Br. J. Pharmacol. (1988), **95**, 1292–1302

Biphasic effects of intra-accumbens histamine administration on spontaneous motor activity in the rat; a role for central histamine receptors

L.J. Bristow & [1]G.W. Bennett

Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH

1 The effect of intra-accumbens injection of histamine and related compounds on the spontaneous motor activity of the rat has been investigated.

2 Microinjections of histamine (1–200 μg) induced dose-dependent, biphasic changes in rat activity consisting of an initial brief hypoactivity response followed by a marked hyperactivity phase. The histamine metabolite, n-tele-methylhistamine was without effect.

3 Pretreatment with the H_1-receptor antagonist mepyramine (10 μg) blocked the hypoactivity response and markedly attenuated histamine-induced hyperactivity. In contrast, pretreatment with the H_2-receptor antagonist SKF93479 had no effect on histamine-induced behaviour.

4 Microinjection of the H_1-receptor agonist 2-thiazolylethylamine induced a marked hyperactivity response, but unlike the response to histamine, there was no initial hypoactivity. The H_2-receptor agonist dimaprit had no apparent behavioural effects following intra-accumbens injection.

5 Intra-accumbens injection of the non-selective histamine agonists n_α-methylhistamine or n_α, n_α-dimethylhistamine induced both marked hypoactivity and hyperactivity responses which were comparable with the effects of histamine.

6 The present results demonstrate a histamine, H_1-receptor-mediated arousal in the nucleus accumbens which follows transitory hypoactivity, possibly due to activation of presynaptic H_3-receptors.

Introduction

Although there is now substantial evidence supporting a role for histamine as a neurotransmitter/neuromodulator in the CNS (Schwartz, 1977; Hough & Green, 1984; Prell & Green, 1986; Pollard & Schwartz, 1987), the central functions of the amine remain uncertain (Cook, 1984; Mazurkiewicz-Kwilecki, 1984). Several reports do, however, suggest that it may modulate arousal although results from previous behavioural studies are somewhat conflicting. On the one hand, microinjections of histamine into the cerebral ventricles of the rat induce sedation (Calcutt & Reynolds, 1976; Onodera & Ogura, 1982) and catalepsy following higher doses (Nowak *et al.*, 1977). Similar sedative responses are also observed following microinjection of histamine into the ventral hippocampus (Alvarez & Guerra, 1982; Alvarez & Banzan, 1986). In contrast, Kalivas (1982) reported that i.c.v. histamine injection resulted in a marked increase in rat activity and suggested that this increased arousal was consistent with the well known clinical observation that H_1-receptor antagonists are sedative in man (Carruthers *et al.*, 1978; Nicholson, 1983; Levander *et al.*, 1985). Furthermore, CNS depressants such as barbiturate anaesthetics have been shown to decrease histamine turnover (Pollard *et al.*, 1974) and a clear variation in histamine turnover, with increased release occurring during the dark (active) phase has been demonstrated in rat hypothalamus (Schwartz *et al.*, 1979). These observations suggest that histamine may alter spontaneous motor activity but the site and mechanism of action remain equivocal.

[1] Author for correspondence.

The present study was designed to examine behavioural changes following microinjections of histamine into the rat nucleus accumbens. The accumbens was chosen for several reasons; firstly, substantial levels of histamine have been measured in this regior (Bennett *et al.*, 1983) and more recently, immunohistochemical studies have suggested a histaminergic pathway arising from cell bodies in the posterior hypothalamus and terminating in the accumbens (Steinbusch & Mulder, 1985; Pollard & Schwartz, 1987). Thirdly, histamine agonists have been shown to alter neuronal firing in rabbit accumbens and activation of adenylate cyclase in this region appears to be mediated by both H_1- and H_2-receptors (Chronister *et al.*, 1982). Finally, it is well established that the nucleus accumbens plays an important role in the control of motor activity (Mogenson *et al.*, 1980). We have also investigated the behavioural effects induced by intra-accumbens administration of a range of histamine receptor agonists and antagonists in an attempt to evaluate the types of receptor mediating the observed behavioural responses.

Methods

Implantation of guide cannulae

Male Wistar rats (270–300 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and bilaterally implanted with stainless steel guide cannulae (23 gauge, 15 mm length) according to Paxinos & Watson (1982) (A, +1.7; L, ±1.4; V, −3, i.e. cannulae tips 4 mm above accumbens). A minimum of 7 days was allowed before commencing experiments, during which cannulae were kept patent by a removable stainless steel stylet (31 gauge, 15 mm length).

Activity measurements

Activity changes following drug administration were monitored with an Actimat (Kinson electronics) doppler shift radar activity meter (Marsden & King, 1979). Rats were individually placed in a sound proof enclosure in their normal plastic housing units and their movements monitored with the radar module positioned above this area. The frequency of the reflected waves is linearly related to the speed of movement of the rat and wave amplitude is proportional to the amount of body area involved. Using a frequency band of 0.4–4 Hz it is possible to monitor low speed activity consisting of movements of the head and body without actual locomotion. Similarly, 4–100 Hz represents high speed activity composed mainly of normal exploratory behaviour. Activity is quantified as the seconds spent in either of these frequency bands and is expressed as activity counts with one count representing one second of activity. Behaviour was monitored in 15 min intervals for up to 2 h and activity scores recorded on a microprocessor controlled printer.

Behavioural observations

In addition to automated measurements, rats were observed and their behaviour scored according to the procedure of Kalivas (1982). Rats scored 1 for the presence, and 0 for the absence of each of 6 different behavioural parameters every 100 s. These parameters were: (1) sleep or motionless, (2) movement of head/forepaw, (3) locomotion, (4) rearing, (5) sniffing and (6) grooming. A range of scores from 0–9 per 15 min of the 2 hour observation period were obtained for each parameter by this method.

Experimental procedures

All experiments were carried out in the light phase (9 h 00 min–18 h 00 min) and rats were familiarised with the experimental environment and the operator on at least two occasions before drug administration. On test days, individual rats were placed in the enclosure and allowed 1 h to habituate; they were then removed, lightly restrained by hand and bilaterally microinjected over a 1 min period. The injection needle was removed after a further 30 s, rats were returned to the enclosure and activity monitored after a 1 min recovery period. Each rat was given an initial saline control injection, followed by a maximum of 4 randomized treatments with 4 days recovery allowed between successive tests. The doses of histamine receptor agonist or antagonist drugs administered per cannulae are expressed in μg, followed by the injection volume, and all were given directly into the accumbens. Data from *in vitro* studies reporting the potency of histamine receptor agonist compounds compared to the amine itself have been used to estimate doses required for each individual drug (see Ganellin, 1982).

Drugs

Drugs were obtained from the following sources; dimaprit dihydrochloride, n_α-methylhistamine dihydrochloride, n_α, n_α-dimethylhistamine dihydrochloride, n-tele-methylhistamine dihydrochloride, SKF 93479 (2-(2-[5-(dimethylaminomethyl)furan-2-ylmethylthio]ethylamino)-5-(6-methylpyrid-3-ylmethyl)-pyrimidin-4-one trihydrochloride), 2-thiazolylethylamine dihydrochloride (Smith, Kline

and French); histamine dihydrochloride, mepyramine maleate (Sigma). All histamine-receptor agonist or antagonist drugs were dissolved in 0.9% saline and the pH adjusted to 7.4.

Histological verification

On completion of the experiment, rats were injected centrally with pontamine sky blue (1% w/v) in a volume and manner identical to drug administration. Brains were then removed, frozen, sectioned and injection sites verified histologically.

The distribution of histamine in the brain following intra-accumbens administration was also investigated. Rats were bilaterally microinjected with a 1:1 by volume solution containing 'cold' histamine and [^{3}H]-histamine (10 Ci mmol^{-1}, Amersham), final dose 10 μg μl^{-1}. This was administered in a volume and manner identical to drug administration in behavioural tests, i.e. 1 μl min^{-1} per cannula. Rats were killed 10 min later, the brains removed and sectioned according to Craigies Anatomical Atlas (1963). A transverse slice (A33–28) was removed and dorso-ventrally divided into nucleus accumbens and caudate/septum pieces (Lighton *et al.*, 1984). The hypothalamus, cerebellum, brain stem and the remaining brain tissue were also taken. Tissue samples were sonicated in 1 ml 90% methanol and counted following addition of 15 ml scintillation fluid.

Data analysis

Histamine-induced changes in activity counts were compared to saline controls by an appropriate analysis of variance test. Thus data represented as time courses were analysed by a 2 way analysis of variance for repeated measures (BMDP(2PV), BMDP statistical package). The results of this analysis, i.e. F values, degrees of freedom (d.f.) and *P* values are presented where appropriate. In addition, data are presented at specific time points and were analysed by one way analysis of variance using Peritz's F-test for multiple comparisons (Harper, 1984).

Results

Histological verification of injection sites

The location of central injection sites for 19 representative animals is shown in Figure 1. Rats were only included in these studies following verification of dye in the region of the nucleus accumbens.

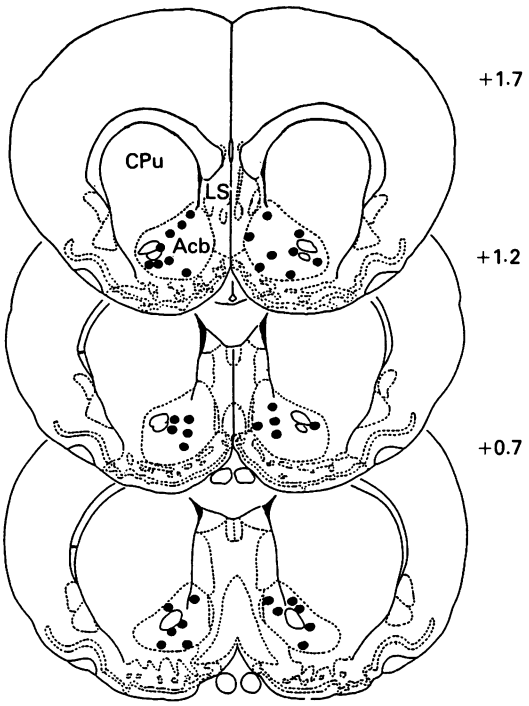

Figure 1 Diagrams representing the location of injection sites in the nucleus accumbens (●, $n = 19$) according to the atlas of Paxinos & Watson. Rats were bilaterally microinjected with pontamine sky blue (1% w/v), killed and the brains removed and frozen. Subsequent thionine staining of 20 μm sections taken using a cryostat microtome enabled identification of injection sites; Acb, nucleus accumbens; LS, lateral septal nuclei; CPu, caudate putamen. Numbers relate to the distance anterior from bregma.

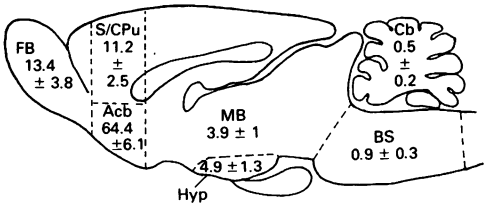

Figure 2 The distribution of radioactivity following bilateral microinjection of a behaviourally active histamine dose (10 μg μl^{-1}) containing [^{3}H]-histamine (10 Ci mmol^{-1}) into the nucleus accumbens. Rats were injected in a manner identical to drug administration in behavioural tests (i.e. 1 μl min^{-1} per cannula) and killed 10 min later. The following tissue regions were taken: FB, forebrain; Acb, nucleus accumbens; S/CPu, septum/caudate; MB, midbrain; Hyp, hypothalamus; BS, brain stem; Cb, cerebellum. Results are expressed as the mean % of the total d.p.m. found in the brain ($n = 7$, ± s.e.mean).

The distribution of tritium in rat brain following intra-accumbens injection of a behaviourally active histamine dose (10 μg) containing [^{3}H]-histamine is shown in Figure 2. The majority of radioactivity was found in the ventral slice containing the nucleus accumbens. Percentage distribution was as follows: nucleus accumbens 64.4%; septum/caudate 11.2%; forebrain 13.4%; hypothalamus 4.9%; midbrain 3.9%; cerebellum 0.5% and brainstem 0.9%.

Effect of control saline injections on total activity counts

Following bilateral microinjection of saline (2 × 1 μl), rats showed marked exploratory activity during the first 15 min period on return to the Actimat (Figure 3). For the remainder of the 2 h experimental period, however, rats showed few signs of arousal and often stayed to one side of the cage, usually sleeping for long periods. This is reflected by the low activity counts.

Effect of intra-accumbens histamine administration on total, low frequency and high frequency counts

Bilateral microinjection of histamine (20 μg μl^{-1}, 2 × 1 μl) markedly altered spontaneous rat activity (Figure 3a). In the first 15 min, rats showed a marked reduction in activity counts compared to saline controls. This was then followed by an increase in activity counts which peaked at 45–60 min and lasted the duration of the experiment. Comparison of activity counts recorded in 15 min intervals following histamine or saline administration using 2 way analysis of variance showed a significant difference between treatments ($F = 30.66$; d.f., 1,7; $P = 0.0009$), a significant effect of time ($F = 3.41$; d.f., 7,49; $P = 0.0048$) and a significant interaction between treatment and time ($F = 11.95$; d.f., 7,49; $P < 0.0001$). Subdivision of activity counts into those representing low speed activity (i.e. small body movements, Figure 3b and high speed activity (i.e. locomotion, Figure 3c) showed a similar biphasic pattern of histamine-induced changes for both these components. However, changes occurring in the low frequency band accounted for some 90% of the total activity counts recorded in 2 h.

Hypo- and hyperactivity responses following intra-accumbens histamine administration were dose-dependent (Figure 4). Activity counts obtained during the first 15 min represent the hypoactivity response (Figure 4a). The lowest dose investigated (1 μg, 2 × 1 μl) did not alter activity counts compared to saline controls. In contrast, 10 μg, 20 μg, 50 μg and 200 μg histamine induced a marked, dose-

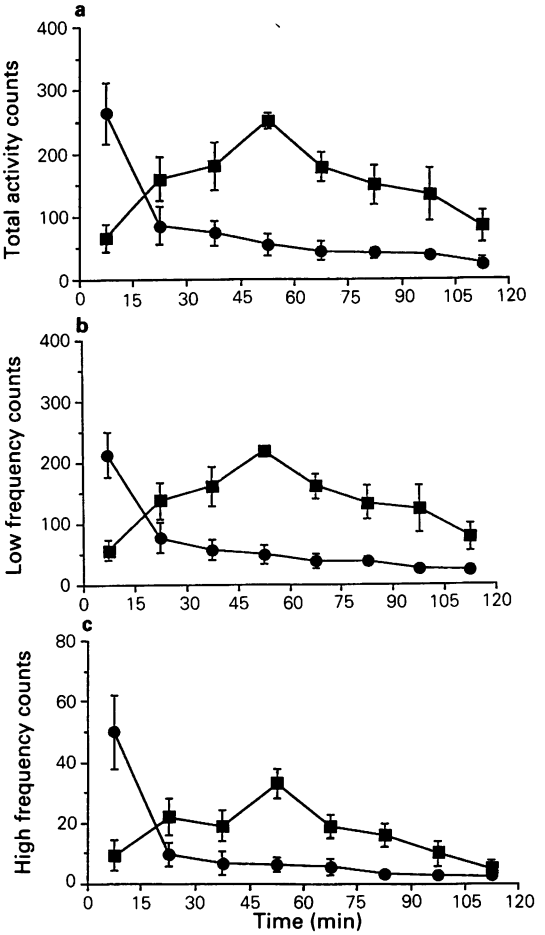

Figure 3 Effect of intra-accumbens microinjection of 0.9% saline (2 × 1 μl) (●) or histamine (20 μg, 2 × 1 μl) (■) on (a) total activity counts, (b) low frequency counts and (c) high frequency counts accumulated in 15 min intervals. Individual rats were allowed 1 h acclimatisation to the Actimat system after which they were removed, bilaterally microinjected (1 μl min^{-1} per cannula) and activity recorded after a 1 min recovery period. The system consists of a radar module which emits a low energy microwave beam, into a soundproof enclosure. Changes in rat movement alter both the frequency and amplitude of the reflected waves. Activity is expressed as activity counts, with one count representing one second of activity. Total counts (a) which represent activity scores across all frequency wavelengths can be subdivided into those occurring in a frequency band of 0.4–4 Hz (low speed activity, b) or 4–100 Hz (high speed activity, c). Low speed activity consists largely of movements of the head and body without actual locomotion whereas high speed activity represents normal exploratory movement around the cage. Points represent means ($n = 8$) with s.e.mean shown by vertical bars and data were analysed by 2 way analysis of variance for repeated measures (see Methods).

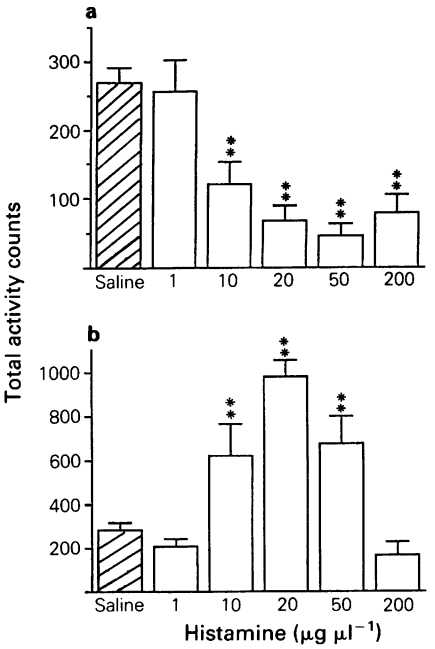

Figure 4 Effect of different histamine doses (1–200 μg) on (a) hypoactivity (i.e. total activity counts obtained during 0–15 min) and (b) hyperactivity (i.e. total activity counts obtained between 15–120 min). Amount of histamine administered per cannula is shown in μg and is given in 1 μl bilaterally ($n = 7$–10). Hatched columns show activity counts for saline controls ($n = 19$). Injections were administered over a 1 min period and the needle removed after a further 30 s. Activity was recorded after a 1 min recovery period. Histamine treatments were randomized and a minimum of 4 days allowed between successive tests. Columns represent means with s.e.mean shown by vertical bars; data were analysed by one way analysis of variance using Peritz's F-test for multiple comparisons, $^{**}P < 0.01$ compared to saline controls.

related hypoactivity. The remaining activity counts recorded (15–120 min) represent histamine-induced hyperactivity responses and again, the lowest dose investigated (1 μg) did not alter activity counts compared to saline controls. In contrast, 10 μg, 20 μg and 50 μg histamine induced a marked increase in activity counts although the response following 50 μg was markedly less than that following 20 μg histamine. The much higher dose of 200 μg histamine failed to induce any changes in activity counts compared to saline controls (Figure 4b).

Histamine (20 μg μl^{-1}, 2 × 1 μl)-induced hyperactivity responses varied according to the testing time. Thus rats microinjected at 16 h 00 min (i.e. activity measurements from 16 h 00 min–18 h 00 min) showed a significantly ($P < 0.05$) greater hyperactivity response than those microinjected at 10 h 00 min (Figure 5). In contrast, the transitory hypoactivity response observed in the first 15 min following histamine administration did not vary with the testing time (Figure 5). Activity counts recorded from 15–120 min following saline control injections were not significantly different (10 h 00 min, counts = 293 ± 64; 16 h 00 min, counts = 435 ± 63).

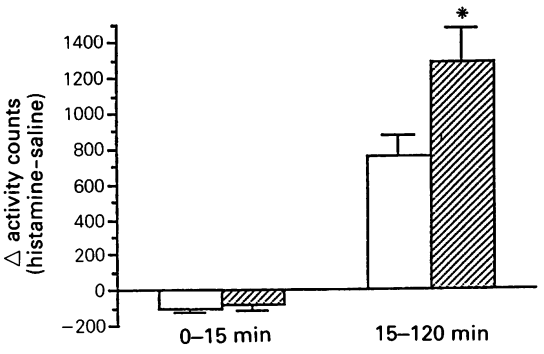

Figure 5 Activity changes following intra-accumbens injection of histamine (20 μg μl^{-1}, 2 × 1 μl) at 10 h 00 min (open columns, $n = 9$) and 16 h 00 min (hatched columns, $n = 5$). Results are expressed as Δ activity counts i.e. the difference between counts following histamine injection and counts following saline control injections for each rat. Data are presented at 2 time points, 0–15 min and 15–120 min. Columns represent mean Δ activity counts with s.e.mean shown by vertical lines, data were analysed by one way analysis of variance using Peritz's F-test, $^{*}P < 0.05$ compared to the response measured at 10 h 00 min.

Effect of intra-accumbens injection of n-tele-methylhistamine on accumulated activity counts

The specificity of histamine-induced changes in behaviour was examined by use of the major histamine metabolite, n-tele-methylhistamine which has been reported to be inactive at both H_1- and H_2-receptors (Black *et al.*, 1972). Following intra-accumbens injection (20 μg, 2 × 1 μl), rats did not show any significant changes in behaviour compared to saline controls (Figure 6; $F_{treatment} = 0.21$; d.f., 1,5; $P = 0.66$; $F_{time} = 19.88$; d.f., 7,35; $P < 0.0001$; $F_{interaction} = 0.62$; d.f., 7,35; $P = 0.73$).

Effect of intra-accumbens histamine administration on observed behaviour

The effect of intra-accumbens histamine administration on individual behavioural parameters was

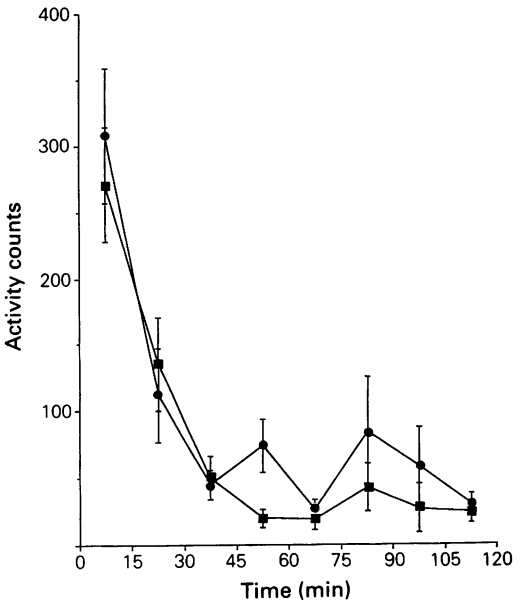

Figure 6 Effect of intra-accumbens injection of n-telemethylhistamine on total activity counts accumulated in 15 min intervals: saline (●)(2 × 1 μl) $n = 6$; n-telemethylhistamine (■) (20 μg, 2 × 1 μl) $n = 6$. Points represent means with s.e.mean shown by vertical bars.

monitored as described in Methods. The hypoactivity response induced by histamine (20 μg, 2 × 1 μl) was associated with a significant ($P < 0.01$) increase in scores for the sleep/motionless category together with significant reductions in head/forepaw movement ($P < 0.05$), locomotion ($P < 0.01$), rearing ($P < 0.01$) and sniffing ($P < 0.05$) (Table 1a). In contrast, histamine (20 μg, 2 × 1 μl)-induced hyperactivity was associated with a significant ($P < 0.01$) reduction in scores for the sleep/motionless category, together with significant increases in head/forepaw movement ($P < 0.01$), sniffing ($P < 0.02$) and grooming ($P < 0.01$) (Table 1b). Although there was also an increase in locomotor activity this just failed to reach significance.

Effect of pretreatment with mepyramine on histamine-induced behavioural changes

To investigate any involvement of H_1-receptors in histamine-induced behavioural changes, rats were pretreated with the H_1-receptor antagonist mepyramine (1 μg or 10 μg, 2 × 0.5 μl) 10 min before histamine administration (20 μg, 2 × 0.5 μl). Pretreatment with the low dose of mepyramine (1 μg) did not alter histamine-induced hypoactivity (counts from 0–15 min, Figure 7a) or hyperactivity responses represented by counts accumulated from 30–90 min (Figure 7b). In contrast, the higher dose of mepyramine (10 μg) blocked the hypoactivity response in the first 15 min (Figure 7a) and markedly attenuated histamine-induced hyperactivity responses (Figure 7b). Mepyramine itself had no effect on behavioural activity compared to saline controls.

Effect of pretreatment with SKF 93479 on histamine-induced behavioural changes

To investigate the possible involvement of H_2-receptors, rats were pretreated with the H_2-receptor antagonist SKF93479 (1 μg or 10 μg, 2 × 0.5 μl) 10 min before histamine treatment (20 μg, 2 × 0.5 μl). In contrast to mepyramine, pretreatment with SKF93479 did not alter histamine-induced hypoactivity (Figure 8a) or hyperactivity responses (Figure 8b) represented by counts accumulated from 30–90 min and was itself without effect.

Effect of intra-accumbens administration of 2-thiazolylethylamine or dimaprit on accumulated activity counts

To characterize further the nature of the receptors mediating histamine-induced changes in behaviour, rats were microinjected with the highly selective (Parsons *et al.*, 1977) H_2-receptor agonist dimaprit or the relatively selective H_1-receptor agonist (Ganellin, 1982) 2-thiazolylethylamine (Figure 9). Intra-accumbens dimaprit administration (20 μg or 50 μg, 2 × 1 μl) did not induce any significant changes in activity counts compared to saline controls (Figure 9). In contrast, 2-thiazolylethylamine (100 μg or 200 μg, 2 × 1 μl) induced a marked hyperactivity response (Figure 9b), which at the higher dose, was immediate in onset (Figure 9a). Unlike the behavioural changes induced by intra-accumbens histamine administration (Figure 3), neither 2-thiazolylethylamine nor dimaprit induced an initial hypoactivity response (Figure 9a).

Effect of intra-accumbens injection of n_α-methylhistamine or n_α, n_α-dimethylhistamine on accumulated activity counts

N_α-methylhistamine and n_α, n_α-dimethylhistamine are potent agonists at the H_3-receptor but also less potent agonists at H_1- and H_2-receptors (Arrang *et al.*, 1983). Intra-accumbens administration of either of these compounds (10 μg, 2 × 1 μl) induced similar behavioral responses to those seen following histamine treatment i.e. an initial hypoactivity response (Figure 10a) followed by a marked hyperactivity phase (Figure 10b).

Table 1 Effect of 0.9% saline (2 × 1 μl) or histamine (20 μg, 2 × 1 μl) on observed behaviour during hypoactivity (0–15 min, (a)) and hyperactivity (15–60 min, (b)) phases

(a) *Hypoactivity phase*

Behaviour	*Saline*	*Histamine* (20 μg)
Sleep motionless	2.5 ± 0.6	7.6 ± 0.7**
Head forepaw movement	5 ± 0.75	2.4 ± 0.7*
Locomotion	3.3 ± 0.8	0.14 ± 0.14**
Rearing	1 ± 0.3**	0
Sniffing	3 ± 0.9*	0.75 ± 0.4
Grooming	0.8 ± 0.3	1 ± 0.4

(b) *Hyperactivity phase*

Behaviour	*Saline*	*Histamine* (20 μg)
Sleep motionless	24 ± 1.3	10.5 ± 2.6**
Head forepaw movement	3 ± 1.2	16.4 ± 2.5**
Locomotion	0.4 ± 0.3	1.9 ± 0.6
Rearing	0.25 ± 0.16	1.75 ± 1
Sniffing	1.6 ± 0.75	10.25 ± 2.6*
Grooming	0.9 ± 0.3	4.6 ± 0.8**

Rats scored 1 for the presence and 0 for the absence of each of the 6 behavioural parameters. These were rated every 100 s thus the maximum score/parameter from 0–15 min is 9 and from 15–60 min is 36. Scores for individual rats were summated and results for each behaviour expressed as means ± s.e.mean ($n = 8$); ** $P < 0.01$, * $P < 0.05$ (Wilcoxon signed rank sum test).

Discussion

Microinjections of histamine into the nucleus accumbens induced marked changes in spontaneous motor activity. These changes were biphasic, consisting of an initial, dose-dependent hypoactivity response followed by a marked hyperactivity phase. The hyperactivity dose-response curve was bell shaped and the disappearance of hyperactivity at high doses of histamine was possibly due to cataleptic effects (Nowak *et al.*, 1977). Using the doppler shift analysis method, subdivision of total activity counts showed that those occurring in the low frequency wavelengths (0.4–4 Hz), which represent small body movements (Marsden & King, 1979), account for 90% of the total changes in activity and thus suggest that histamine-induced behaviour consists mainly of non-locomotory movements. This is supported by direct observation which shows both hypo- and hyperactivity phases to be associated with changes in head/forepaw movement, rearing, sniffing and grooming. Although the contribution of high frequency counts, which represent large body movements, was small, significant biphasic changes were also observed following histamine treatment suggesting histamine-induced changes in locomotor activity. Behavioural observations confirmed that histamine induces hypolocomotion during the first 15 min and increased sniffing, grooming and small body movements from 15–60 min.

The histamine-induced hyperactivity seen in our studies is consistent with other work supporting a central arousal role for this amine. Thus, H_1-receptor agonists have been shown to increase wakefulness and decrease rapid eye movement (REM) and non-REM sleep in rats (Monti *et al.*, 1986) whereas H_1-receptor antagonists induce sedation in man (Levander *et al.*, 1985). Furthermore, histamine-induced hyperactivity responses were markedly enhanced when measured in the late afternoon, consistent with the circadian changes in histamine turnover reported in the hypothalamus (Schwartz *et al.*, 1979). The discrepancies seen between our results and those studies reporting that histamine induced sedation (Calcutt & Reynolds, 1976; Onodera & Ogura, 1982; Alvarez & Banzan, 1986) may relate to different routes of drug administration and possible regionally selective sites of action. Alternatively, the differences may be technical, and result as a consequence of both the time of behavioural testing and the type of activity meters used. Thus Alvarez & Banzan tested rat behaviour 5 min after histamine administration and for a total of 5 min. The data presented by these authors would thus correspond to the initial hypoactivity response seen in our experiments and the short observation time may account for the apparent absence of hyperactivity in their study. With respect to the effects of different metering systems, open field tests (where the number of blocks traversed by the rat are counted) and photocell counters (Onodera & Ogura, 1982) may not be capable of monitoring histamine-induced hyperactivity since the evidence from the present studies suggest that the locomotor component of this response is small with respect to the overall activity changes (Figure 3, Table 1).

To examine the specificity of histamine-induced changes in behaviour, rats were microinjected with the major metabolite n-tele-methylhistamine (Schwartz *et al.*, 1971; Schayer & Reilly, 1973) which is reported to be inactive at central histamine receptors (Black *et al.*, 1972; Arrang *et al.*, 1983). In contrast to the effects induced by histamine, n-tele-methylhistamine did not alter spontaneous activity compared to saline controls. This would suggest that histamine-induced behavioural changes are receptor-mediated and are not a consequence of non-specific effects due to osmolality or pharmacological properties of the imidazole nucleus.

The nature of the histamine receptors initiating these changes in behaviour was further investigated

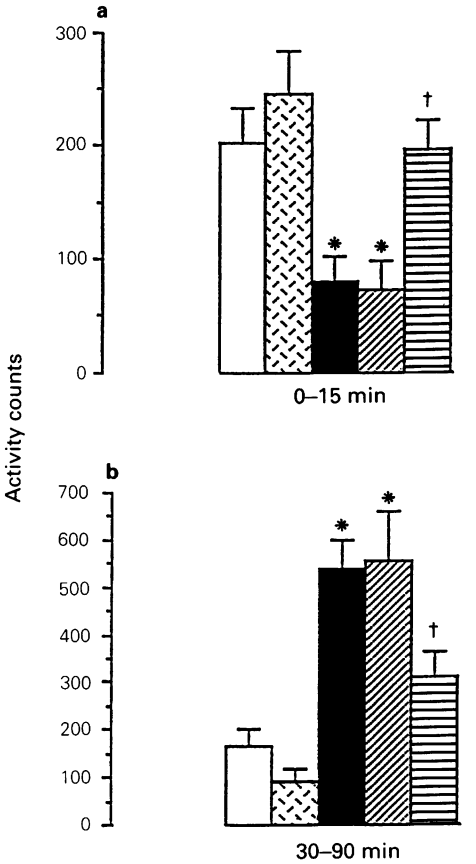

Figure 7 Effect of pretreatment with mepyramine on histamine-induced changes in activity represented by (a) activity counts obtained from 0–15 min and (b) activity counts obtained from 30–90 min; (open columns) saline (2 × 0.5 μl) + saline (2 × 0.5 μl), $n = 10$; (stippled columns) mepyramine (10 μg, 2 × 0.5 μl) + saline (2 × 0.5 μl), $n = 9$; (solid columns) saline (2 × 0.5 μl) + histamine (20 μg, 2 × 0.5 μl), $n = 10$; (hatched columns) mepyramine (1 μg, 2 × 0.5 μl) + histamine (20 μg, 2 × 0.5 μl), $n = 9$; (horizontally lined columns) mepyramine (10 μg, 2 × 0.5 μl) + histamine (20 μg, 2 × 0.5 μl), $n = 9$. Individual rats were allowed 1 h acclimatization to the Actimat system after which they were removed and pretreated with mepyramine 10 min before subsequent histamine treatment. After a 1 min recovery period, activity was recorded in 15 min intervals for 2 h. Columns represent mean activity counts with s.e.mean shown by vertical bars; data were analysed by one way analysis of variance using Peritz's F-test for multiple comparisons; * $P < 0.05$ compared to saline controls, † $P < 0.05$ compared to saline + histamine treatment.

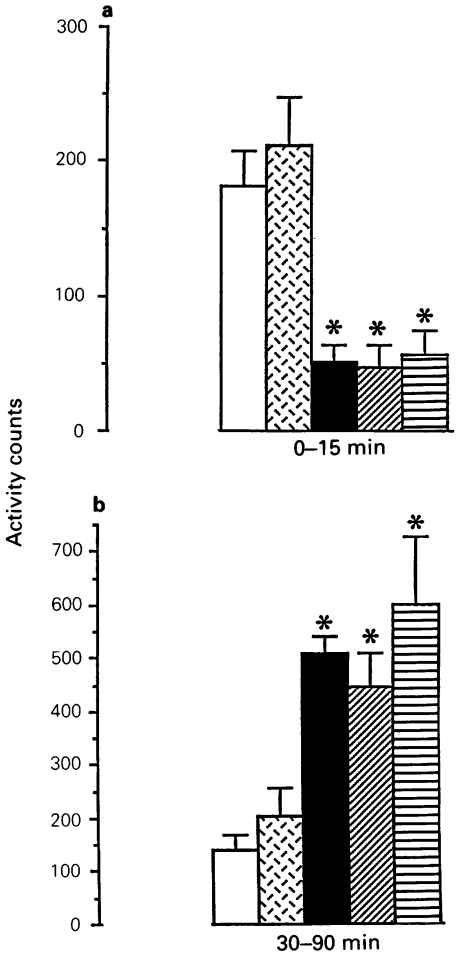

Figure 8 Effect of pretreatment with SKF 93479 on histamine-induced changes in activity represented by (a) activity counts obtained from 0–15 min and (b) activity counts obtained from 30–90 min; (open columns) saline (2 × 0.5 μl) + saline (2 × 0.5 μl), $n = 16$; (stippled columns) SKF 93479 (10 μg, 2 × 0.5 μl) + saline (2 × 0.5 μl), $n = 7$; (solid columns) saline (2 × 0.5 μl + histamine (20 μg, 2 × 0.5 μl), $n = 16$; (hatched columns) SKF 93479 (1 μg, 2 × 0.5 μl) + histamine (20 μg, 2 × 0.5 μl), $n = 9$; (horizontally lined columns) SKF 93479 (10 μg, 2 × 0.5 μl) + histamine (20 μg, 2 × 0.5 μl), $n = 7$. Individual rats were allowed 1 h acclimatization to the Actimat system after which they were removed and pretreated with SKF 93479 10 min before subsequent histamine treatment. After a 1 min recovery period, activity was recorded in 15 min intervals for 2 h. Columns represent mean activity counts with s.e.mean shown by vertical bars; data were analysed by Peritz's F-test for multiple comparisons, * $P < 0.05$ compared to saline controls.

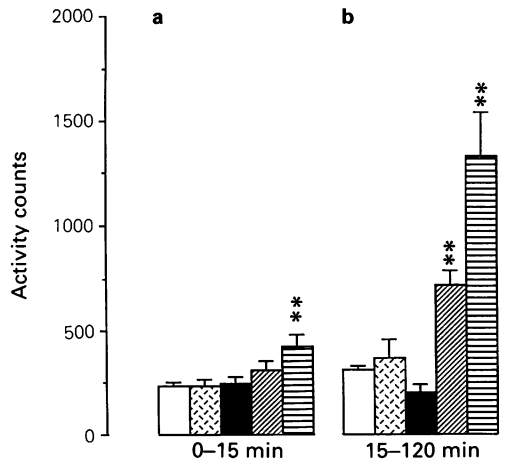

Figure 9 Effect of intra-accumbens microinjection of (open columns) saline (2 × 1 μl), $n = 28$; (stippled columns) dimaprit (20 μg, 2 × 1 μl), $n = 10$; (solid columns) dimaprit (50 μg, 2 × 1 μl), $n = 7$; (hatched columns) 2-thiazolylethylamine (100 μg, 2 × 1 μl), $n = 9$; (horizontally lined columns) 2-thiazolylethylamine (200 μg, 2 × 1 μl), $n = 8$. Columns represent mean activity counts with s.e.mean shown by vertical bars recorded from (a) 0–15 min and (b) 15–120 min; data were analysed by one way analysis of variance using Peritz's F-test for multiple comparisons, ** $P < 0.01$ compared to saline controls.

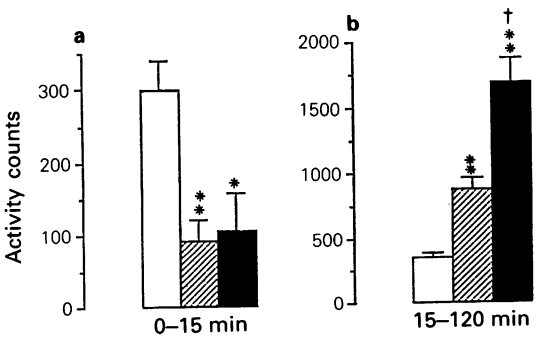

Figure 10 Effect of intra-accumbens administration of saline, n_α-methylhistamine or n_α, n_α-dimethylhistamine on activity counts recorded from (a) 0–15 min and (b) 15–120 min: (open columns) saline (2 × 1 μl) $n = 15$; (hatched columns) n_α-methylhistamine (10 μg, 2 × 1 μl) $n = 7$; (solid columns) n_α, n_α-dimethylhistamine (10 μg, 2 × 1 μl) $n = 8$. Columns represent mean activity counts with s.e.mean shown by vertical bars; data were analysed by one way analysis of variance using Peritz's F-test for multiple comparisons, ** $P < 0.01$ compared to saline controls.

by pretreating rats with the H_1-receptor antagonist mepyramine or the H_2-receptor antagonist SKF93479 (Blakemore *et al.*, 1981). Mepyramine (10 μg) blocked the hypoactivity response and significantly attenuated the hyperactivity phase suggesting that both components of histamine-induced behaviour are mediated via H_1-receptors. This was further substantiated following pretreatment with the H_2 antagonist SKF93479 which did not alter either the hypo- or hyperactivity response to histamine suggesting that H_2-receptors are not involved. Certain histamine related compounds are, however, notable for their lack of specificity. For example, although mepyramine is highly selective for the H_1-receptor when used at nanomolar concentrations *in vitro*, it also acts on H_2- and muscarinic receptors, has local anaesthetic properties and can block uptake sites for catecholamines and 5-hydroxytryptamine (Schwartz, 1979; Hough & Green, 1984; Prell & Green, 1986). It is thus possible that the reduction in histamine-induced behaviours following pretreatment with mepyramine could be due to a number of actions other than its antagonistic effects at H_1 receptors.

Since these results do not provide conclusive evidence for the involvement of histamine receptors, we investigated the effect of intra-accumbens administration of histamine agonists. Dimaprit is a selective H_2-receptor agonist (Parsons *et al.*, 1977) with negligible affinity for H_1- or muscarinic receptors. Intra-accumbens administration of dimaprit did not induce any significant changes in activity compared to saline controls thus providing further evidence for the lack of involvement of H_2-receptors. In contrast, treatment with the relatively selective H_1 agonist 2-thiazolylethylamine induced a marked hyperactivity response which at the higher dose (200 μg) was immediate in onset. This would support the suggestion that histamine-induced hyperactivity is mediated via H_1-receptors and is in agreement with the finding that mepyramine pretreatment attenuates this response. Unlike the behavioural changes induced by histamine, however, thiazolylethylamine did not induce an initial hypoactivity response.

The latter observation has led us to consider the possibility of intra-accumbens histamine interacting with a further receptor type, the H_3-receptor. There is now substantial evidence that histamine released into the synaptic cleft can inhibit both its further release and synthesis by acting on presynaptic autoreceptors (Arrang *et al.*, 1983; Schwartz *et al.*, 1986). Furthermore, autoradiographic studies have now shown the presence of H_3-receptors in the region of the accumbens (Arrang *et al.*, 1987). Two compounds, n_α-methylhistamine and n_α, n_α-dimethylhistamine are reported to be potent H_3-receptor agonists, although they are not selective and have significant agonist actions at both H_1- and

H_2-receptors (Arrang *et al.*, 1983). Intra-accumbens microinjections of either of these compounds induced identical activity changes to those seen following histamine treatment i.e. an initial hypoactivity response followed by a marked hyperactivity. The effect of treatment with 2-thiazolylethylamine and mepyramine would suggest that the hyperactivity induced by these methyl derivatives results from agonist actions at the H_1-receptor. In contrast, the initial hypoactivity response may be mediated via H_3-receptors since it is not apparent following treatment with either H_1 or H_2 agonists. It is interesting that the affinity of histamine for the H_3-receptor is reported to be 100 fold higher than its affinity for either of the postsynaptic receptors (Schwartz *et al.*, 1986). It is thus possible that the immediate hypoactivity response results from histamine acting at H_3-receptors to reduce endogenous histamine release. Subsequent accumulation of exogenously applied histamine in the synaptic cleft may then reach sufficient concentrations to activate postsynaptic H_1-receptors and induce a hyperactivity response. One question remaining is why mepyramine at high doses blocks the initial hypoactivity induced by histamine. Two possible explanations for this observation are (a) mepyramine can inhibit histamine methyltransferase so inhibition of histamine metabolism may mask any reduction in histamine release following H_3-receptor stimulation (Netter & Bodenschatz, 1967; Pollard *et al.*, 1973) and (b) mepyramine itself at high doses may be antagonistic at the H_3-receptor. Although our studies would predict the involvement of H_3-receptors in histamine-induced hypoactivity, the compounds used are not selective for this receptor type. Recently, however, two highly selective and potent compounds have been developed, $(r)_\alpha$-methylhistamine, an H_3 agonist and thioperamide, an H_3 antagonist (Arrang *et al.*, 1987). The use of these compounds in recent studies in our laboratory has further substantiated a role for H_3-receptors in mediating the hypoactivity response to histamine (Bristow & Bennett, 1988).

In conclusion, the present studies demonstrate marked behavioural changes following intra-accumbens histamine administration in the rat. Activity changes following treatment with histamine compounds suggest that these hypo- and hyperactivity responses may result from actions at H_3- and H_1-receptors respectively. It remains to be determined whether these changes occur as a direct effect of histamine acting at its receptors or whether this interaction induces a series of secondary events which themselves induce these behavioural effects.

We thank Smith, Kline and French for their generous supply of histaminergic compounds. L.J.B. is an SERC/CASE student with SK&F.

References

ALVAREZ, E.O. & BANZAN, A.M. (1986). Histamine in dorsal and ventral hippocampus: II Effects of H_1 and H_2 histamine antagonists on exploratory behaviour in male rats. *Physiol. Behav.*, **37**, 39–45.

ALVAREZ, E.O. & GUERRA, F.A. (1982). Effects of histamine microinjections into the hippocampus on open field behaviour in rats. *Physiol. Behav.*, **28**, 1035–1040.

ARRANG, J.M.; GARBARG, M. & SCHWARTZ, J.C. (1983). Autoinhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature*, **302**, 832–837.

ARRANG, J.M., GARBARG, M., LANCELOT, J.C., LECOMTE, J.M., POLLARD, H., ROBBA, M., SCHUNACK, W. & SCHWARTZ, J.C. (1987). Highly potent and selective ligands for histamine receptors – H_3. *Nature*, **327**, 117–123.

BENNETT, G.W., COOPER, S., EGLEN, A. & MARSDEN, C.A. (1983). The effect of N-acetylhistamine on regional TRH levels in rat brain and spinal cord. *Br. J. Pharmacol.*, **80**, 641P.

BLACK, J.W., DUNCAN, W.A.M., DURANT, G.J., GANELLIN, C.R. & PARSONS, M.E. (1972). Definition and antagonism of histamine H_2 receptors. *Nature*, **236**, 385–390.

BLACKMORE, R.C., BROWN, T.H., DURANT, G.J., GANELLIN, C.R., PARSONS, M.E., RASMUSSEN, A.C. & RAWLINGS, D.A. (1981). SKF 93479, a potent and long acting histamine H_2 receptor antagonist. *Br. J. Pharmacol.*, **74**, 200P.

BRISTOW, L.J. & BENNETT, G.W. (1988). A role for histamine H_3 receptors in histamine induced hypoactivity in the rat. *Br. J. Pharmacol.*, **94**, 319P.

CALCUTT, C.R. & REYNOLDS, J. (1976). Some behavioural effects following intracerebroventricular (icv) injection in rats of histamine H_1 and H_2 receptor agonists and antagonists. *Neuroscience Letters*, **3**, 82–83.

CARRUTHERS, S.G., SHOEMAN, D.W., HIGNITE, C.E. & ARZNOFF, D.L. (1978). Correlation between plasma diphenhydramine level and sedative and antihistamine effects. *Clin. Pharmacol. Ther.*, **23**, 375–382.

CHRONISTER, R.B., PALMER, G.C., DEFRANCE, J.F., SIKES, R.W. & HUBBARD, J.I. (1982). Histamine: correlative studies in nucleus accumbens. *J. Neurobiol.*, **13**, 23–37.

COOK, D.A. (1984). Recent advances in histamine research; closing remarks. *Can. J. Physiol. Pharmacol.*, **62**, 738–740.

CRAIGIES, J.R.M. (1963). *Neuroanatomy of the Rat*, ed. Zernon, W. New York: Academic Press.

GANELLIN, C.R. (1982). Chemistry and structure-activity relationships of drugs acting at histamine receptors. In *Pharmacology of Histamine Receptors*. ed. Ganellin, C.R. & Parsons, M.E. pp. 10–102. Bristol: John Wright.

HARPER, J.F. (1984). Peritz's F-test: Basic program of a robust multiple comparisons test for statistical analysis of all differences among means. *Comput. Biol. Med.*, **14**, 437–445.

HOUGH, L.B. & GREEN, J.P. (1984). Histamine and its receptors in the nervous system. In *Handbook of Neurochem.*, Vol. 6, ed. Lajtha, A. pp. 145–211. New York: Plenum Press.

KALIVAS, P.W. (1982). Histamine induced arousal in the conscious and pentobarbital pretreated rat. *J. Pharmacol. Exp. Ther.*, **222**, 37–42.

LEVANDER, S., HAGERMARK, O. & STAHLE, M. (1985). Peripheral antihistamines and central sedative effects of three H_1 receptor antagonists. *Eur. J. Clin. Pharmacol.*, **28**, 523–529.

LIGHTON, C., MARSDEN, C.A. & BENNETT, G.W. (1984). The effects of 5,7-dihydroxytryptamine and p-chlorophenylalanine on thyrotrophin releasing hormone in regions of the brain and spinal cord of the rat. *Neuropharmacol.*, **23**, 55–60.

MARSDEN, C.A. & KING, B. (1979). The use of doppler shift radar to monitor physiological and drug induced activity patterns in the rat. *Pharmacol. Biochem. Behav.*, **10**, 631–635.

MAZURKIEWICZ-KWILECKI, I.M. (1984). Possible role of histamine in brain function: neurochemical, physiological and pharmacological indications. *Can. J. Physiol. Pharmacol.*, **62**, 709–714.

MOGENSON, G.J., JONES, D.L. & YIM, C.Y. (1980). From motivation to action: functional interface between the limbic system and the motor system. *Prog. Neurobiol.*, **14**, 69–97.

MONTI, J.M., PELLEJERO, T. & JANTOS, H. (1986). Effects of H_1 and H_2 histamine receptor agonists and antagonists on sleep and wakefullness in the rat. *J. Neural. Transm.*, **66**, 1–11.

NETTER, K.J. & BODENSCHATZ, K. (1967). Inhibition of histamine – N-methylation by some antihistamines. *Biochem. Pharmacol.*, **16**, 1627–1631.

NICHOLSON, A.N. (1983). Antihistamines and sedation. *The Lancet*, **ii**, 211–212.

NOWAK, J.Z., PILC, A., LEBRECHT, U. & MASLINSKI, C. (1977). Does histamine interact with cholinergic neurones in its cataleptogenic action in the rat? *Neuropharmacol.*, **16**, 841–847.

ONODERA, K. & OGURA, Y. (1982). The effect of intraventricular injection of histamine on the behaviour of mice and rats. In *Advances in Histamine Research*; Advances in the Biosciences, vol. 33, ed. Uvnas, B. pp. 127–136. New York: Pergamon Press.

PARSONS, M.E., OWEN, D.A.A., GANELLIN, C.R. & DURANT, G.J. (1977). Dimaprit – (s-(3-(N,N-dimethylamino)propyl)isothiourea) – A highly specific histamine H_2 receptor agonist. Part 1. Pharmacology. *Agents and Actions*, **7**, 31–37.

PAXINOS, G. & WATSON, C. (1982). The Rat Brain in Stereotaxic Coordinates. Sydney, Australia: Academic Press.

POLLARD, H., BISCHOFF, S. & SCHWARTZ, J.C. (1973). Modifications of brain HA metabolism induced by antihistamines. *Agents and Actions*, **3**, 190–191.

POLLARD, H., BISCHOFF, S. & SCHWARTZ, J.C. (1974). Turnover of histamine in rat brain and its decrease under barbiturate anaesthesia. *J. Pharmacol. Exp. Ther.*, **190**, 88–99.

POLLARD, H. & SCHWARTZ, J.C. (1987). Histamine neuronal pathways and their functions. *TINS*, **10**, 86–89.

PRELL, G.D. & GREEN, J.P. (1986). Histamine as a neuroregulator. *Ann. Rev. Neurosci.*, **9**, 209–254.

SCHAYER, R.W. & REILLY, M.A. (1973). Formation and fate of histamine in rat and mouse brain. *J. Pharmacol. Exp. Ther.*, **184**, 33–40.

SCHWARTZ, J.C. (1977). Histaminergic mechanisms in brain. *Ann. Rev. Pharmacol. Toxicol.*, **17**, 329–339.

SCHWARTZ, J.C. (1979). Minireview: Histamine receptors in brain. *Life Sci.*, **25**, 895–912.

SCHWARTZ, J.C., ARRANG, J.M. & GARBARG, M. (1986). Three classes of histamine receptors in brain. *TIPS*, **7**, 24–28.

SCHWARTZ, J.C., BARBIN, G., BAUDRY, M., GARBARG, M., MATRES, M.P., POLLARD, H. & VERDIERE, M. (1979). Metabolism and functions of histamine in the brain. In *Current Developments in Psychopharmacology*, vol. 5. ed. Essman, W.B. & Valzelli, L. pp. 173–261. New York: SP Medical and Scientific Books.

SCHWARTZ, J.C., POLLARD, H., BISCHOFF, S., REHAULT, M.C. & VERDIERE-SAHUQUE, M. (1971). Catabolism of ^{3}H-histamine in the rat after intracisternal administration. *Eur. J. Pharmacol.*, **16**, 326–335.

STEINBUSCH, H.W.M. & MULDER, A.H. (1985). Localization and projections of histamine immunoreactive neurones in the central nervous system of the rat. In *Frontiers in Histamine Research.* Advances in the Biosciences, vol. 51, ed. Ganellin, C.R. & Schwartz, J.C. pp. 119–130. New York: Pergamon Press.

(Received February 11, 1988
Revised July 24, 1988
Accepted August 1, 1988)