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Inhibition by cations of antagonist binding to histamine H_1-receptors: differential effect of sodium ions on the binding of two radioligands

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1 Measurements have been made of the inhibition by mono- and divalent cations of the binding of [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP) to histamine H_1-receptors in homogenates of guinea-pig cerebellum.

2 The binding of [^{3}H]-QMDP was inhibited by monovalent cations with an order of potency $Li^+ = Na^+ > K^+ > Cs^+ = Rb^+$. The IC_{50} for Li^+ was 39 mM, but that for K^+ was 132 mM. Hill coefficients for inhibition curves for Li^+ and Na^+ were < 1.

3 Divalent cations also inhibited the binding of [^{3}H]-QMDP. The most potent cations examined were Hg^{2+}, Cd^{2+} and Zn^{2+}, with IC_{50} values of 5, 17 and 41 μM, respectively. Ca^{2+} and Mg^{2+} were relatively weak inhibitors (IC_{50} 12 and 34 mM, respectively). The potency of Ni^{2+}, Co^{2+} and Mn^{2+} was intermediate between these groups. Hill coefficients for inhibition curves for Hg^{2+}, Cd^{2+} and Zn^{2+} were > 1, but Hill coefficients for the other cations were < 1.

4 Both mono- and divalent cations also inhibited the binding of [^{3}H]-mepyramine. The divalent cations were approximately equipotent in inhibiting the binding of [^{3}H]-QMDP and [^{3}H]-mepyramine. The same was true for Li^+. However, Na^+ was markedly more effective against [^{3}H]-QMDP binding than against the binding of [^{3}H]-mepyramine.

5 The effect of 40 mM Li^+ on the parameters of binding of [^{3}H]-mepyramine was to increase the best-fit value of the concentration giving half-maximal binding EC_{50}, by approximately 2 fold without having any significant effect on the maximum amount of binding. Cd^{2+} (15 μM) caused both an increase in EC_{50} and a decrease in B_{max} (32 ± 4% inhibition). Na^+, 100 mM, had no significant effect on either EC_{50} or B_{max} for [^{3}H]-mepyramine binding.

Keywords: Histamine H_1-receptors; [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine; [^{3}H]-mepyramine; mercury; cadmium; zinc; nickel; cobalt; sodium; lithium

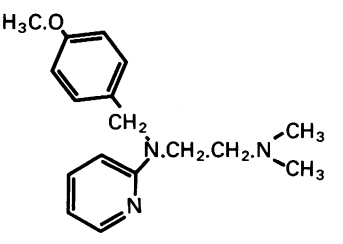
Introduction

The binding of histamine and other agonists to histamine H_1-receptors is inhibited by Li^+ and Na^+, and potentiated by Mn^{2+} and Mg^{2+} (Chang & Snyder, 1980; Toll & Snyder, 1982). In contrast, the binding of [^{3}H]-mepyramine, an antagonist, is reported to be relatively unaffected by these cations (Chang & Snyder, 1980). However, this does not appear to be a general rule for H_1-antagonists, since in the course of an investigation of the binding properties of a new quaternary radioligand for the H_1-receptor, [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP), we observed that the receptor binding of [^{3}H]-QMDP was reduced by 100 mM NaCl (Treherne & Young, 1988a). We describe here the results of a more extensive study of the effect of cations on the binding of [^{3}H]-QMDP and [^{3}H]-mepyramine. Both of these compounds have the 'classical' antihistamine structure (Figure 1), are a similar size and have a similar affinity for the H_1-receptor, K_d *circa* 1 nM (Treherne & Young, 1988a). We show that the binding of both [^{3}H]-QMDP and [^{3}H]-mepyramine is inhibited by a range of mono- and divalent cations, but that Na^+ distinguishes between the binding of the two radioligands. Some of these results have been presented in preliminary form to the British Pharmacological Society (Treherne *et al.*, 1989).

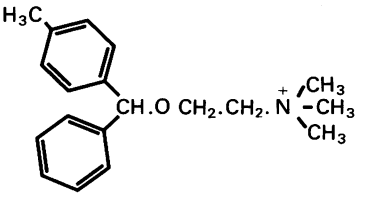
Methods

Preparation of cerebellar membranes

Cerebella from Dunkin-Hartley strain guinea-pigs (males, 250–600 g; Tucks, Battlebridge, Essex) were washed in 50 mM 2-amino-2-hydroxymethylpropan-1,3-diol hydrochloride (Tris-HCl) buffer, pH 7.5, containing 1 mM ethylenediaminetetracetic acid (EDTA), and then homogenized in 10 volumes of the same buffer in a Teflon-glass homogenizer with a motor-driven pestle (3,000 r.p.m., 5 or 6 up and down strokes). The homogenate was centrifuged at 17,000 *g* for 30 min and the pellet then resuspended in Tris buffer, but without EDTA, and centrifuged again at 17,000 *g* for 30 min. The resulting pellet was suspended in 50 mM Tris buffer, pH 7.5, to give a final protein concentration of 6–10 mg ml^{-1} and



Mepyramine



QMDP

Figure 1 Structures of mepyramine and (+)-N-methyl-4-methyldiphenhydramine (QMDP).

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was stored at −20°C until required for use. Protein was determined essentially as described by Lowry et al. (1951) with bovine serum albumin used as a standard.

Measurement of the binding of [^{3}H]-mepyramine and [^{3}H]-QMDP

Incubations in 50 mM Tris-HCl buffer, pH 7.5, contained [^{3}H]-mepyramine or [^{3}H]-QMDP (both usually in the concentration range 0.4–0.7 nM for inhibition curves), metal salt (where appropriate) and homogenate (0.19–0.27 mg protein) in a total volume of 1.00–1.11 ml (4–5 replicates at each inhibitor concentration, 8–16 replicates in the absence of metal ions, spread through the experiment). A set of incubations with 100 mM NaCl was included occasionally in experiments with other metal salts in order to check the consistency of the effects of ions. Non-H_1-receptor binding (non-specific binding) was determined as the binding insensitive to inhibition by 1 μM temelastine, which has a low affinity for secondary, non-H_1-receptor, binding sites for [^{3}H]-QMDP (Treherne & Young, 1988a). The dissociation constant for temelastine binding to the H_1-receptor in 50 mM Tris-buffer, determined in one experiment from inhibition of [^{3}H]-mepyramine binding, was 0.70 ± 0.16 nM, similar to the values 0.63 ± 0.04 and 0.83 ± 0.07 nM determined in 50 mM Na-K phosphate buffer from inhibition of the binding of [^{3}H]-mepyramine and [^{3}H]-QMDP, respectively (Treherne & Young, 1988a). The extent of the non-specific binding at 0.4–0.6 nM ^{3}H-ligand in 50 mM Tris-HCl buffer was usually 25–30% of total binding with [^{3}H]-QMDP and 9–16% with [^{3}H]-mepyramine. The temelastine-insensitive binding was always measured at each metal ion concentration. Equilibration was for 60 min at 30°C and was terminated by filtration through Whatman GF/B glass fibre paper, pre-soaked in 0.3% (w/v) polyethylenimine for 3–5 h, using a Brandel (Gaithersburg, Md, U.S.A.) cell harvester. The filters were washed with ice-cold buffer and then transferred to scintillation insert vials containing 4.8 ml scintillator and allowed to stand overnight before determination of tritium by liquid scintillation counting. The scintillator was Quickszint 212 (Zinnser)/water, 95:5 (v/v) or Emulsifier-Safe (Packard).

Analysis of data

To obtain unbiased estimates ± estimated s.e. of IC_{50}, the concentration of cation producing 50% inhibition of the temelastine-sensitive binding of [^{3}H]-QMDP, and n, the Hill coefficient, the temelastine-sensitive binding of the radioligand was assumed to follow a Hill equation (logistic equation), and the inhibition data were fitted to the equation: Percentage of uninhibited binding = $100/((M/IC_{50})^n + 1)$, where M is the concentration of cation. The fitting was carried out using the Harwell Library non-linear regression routine VB01A. All points were weighted according to the reciprocal of the variance associated with them.

In experiments in which the effect of metal cations on the parameters of [^{3}H]-mepyramine binding were measured, curves of the specific binding both in the presence and in the absence of a fixed concentration of the ion were always determined in the same experiment. The curves were first fitted to a Hill equation: $B = B_{max} \cdot C^n/(C^n + EC_{50}^n)$, where B is the amount of specific binding of [^{3}H]-mepyramine, B_{max} the maximum binding capacity, C the concentration of [^{3}H]-mepyramine, EC_{50}, the concentration of [^{3}H]-mepyramine giving half-maximal binding and n the Hill coefficient, in order to check that the Hill coefficients did not differ significantly from unity. The curves in the presence and absence of the metal ion were then fitted simultaneously to the Hill equation without any constraints and with n constrained to be the same for both curves (see Crawford & Young, 1990, for details), in order to test for any significant difference between the values of n in the presence and absence of the metal cation. One experiment in which there was a significant difference in the Hill coefficient was discarded (1 out of 17 experiments). Two further experiments in which n was closely similar for the two curves, but significantly less than unity, were also discarded, although the ratios of EC_{50} and B_{max} (both for $CdCl_2$) were in the range of the values given in Table 2. Best-fit values of EC_{50} and B_{max} for the remaining curves were then obtained by fitting each curve to an hyperbola: $B = B_{max} \cdot C/(C + EC_{50})$. In the absence of inhibitors, EC_{50} is equivalent to the dissociation constant, K_d.

Drugs

[^{3}H]-mepyramine (26 Ci mmol^{-1}) was obtained from Amersham International. [^{3}H]-QMDP was synthesized and purified as described previously (Treherne & Young, 1988a). Metal ion salts were all analar grade and all were chloride salts, except for zinc, which was the sulphate. Magnesium chloride was dried *in vacuo* before use. Temelastine was a kind gift from Smith, Kline & French Research Ltd.

Results

Inhibition of [^{3}H]-QMDP binding by monovalent cations

NaCl inhibited both the temelastine-insensitive (non-specific) and total binding of [^{3}H]-QMDP to cerebellar membranes, measured in 50 mM Tris-HCl buffer, but with a greater effect on the total binding (Figure 2), in accord with earlier observations of the effect on the binding of [^{3}H]-QMDP in Na-K phosphate buffer of the addition of further NaCl (Treherne & Young, 1988a). The greater effect of NaCl on the total binding indicates that the temelastine-sensitive component (H_1-receptor binding), the difference between the two curves, is also inhibited. A curve of the inhibition by NaCl of the H_1-receptor binding component, constructed from the combined data from 8 experiments, is shown in Figure 3.

The temelastine-sensitive binding of [^{3}H]-QMDP was also inhibited by other monovalent ions (Figure 3). LiCl was practically equipotent with NaCl, but KCl was much less effective, as was CsCl and (not shown) RbCl. Unbiased estimates of the values of IC_{50} and the Hill coefficient, obtained by fitting the curves assuming that the binding follows a Hill equation, are set out in Table 1. For both LiCl and NaCl the Hill coefficients were significantly less than unity. The difference in potency of the metal chlorides indicates that the inhibition is not just due to an increase in the ionic strength of the medium

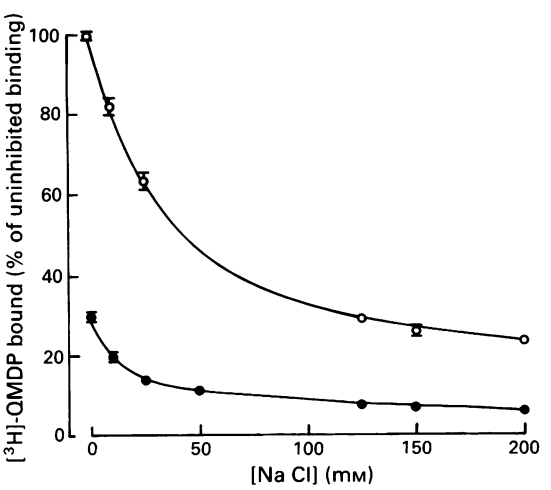

Figure 2 Inhibition by NaCl of the total and non-specific binding of 0.52 nM [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP). Each point is the mean of 5 replicate determinations from a single experiment; vertical bars show s.e.mean. The experiment was repeated 8 times with similar results. The lines through the points have been drawn by inspection. (○) Total binding of [^{3}H]-QMDP; (●) binding in the presence of 1 μM temelastine.

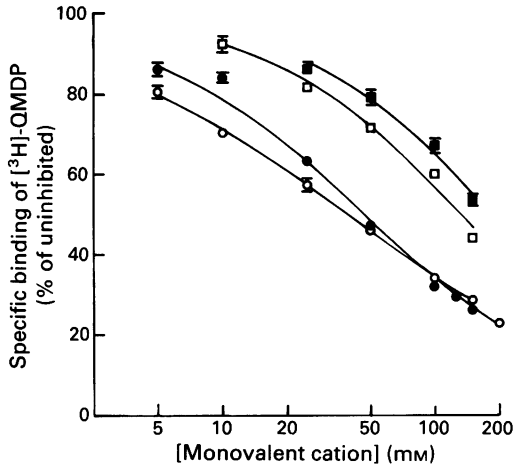

Figure 3 Inhibition of [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP) binding to the histamine H_1-receptor by monovalent cations. Points are the difference between the binding of 0.39–0.71 nM [^{3}H]-QMDP in the absence and presence of 1 μM temelastine and are the combined values (weighted means with s.e.mean shown by vertical bars) from 2 (CsCl), 4 (LiCl), 5 (KCl) or 8 (NaCl) experiments. The position of the inhibition curve for RbCl was similar to that for CsCl. The lines drawn were obtained by fitting the data to a Hill equation (see Methods). Best-fit values of IC_{50} are given in Table 1. (○) LiCl; (●) NaCl; (□) KCl; (■) CsCl.

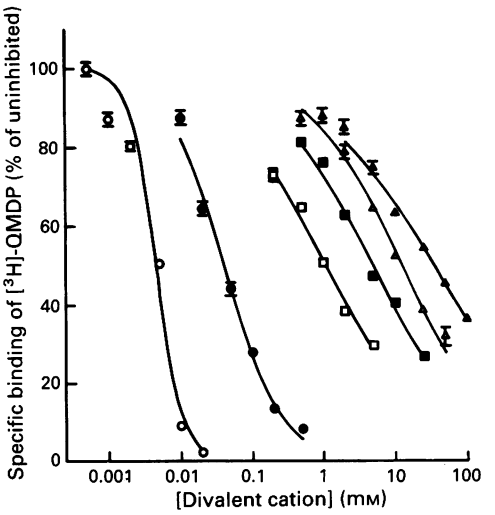

Figure 4 Inhibition of [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP) binding to the histamine H_1-receptor by divalent cations. Points are the difference between the binding of 0.37–0.70 nM [^{3}H]-QMDP in the absence and presence of 1 μM temelastine and are the combined values (weighted means with s.e.mean shown by vertical bars) from 4 ($MgCl_2$), 5 ($CaCl_2$) or 6 ($HgCl_2$, $MnCl_2$ $NiCl_2$, $ZnSO_4$) experiments. The lines drawn were obtained by fitting the data to a Hill equation (see Methods). Best-fit values of IC_{50} are given in Table 1. Curves for $CdCl_2$ and $CoCl_2$ have been omitted for clarity. The curve for $CdCl_2$ is shown in Figure 5. (○) $HgCl_2$; (●) $ZnSO_4$; (□) $NiCl_2$; (■) $MnCl_2$; (△) $CaCl_2$; (▲) $MgCl_2$.

and makes it probable that it is largely or wholly a property of the cation. Direct comparison in 3 experiments of the inhibitory effects of NaCl, Na_2SO_4 and $NaNO_3$, equimolar with respect to Na^+, failed to show any consistent difference between the different salts.

The temelastine-insensitive binding of [^{3}H]-QMDP was inhibited by Li^+, K^+, Cs^+ and Rb^+ in the same way as by Na^+ (cf. Figure 2) and with a similar potency. The initial drop in the non-specific binding may represent inhibition by the monovalent cations (IC_{50} 15–30 mM) of a medium-affinity, temelastine-insensitive binding site for [^{3}H]-QMDP (Treherne & Young, 1988a), since this component was not apparent in experiments with [^{3}H]-mepyramine.

Table 1 IC_{50} values and Hill coefficients for inhibition of [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP) binding by mono- and divalent cations

Inhibitor	IC_{50} (μM)	n_H
$HgCl_2$	5 ± 1	2.38 ± 0.43
$CdCl_2$	14 ± 3	0.96 ± 0.05
$ZnSO_4$	41 ± 3	1.09 ± 0.13
$NiCl_2$	1,100 ± 90	0.61 ± 0.05
$CoCl_2$	1,900 ± 200	0.54 ± 0.04
$MnCl_2$	5,000 ± 200	0.63 ± 0.04
$CaCl_2$	12,400 ± 600	0.67 ± 0.03
$MgCl_2$	33,800 ± 2,400	0.52 ± 0.04
LiCl	39,000 ± 800	0.68 ± 0.01
NaCl	46,900 ± 2,100	0.86 ± 0.02
KCl	132,000 ± 11,000	0.97 ± 0.10
CsCl	>150,000	
RbCl	>150,000	

Values of IC_{50}, the concentration of metal salt producing 50% inhibition of the binding of [^{3}H]-QMDP sensitive to 1 μM temelastine, and n_H, the Hill coefficient, ± estimated s.e., were obtained by fitting inhibition curves derived from the combined data from 3–6 experiments with each inhibitor (cf. Figures 3, 4 and 5) to a Hill equation (see Methods). The concentration of [^{3}H]-QMDP present was normally 0.4–0.6 nM.

Inhibition of [^{3}H]-QMDP binding by divalent cations

The conclusion that the inhibition of [^{3}H]-QMDP binding is largely due to the cation was strengthened by the wide variation in potency of a series of divalent ions (Figure 4). $HgCl_2$ was a highly potent inhibitor of the temelastine-sensitive binding of [^{3}H]-QMDP, with an IC_{50} of 5 μM, whereas $CaCl_2$ and $MgCl_2$ were much less potent, with IC_{50} values of 13 and 37 mM, respectively (Table 1). The order of potency was $Hg^{2+} > Cd^{2+} > Zn^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Ca^{2+} > Mg^{2+}$. All of the divalent cation salts examined were chlorides, except for Zn^{2+}, which was the sulphate. The difference in the slopes of the inhibition curves for the potent and weaker divalent cations, evident in Figure 4, is reflected in the best-fit values of the Hill coefficients (Table 2).

The temelastine-insensitive, non-specific, binding of [^{3}H]-QMDP was also inhibited by the divalent cations. As with the monovalent ions, there appeared to be an initial more rapid decline in the temelastine-insensitive binding, but the IC_{50} for this component, where the concentrations of the ions were high enough for it to be defined, varied from approximately 0.1 mM (Zn^{2+}) to approximately 3 mM (Mg^{2+}).

Comparison of the effects of ions on the binding of [^{3}H]-QMDP and [^{3}H]-mepyramine

The divalent ions were effective inhibitors of the binding of [^{3}H]-mepyramine as well as that of [^{3}H]-QMDP. The effect of Cd^{2+} and Ca^{2+} on the temelastine-sensitive binding of [^{3}H]-QMDP and [^{3}H]-mepyramine is shown in Figure 5. The other divalent ions (except Co^{2+}, which was not tested) were similarly practically equipotent against the two ^{3}H-ligands, the biggest difference being with Ni^{2+}, which was 2 fold more potent in inhibiting the binding of [^{3}H]-QMDP than that of [^{3}H]-mepyramine.

Of the monovalent cations, Li^+ was virtually equipotent in inhibiting the binding of [^{3}H]-QMDP and [^{3}H]-mepyramine (Figure 6a). However, Na^+, which was practically equipotent with Li^+ in inhibiting the binding of [^{3}H]-QMDP (Figure 3), was strikingly more effective in inhibiting the binding of [^{3}H]-QMDP than that of [^{3}H]-mepyramine (Figure 6b). The mean

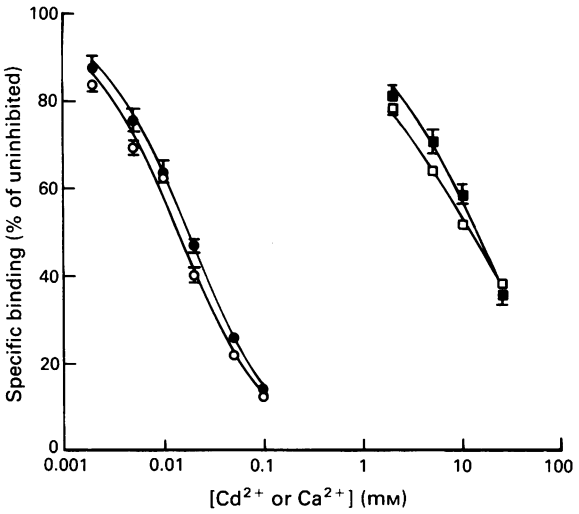

Figure 5 Comparison of the effect of Cd^{2+} and Ca^{2+} on the binding of [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP) and [^{3}H]-mepyramine to the histamine H_1-receptor. Points are the difference in the binding of 0.53–0.72 nM [^{3}H]-QMDP or 0.36–0.80 nM [^{3}H]-mepyramine in the absence and presence of 1 μM temelastine and are the combined values (weighted means with s.e.mean shown by vertical bars) from 4–5 experiments with [^{3}H]-QMDP (○, □) and 2–3 experiments with [^{3}H]-mepyramine (●, ■). The lines drawn were obtained by fitting the data to a Hill equation (see Methods). (○, ●) $CdCl_2$; (□, ■) $CaCl_2$.

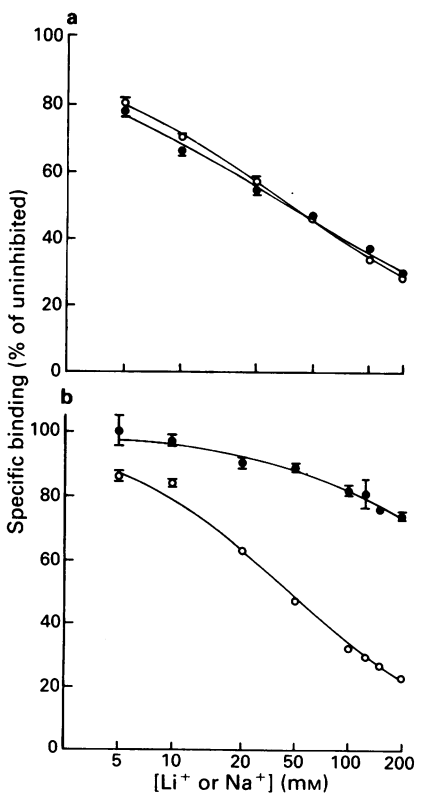

Figure 6 Comparison of the effect of LiCl (a) and NaCl (b) on the binding of [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP) and [^{3}H]-mepyramine to the histamine H_1-receptor. Points are the difference in the binding of the ^{3}H-ligand in the absence and presence of 1 μM temelastine. The lines drawn were obtained by fitting the data to a Hill equation (see Methods). (a) LiCl: combined data (weighted means with s.e.mean shown by vertical bars) from 4 experiments with 0.57–0.71 nM [^{3}H]-QMDP (○) and 3 experiments with 0.42–0.69 nM [^{3}H]-mepyramine (●). (b) NaCl: combined data (weighted mean with s.e.mean shown by vertical bars) from 8 experiments with 0.39–0.67 nM [^{3}H]-QMDP (○) and 4 experiments with 0.34–0.69 nM [^{3}H]-mepyramine (●).

effect of 100 mM Na^+ was 69 ± 1% inhibition of the binding of [^{3}H]-QMDP (25 measurements) and 13 ± 2% inhibition of the binding of [^{3}H]-mepyramine (13). K^+, a weak inhibitor of [^{3}H]-QMDP binding (Figure 3), was slightly less effective against [^{3}H]-mepyramine, but the difference was comparatively small (28 ± 1 (5 measurements) and 19 ± 1% (3) inhibition by 50 mM K^+ of [^{3}H]-QMDP and [^{3}H]-mepyramine binding, respectively, and 40 ± 1 (4) and 35 ± 3% (3) inhibition by 100 mM K^+).

The selective action of Na^+ appears to be related to changes in [^{3}H]-QMDP or [^{3}H]-mepyramine binding, rather than to an effect on the binding of 1 μM temelastine, which was used to define H_1-receptor binding. In two experiments a direct comparison was made of the effect of 100 mM Li^+ and 100 mM Na^+ on the level of the temelastine-insensitive binding of both [^{3}H]-QMDP and [^{3}H]-mepyramine. In both experiments the levels of binding were similar for a given radioligand, consistent with the lack of any selective effect of Na^+ on temelastine binding. This is in accord with the similar dissociation constants determined for temelastine in 50 mM Tris and 50 mM Na-K phosphate buffers (see Methods).

Effect of Cd^{2+}, Li^+ and Na^+ on the parameters of [^{3}H]-mepyramine binding

As a first step towards investigating the mechanism of the inhibitory action of the cations, measurements were made of the effect on the parameters of [^{3}H]-mepyramine binding of Cd^{2+}, as a representative potent divalent cation, Li^+, which does not distinguish [^{3}H]-QMDP and [^{3}H]-mepyramine, and Na^+, which gives only a very modest inhibition of the binding of [^{3}H]-mepyramine. The concentrations of Cd^{2+} and Li^+ chosen, 15 μM and 40 mM, were those which gave approximately 50% inhibition in the inhibition experiments (Figures 5 and 6a). The effect of Cd^{2+} was to cause an approximately two fold increase in the EC_{50} for [^{3}H]-mepyramine binding, together with a statistically significant decrease in B_{max}, the maximum binding (Table 2). In 4 out of 5 experiments with Li^+ there was a small numerical decrease in B_{max}, but overall the reduction was not statistically significant, whereas the EC_{50} was increased by a factor of 2.3. Na^+, 100 mM, had no significant effect on either EC_{50} or B_{max} for [^{3}H]-mepyramine binding (Table 2), in agreement with the observation of Chang & Snyder (1980).

The EC_{50} for [^{3}H]-mepyramine binding in the absence of added ions, equivalent to the dissociation constant, K_d, was 0.72 ± 0.09 nM (Table 2). A similar value, 0.59 ± 0.02 nM was obtained in a single experiment in which the inhibition of the binding of 0.55 nM [^{3}H]-mepyramine by mepyramine was

Table 2 Effect of $CdCl_2$, LiCl and NaCl on the parameters of [^{3}H]-mepyramine binding

	Change in parameter (% of value in the absence of metal ion)	
Metal salt	EC_{50}	B_{max}
$CdCl_2$ 15 μM	189 ± 23 (5)*	68 ± 4 (5)*
LiCl 40 mM	230 ± 25 (5)*	91 ± 4 (5)
NaCl 100 mM	145 ± 29 (4)	108 ± 6 (4)

The binding of [^{3}H]-mepyramine sensitive to 1 μM temelastine was measured in the presence and in the absence of the metal ion in each experiment. The best-fit values of EC_{50} and B_{max} for each curve were obtained as described under Methods. Values are the mean ± s.e.mean of the percentages from the number of individual experiments shown in parentheses. The mean value of EC_{50} for [^{3}H]-mepyramine in the absence of metal salt, equivalent to the dissociation constant, K_d, was 0.72 ± 0.09 nM (14).

* Significantly different from 100 ($P < 0.05$), assuming that the means of the ratios from different experiments are normally distributed.

measured. Both of these values are similar to the mean value for K_d, 0.63 nM, obtained from several studies in which measurements were made in 50 mM Na-K phosphate buffer (37.8 mM Na_2HPO_4, 12.2 mM KH_2PO_4) (Aceves *et al.*, 1985), consistent with the lack of any marked effect of Na^+ on [^{3}H]-mepyramine binding. The mean value for K_d for [^{3}H]-QMDP determined from binding curves in 50 mM Tris-HCl buffer (in the absence of metal ions) was 0.55 ± 0.11 nM (6 measurements). This is less than the value, 0.88 ± 0.02 nM, measured in 50 mM Na-K phosphate buffer (Treherne & Young, 1988a), although it should be noted that the cerebellar homogenate in those experiments had not been washed in EDTA.

Discussion

The two main findings of this study are (i) that the binding of antagonists to the histamine H_1-receptor is inhibited by cations and (ii) that sodium ions distinguish between the binding of [^{3}H]-QMDP and [^{3}H]-mepyramine. The relatively weak effect of Na^+ on [^{3}H]-mepyramine binding and the use of a single, 1 mM, concentration of Ca^{2+} and Mg^{2+} are probably the main reasons why the effect of cations was missed in the earlier study (Chang & Snyder, 1980), although the small inhibitory effect reported with 5 and 10 mM Mn^{2+} contrasts with our observation (Table 1) that the IC_{50} for Mn^{2+} is *circa* 5 mM and is similar for [^{3}H]-QMDP and [^{3}H]-mepyramine. However, there were some methodological differences, in that the earlier study used membrane preparations from guinea-pig whole brain, rather than from cerebellum, and there was no wash with EDTA. No measurements were made in the earlier paper of the most striking group of inhibitors namely the divalent cations from group IIB; Zn^{2+}, Cd^{2+} and Hg^{2+}.

The high potency of the divalent cations from group IIB raises the question of whether the mechanism of action is the same as for the much weaker divalent cations of group IIA, Mg^{2+} and Ca^{2+}. It is notable that the slope of the inhibition curves for the group IIB cations and those from the other divalent cations appear to fall into two distinct groups, with the Hill coefficients for Zn^{2+}, Cd^{2+} and Hg^{2+} being $\geqslant 1$ and the Hill coefficients for the others being < 1. The notable chemical property of the group IIB cations, especially Hg^{2+}, is the ready formation of complexes with nitrogen and sulphur electron donors. Thiol groups are one obvious target, but the thiol group reagent N-ethylmaleimide, which shifts the IC_{50} for curves of histamine inhibition of [^{3}H]-mepyramine binding to lower values and changes the Hill coefficient, has been reported not to have an effect on the binding of [^{3}H]-mepyramine itself (Yeramian *et al.*, 1985). Interestingly, two organic mercurials, *p*-chloromercuriphenylsulphonic acid (1 mM) and mersalyl (0.1 mM), examined in that study did cause a marked inhibition of [^{3}H]-mepyramine binding (Yeramian *et al.*, 1985). We have not yet investigated whether thiol group reagents alter the inhibitory action of the group IIB cations, but in an early experiment, carried out with a membrane preparation which had not been washed with EDTA, pretreatment with 1 mM N-ethylmaleimide had no significant effect on either the EC_{50} or B_{max} for [^{3}H]-QMDP binding to cerebellar membranes, measured in 50 mM Tris buffer. Similarly, 1,4-dithiothreitol, the disulphide bond reducing agent, which changes curves of histamine inhibition of [^{3}H]-mepyramine binding to homogenates of guinea-pig cerebellum in the same way as N-ethylmaleimide, but which again has no effect on the parameters of [^{3}H]-mepyramine binding (Donaldson & Hill, 1986) caused only a 10% inhibition of [^{3}H]-QMDP binding at a concentration of 1 mM and did not distinguish between the binding of [^{3}H]-QMDP and [^{3}H]-mepyramine (Treherne & Young, unpublished observations).

The inhibitory action of less potent cations, such as Ca^{2+}, Mg^{2+} or Li^+, seems much less likely to involve complex formation with thiol or amino functions, but any proposed mechanism has to encompass both this inhibition, which is not radioligand-selective, with the selective action of Na^+ in distinguishing between the binding of [^{3}H]-QMDP and [^{3}H]-mepyramine. Modulation of antagonist binding by Na^+ has not been reported previously for the H_1-receptor, but it is well established for other receptors. Selective actions of Na^+ on both agonist and antagonist binding, with different antagonists being affected to differing extents, was reported in the original study on opiate binding by Pert & Snyder (1974) and have been confirmed in more recent investigations (Paterson *et al.*, 1986; Kosterlitz *et al.*, 1987; Nijssen & Childers, 1987). Similarly Na^+ affects the binding of different antagonists to the dopamine D_2 receptor to differing degrees (Jarvie *et al.*, 1987; Urwyler, 1987) and there is an interesting analogy with the present results in that certain permanently uncharged analogues of dopamine, which have no D_2-agonist action, inhibit the binding of [^{3}H]-spiperone to D_2-receptors, but this inhibition is much reduced in the presence of 125 mM NaCl (Wallace *et al.*, 1988).

The usual explanation for the effect of Na^+ is that it induces a conformational change in the receptor such that the proportion in a state which has a high affinity for agonists is reduced. However, the connection, if any, between ion effects on agonist and on antagonist binding at the H_1-receptor remains to be established. Both Na^+ and Li^+ reduce H_1-agonist inhibition of [^{3}H]-mepyramine binding (Chang & Snyder, 1980; Toll & Snyder, 1982), but only Na^+ distinguishes between the binding of [^{3}H]-QMDP and [^{3}H]-mepyramine. However, a Na^+-induced conformational change is most likely to be the basis of the differential effect on the binding of [^{3}H]-QMDP and [^{3}H]-mepyramine. If Na^+ were acting directly at the antagonist binding site to inhibit [^{3}H]-QMDP binding, then it would be difficult to understand why it should be relatively ineffective in inhibiting the binding of [^{3}H]-mepyramine. There is also some indirect evidence that the site at which Na^+ acts is not accessible from the extracellular medium. The K_d for QMDP inhibition of histamine-induced contraction of longitudinal muscle strips from guinea-pig small intestine suspended in Krebs-Henseleit medium (containing 143 mM Na^+) is 0.56 nM (Treherne & Young, 1988a) and is closely similar to the value, 0.55 nM, determined from the binding of [^{3}H]-QMDP in 50 mM Tris buffer (see Results).

Considerations of the site of action of Na^+ and the other cations are complicated by the fact that the histamine H_1-receptor belongs to the family of G-protein-coupled receptors (Claro *et al.*, 1989; Hill, 1990), consistent with the effect of GTP on histamine inhibition of [^{3}H]-mepyramine binding (Chang & Snyder, 1980). Ions are known to influence receptor-G-protein interactions and the complex effects of Mg^{2+} in particular are well documented (Gilman, 1987). Na^+ can also modulate receptor-G-protein interaction, since the reduction of GTPase activity in NG108-15 cell membranes by an opiate antagonist (but not all antagonists) can be regulated by Na^+ (Costa *et al.*, 1990). However, Na^+ modulation of [^{3}H]-SCH 23390 binding to dopamine D_1-receptors in rat striatal membranes does not require a functional coupled G-protein (Urwyler, 1989), implying a direct action on the receptor macromolecule. The same could well be true for the H_1-receptor, since the effects of Na^+, Mn^{2+} and Mg^{2+} on the parameters of H_1-agonist inhibition of [^{3}H]-doxepin binding in guinea-pig brain membranes are retained in digitonin-solubilized preparations, whereas the effect of GTP on histamine binding is lost (Toll & Snyder, 1982).

Whether Li^+ or Ca^{2+} or Mg^{2+} act at the same site as Na^+ is not clear, although if they did it would mean that they caused a change to a different conformation which bound neither [^{3}H]-QMDP nor [^{3}H]-mepyramine. The evidence available does not support any simple mechanism of action. The difference in potency among mono- or divalent cation chlorides argues against any effect due to an increase in the ionic strength of the medium or to a change in membrane surface charge, which appears to be the basis of the effects of ions on binding to muscarinic receptors (Birdsall *et al.*, 1979)

and κ-opioid receptors (Sargent et al., 1988). A simple competitive action of Li^+ at the antagonist binding site is consistent with the decrease in the EC_{50} for [^{3}H]-mepyramine binding in the presence of Li^+ (Table 2), and the lack of a significant effect on B_{max} (Table 2). However, it is not consistent with a Hill coefficient significantly less than unity (Table 1). Conversely, the Hill coefficient for the Cd^{2+} inhibition curve is near unity, but the decrease in B_{max} is inconsistent with a competitive mechanism of action. This difference in the effects of two cations gives some support to the proposition that the sites or mechanisms of action may differ. The decrease in B_{max} caused by Cd^{2+} is also inconsistent with a simple allosteric action to stabilize a changed conformation of the receptor, unless the conformational change is very slow. We have not investigated the kinetics of the action of the cations at the H_1 receptor, but it may be noted that the Na^+ induced increase in [^{3}H]-naloxone binding to rat brain membranes at 25°C requires 30–40 min to reach maximum effect (Nijssen & Childers, 1987). In earlier studies we failed to detect any slow conformational changes induced by the binding of either [^{3}H]-mepyramine (Wallace & Young, 1983) or [^{3}H]-QMDP (Treherne & Young, 1988b).

The accessibility or otherwise from the extracellular medium of the site for the cations other than Na^+ is also uncertain. The IC_{50} for Li^+ inhibition of histamine-induced contraction of guinea-pig intestinal muscle strips, measured in the presence of approximately 140 mM Na^+, is 44 mM (Hori et al., 1989), which is close to the IC_{50} for inhibition of [^{3}H]-QMDP binding, 39 mM (Table 1). However, the IC_{50} values for Li^+ against carbachol- and K^+-induced contraction of the muscle strips were also in the range of 40–45 mM and the degree of inhibition correlated well with the concentration of intracellularly accumulated Li^+, which appeared to have direct effects on the contractile proteins (Hori et al., 1989). Clearly, evaluation of the inhibitory effects of ions on tissue responses remote from receptor activation presents difficulties, particularly with the divalent cations such as Ni^{2+} which are also channel blockers.

In assessing the data on ion effects presented above it must be borne in mind that all of our measurements have been made in 50 mM Tris-HCl buffer. We have used this buffer in order to be able to relate our results to those from the earlier studies on the action of cations at the H_1-receptor, but the Tris cation can itself have effects on antagonist binding. This is illustrated by a study of the effect of cations on the binding of [^{3}H]-nitrendipine, a dihydropyridine L-type calcium channel antagonist, in which 50 mM Tris was shown to obscure the effect of 100 mM Na^+ (Bolger & Skolnick, 1986). This example is given some relevance by the observation that the IC_{50} values for inhibition of the binding of another dihydropyridine derivative, [^{3}H]-(+)-PN 200-110, to portal vein membranes by Cd^{2+}, Ni^{2+} and Co^{2+} (Dacquet et al., 1989) are similar to those for inhibition of [^{3}H]-QMDP binding. However, although there are some interesting analogies between binding to L-type Ca^{2+} channels and to H_1-receptors, the two seem to be clearly distinguished by La^{3+}, which is a potent inhibitor of [^{3}H]-(+)-PN 200-110 binding (IC_{50} 10 μM), but which in preliminary measurements we have found to be much less effective against [^{3}H]-QMDP binding (IC_{50} *circa* 1 mM). Dihydropyridines themselves are weak inhibitors of [^{3}H]-QMDP binding (Treherne & Young, 1988a).

The structural features which are important in distinguishing the binding of [^{3}H]-QMDP and [^{3}H]-mepyramine in the presence of Na^+ remain undefined . However, the most notable difference between the two molecules is that [^{3}H]-QMDP is a quaternary amine and therefore permanently positively charged, whereas mepyramine is a tertiary amine, although largely charged at physiological pH. The quaternary derivative of histamine, N^α,N^α,N^α-trimethylhistamine, is a very weak agonist, but N^α,N^α-dimethylhistamine is an effective H_1-receptor agonist and the suggestion has been made that the important difference is that at physiological pH the tertiary amine possesses a transferable proton (Ganellin, 1982). How relevant this analogy is to apparent differences in antagonist binding will be more apparent when a range of H_1-antagonist structures have been screened.

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