to interact with G-proteins by having a higher affinity for 'uncoupled' receptors (Costa *et al.*, 1990;1992; Lefkowitz *et al.*, 1993). Furthermore, the binding of this type of antagonist, but not neutral antagonists (which have similar affinity for uncoupled and G-protein coupled receptors), can be markedly altered by sodium ions and guanine nucleotides (Costa *et al.*, 1990). Interestingly, Arrang *et al.* (1990) found no evidence for differential binding of thioperamide to H_3-receptors when ^{3}H-agonist binding to rat brain membranes was monitored in 50 mM Na/K phosphate buffer.

In this study we have directly compared the effect of sodium ions and guanine nucleotides on the binding characteristics of thioperamide, another H_3-selective antagonist, clobenpropit (Van der Goot *et al.*, 1992) and N$^\alpha$-methylhistamine in rat cerebral cortex.

Methods

Tissue preparation

Cerebral cortices were dissected from either male Hooded Lister rats (300-400g) or frozen male Hooded Lister rat brains and disrupted by hand with a ground glass homogenizer in 20 volumes of Tris HCl buffer (50 mM, pH 7.4), unless otherwise stated. Homogenates were centrifuged twice at 20,000*g* for 10 min. The final pellet was resuspended in 10 volumes of buffer and stored as aliquots at

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−20°C. Samples were used within one month of preparation and protein concentration was determined by the method of Bradford (1976).

[^{3}H]-N$^\alpha$-methylhistamine binding assay

Homogenate (0.4–1.0 mg) was incubated with 1 nM [^{3}H]-N$^\alpha$-methylhistamine (84.1 Ci mmol^{-1}) in a total volume of 1 ml, 50 mM Tris HCl, pH 7.4, with or without competing ligands. Assays were performed in quadruplicate. Non-specific binding was defined in the presence of thioperamide 10 μM. Tubes were incubated for 60 min at 37°C then filtered through polyethylenimine (0.3%) pretreated Whatman GF/B filters (Burns *et al.*, 1983), using a Brandel cell harvester. Filters were washed twice with 4 ml ice-cold buffer and filter-bound radioactivity determined by liquid scintillation counting (45% efficiency).

Materials and chemicals

[^{3}H]-N$^\alpha$-methylhistamine (specific activity 84.1 Ci mmol^{-1}) was obtained from NEN DuPont. (Herts, England). Betahistine, histamine, mepyramine, guanosine 5'triphosphate (GTP), guanosine 5'-*o*-(3-thiotriphosphate) (GTPγS) and 5'-guanylimidodiphosphate (GppNHp) were purchased from Sigma Chemical Co. (Dorset, England). Burimamide, norburimamide, N$^\alpha$-methylhistamine, R$^\alpha$-methylhistamine, SKF 91486b (3-(imidazole-4-yl)-propylguanidine sulphate) and thioperamide were obtained from Smith-Kline French laboratories (Herts, England) and tiotidine from ICI Pharmaceutical (Macclesfield, England). Gifts of clobenpropit and phenylbutanoylhistamine from Glaxo Group Research (Herts, England) are gratefully acknowledged. Frozen male Hooded Lister rat brains were purchased from Charles River U.K. Ltd.

Data analysis

Curves for the specific binding of [^{3}H]-N$^\alpha$-methylhistamine at different concentrations of ^{3}H-ligand were fitted by the non-linear regression program Inplot4 (ISI). The equation fitted was:

$$\text{Specific binding} = \frac{B_{max} \times L}{(L^n + K_d^n)}$$

where B_{max} is the maximal specific binding site capacity, L is the concentration of [^{3}H]-N$^\alpha$-methylhistamine, K_d is the dissociation constant and n is the Hill coefficient.

Curves of inhibition of [^{3}H]-N$^\alpha$-methylhistamine were initially fitted to a logistic equation using Inplot4. The equation fitted was:

$$\%\text{ inhibition of specific binding} = \frac{100 \times D^n}{D^n + (IC_{50})^n}$$

where D is the concentration of competing ligand, IC_{50} is the concentration of non-radioactive inhibitor producing 50% inhibition of the specific binding of 1 nM [^{3}H]-N$^\alpha$-methylhistamine and n is the Hill coefficient. Apparent dissociation constants (K_i) were determined using the expression derived from the Cheng & Prusoff equation (1973):

$$K_i = IC_{50}/(1 + L/K_d)$$

where L is the radioligand concentration and K_d is its dissociation constant.

To determine whether the inhibition curves for each inhibitor deviated significantly from those expected for binding to a single site, the analysis was repeated with the Hill coefficient constrained to a value of unity. Statistical analysis of the change in residual sums of squares obtained from the two fits was analysed using the *F*-variance ratio test i.e. $F = (R_2\text{-}R_1)\ (df_2\text{-}df_1)/R_1\text{-}df_1$, where R_2 and R_1 represent the residuals with and without the constraint on the Hill coefficient, and df_2 and df_1 are the respective degrees of freedom.

Data for inhibitors with a Hill coefficient significantly less than unity were also fitted to a two site model:

$$\%\text{ inhibition of specific binding} = \frac{C}{(D + IC_{50}H)} + \frac{(100\text{-}C)}{(D + IC_{50}L)}$$

where C is the percentage of high affinity sites, D is the concentration of inhibitor and $IC_{50}H$ and $IC_{50}L$ are the respective IC_{50} values for the high and low affinity sites.

Data are expressed as mean ± s.e.mean and *n* represents the number of separate individual experiments performed.

Results

Characterization of [^{3}H]-N$^\alpha$-methylhistamine binding

[^{3}H]-N$^\alpha$-methylhistamine specific binding to rat cerebral cortical membranes in 50 mM Tris HCl buffer (pH 7.4), was rapid ($t_{1/2} < 5$ min), and saturable (i.e. equilibrium was reached within 20 min and binding remained stable for at least 1 h). Results were consistent with either fresh or frozen tissue. Scatchard analysis of saturation binding data (Figure 1) disclosed a single class of high affinity sites ($K_d = 2.06 \pm 0.17$ nM, $B_{max} = 65.6 \pm 9.8$ fmol mg^{-1} protein; $n = 6$). Results from competition studies, using Tris HCl (50 mM, pH 7.4) buffer showed that inhibition of [^{3}H]-N$^\alpha$-methylhistamine binding by a range of compounds (Figure 2)

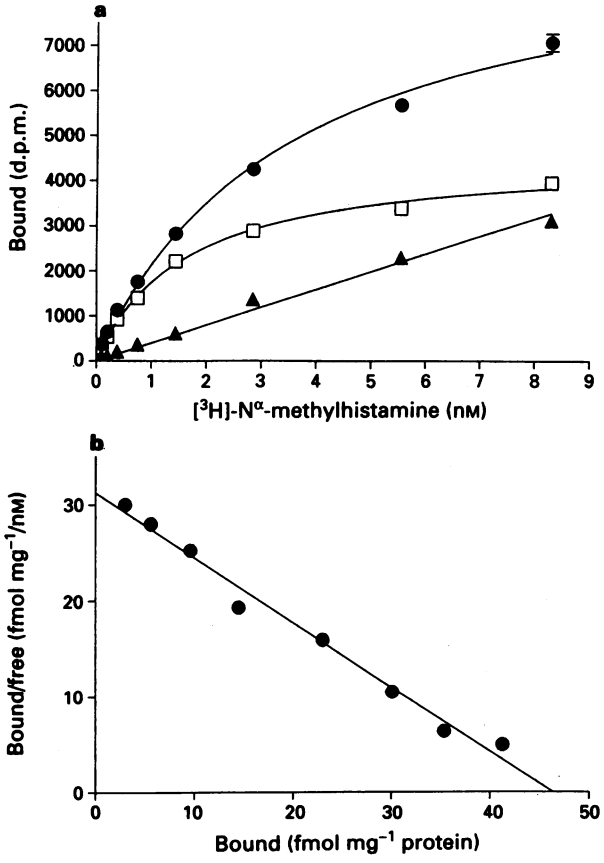

Figure 1 Saturation of [^{3}H]-N$^\alpha$-methylhistamine binding at equilibrium. (a) Binding isotherm, (□) specific binding; (●) total binding; (▲) non-specific binding. Non-specific binding was determined in the presence of thioperamide 10 μM. (b) Scatchard analysis of the specific binding data shown in (a). Data shown are representative of three separate experiments. Each point is the average of four determinations.

were characteristic of histamine H_3-receptor binding. Corresponding IC_{50} and K_i values are shown in Table 1. With the exception of thioperamide, all of the compounds inhibited [^{3}H]-N$^\alpha$-methylhistamine binding with Hill coefficients (n_H), not significantly different from unity. However, thioperamide gave a shallow slope ($n_H = -0.73 \pm 0.02$), and the data fitted significantly better to a two component competition model than with a sigmoidal competition curve ($P<0.05$). With this analysis, thioperamide discriminated between two binding sites, one of high affinity ($IC_{50\ 1} = 2.75 \pm 0.87$ nM) and one of low affinity ($IC_{50\ 2} = 101.6 \pm 12.0$ nM) in the ratio 24:76 ± 2% (high:low) ($n = 15$). This heterogeneity in binding shown by thioperamide was subsequently lost if the experiments were performed in 50 mM sodium/potassium phosphate buffer (Table 2).

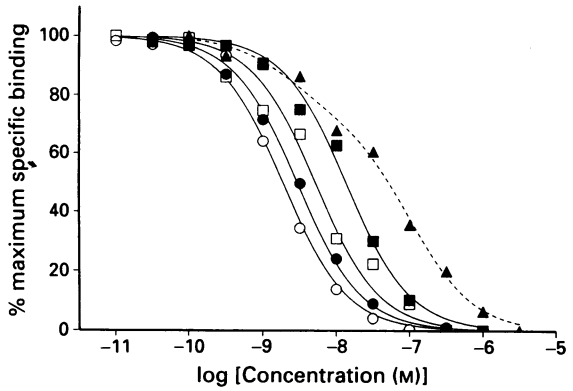

Figure 2 Competition binding with [^{3}H]-N$^\alpha$-methylhistamine in rat cerebral cortical membranes. Non-specific binding was determined in the presence of thioperamide 10 μM. Specific binding was defined as the difference between total and non-specific binding. Data are expressed as percentage maximum specific binding. Each curve represents the mean of three separate experiments and the s.e.mean was less than 5% in all cases: (○) clobenpropit; (●) N$^\alpha$-methylhistamine; (□) R$^\alpha$-methylhistamine; (■) SKF 91486b; (▲) thioperamide.

Table 1 Inhibition of [^{3}H]-N$^\alpha$-methylhistamine binding in rat cerebral cortex by various compounds acting at histamine receptors

	IC_{50} (nM)	K_i (nM)
Clobenpropit	1.6	1.1
N$^\alpha$-methylhistamine	4.8	3.2
R$^\alpha$-methylhistamine	7.6	5.1
SKF 91486 b	16	11
Histamine	20	14
Thioperamide*	65	48
Burimamide	340	230
Phenylbutanoylhistamine	750	500
Norburimamide	8,900	6,000
Betahistine	50,000	34,000
Mepyramine	42,000	28,000
Tiotidine	>100,000	>67,000

Each IC_{50} value is the average of between three and six separate experiments. K_i values were calculated using Cheng & Prussoff equation (1973). *$n_H = -0.73 \pm 0.02$, significantly different from unity ($P<0.05$).

Effects of sodium ions

Competition binding experiments were performed with three H_3-selective ligands, thioperamide, a second antagonist, clobenpropit and the agonist N$^\alpha$-methylhistamine, in the presence of 0, 50 and 100 mM NaCl. Specific binding of 1 nM [^{3}H]-N$^\alpha$-methylhistamine was decreased by 37.9 ± 4.4% and 47.9 ± 0.8% in the presence of 50 and 100 mM NaCl respectively ($n = 4$). Sodium ions had no effect on the binding characteristics of clobenpropit or N$^\alpha$-methylhistamine. However, with thioperamide (Figure 3), the inhibition curve was shifted to the left and the Hill coefficient was increased to unity i.e. towards the high affinity site. (Data are summarized in Table 2). In saturation studies, the presence of 100 mM NaCl produced a 31 ± 6% reduction in receptor density (i.e. B_{max} decreased from 86.0 ± 0.7 fmol mg^{-1} to 59.3 ± 4.8 fmol mg^{-1} protein) without significantly altering the apparent affinity for [^{3}H]-N$^\alpha$-methylhistamine ($K_d = 2.02 \pm 0.09$ nM and 2.15 ± 0.13 nM in the absence and presence of 100 mM NaCl respectively) ($n = 3$).

Effects of guanylnucleotides

We investigated the effects of guanosine triphosphate (GTP) and the two non-hydrolysable analogues guanosine 5′-*o*-(3-thiotriphosphate) (GTPγS) and 5′-guanylimidodiphosphate (GppNHp) on [^{3}H]-N$^\alpha$-methylhistamine binding. The order of potency for inhibition of binding was GTPγS IC_{50} = (0.2 ± 0.0 μM) > GppNHp (1.5 ± 0.1 μM) > GTP (39.2 ± 0.6 μM) ($n = 3$). All three compounds were able to displace only 58 ± 2% of specific binding (Figure 4). The presence of

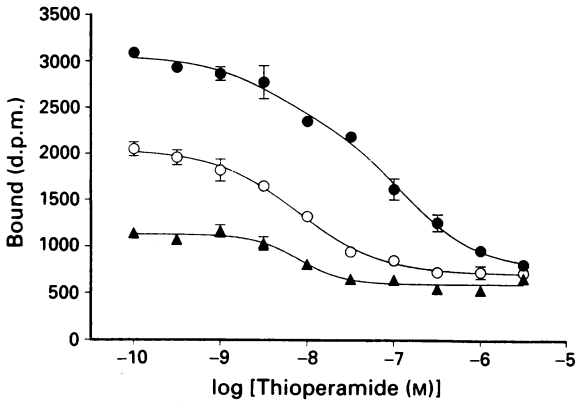

Figure 3 Competition binding curves for thioperamide were performed in the absence (●), and presence of 50 mM (○) and 100 mM (▲) NaCl. Non-specific binding was determined with thioperamide 10 μM. Values are the mean ± s.e.mean of three individual experiments.

Table 2 Comparison between the inhibition binding curves for thioperamide, clobenpropit and N$^\alpha$-methylhistamine in 50 mM Tris HCl ± sodium ions and 50 mM Na/K phosphate buffers

	Tris HCl (50 mM) + 0 mM *NaCl*	*Tris HCl* (50 mM) + 50 mM *NaCl*	*Tris HCl* (100 mM) + 100 mM *NaCl*	*Na/K phosphate* (50 mM)
N$^\alpha$-methylhistamine	6.2 ± 0.2	5.52 ± 0.02	7.7 ± 0.3	4.0 ± 0.6
Clobenpropit	1.3 ± 0.2	1.10 ± 0.10	1.5 ± 0.2	1.1 ± 0.2
Thioperamide	64.9 ± 4.5*	8.40 ± 0.70	7.1 ± 0.4	9.0 ± 0.4

IC_{50} values are expressed as mean ± s.e.mean of three individual experiments. *$n_H = -0.73 \pm 0.02$, significantly different from unity ($P<0.05$).

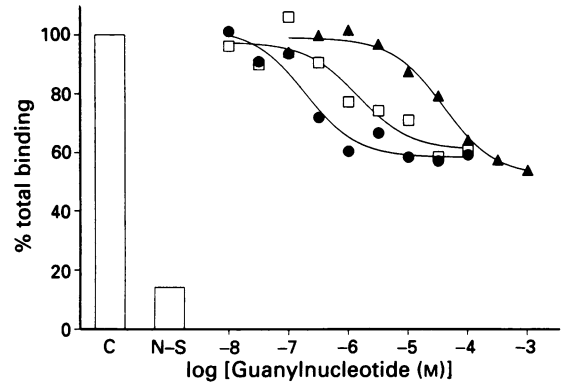

Figure 4 Inhibition of [^{3}H]-N$^\alpha$-methylhistamine binding by guanylnucleotides: (●) GTPγS; (□) GppNHp; (▲) GTP. Data are expressed as percentage total binding. Non-specific binding (15% total binding) was determined in the presence of thioperamide 10 μM. Values represent the mean of three separate experiments and s.e.mean was less than 5% in all cases.

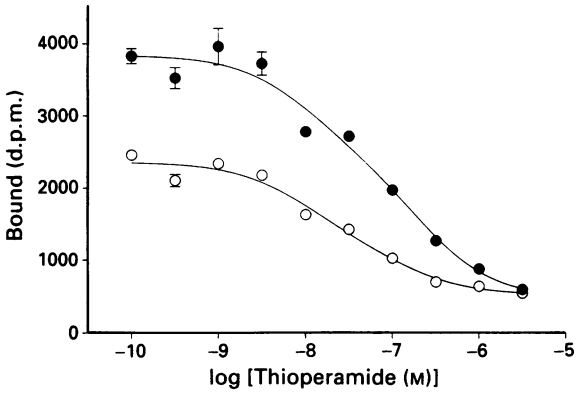

Figure 5 Inhibition of [^{3}H]-N$^\alpha$-methylhistamine binding by thioperamide in the absence (●) and presence (○) of GTPγS (3×10^{-5} M). Data shown are from a single experiment which is representative of three separate experiments. Data points are the mean ± s.e.mean of quadruplet determinations.

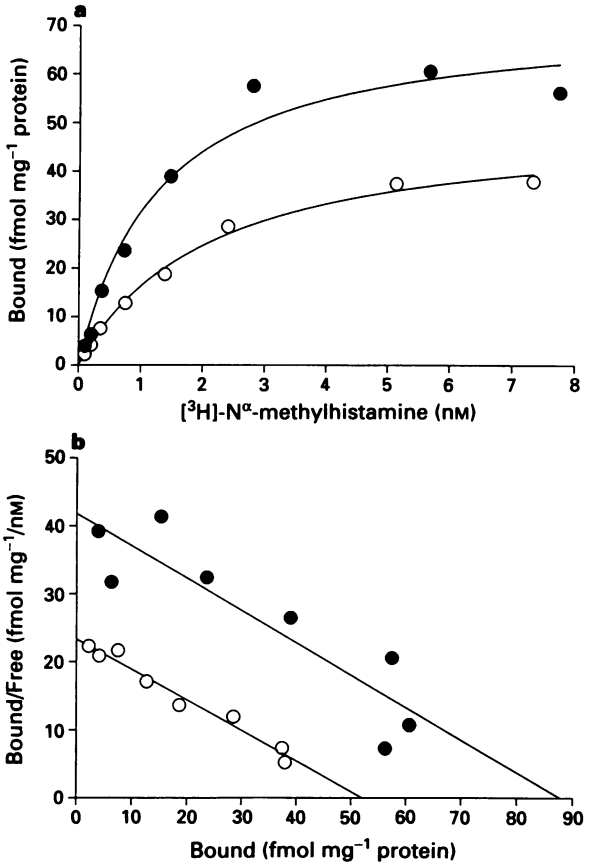

Figure 6 Saturation binding experiments in the absence (●) and presence (○) of GTPγS (3×10^{-5} M). (a) Specific binding, determined from the difference between total binding (in the absence of) and non-specific binding (in the presence of) thioperamide 10 μM. (b) Scatchard analysis of the specific binding shown in (a). Data shown represent one of two experiments.

GTPγS (3×10^{-5}M) produced a decrease in specific binding, an increase in Hill coefficient towards unity and a shift in IC_{50} towards the high affinity site of the thioperamide concentration-response curve (Figure 5), i.e. a single site with an $IC_{50} = 22.1 \pm 0.5$ nM was observed when GTPγS is present, compared with two sites ($IC_{50\ 1} = 1.98 \pm 0.68$ nM (25%); $IC_{50\ 2} = 104.7 \pm 35.5$ nM (75%) in the control ($n = 3$)). Also, saturation binding in the presence of GTPγS (3×10^{-5}M) produced a 44 ± 3% decrease in B_{max} with no change in K_d for [^{3}H]-N$^\alpha$-methylhistamine ($n = 3$), (Figure 6); these results imply that sodium ions and guanylnucleotides modulate thioperamide binding in the same way.

Discussion

High affinity specific binding of [^{3}H]-N$^\alpha$-methylhistamine to membranes of rat cerebral cortex was saturable and consistent with the labelling of a single class of histamine H_3-receptors. The apparent dissociation constants obtained for a range of non-radioactive H_3-receptor antagonists were consistent with those values obtained from antagonism of histamine H_3-receptor-mediated functional responses (Arrang *et al.*, 1983; 1985; 1987a,b; Van der Werf *et al.*, 1987; Van der Vliet *et al.*, 1988; Schlicker *et al.*, 1988; Hew *et al.*, 1990; Clapham & Kilpatrick, 1992; Taylor & Kilpatrick, 1992). With the exception of the H_3-antagonist thioperamide, the inhibition of [^{3}H]-N$^\alpha$-methylhistamine binding by all other compounds (both agonists and antagonists) was consistent with a simple mass action equilibrium with a single class of non-interacting H_3-receptor sites. In the case of thioperamide, however, the inhibition curve obtained had a Hill coefficient significantly less than unity and could be described by a two-component model comprising high affinity ($IC_{50} = 2.75$ nM) and low affinity ($IC_{50} = 101.6$ nM) sites in the ratio 24:76% (high:low). This heterogeneity in binding shown by thioperamide was subsequently lost if the experiments were performed in 50 mM sodium/potassium phosphate buffer. These observations are consistent with the observations of both West *et al.* (1990) and Arrang *et al.* (1990) who made measurements in either Tris buffer of sodium/potassium phosphate buffer respectively. These data suggest that the ionic composition of the incubation media may alter the conformation of the H_3-receptor.

In the present study we have evaluated the effect of sodium ions on the binding characteristics of thioperamide, the agonist N$^\alpha$-methylhistamine and a second high affinity H_3-antagonist, clobenpropit. Specific binding of 1nM [^{3}H]-N$^\alpha$-methylhistamine was decreased by 40–50% in Tris buffer containing 50 or 100 mM NaCl. Sodium ions had no effect on the binding characteristics of the clobenpropit or N$^\alpha$-methylhistamine. However, with thioperamide, the inhibition curve was shifted to the left and the Hill coefficient was increased to unity i.e. towards the high affinity site. The IC_{50} obtained for thioperamide in the presence of sodium ions was 8 nM. This would suggest that sodium ions can alter the conformation of the H_3-receptor in such a way that one binding site (for which thioperamide has lower affinity, but

which clobenpropit is unable to resolve because of its equal affinity for both conformations) is lost.

This selective effect of sodium ions on the binding of only certain antagonists has also been observed in studies of the binding of opioid ligands in NG108-15 cells (Costa *et al.*, 1992). In the NG108-15 cell line the binding of classical 'neutral' opiate receptor antagonists was not affected by sodium ions and was consistent with binding to a single population of high affinity sites (Costa *et al.*, 1992). In contrast, however, the binding of the putative 'negative' opiate receptor-antagonist, ICI 174864 (which via its proposed negative receptor efficacy is capable of reducing basal GTPase activity in the absence of any agonist occupancy of the receptor; Costa *et al.*, 1990), indicated the presence of two affinity states (Costa *et al.*, 1992). Furthermore, in the presence of sodium ions the proportion of low affinity sites was markedly reduced (Costa *et al.*, 1992). In the NG108-15 cell membranes, the most likely explanation for these effects is that there is spontaneous coupling of opiate receptors to G-proteins (leading to some degree of agonist-independent constitutive receptor activity) which can be prevented by the presence of sodium ions (Costa *et al.*, 1990; Lefkowitz *et al.*, 1993). If 'negative' antagonists such as ICI 174864 possess negative efficacy by virtue of a higher affinity for un-coupled relative to that for G-protein-coupled receptors, then the presence of sodium ions would tend to stabilize the binding of this type of antagonist into a single high affinity state. This is precisely what was observed with thioperamide at the H_3-receptor in the present study.

Evidence for the linkage of H_3-receptors to their effector systems via G-proteins has been provided by the observation in this and other studies (Arrang *et al.*, 1990; West *et al.*, 1990; Kilpatrick & Michel, 1991; Zweig *et al.*, 1992) that ^{3}H-agonist binding can be modulated by guanylnucleotides. West *et al.* (1990) reported a decrease in both B_{max} and K_d of [^{3}H]-N$^\alpha$-methylhistamine in the presence of GTPγS. In the present study we have observed that approximately 40% of [^{3}H]-N$^\alpha$-methylhistamine specific binding sites in rat cerebral cortical membranes were GTP-sensitive (seen by a reduction in B_{max} in the presence of GTPγS), suggesting that they are typically linked to a G-protein. In most G-protein-coupled receptor systems, the effect of added GTPγS is to shift the resting equilibrium between high affinity G-protein-coupled receptors and low affinity uncoupled receptors in favour of the low affinity sites. However, since we are monitoring high affinity ^{3}H-agonist binding over a fairly narrow concentration-range, this type of behaviour would be seen as a reduction in apparent B_{max} (i.e. the low affinity sites would no longer be detectable). It is clear in our study, however, that the remainder of the [^{3}H]-N$^\alpha$-methylhistamine binding sites were unaffected by GTP, yet still had high affinity for the ^{3}H-agonist ligand. Another feature of the studies performed in the presence of GTPγS is that the guanine nucleotide appeared to modulate the binding characteristics of thioperamide and [^{3}H]-N$^\alpha$-methylhistamine in a similar way to that produced by sodium ions. Thus, in the presence of GTPγS, the binding of thioperamide appeared to be to a homogeneous population of sites with an IC_{50} of 20 nM and the B_{max} for [^{3}H]-N$^\alpha$-methylhistamine was reduced by a similar amount to that produced by sodium ions.

The molecular nature of the high affinity GTP-insensitive ^{3}H-agonist H_3-receptor sites remains to be established, although some insight may be provided by the ternary complex model recently developed by Lefkowitz and colleagues (1993) to account for constitutive receptor activity. In this model, it is proposed that a receptor can exist (in terms of agonist affinity) in a low affinity state R or in a high affinity intermediate form R* which is the only form capable of interacting directly with the G-protein (and can do this even in an agonist-unoccupied state).

$$R \rightleftharpoons R^* \rightleftharpoons R^*G$$

Ligands with differential affinity for each conformation e.g. agonists, can then alter the proportions of each isomer at equilibrium. R* provides a possible explanation for high affinity H_3-agonist binding in the presence of guanine nucleotides if a significant proportion of the H_3-receptors present in rat cerebral cortex are able to isomerise spontaneously into the active conformation R*. This would lead to a higher affinity for H_3-agonists but a reduced signal to noise ratio owing to a higher inherent basal receptor activation (due to the potential for R* to couple to G-proteins to form R*G in the absence of agonist; Lefkowitz *et al.*, 1993). These properties are not, however, incompatible with those expected for an autoreceptor function. If this is the case, then a corollary of this putative mechanism is that sodium ions and guanine nucleotides prevent the formation of R*-G-protein complexes (R*G) and reduce the number of receptors in the R* conformation (i.e. convert a proportion of them to uncoupled low affinity R forms of the receptor). Furthermore, the data obtained with thioperamide would indicate that thioperamide has a higher affinity for the R* form compared to the G-protein-coupled R*G conformation which could convey a degree of 'negative' efficacy. It is assumed that any binding to R which has a low agonist affinity would not be detectable within the concentration range of [^{3}H]-N$^\alpha$-methylhistamine used in these studies.

There is no evidence available at the present time to indicate whether thioperamide is capable of expressing 'negative' efficacy or not. This would be largely dependent upon the extent to which H_3-receptors in different cells and tissues are constitutively active. Thioperamide has been observed to elicit a concentration-dependent increase in [^{3}H]-histamine release in rat cerebral cortical slices (Arrang *et al.*, 1983) but this could simply be due to antagonism of endogenously released histamine exerting a negative feedback effect on [^{3}H]-histamine release. Most information on constitutive activity and the evaluation of the potential for established receptor-antagonists (e.g. prazosin) to possess negative efficacy has come from mutagenesis studies of cloned receptors (Kjelsberg *et al.*, 1992; Lefkowitz *et al.*, 1993; Samama *et al.*, 1993a,b; Tian *et al.*, 1993; Chidiac *et al.*, 1993). Consequently, the resolution of this issue may have to await similar mutagenesis studies on the histamine H_3-receptor.

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