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HISTAMINE H_1-AGONIST POTENTIATION OF ADENOSINE-STIMULATED CYCLIC AMP ACCUMULATION IN SLICES OF GUINEA-PIG CEREBRAL CORTEX: COMPARISON OF RESPONSE AND BINDING PARAMETERS

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1 A range of histamine analogues have been examined as potentiators of the adenosine-stimulated accumulation of cyclic adenosine 3',5'-monophosphate (cyclic AMP) in slices of guinea-pig cerebral cortex. Dose-response curves were constructed for the 6 most active compounds and characterized in terms of the IC_{50}, the slope and the maximum response attainable relative to that of histamine.

2 Histamine, 2-thiazolylethylamine and N^{α}-methylhistamine produced a maximal or near maximal response. N^{α},N^{α}-dimethylhistamine and 2-methylhistamine appear to be partial agonists.

3 The response to all the agonists was practically abolished by mepyramine 1 μM, indicating that the response is mediated largely or wholly via histamine H_1-receptors.

4 The relative potencies of the agonists on cyclic AMP accumulation were in general similar to relative potencies in causing contraction of intestinal smooth muscle. The biggest difference was observed with N^{α}-methylhistamine.

5 The histamine analogues were also examined as inhibitors of [^{3}H]-mepyramine binding in homogenates of guinea-pig cerebral cortex. The inhibition curves were characterized in terms of IC_{50}, the slope and the maximum percentage inhibition. This last value was compared with the inhibition produced by promethazine 2 μM.

6 For the 6 most potent agonists, the EC_{50} for cyclic AMP accumulation was compared with the IC_{50} against [^{3}H]-mepyramine binding, corrected for inhibition of non-receptor binding and for competition with [^{3}H]-mepyramine. With the possible exception of 2-pyridylethylamine, the values did not differ by more than a factor of 3.

Introduction

There is now strong evidence for the presence of histamine H_1-receptors in mammalian brain (for reviews see Schwartz, 1979; Schwartz, Pollard & Quach, 1980). The identification, binding characteristics and localization of these receptors have been inferred in part from binding studies with ^{3}H-labelled antagonists, especially [^{3}H]-mepyramine (Hill, Emson & Young, 1978; Tran, Chang & Snyder, 1978; Palacios, Wamsley & Kuhar, 1981). Antagonist affinities derived from inhibition of [^{3}H]-mepyramine binding compare closely with those measured on peripheral smooth muscle and in two studies more direct comparison has been made between affinities from binding and from antagonism of an H_1-mediated functional response in slices from the same brain region (Quach, Duchemin, Rose & Schwartz, 1980; Hill, Daum & Young, 1981). In contrast, the only attempt to correlate quantitatively the properties of H_1-agonist binding in brain, as mirrored by the inhibition of [^{3}H]-mepyramine binding, with parameters of agonist function appears to be that of Kandel and coworkers (Kandel, Steinberg, Wells, Kandel & Gornall, 1980). However, the functional responses selected by these workers were measured on peripheral preparations and there is evidence that the relative potency of H_1-agonists can vary between tissues, e.g. between intestinal (Durant, Ganellin & Parsons, 1975) and airway (Duncan, Brink, Adolphson & Douglas, 1980) smooth muscle. Ideally agonist binding and function should be measured on the same tissue. The difficulty in guinea-pig brain has been the lack, until recently, of a selective H_1 functional response, but in our experience the potentiation by histamine of the adenosine-stimulated accumulation of cyclic adenosine 3',5'-monophosphate (cyclic AMP) in slices of guinea-pig cerebral cortex is eminently suitable for this type of quantitative study (Hill *et al.*, 1981).

Effects of histamine on cyclic AMP levels in tissue

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slices are mediated both by H_1- and H_2-receptors. Whereas the H_2-action seems to occur via receptors directly coupled to the cyclase (Hegstrand, Kanof & Greengard, 1976; Green, Johnson, Weinstein & Maayani, 1977), there is evidence that the H_1-action (reviewed in Daly, 1977) is indirect and depends on the presence of a directly acting agonist, such as adenosine or indeed histamine (H_2) itself (Palacios, Garbarg, Barbin & Schwartz, 1978; Daly, Padgett, Nimitkitpaisan, Creveling, Cantacuzene & Kirk, 1980). However, in slices of guinea-pig cerebral cortex there appears to be little or no H_2-component (Chasin, Mamrak, Samaniego & Hess, 1973; Hill *et al.*, 1981; but compare Baudry, Matres & Schwartz, 1975; Dismukes, Rogers & Daly, 1976; Daly *et al.*, 1980) and the response to histamine added alone seems to depend on the presence of endogenous adenosine, since it is abolished by adenosine deaminase treatment (Schwabe, Ohga & Daly, 1978; Hill *et al.*, 1981).

In this paper we describe a characterization of the action of agonists as potentiators of the adenosine-stimulated accumulation of cyclic AMP and a comparison with their potencies as inhibitors of [^{3}H]-mepyramine binding.

Methods

Preparation of cortical slices

Hartley-strain guinea-pigs of either sex (300–500 g) were killed by cervical dislocation and decapitation and the cerebral cortex quickly dissected out on ice. Slices (300 × 300 μm) were cross-cut with a McIlwain tissue chopper and incubated at 37°C in Krebs-Henseleit medium (75 ml per g of tissue) constantly gassed with O_2:CO_2 (95:5) in a shaking water bath. After 10 min the Krebs medium was decanted and replaced with one containing adenine 4 μM and the incubation continued for a further 20 min. At the end of this preliminary incubation, slices were washed with fresh Krebs medium, then suspended in Krebs medium at a concentration of 100 mg, wet weight, per ml.

Drug treatment of slices and assay of cyclic AMP

Aliquots (50 μl, 5 mg wet weight) of cortical slice suspension were added to 240 μl of Krebs medium, or Krebs medium containing antagonist drug, in 1.5 ml microfuge tubes (Hughes & Hughes). The tubes were gassed with O_2:CO_2 (95:5), capped and incubated for 20 min at 37°C in a shaking water bath. Agonists were added after this step in 10 μl of medium, the tubes were gassed again with O_2:CO_2 (95:5) and the incubation continued for a further 10 min. Tissue cyclic AMP was released by heating the samples on a boiling water bath for 10 min and the tissue debris then removed by centrifugation at approx. 8,700 *g* for 2 min in a Beckman microfuge B. Duplicate 20 μl samples were taken for cyclic AMP determination by a sensitive protein binding assay (Brown, Ekins & Albano, 1972). The tissue pellets were solubilized by heating in 1 ml of 1 M NaOH, and the protein concentration determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Measurement of inhibition of [^{3}H]-mepyramine binding

Cerebral cortices from 2–6 guinea-pigs (Hartley strain) were homogenized in 5 volumes of 50 mM Na-K phosphate buffer, pH 7.4, with a motor driven (Tri-R Stir, setting 4) teflon pestle and glass homogenizer and then centrifuged at 18,000 *g* for 15 min. The pellet was washed by resuspension in 5 mM Na-K phosphate buffer, pH 7.4 and recentrifugation at 18,000 *g* for 15 min. The pellet was finally resuspended in 5 mM phosphate buffer (4 ml per guinea-pig used), divided into smaller samples and stored frozen at −20°C until required for use.

Binding assays were carried out in modified Krebs-phosphate buffer, pH 7.2 (containing (mM): NaCl 133, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, Na_2HPO_4 5 and glucose, 5.5) or Krebs-HEPES buffer, pH 7.4 (containing (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, HEPES 25 and glucose 5.5). Each tube contained [^{3}H]-mepyramine 1 nM, 40 μl homogenate (usually 0.5–0.6 mg protein), inhibitor where appropriate and buffer to a final volume of 1 ml. Incubations, 37°C for 12 min, were terminated by addition of 4 ml ice-cold buffer containing mepyramine 1 μM and filtered immediately through a Whatman GF/B filter under vacuum (c. 300 mmHg). The filters were washed twice with 4 ml ice-cold buffer containing mepyramine 1 μM and transferred to scintillation vials. Scintillator (toluene:ethoxyethanol:butyl PBD, 67:33:0.6 v/v/w) was added and the vials shaken vigorously to break up the filters. Tritium was determined by liquid scintillation counting and ct/min converted into d/min by the channels ratio method using an external radioactive source.

The use of 1 μM mepyramine in the diluting buffer and wash solution, a procedure which significantly reduces the non-specific binding of [^{3}H]-mepyramine to glass fibre filters, is based on the observation (Wallace & Young, 1981) that at low temperatures the dissociation of mepyramine from the histamine H_1-receptor is very slow. The rate is not increased in the presence of 1 μM mepyramine. Hence the displacement of receptor-bound [^{3}H]-mepyramine by non-radioactive mepyramine will be negligible in the few seconds required for filtration and washing.

Analysis of data

Concentration-response curves for agonist stimulation of cyclic AMP accumulation were either drawn by inspection or, where the data were sufficient, fitted to a Hill equation using the Harwell Library non-linear regression programme VBO1A. The actual equation fitted was:

$$\text{Stimulation of cyclic AMP production} = \frac{ST_{max} \cdot D^n}{D^n + (EC_{50})^n}$$

where D is the agonist concentration, n is the Hill coefficient, EC_{50} is the concentration of agonist giving half-maximal stimulation and ST_{max} is the maximal stimulation. Each point was weighted according to the reciprocal of the variance associated with it. Repeated trials were made with different initial parameter estimates and the final best-fit values defined as those that were associated with the lowest residual. A Hill equation was chosen solely as a convenient way of testing whether the curves differed significantly from simple rectangular hyperbolae ($n = 1$).

Curves of inhibition of [^{3}H]-mepyramine binding were analysed similarly by fitting the equation:

$$\text{\% of uninhibited binding of [}^3\text{H]-mepyramine} = \frac{100-NS}{(A^n/IC_{50}{}^n + 1)} + NS$$

where A is the concentration of inhibitor and NS is the percentage of inhibitor-insensitive binding. Assuming a simple competition between the inhibitor and [^{3}H]-mepyramine, the IC_{50} will be shifted from the concentration it would have occupied in the absence of the ^{3}H-ligand by a factor ($M \cdot K_{mep} + 1$), where M is the concentration of [^{3}H]-mepyramine and K_{mep} its affinity constant. K_{mep} was taken to be $1.5 \times 10^9\ M^{-1}$, the mean of measurements on inhibition of cyclic AMP accumulation and inhibition of [^{3}H]-mepyramine binding (Hill *et al.*, 1981).

Where the data were insufficient for analysis of inhibition curves in this way, the IC_{50} was measured as the concentration of inhibitor causing 50% inhibition of the binding of [^{3}H]-mepyramine sensitive to 2 μM promethazine.

Drugs

[^{3}H]-mepyramine (specific activity 24.1 Ci/mmol) was purchased from the Radiochemical Centre, Amersham.

Adenine, adenosine and HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid) were obtained from Sigma, mepyramine maleate and promethazine hydrochloride from May & Baker and histamine dihydrochloride from BDH. Gifts of dimaprit, 2-methylhistamine, 4-methylhistamine, N^{α}-methylhistamine (4-(2-methylaminoethyl)-imidazole), N^{α},N^{α}-dimethylhistamine, N^{τ}-methylhistamine (1-methyl-4-(2-aminoethyl)imidazole), 2-pyridylethylamine (2-(2-aminoethyl)pyridine), 4-pyridylethylamine (4-(2-aminoethyl)pyridine), SKF 91488 and 2-thiazolylethylamine (2-(2-aminoethyl)thiazole), (all from Smith, Kline and French) and N-methyl-2-pyridylethylamine (2-(2-N-methylaminoethyl)pyridine) (Betahistine) (Duphar Laboratories) are gratefully acknowledged. All the histamine analogues were in the form of the dihydrochloride salt, except for SKF91488 which was the dihydrobromide. N^{α},N^{α}-dimethylhistamine was dried overnight at 41°C over P_2O_5 *in vacuo* (0.1 mmHg) before use.

Table 1 Effect of histamine H_1- and H_2-agonists on the accumulation of cyclic AMP in slices of guinea-pig cerebral cortex in the presence and absence of adenosine

Addition	*Accumulation of cyclic AMP* (pmol/mg protein)
None	16.9 ± 3.2
Adenosine	56.2 ± 7.2
Histamine	47.5 ± 7.0
2-Thiazolylethylamine	41.6 ± 8.7
Dimaprit	14.5 ± 2.6
Histamine + adenosine	179.1 ± 14.6
2-Thiazolylethylamine + adenosine	169.2 ± 28.0
Dimaprit + adenosine	54.0 ± 9.7

Incubations were as described under Methods. Histamine analogues were present at a concentration of 1 mM and adenosine at 0.1 mM. The values (means ± s.e.mean of 6 replicate determinations) are taken from a single experiment.

Results

Potentiation of adenosine-stimulated accumulation of cyclic AMP by histamine analogues

Typical responses for the potentiation by histamine and two analogues are shown in Table 1. Histamine and 2-thiazolylethylamine, a selective H_1-agonist, potentiated the response, whereas dimaprit, a specific H_2-agonist was without significant effect. Qualitatively similar results were obtained in a second experiment carried out in the same way, but in a third experiment 1 mM dimaprit added alone did produce a significant response. The extent of the potentiation by histamine of the effect of 0.1 mM adenosine was normally 3–7 fold, but on occasions larger stimulations of up to 23 fold were observed. The mean potentiation over 56 experiments was 6.6 ± 0.6 fold.

Dose-response curves for histamine, 2-thiazolylethylamine and 2-pyridylethylamine are shown in Figure 1a. The three curves appear to be parallel, but whereas 2-thiazolylethylamine produced the same maximum response as histamine, it is uncertain whether this would have been so for 2-pyridylethylamine. At concentrations > 1 mM responses to agonists were often markedly less than at lower concentrations and in view of the variety of non-selective inhibitory effects which can occur in this very high concentration range, all response measurements were made at concentrations ≤ 1 mM. However, two of the compounds tested, 2-methylhistamine and N^{α},N^{α}-dimethylhistamine, clearly did not produce the same maximum response as histamine and thus appeared to be partial agonists. This effect is illustrated for N^{α},N^{α}-dimethylhistamine in Figure 1b, where a comparison is made with the dose-response curves for histamine and N^{α}-methylhistamine.

In order to make a more quantitative comparison of agonist responses the dose-response curves were fitted to a Hill equation (see Methods) and the best-fit values obtained for the Hill coefficient (n_H), the EC_{50} and the maximum stimulation (Table 2). The data for 2-pyridylethylamine were inadequate for analysis in this way. Of the compounds tested only 3

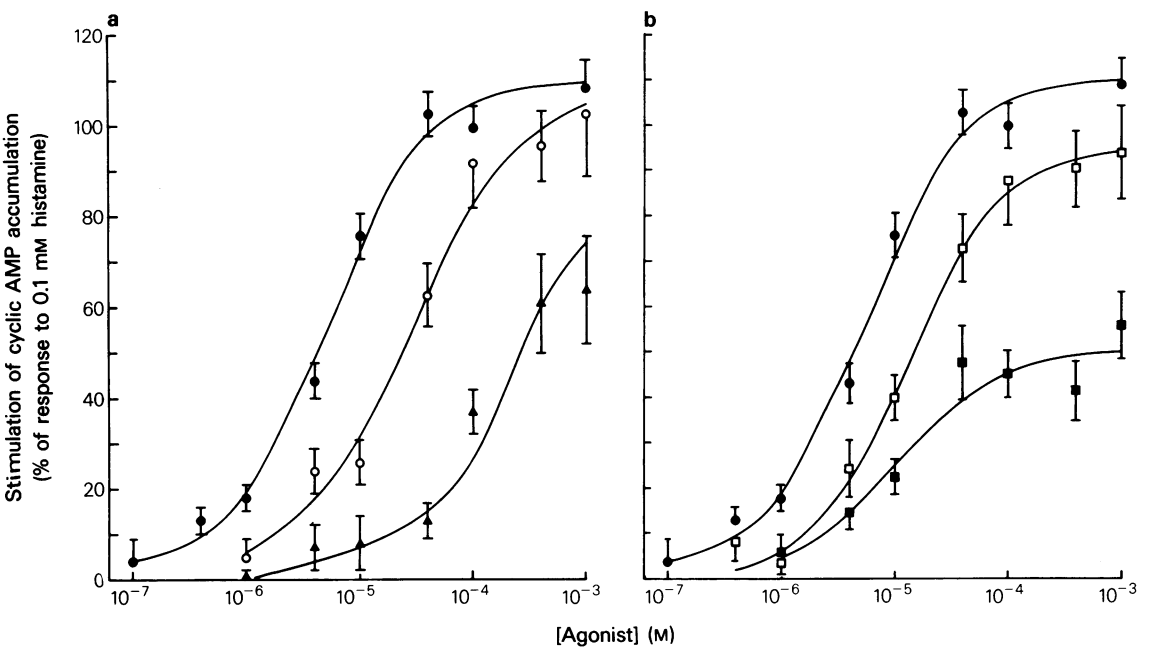

Figure 1 Potentiation of the adenosine-stimulated accumulation of cyclic AMP in guinea-pig cerebral cortical slices by histamine H_1-agonists. Incubations, containing 0.1 mM adenosine were as described under Methods. Adenosine and the H_1-agonist were added simultaneously. To normalise responses from different slice preparations, responses are expressed as a percentage of that produced by 0.1 mM histamine, which was measured in all experiments. Each point represents the combined mean from 15 (histamine) or 3–4 (other agonists) separate experiments; vertical lines show s.e.mean. The curves drawn are weighted best-fit lines to a Hill equation (see Methods), except that for 2-pyridylethylamine, which was drawn by inspection. (a) (●) Histamine; (⊘) 2-thiazolylethylamine; (▲) 2-pyridylethylamine. (b) (●) Histamine; (□) N^{α}-methylhistamine; (■) N^{α},N^{α}-dimethylhistamine.

Table 2 Characteristics of histamine H_1-agonists as potentiators of the adenosine-stimulated accumulation of cyclic AMP in slices of guinea-pig cerebral cortex

Agonist	n	EC_{50} (μM)	n_H
Histamine	15	5.1 ± 1.0	0.97 ± 0.12
2-Thiazolylethylamine	3	28 ± 12	0.85 ± 0.18
2-Pyridylethylamine	3	(320)*	–
N^{α}-methylhistamine	4	13 ± 3	1.05 ± 0.15
N^{α},N^{α}-dimethylhistamine	3	11 ± 4	0.96 ± 0.30
2-Methylhistamine	4	16 ± 4	1.20 ± 0.27

Compounds giving a small potentiation (< 21% of response to histamine)†
Betahistine, 4-methylhistamine. N^{τ}-methylhistamine, pargyline, 4-pyridylethylamine
No significant response†
SKF 91488

Best-fit values ± estimated s.e. of EC_{50} (concentration of agonist producing a half-maximal response), n_H (Hill coefficient) and the maximum response (expressed as a percentage of the response to 0.1 mM histamine, cf. legend to Figure 1), were obtained from weighted non-linear regression analysis of dose-response curves as described under Methods. *n* is the number of separate dose-response curves which were combined for the analysis.
*Estimated EC_{50}, assuming a maximum response equal to that of histamine.
†Weakly-active and inactive compounds were each tested at concentrations of 0.01, 0.1 and 1 mM.

produced a maximum or near maximum response, histamine, 2-thiazolylethylamine and N^{α}-methylhistamine. 2-Methylhistamine, like N^{α},N^{α}-dimethylhistamine, produced only 46% of the maximum response to histamine. Interestingly, all the curves approximated well to rectangular hyperbolae (Hill coefficient not significantly different from unity).

The position of the dose-response curve for histamine was little altered when the incubation was carried out in the presence of 10 μM SKF 91488, a non-competitive inhibitor of histamine-*N*-methyltransferase (Beaven & Shaff, 1979). The EC_{50} from the combined results from 3 experiments was 8 μM. In the presence of a higher concentration of SKF 91488, 0.64 mM, the dose-response curve for histamine was more clearly shifted to the right (EC_{50} 11 μM, 3 experiments).

To ascertain that the response to each of the agonists was mediated by H_1-receptors, measurements were made of the inhibition produced by 1 μM mepyramine (Table 3). In every case the response to a dose of agonist which otherwise produced a near maximum stimulation was virtually abolished. The smaller response, relative to histamine, of the two partial agonists and 2-pyridylethylamine is reflected in the large errors associated with the percentage inhibition.

The relative potencies of the agonists (relative EC_{50} values) as potentiators of adenosine-stimulated cyclic AMP accumulation are set out in Table 4 and compared with their relative potencies in producing contraction of the longitudinal muscle of guinea-pig ileum. The correspondence is generally good, although N^{α},N^{α}-dimethylhistamine and 2-methylhistamine both produced the same maximum contraction of the muscle as histamine. The agonist

Table 3 Sensitivity of agonist potentiation of adenosine-stimulated cyclic AMP accumulation to inhibition by mepyramine

Agonist	*Conc.* (mM)	*Inhibition by 1 μM mepyramine* (%)
Histamine	0.1	95 ± 7
2-Thiazolylethylamine	0.4	97 ± 7
2-Pyridylethylamine	1	111 ± 20
N^{α}-methylhistamine	0.1	106 ± 7
N^{α},N^{α}-dimethylhistamine	0.1	92 ± 14
2-Methylhistamine	0.1	98 ± 16

The inhibition is calculated from the ratio of the difference in the amount of cyclic AMP measured (agonist + adenosine − adenosine alone) in the presence and absence of 1 μM mepyramine. The approximate error of the ratio takes into account the error on each of the measured quantities. The mepyramine was added 20 min before the agonist. The concentrations of agonists were chosen to be just below the top of the dose-response curve.

Table 4 Relative potencies of agonists in potentiating cyclic AMP accumulation: comparison with potencies for contraction of the longitudinal muscle from guinea-pig intestine

Agonist	*Relative potency cyclic AMP*	*(Histamine = 100) longit. muscle*
Histamine	100	100
2-Thiazolylethylamine	18	22 (14)
2-Pyridylethylamine	(2)*	10 (4)
N^{α}-methylhistamine	39	134 (7)‡
N^{α},N^{α}-dimethylhistamine	46†	83 (5)‡
2-Methylhistamine	32†	20 (3)

Relative potencies for agonist potentiation of adenosine-stimulated cyclic AMP accumulation were determined from EC_{50} values (Table 2). Agonist-induced contraction of longitudinal muscle strips from guinea-pig small intestine was measured isotonically in Krebs-Henseleit medium at 30°C in a conventional organ bath. The number of determinations is given in parentheses.

†Partial agonist

*Assuming maximum response to be the same as that of histamine. If the response at 1 mM is maximal (cf. Figure 1a), then the relative potency rises to approx. 5.

‡Hill, Neale & Poniatowska, unpublished observations.

for which the relative potency differs most markedly between the two responses is N^{α}-methylhistamine. N-Methyl-2-pyridylethylamine (betahistine) gave the same maximum muscle contraction as histamine, but had only 9% of the activity on the cyclic AMP response.

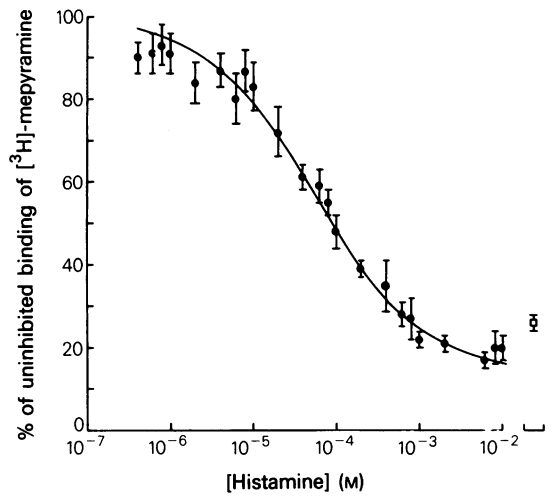

Figure 2 Inhibition by histamine of the binding of 1 nM [^{3}H]-mepyramine to a washed fraction from guinea-pig cerebral cortex. Measurements were made in Krebs-Hepes buffer as described under Methods. Each point is the percentage of the amount of [^{3}H]-mepyramine bound in the presence of histamine (5 replicate determinations at each concentration) compared with that in its absence (30 replicates); vertical lines show approx. s.e.mean. The curve drawn through the points is a weighted best-fit to a Hill equation (see Methods). The open square (□) indicates the inhibition produced by 2 μM promethazine (15 replicates) in the same experiment.

Inhibition of [^{3}H]-mepyramine binding

All the compounds tested inhibited the binding of [^{3}H]-mepyramine to a washed homogenate of guinea-pig cerebral cortex. A representative inhibition curve, that given by histamine, is shown in Figure 2. For quantitative analysis the curves were fitted to a Hill equation (see Methods) and the best-fit values obtained for the Hill coefficient, the percentage of the binding sensitive to inhibition by the test inhibitor and the IC_{50} (the concentration of inhibitor giving 50% inhibition of the inhibitor-sensitive binding). Measurement was also made in each experiment of the percentage of the binding of [^{3}H]-mepyramine sensitive to inhibition by 2 μM promethazine. The values obtained are set out in Table 5. For some of the weaker inhibitors, $IC_{50} > 1$ mM, the data were inadequate for treatment in this way and the IC_{50} was measured as the concentration producing 50% inhibition of the promethazine-sensitive binding (Table 5).

Similar results were obtained, where comparison was made, whether measurements were made in Krebs-phosphate medium or in Krebs-HEPES medium, which has the advantage of having a greater buffering capacity.

The percentage of non-receptor binding, as defined by 2 μM promethazine varied markedly between experiments, which were carried out over a 12 month period. Whether this is an artifact of the method of preparation of the homogenate or whether it represents a genuine variation in the numbers of H_1-receptors is not known. For the majority of compounds the maximum level of inhibition was greater than that produced by 2 μM promethazine. This effect will contribute to some extent to the low values of the Hill coefficients, which for most curves were signific-

Table 5 Inhibition of [^{3}H]-mepyramine binding by histamine receptor agonists and related compounds

Medium / *Agonist*	n	IC_{50} (μM)	n_H	*Non-specific binding (%) defined by* *Inhibitor*	*Promethazine* (2 μM)
Krebs-phosphate					
Histamine	26	46± 3	0.87±0.06	14±1	22±1
2-Thiazolylethylamine	23	59± 7	0.88±0.08	22±2	22±1
2-Pyridylethylamine	23	83± 9	0.80±0.06	8±2	15±1
Krebs-HEPES					
Histamine	23	65± 5	0.64±0.05	13±2	23±2
2-Thiazolylethylamine	23	58± 7	0.75±0.07	23±2	26±2
2-Pyridylethylamine	23	94± 9	0.84±0.06	21±2	26±1
N^{α}-methylhistamine	25	45± 4	0.75±0.06	14±2	24±2
N^{α},N^{α}-dimethylhistamine	26	33± 1	0.86±0.03	17±1	21±1
2-Methylhistamine	26	104± 5	0.76±0.03	15±1	22±1
Betahistine	17	100±10	1.22±0.14	30±1	39±2
Pargyline	7	550			
4-Methylhistamine	7	1000			
4-Pyridylethylamine	7	1200			
SKF 91488	7	1400			
Dimaprit	7	1700			
N^{γ}-methylhistamine	24	3000			

Where the data were adequate values ± estimated s.e. of IC_{50}, n_H (Hill coefficient) and the % of inhibitor-insensitive binding were obtained by fitting a Hill equation to the inhibition curves (e.g. Figure 2) using weighted non-linear regression analysis (see Methods). *n* is the number of points on each curve. The % of the binding of 1 nM [^{3}H]-mepyramine insensitive to inhibition by 2 μM promethazine was measured in every experiment and for the less potent compounds, where the foot of the curve was insufficiently well defined for the curve-fitting procedure, the IC_{50} represents the concentration required for 50% inhibition of the promethazine-sensitive binding.

antly < 1, and seems likely to be due to some inhibition of non-receptor binding of [^{3}H]-mepyramine by the very high concentrations of agonists needed to define the foot of the binding curve.

The position of the inhibition curve for histamine, measured in Krebs-HEPES medium, was not significantly altered by the presence of 10 μM SKF 91488. In one experiment in which the central portion of the inhibition curve for histamine was measured in the presence and absence of SKF 91488 on the same homogenate, the IC_{50}s for the promethazine-sensitive binding were 44 μM (no SKF 91488) and 32 μM (SKF 91488 present).

2-Thiazolylethylamine interacts with monoamine oxidase (Callingham, Lyles & Mackey, 1981) and is a substrate for it (G.A. Lyles, personal communication). However, treatment of the cortical homogenate with 0.1 mM pargyline for 30 min at 37°C, followed by centrifugation and resuspension of the pellet to remove most of the unbound pargyline, had no influence on the IC_{50} for 2-thiazolylethylamine inhibition of [^{3}H]-mepyramine binding, compared with an untreated control.

Comparison of parameters of agonist binding and response

In Table 6 comparison is made between the EC_{50} values for agonists in stimulating the adenosine-induced accumulation of cyclic AMP and adjusted IC_{50} values for the inhibition of [^{3}H]-mepyramine binding. The IC_{50} values for the inhibitor-sensitive binding (Table 5) have been corrected for inhibition of promethazine-insensitive binding by taking the level of non-specific binding to be that defined by promethazine. Correction has also been made for the effect of competition with [^{3}H]-mepyramine (see Methods). As discussed below, neither the EC_{50} nor the adjusted IC_{50} may accurately reflect the true equilibrium constants.

Discussion

The utility of the H_1-agonist potentiation of the adenosine-stimulated accumulation of cyclic AMP for quantitative studies on H_1-receptor activation in guinea-pig cerebral cortex is borne out by the results presented here. In agreement with our earlier study on H_1-antagonists (Hill *et al.*, 1981), there is little evidence of any H_2-coupled adenylate cyclase activity, although in one experiment dimaprit, which appears to have virtually no H_1-activity, did produce a small response. Previous studies on guinea-pig cerebral cortical slices have reported variously on the presence of an H_2-component that is absent (Chasin

Table 6 Comparison of the potency of agonists as potentiators of the adenosine-stimulated accumulation of cyclic AMP and as inhibitors of [^{3}H]-mepyramine binding

Agonist	*EC_{50} for accumulation of cyclic AMP* (μM)	*IC_{50} (corr.) for inhibition of [^{3}H]-mepyramine binding* (μM)
Histamine	5	16
2-Thiazolylethylamine	28	23
2-Pyridylethylamine	(320)*	40
N^{α}-methylhistamine	13	13
N^{α},N^{α}-dimethylhistamine	11†	12
2-Methylhistamine	16†	35

EC_{50} values are taken from Table 2. The IC_{50} (corr.) is the concentration of agonist required for 50% inhibition of the promethazine-sensitive binding of 1 nM [^{3}H]-mepyramine, corrected for competition with [^{3}H]-mepyramine (see Methods).

†Partial agonist.

*EC_{50} obtained assuming same maximal response as histamine. If the response given by 1 mM 2-pyridylethylamine is maximal, the EC_{50} decreases to approx. 100 μM (cf. Figure 1a).

et al., 1973), small (Dismukes *et al.*, 1976) or substantial (Baudry *et al.*, 1975; Daly *et al.*, 1980). In homogenates of cerebral cortex the presence of an H_2-stimulated adenylate cyclase is well established (Hegstrand *et al.*, 1976; Green *et al.*, 1977; Coupet & Szuchs-Myers, 1981), although it should be noted that these measurements were made in the presence of a phosphodiesterase inhibitor. We cannot resolve all these differences, but it is possible that a small H_2-component, such as that detected in one out of three experiments with dimaprit, could have been present in other experiments, but swamped by the very much larger effect produced by the adenosine and H_1-agonist combination. However, for the purpose of the present study the virtual absence of an H_2-component avoids complications such as those encountered in the quantitative analysis of the H_1- + H_2-response in hippocampal slices (Palacios *et al.*, 1978).

One interesting finding to emerge from the comparison between agonists of their action on cyclic AMP levels is that 2-methylhistamine and N,N-dimethylhistamine appear to be partial agonists. 2-Pyridylethylamine might also be a partial agonist and this would be consistent with the very weak response, approximately 10% of that given by histamine, produced by the N-methyl derivative, betahistine (cf. histamine and N^{α}-methylhistamine). All of these compounds produce a maximum contraction of the ileum, but it is interesting to note that the N,N-diethyl derivative of 2-pyridylethylamine does not (Cook, Iwanow, Kenakin & Krueger, 1982). N^{α}-methylhistamine and N^{α},N^{α}-dimethylhistamine were included in an early study of stimulation of adenylate cyclase by histamine analogues in adenine-labelled guinea-pig cerebral cortical slices (Shimizu, Creveling & Daly, 1970) and although no dose-response curves were recorded, the data for the effect of 0.1 mM concentrations approximate to those reported here. Interestingly the dose-response curves for cyclic AMP accumulation for histamine, N^{α}-methylhistamine and N^{α},N^{α}-dimethylhistamine (Figure 1b) are closely similar to those for the corresponding derivatives of 5-hydroxytryptamine on the adenylate cyclase of *Fasciola hepatica* (Northup & Mansour, 1978. Note that in the legend to their Figure 3 the filled and open squares should be interchanged).

The relative potencies of the agonists which produced an appreciable increase in cyclic AMP levels were mostly similar to their relative potencies in causing contraction of intestinal smooth muscle, the only marked discrepancy being with N^{α}-methylhistamine and to a lesser extent with the N^{α},N^{α}-dimethyl analogue. N^{α}-methylhistamine seems to have been little studied previously, but our value for its potency on longitudinal muscle is in accord with the report of Vartiainen (1935) that it was more active than histamine (up to 2 fold greater). Reported relative potencies for N^{α},N^{α}-dimethylhistamine on contraction vary somewhat: 37 (Vartiainen, 1935), 75 (Huebner, Turner & Scholz, 1949), 80 (Craver, Barrett, Cameron & Harrold, 1951) and 44 (Durant *et al.*, 1975). These compare with our figures of 83 on the muscle and 46 on cyclic AMP accumulation. Otherwise our values on the muscle strip are in good agreement with those of Durant *et al.* (1975) and Lee & Jones (1949) on ileal segments. Interestingly the relative potencies of histamine, 2-thiazolylethylamine and 2-methylhistamine on H_1-induced glycogenolysis in mouse cerebral cortical slices (100:30:12) (Quach *et al.*, 1980) are reasonably comparable with the values on cyclic AMP accumulation in the guinea-pig, but

there is no indication that 2-methylhistamine is a partial agonist on the glycogenolysis response.

With regard to the inhibition of [^{3}H]-mepyramine binding, the corrected IC_{50} (apparent *Ki*) values for histamine, 2-pyridylethylamine and betahistine are in good agreement with the values measured on whole brain homogenates in Tris buffer containing 100 mM NaCl by Chang & Snyder (1980). In contrast much lower potencies were reported for four of the agonists as inhibitors of [^{3}H]-mepyramine binding to a P2 fraction from guinea-pig cerebral cortex measured in Krebs-Henseleit medium (Kandel *et al.*, 1980). The reason for this discrepancy is not apparent. On homogenates of guinea-pig intestinal smooth muscle histamine, 2-thiazolylethylamine and 2-pyridylethylamine appeared to be more potent than on the cortical homogenate (Hill & Young, 1981).

With the notable exception of 2-pyridylethylamine, there is a surprisingly good correlation between the EC_{50} values for the cyclic AMP response and the corrected IC_{50} for inhibition of [^{3}H]-mepyramine binding (Table 6), even though there are problems in relating both of these parameters to an equilibrium constant. It also has to be borne in mind that the cyclic AMP response was measured on tissue slices and the binding on a homogenate, although the temperature and period of incubation with the agonist were virtually the same. In the media employed for the binding the membrane fragments presumably reseal to form vesicles, but whether agonist binding is the same as in the intact tissue remains to be established. The interpretation of the inhibition curves is also complicated by the fact that the majority are characterized by Hill coefficients less than unity. Whether this is a consequence of complex kinetics at a homogeneous population of receptors or alternatively represents multiple receptor populations is unknown. Certainly there is evidence that H_1-receptors occur both in neuronal tissue and on cerebral microvessels (Peroutka, Moskowitz, Reinhard & Snyder, 1980), but there is no indication that the binding properties of these two populations differ.

Interpretation of the EC_{50} values also poses problems. The mechanism by which H_1-agonists potentiate the accumulation of cyclic AMP remains to be established, but is almost certainly indirect and could possibly involve the intermediacy of Ca^{2+} ions (Schwabe *et al.*, 1978). Thus the response is probably remote from receptor activation and the reasonably good approximation of agonist dose-response curves to simple hyperbolae (Table 2) could be fortuitous. However, Strickland & Loeb (1981) have recently presented a simple model for responses involving secondary mediators and, making certain simplifying assumptions, have shown that the resultant dose-response curve can be shifted to the left and parallel to that for receptor occupancy. In effect this would represent 'spare receptors' and it is possible that the data for histamine, $EC_{50} <$ corr. IC_{50}, might be explained in this way. However, this would not hold for either 2-thiazolylethylamine of N^{α}-methylhistamine where the EC_{50} for response is closely similar to the corrected IC_{50} for inhibition of [^{3}H]-mepyramine binding.

There is a further problem in comparison of the EC_{50} and IC_{50} values in that one measures receptor activation and the other measures receptor occupancy. Agonists must produce a conformation change in the receptor, so that a minimal scheme to describe receptor activation would be:

$$D + R \rightleftharpoons DR \rightleftharpoons DR'$$

Thus response would be a measure of DR' and binding of DR + DR'. For strong agonists the equilibrium between DR and DR' would presumably lie far to the right, but the behaviour of partial agonists is most simply explained if an appreciable proportion of DR is present at equilibrium. Thus for a partial agonist it might be anticipated that the corrected IC_{50} would be lower than EC_{50}. This is not observed for either N^{α},N^{α}-dimethylhistamine or 2-methylhistamine, although the agreement for the former might be considered quite reasonable. In any case it is not established that either of these amines is a true partial agonist. It is possible that at high concentrations both compounds could have some other, depressant, action on cyclic AMP levels.

Even with all the above caveats, the similarity of the EC_{50} for response and the corrected IC_{50} for binding is notable. The only compound for which there could be a large discrepancy is 2-pyridylethylamine. The difficulty here lies in the uncertainty of the EC_{50}. If the response is in fact maximal at 1 mM then the EC_{50} would be somewhere near 100 μM. This is within a factor of 2.5 of the IC_{50}, but in the opposite direction to both histamine and 2-methylhistamine. It is interesting to note that the N,N-diethyl derivative of 2-pyridylethylamine behaves in an anomalous fashion as an apparent partial agonist on the guinea-pig ileum (Cook *et al.*, 1982). It may be, of course, that the apparent discrepancy with 2-pyridylethylamine is a consequence of the fact that changes in cyclic AMP are a step or steps removed from receptor occupancy. A better comparison between agonist binding and agonist response may be obtained when it becomes possible to measure a functional response more closely linked to receptor activation.

In summary, this study has shown that parameters of agonist binding may be useful in predicting agonist potency on central H_1-mediated responses for which the 'receptor reserve' is small. Further, the study of such responses is more likely to reveal partial agonist

properties than the use of the guinea-pig ileum, where the 'receptor reserve' is large.

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