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STUDIES ON THE MECHANISM OF HISTAMINE-INDUCED RELEASE OF NORADRENALINE AND 5-HYDROXYTRYPTAMINE FROM SLICES OF RAT CEREBRAL CORTEX

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Abstract—The effect of histamine on the release of endogenous noradrenaline and 5-hydroxytryptamine (5-HT) has been examined in slices of rat cerebral cortex. Histamine was found to produce a marked release of both amines from rat cerebral cortex at concentrations between 0.1 and 1 mM. This response to histamine was relatively resistant to removal of calcium ions from the incubation medium when compared to the release evoked by depolarising potassium stimuli. The response to 1 mM histamine was not, however, significantly inhibited by the H_1-antagonist mepyramine (1 μM) or the H_2-antagonist cimetidine (100 μM). Furthermore, impromidine which is both a potent H_2-agonist and a potent H_3-antagonist was without effect on the basal and histamine-stimulated release of endogenous noradrenaline and 5-HT. The response to histamine was, however, significantly attenuated by nisoxetine, fluoxetine and imipramine which are inhibitors of the amine uptake systems. The results of this study show that high concentrations (0.1 to 1 mM) of histamine can produce a marked increase in the release of endogenous 5-HT and noradrenaline from rat cerebral cortex, apparently via a non-receptor mechanism. This effect will need to be borne in mind in interpreting biochemical and behavioural responses to histamine in this concentration range.

There is accumulating evidence that favours the suggestion that histamine may have a role as a neurotransmitter or neuromodulator in the mammalian central nervous system [1–3]. Three different receptor subtypes for histamine have been identified in mammalian brain [4, 5]. Histamine H_1-receptors are linked to the production of inositol phosphates and diacylglycerol [6, 7], H_2-receptors are coupled via the N_s regulatory protein to adenylate cyclase [8, 9] and H_3-receptors are present on histaminergic nerve-endings and control the synthesis and release of histamine [10–12]. However, as yet, very little is known concerning the physiological functions of this biogenic amine in the central nervous system.

It has been shown that histamine, when injected into appropriate regions of mammalian brain, can affect a number of central processes including locomotor activity [13], thermoregulation [14, 15], cardiovascular regulation [16, 17] and the hypothalamo-hypophysial hormone-secreting systems [3]. It is possible that some of these effects of histamine are indirectly mediated by the release of other neurotransmitter substances.

Subramanian and Mulder [18] have reported that histamine can induce a slow dose-dependent stimulation of [^{3}H]catecholamine efflux from a number of regions of rat brain including hypothalamus, striatum and cerebral cortex. A similar effect of histamine on the release of both [^{3}H]noradrenaline and [^{3}H]5-hydroxytryptamine (5-HT)† has been demonstrated in synaptosomal preparations of rat brain following prelabelling with tritiated neurotransmitters [19].

It is generally assumed that the measurement of the release of radiolabelled neurotransmitters is an accurate marker for the release of endogenous substances. However, it has recently been shown [20] that major differences can occur between the release of endogenous and radiolabelled dopamine from rat striatal slices induced by various chemical stimuli. In this paper we report the effect of histamine on the release of endogenous catecholamines and indoleamines from slices of rat cerebral cortex. A preliminary account of this work has been presented to the British Pharmacological Society [21].

MATERIALS AND METHODS

Measurement of endogenous amine release. The cerebral cortex from three rats (Wistar 250 g, males) was dissected out on ice and sliced in two directions (300 × 300 μm) with a McIlwain tissue chopper. Slices were washed three times in Krebs Henseleit medium (mM): 118 NaCl; 4.7 KCl; 1.2 $MgSO_4$; 2.5 $CaCl_2$; 1.2 KH_2PO_4; 25 $NaHCO_3$ and 5.5 glucose, pH 7.4, containing 50 μM pargyline and then preincubated for 40 min in a shaking water bath at 37° under an atmosphere of O_2/CO_2 (95:5). Where appropriate, antagonists were added for the final 20 min of this preincubation period. The slices were then allowed to settle under gravity and 100 μl (1.9–2.9 mg protein) of the gravity packed slices were added to 680 μl of Krebs Henseleit medium, con-

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† Abbreviations used: 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindole acetic acid; DHBA, dihydroxybenzylamine.

taining antagonist where appropriate, in Beckman biovials. The samples were gassed with O_2/CO_2 (95:5) and incubated for 10 min at 37°. Agonists were added in 20 μl of Krebs Henseleit medium and the incubation continued for a further 20–40 min at 37°. For incubations in high KCl solutions (56 mM), slices were transferred to high KCl medium where the sodium concentrations had been appropriately reduced to maintain isotonicity. In studies of the effect of histamine on K^+-stimulated amine release, experiments were performed in a low calcium Krebs Henseleit medium containing 1.2 mM $CaCl_2$, pargyline (50 μM) and imipramine (10 μM). Slices were incubated for 10 min in the presence or absence of histamine prior to the addition of KCl (final concentration 35 mM) for a further 5 min. Incubations were terminated by centrifugation at 1000 *g* for 5 min and the supernatant from each incubation was split into two, acidified and stored at −20° until assay of indoleamines and catecholamines respectively. Tissue pellets were solubilised by heating in 1 M NaOH and the protein content of each incubation determined as described previously [22].

Each experiment comprised 30–36 separate incubations; 5–8 separate incubations were included for measurement of the basal efflux and for each treatment group. Values for the endogenous amine outflow from brain slices in each incubation are expressed with respect to the protein content of that incubation. Results are expressed as mean ± SE. The stimulated release of catecholamines and indoleamines in a given experiment was calculated by subtracting the basal outflow from the total release occuring in response to histamine or potassium chloride. Values for 5-HT and noradrenaline release were obtained from the same incubations. Statistical analysis was by one-way analysis of variance, paired *t*-test or Wilcoxin signed rank test.

Electrochemical measurement of indoleamines and catecholamines. Supernatants were assayed for indoleamines and catecholamines by HPLC with electrochemical detection [23]. Separation of indoleamines was achieved by reverse phase HPLC using a 25 cm × 4.6 mm column (Sherisorb 50DS), a mobile phase of 0.1 M sodium acetate and 0.1 M citric acid containing 1 mM EDTA and 10% methanol (v/v), pH 4.0 and a flow rate of 0.6 ml/min [23]. 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) peaks in samples were verified by co-elution with authentic 5-HT and 5-HIAA in this (retention times; 10.8 min 5-HT, 16.7 min 5-HIAA) and two other solvent systems: (i) the ion-pair catecholamine system described below (retention times; 80.8 min 5-HT, 49.1 min 5-HIAA) and (ii) 0.15 M NaH_2PO_4, 0.1 mM EDTA, 12% methanol (v/v), 0.5 mM sodium octyl sulphate, pH 3.5, Spherisorb 50DS column (retention times; 21.5 min 5-HT, 16.7 min 5-HIAA).

Separation of catecholamines was achieved by reverse phase ion-pair HPLC on a 25 cm × 4.6 mm Sperisorb 50DS2 column [24]. The mobile phase was 35 mM citric acid, 12.5 mM Na_2HPO_4, 0.25 mM sodium octyl sulphate, 0.05 mM EDTA containing 6% methanol (v/v), pH 3.5 and the flow rate was set at 0.7 ml/min. Catecholamines were extracted with alumina [25] prior to HPLC analysis in order to remove exogenous histamine which was electrochemically active at high concentrations and interfered with the analysis of noradrenaline. This process also removed 5-HT and 5-HIAA which were otherwise retained on the reverse phase column for over an hour. Peak areas were corrected for recovery on the alumina extraction using the internal standard dihydroxybenzylamine (DHBA). Verification of noradrenaline peaks was demonstrated by co-elution with authentic noradrenaline in this (retention time 7.6 min) and a second HPLC solvent system (0.1 M NaH_2PO_4, 1 mM EDTA, 1.6 mM sodium octyl sulphate, 8.5% methanol (v/v), pH 3.6, Sperisorb 50DS2 column, retention time 4.5 min).

Chemicals. Histamine dihydrochloride was obtained from BDH and Sigma. Gifts of impromidine trihydrochloride, cimetidine (both from Smith Kline & French), fluoxetine hydrochloride, nisoxetine hydrochloride (both from Eli Lilly) and imipramine hydrochloride (Courtin and Warner) are gratefully acknowledged. All other drugs and analytical chemicals were purchased from Sigma or Fisons Pharmaceuticals.

RESULTS

Histamine-stimulated release of endogenous amines

K^+ (35 mM) stimulated the release of both 5-HT and noradrenaline from slices of rat cerebral cortex.

Table 1. Effect of calcium-free media on the stimulated release of endogenous noradrenaline and 5-HT elicited by histamine and KCl

Addition	Stimulation of 5-HT or noradrenaline release in calcium-free medium (% of response in the presence of 2.5 mM calcium) Noradrenaline	(N)	5-HT	(N)
56 mM KCl	46.2 ± 7.8	(9)	33.9 ± 7.8	(9)
1 mM Histamine	81.5 ± 18.8†	(8)	86.4 ± 7.7*†	(8)

Values represent mean ± SE. The number of experiments (N) is given in parentheses. In each experiment six separate determinations were made of basal, KCl- and histamine-stimulated amine efflux.

* $P < 0.005$ compared with KCl-stimulated (analysis of variance).

† Not significantly different from the values obtained in the presence of calcium (paired *t*-test and Wilcoxon signed rank test).

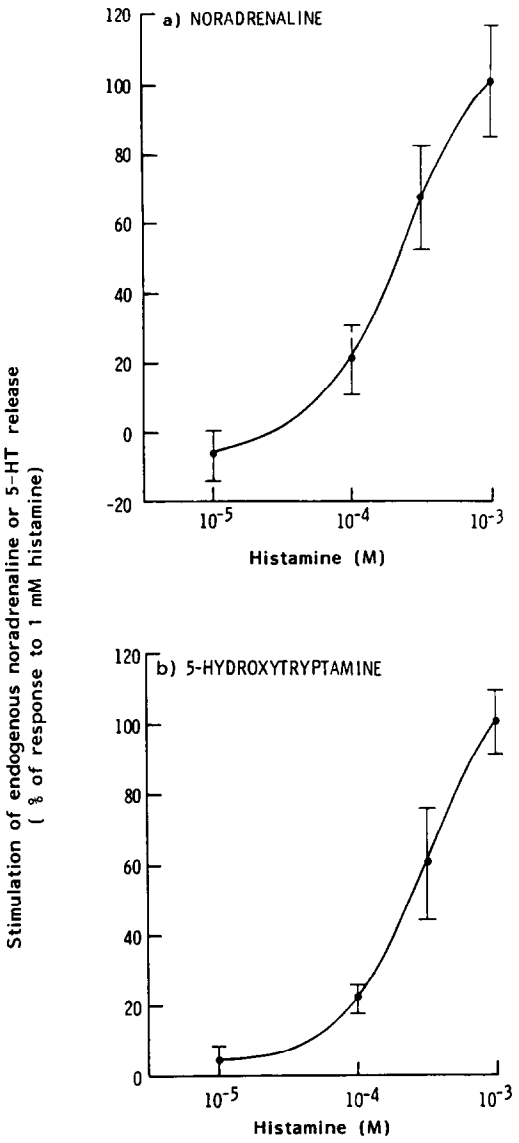

Fig. 1. Concentration–response curves for the release of endogenous noradrenaline (a) and 5-HT (b) from slices of rat cerebral cortex by histamine. Values represent the mean ± SE of six separate incubations made in each of six separate experiments. To normalise values from different slice preparations, values (stimulated minus basal) are expressed as the percentage of the stimulation produced by 1 mM histamine. Values for noradrenaline and 5-HT release were determined in supernatants from the same incubations.

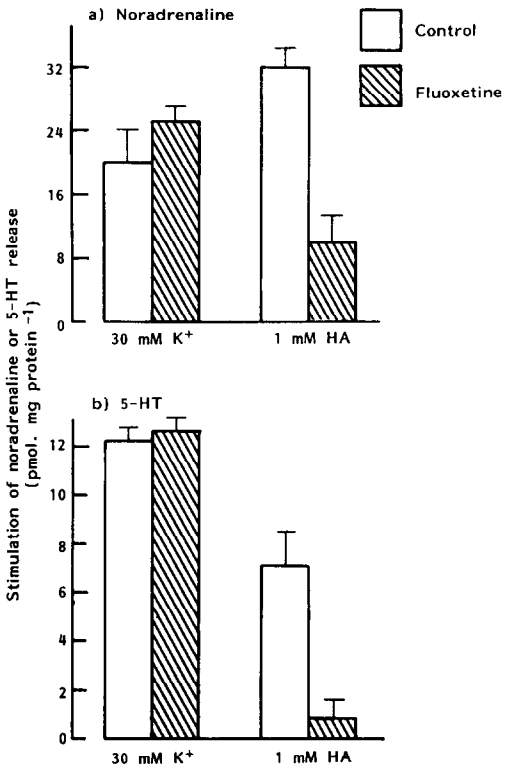

Fig. 2. Effect of the uptake-inhibitor fluoxetine (50 μM) on the histamine-induced release of endogenous noradrenaline (a) and 5-HT (b) from slices of rat cerebral cortex. Values represent mean ± SE of six separate incubations. Noradrenaline and 5-HT release were determined simultaneously. Each point represents the difference between the histamine- or KCl-stimulated and basal effluxes of the respective amine. The mean basal effluxes of noradrenaline and 5-HT in this experiment were 6.1 ± 1.9 and 6.4 ± 1.9 pmoles/mg protein respectively. In the presence of fluoxetine these were 5.0 ± 0.9 and 16.6 ± 1.4 pmoles/mg protein. Similar data were obtained in three other experiments.

The mean stimulation (stimulated minus basal) of the total outflow of these amines obtained in 24 experiments was 16.0 ± 1.7 and 18.3 ± 2.1 pmoles/mg protein for 5-HT and noradrenaline respectively. Histamine (1 mM) also stimulated the release of endogenous 5-HT (20.2 ± 1.8 pmoles/mg protein, 38 experiments) and noradrenaline (10.5 ± 1.5 pmoles/mg protein, 40 experiments). The basal effluxes of 5-HT and noradrenaline in these experiments were 5.9 ± 0.6 (N = 57) and 7.5 ± 0.7 (N = 68) pmoles/mg protein respectively. Concentration–response experiments indicated that a concentration of 0.1 mM histamine or greater was normally required for the stimulation of endogenous amine release (Fig. 1).

The calcium dependence of the K^+- and histamine-induced release of endogenous 5-HT and noradrenaline is illustrated in Table 1. The K^+-induced release of these amines was markedly reduced by 54–66% following removal of calcium from the incubation medium (Table 1). In contrast, the stimulation of 5-HT and noradrenaline release induced by histamine was much more resistant to removal of extracellular calcium (Table 1).

Effect of histamine-receptor antagonists

In order to determine whether the effect of histamine on the release of endogenous 5-HT and noradrenaline was a receptor-mediated process we have studied the effect of mepyramine, cimetidine and impromidine on this response. Neither the H_1-selective antagonist mepyramine (1 μM) nor the H_2-selec-

Table 2. Influence of histamine-receptor antagonists on the stimulation of noradrenaline and 5-HT release elicited by histamine

	Stimulation of noradrenaline or 5-HT release (pmoles/mg protein)			
	Noradrenaline		5-HT	
Addition	Control	+Antagonist	Control	+Antagonist
A. Mepyramine				
35 mM KCl	12.9 ± 2.3	12.3 ± 1.3	26.4 ± 6.2	21.6 ± 3.4
1 mM HA	9.6 ± 2.7	10.0 ± 0.9	29.2 ± 5.5	19.3 ± 2.8
B. Cimetidine				
35 mM KCl	5.4 ± 0.6	7.5 ± 2.4	22.1 ± 3.5	24.7 ± 4.3
1 mM HA	2.0 ± 0.6	3.6 ± 1.1	24.7 ± 6.9	22.9 ± 3.8
C. Impromidine				
35 mM KCl	16.6 ± 2.9	14.4 ± 1.4	25.5 ± 3.4	23.4 ± 4.2
1 mM HA	8.2 ± 2.8	6.0 ± 2.7	28.0 ± 3.8	25.5 ± 2.7

Mepyramine (1 μM), cimetidine (0.1 mM) or impromidine (4 μM) were added 30 min prior to the addition of potassium chloride or histamine (HA). Values represent mean ± SE of six separate incubations in a single experiment. Stimulations of amine release are expressed as the difference between agonist-stimulated and basal levels. The antagonists had no significant effect on basal amine levels. Analysis of variance showed that there was no significant attenuation of agonist-responses by any of the antagonists. Each experiment with a given antagonist was repeated twice more and similarly showed no significant effect on KCl- or HA-induced monoamine release.

tive antagonist cimetidine (0.1 mM) significantly altered the histamine-stimulated or basal efflux of 5-HT and noradrenaline (Table 2). Similarly, impromidine, which is both a potent H_2-agonist and a potent H_3-antagonist [10] did not alter the basal or agonist-stimulated efflux of these endogenous amines. These results suggest that the effect of histamine on the release of these amines is not mediated by any of the three known classes of histamine receptor.

Effect of catecholamine and indoleamine uptake inhibitors

Inclusion of the 5-HT uptake inhibitor fluoxetine (50 μM) in the incubation medium inhibited the stimulation of noradrenaline and 5-HT release elicited by histamine but was without effect on the response to 30 mM K^+ (Fig. 2). The effect of fluoxetine on K^+- and histamine-stimulated 5-HT release was accompanied by a large increase in the basal efflux of 5-HT from 6.4 ± 1.9 to 16.6 ± 1.4 pmoles/mg protein (N = 6 in each case). No change, however, was observed in the basal efflux of noradrenaline. Lower concentrations of fluoxetine also inhibited significantly ($P < 0.005$; Student *t*-test on differences between stimulated and basal) the response to histamine producing a 73% and 56% inhibition of the stimulation of 5-HT and noradrenaline release respectively (Table 3). A complete inhibition of both 5-HT and noradrenaline

Table 3. Effect of uptake inhibitors on the release of endogenous noradrenaline and 5-HT elicited by histamine (HA, 1 mM)

	Total outflow (pmoles/mg protein) of:			
	Noradrenaline		5-HT	
Inhibitor (μM)	Basal	HA	Basal	HA
A. Imipramine				
(0)	1.5 ± 0.5	9.1 ± 0.8*	3.3 ± 0.7	16.5 ± 1.7*
(10)	2.5 ± 0.6	2.1 ± 0.5†	8.8 ± 1.1	11.3 ± 0.7†
(50)	2.5 ± 0.4	2.0 ± 0.1†	13.0 ± 1.4	14.3 ± 0.6†
B. Fluoxetine				
(0)	1.7 ± 0.3	11.6 ± 0.5*	4.4 ± 0.6	36.5 ± 2.3*
(5)	1.9 ± 0.4	7.7 ± 0.4*	13.5 ± 1.1	28.2 ± 2.0*
(10)	2.5 ± 0.4	6.9 ± 0.3*	18.8 ± 1.3	27.6 ± 1.2*
C. Nisoxetine				
(0)	5.0 ± 0.8	15.5 ± 1.1*	7.9 ± 1.1	25.1 ± 1.7*
(1)	5.4 ± 0.9	5.3 ± 1.0†	10.0 ± 1.5	22.1 ± 1.9*
(10)	8.2 ± 0.7	6.4 ± 1.0†	14.9 ± 0.8	21.0 ± 1.8‡

Values represent mean ± SE of six separate incubations. Two to three other experiments gave similar results with each uptake inhibitor.

* $P < 0.005$.

† Not significant compared to basal (one-way analysis of variance)

‡ $P < 0.01$.

Table 4. Effect of histamine (HA) on the stimulation of noradrenaline and 5-HT release elicited by KCl

Addition	Stimulation of noradrenaline or 5-HT release (pmoles/mg protein): Noradrenaline	5-HT
Basal	1.3 ± 0.1	2.4 ± 0.2
35 mM KCl	7.8 ± 1.0	7.7 ± 1.1
$+10^{-9}$ M HA	7.7 ± 0.9	7.7 ± 1.2
$+10^{-8}$ M HA	7.8 ± 0.9	7.9 ± 0.7
$+10^{-7}$ M HA	8.1 ± 0.9	7.5 ± 0.8
$+10^{-6}$ M HA	7.7 ± 1.0	7.1 ± 0.9
$+10^{-5}$ M HA	8.4 ± 1.3	7.6 ± 1.2

Experiments were performed in low calcium (1.2 mM) medium in the presence of 50 μM pargyline and 10 μM imipramine as described under Methods. Values represent mean ± SE of five separate incubations. Four other experiments gave very similar results. Analysis of variance showed that there was no significant attenuation of KCl-stimulated release by histamine in all five experiments.

release was produced by 10 and 50 μM imipramine (Table 3). The selective noradrenaline uptake inhibitor nisoxetine, however, completely abolished the effect of histamine on noradrenaline release at concentrations at which a significant stimulation of 5-HT release was still observed (Table 3).

Effect of histamine on K^+-evoked release

Recent evidence has suggested that histamine H_3-receptors [10, 11] may be present on catecholamine-containing nerve terminals in the peripheral nervous system and inhibit the release of this amine [26]. In order to determine whether H_3-receptors are present on catecholamine and indoleamine nerve terminals and can inhibit amine release in the central nervous system, experiments were conducted in the presence of 10 μM imipramine to inhibit the releasing effect of histamine. In these experiments, the duration and strength of the depolarising stimulus and the external calcium concentration was also kept low to limit the extent of the inhibitory effects of endogenously released neurotransmitters [27]. However, histamine did not modify the K^+-evoked release of endogenous noradrenaline or 5-HT from slices of rat cerebral cortex (Table 4).

DISCUSSION

The results of this study show that histamine can produce a marked increase in the release of *endogenous* noradrenaline and 5-HT from slices of rat cerebral cortex. This confirms the observations made previously in studies of the release of *exogenous* [^{3}H]noradrenaline and [^{3}H]5-HT from pre-labelled brain slices and synaptosomes [18, 19]. Similar effects of histamine on [^{3}H]noradrenaline release have also been reported in peripheral tissues such as rabbit pulmonary artery [28], rat vas deferens [29] and cat cerebral arteries [30, 31].

Analysis of the concentration–response relationships indicated that histamine was only effective in releasing endogenous noradrenaline and 5-HT at high concentrations with an EC_{50} of *ca* 400 μM. This is somewhat larger than the values obtained for histamine on receptor-mediated biochemical responses such as adenylate cyclase activation (EC_{50} *ca* 100 μM) [9] and inositol phosphate accumulation (EC_{50} 20 μM [32] in rat brain, but amine release may contribute to these responses at higher agonist concentrations. Studies with antagonists of histamine H_1-, H_2- and H_3-receptors provided strong evidence that the amine-releasing effect of histamine was not mediated by one of the three established classes of histamine receptor. Addition of the H_1-selective antagonist mepyramine at concentrations (1 μM) three orders of magnitude higher than its dissociation constant for the H_1-receptor [33] was without significant effect on the histamine-induced release of 5-HT and noradrenaline. Similarly, the H_2-receptor antagonist cimetidine and the H_2-agonist/H_3-antagonist impromidine [10] did not attenuate the effect of histamine on amine release.

The release of catecholamines and indoleamines induced by 56 mM KCl was inhibited by the removal of calcium ions from the incubation medium but was not completely abolished. In our experiments 30–40% of the potassium-induced release of 5-HT and noradrenaline was resistant to calcium removal. Similar results with monoamine neurotransmitters have been obtained by other workers, particularly when high potassium concentrations have been used in conjunction with monoamine oxidase inhibitors [34–36]. The responses to histamine, however, were noticeably more resistant to calcium removal.

The effect of histamine on catecholamine and indoleamine release was significantly inhibited by the uptake inhibitors imipramine, fluoxetine and nisoxetine. In the case of the selective inhibitor of noradrenaline uptake nisoxetine, the fact that complete inhibition of the effect of histamine on noradrenaline release could be produced in the continued presence of a significant effect on 5-HT release (in the same brain slices) suggests that these two effects are not dependent upon one another. Furthermore, the results obtained with these inhibitors of uptake, taken together with the resistance of the histamine responses to calcium removal and histamine-receptor antagonists, suggest that histamine has a tyramine-like effect on the release of noradrenaline and 5-HT. A similar suggestion has

been put forward to account for the effect of histamine on catecholamine release in rabbit pulmonary artery [28]. It is uncertain, however, whether histamine can enter catecholamine and indoleamine nerve terminals in sufficient amounts to actively displace these neurotransmitters since it has recently been shown that [^{3}H]histamine uptake into rat brain slices is not significantly inhibited by the catecholamine and indoleamine neurotoxins 6-hydroxydopamine and 5,7-dihydroxytryptamine [37]. Furthermore, addition of the indirectly acting sympathomimetic tyramine did not enhance the efflux of [^{3}H]histamine from rat brain slices [18]. This suggests that histamine may only enter noradrenaline and 5-HT nerve terminals in small amounts via the monoamine uptake systems. However, these intraneuronal concentrations of histamine may be still sufficient to induce the release of monoamines by interacting with intracellular processes.

In the experiments described in this paper an incubation instead of a superfusion method was used in order to measure endogenous monoamines. Under these conditions reuptake of released amines will take place to a significant extent. Thus, an alternative explanation for a calcium-independent "release" of these neurotransmitters, which is sensitive to uptake inhibitors, is that histamine inhibits the reuptake of noradrenaline and 5-HT into nerve terminals and produces an increase in the levels of these amines in the extracellular medium. However, it has been shown previously [19] that histamine does not inhibit the uptake of [^{3}H]noradrenaline and [^{3}H]5-HT into rat brain synaptosomes at concentrations up to 1 mM. It is also notable in the present study that imipramine and nisoxetine can abolish the effect of histamine at concentrations which do not significantly change the basal accumulation of endogenous noradrenaline (Table 3). If uptake inhibition was primarily involved in the action of histamine, then uptake inhibitors would be expected to reduce the extent of the stimulation of release (total efflux minus basal efflux) by increasing the basal efflux of amines towards the efflux produced by histamine alone. The exact mechanism of the amine-releasing properties of histamine therefore remains to be established but the present study suggests that the high affinity carrier systems for 5-HT and noradrenaline are intimately involved.

In the peripheral nervous system histamine H_3-receptors [10, 11] have been detected on sympathetic nerve terminals. Our preliminary studies of the effect of histamine on the potassium-evoked release of endogenous noradrenaline and 5-HT in rat cerebral cortex, however, failed to detect any inhibitory action even when imipramine was included in the incubation media to inhibit histamine-evoked release. Although the experimental conditions were designed to limit the extent of interference from endogenously released neurotransmitters, superfusion experiments will need to be performed to rule out the possibility of H_3-receptors on catecholamine and indoleamine nerve terminals in rat brain.

It is unlikely that the stimulation of noradrenaline and 5-HT release elicited by histamine is involved in neurotransmission in the mammalian central nervous system, although local synaptic concentrations of histamine as high as 0.1–1 mM cannot be totally ruled out when one considers that the concentration of neurotransmitters, such as acetylcholine, in storage vesicles is *ca* 100 mM [38]. Nevertheless, the high concentrations of histamine required for this response will almost certainly be achieved in the local environment of degranulating mast cells following anaphylactic release of histamine from brain mast cells [38–40]. Thus, the amine-releasing properties of histamine may be very relevant to the consequences of immune reactions involving brain mast cells, where histamine is an important mediator. Furthermore, the possibility of the release of brain noradrenaline and 5-HT will need to be borne in mind in behavioural studies utilising intracerebral application of histamine where large doses of histamine (e.g. 10 μg in 2 μl [41]) are injected into discrete brain regions and where it is not possible to achieve a known concentration of the pharmacological agent a the site of action. For example, it has been suggested that 5-HT released from the hypothalamus by intraventricular injection of histamine participates in the hypothermic action of exogenous histamine in rats [42]. It is therefore possible that non-receptor-mediated catecholamine and indoleamine release contributes to some of the observed behavioural responses to centrally administered histamine.

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REFERENCES

1. Schwartz JC, Arrang JM and Garbarg M, Histamine as a neurotransmitter in the mammalian brain *J Neurochem* **35**: 26–33, 1978.
2. Pollard H and Schwartz JC, Histamine neuronal pathways and their functions. *Trends Neurosci* **10**: 86–89, 1987.
3. Prell GD and Green JP, Histamine as a neuroregulator. *Ann Rev Neurosci* **9**: 209–254, 1986.
4. Schwartz JC, Arrang JM and Garbarg M, Three classes of histamine receptor in brain. *Trends Pharmacol Sci.* **8**: 24–28, 1986.
5. Hill SJ, Histamine receptors branch out. *Nature (Lond)* **327**: 104–105, 1987.
6. Daum PR, Downes CP and Young JM, Histamine-stimulation of inositol-1-phosphate accumulation in lithium treated slices from regions of guinea-pig brain. *J Neurochem* **43**: 25–32, 1984.
7. Donaldson J and Hill SJ, Histamine-induced hydrolysis of polyphosphoinositides in guinea-pig ileum and brain. *Eur J Pharmacol* **124**: 255–265, 1986.
8. Hegstrand LR, Kanof PD and Greengard P, Histamine-sensitive adenylate cyclase in mammalian brain. *Nature (Lond)* **260**: 163–165, 1976.
9. Green JP, Johnson CL, Weinstein H and Maayani S, Antagonism of histamine-activated adenylate cyclase in brain by D-lysergic acid diethylamide. *Proc Natl Acad Sci USA* **74**: 5697–5701, 1977.
10. Arrang JM, Garbarg M and Schwartz JC, Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature (Lond)* **302**: 832–837, 1983.
11. Arrang JM, Garbarg M, Lancelot JC, Lecomte JM, Pollard H and Schwartz JC, Highly potent and selective ligands for histamine H_3-receptors. *Nature (Lond)* **327**: 117–122, 1987.

12. Hill SJ, Histamine receptors in the mammalian central nervous system: Biochemical studies. In: *Progress in Medicinal Chemistry*, Vol. 24 (Eds. Ellis G and West GB), pp. 29–84. Elsevier, Amsterdam, 1987.
13. Kalivas PW, Histamine-induced arousal in the conscious and pentobarbitone-pretreated rat. *J Pharmacol Exp Ther* **222**: 37–42, 1982.
14. Shaw GG, Hypothermia produced in mice by histamine acting on the central nervous system. *Br J Pharmacol* **42**: 205–214, 1971.
15. Lomax P and Green MD, Histamine and temperature regulation. In: *Temperature Regulation and Drug Action.* (Eds Lomax P, Schonbaum E and Jacob J), pp. 85–93. Karger, Basel, 1975.
16. Sinha NL, Gupta ML and Bhargava KP, Effect of histamine and antihistamines on central vasomotor loci. *Eur J Pharmacol* **5**: 235–238, 1969.
17. Finch L and Hicks PE, The cardiovascular effects of intraventricularly administered histamine in the anaesthetised rat. *Naunyn-Schmiedebergs Arch Pharmacol* **293**: 151–157, 1976.
18. Subramanian N and Mulder AH, Modulation by histamine of the efflux of radiolabelled catecholamines from rat brain slices. *Eur J Pharmacol* **43**: 143–152, 1977.
19. Tuomisto J and Tuomisto L, Effects of histamine and histamine antagonists on the uptake and release of catecholamines and 5-HT in brain synaptosomes. *Med Biol* **58**: 33–37, 1980.
20. Hurdon H, Strupish J and Nahorski SR, Differences between the release of radiolabelled and endogenous dopamine from superfused rat brain slices: Effects of depolarising stimuli, amphetamine and synthesis inhibition. *Brain Res* **348**: 309–320, 1985.
21. Crozier CS, Hill SJ, Jenner AJ and Mason R, Effect of histamine on the release of endogenous noradrenaline and 5-hydroxytryptamine from rat cerebral cortical slices. *Br J Pharmacol* **88**: 327P, 1986.
22. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
23. Marsden CA and Joseph MA, Biogenic amines, In: *HPLC of Small Molecules. A Practical Approach* (Ed. Lim CK), pp. 29–47. IRL Press, Oxford, 1986.
24. Kim C, Campanelli C and Khanna JM, Determination of picogram levels of brain catecholamines and indoles by a simplified liquid chromatographic electrochemical detection method. *J Chromatogr* **282**: 151–159, 1983.
25. Reynolds GP, Increased concentrations and lateral asymmetry of amygdala dopamine in schizophrenia. *Nature (Lond)* **305**: 527–528, 1983.
26. Ishikawa S and Sperelakis N, A novel class (H_3) of histamine receptors on perivascular nerve terminals. *Nature (Lond)* **327**: 158–160, 1987.
27. Arrang JM, Garbarg M and Schwartz JC, Auto-regulation of histamine release in brain by presynaptic H_3-receptors. *Neuroscience* **15**: 553–562, 1985.
28. Starke K and Weitzel R, Is histamine involved in the sympathomimetic effect of nicotine? *Nauyn-Schmiedebergs Arch Pharmacol* **304**: 237–248, 1978.
29. MacIntosh CM and Vohra MM, Effect of histamine and H_1 and H_2 agonists on the fractional outflow of radioactivity from the rat vas deferens preloaded with ^{3}H-noradrenaline. *Life Sci* **31**: 2819–2826, 1982.
30. Marco EJ, Balfagon G, Marin J, Gomez B and Lluch S, Indirect adrenergic effect of histamine in cat cerebral arteries. *Nauyn-Schmiedebergs Arch Pharmacol* **312**: 239–243, 1980.
31. Balfagon G, Galvan R and Marco EJ, Mechanism of the indirect adrenergic effect of histamine in cat cerebral arteries. *J Pharm Pharmacol* **36**: 248–252, 1984.
32. Brown E, Kendall DA and Nahorski SR, Inositol phospholipid hydrolysis in rat cerebral cortical slices: 1. Receptor characterisation. *J Neurochem* **42**: 1379–1387, 1984.
33. Hill SJ, Emson PC and Young JM, The binding of ^{3}H-mepyramine to histamine H_1-receptors in guinea-pig brain. *J Neurochem* **31**: 997–1004, 1978.
34. Hery M, Faudon M and Hery F, In vitro release of newly synthesised serotonin from superfused rat suprachisamatic area: ionic dependency, *Eur J Pharmacol* **89**: 9–18, 1983.
35. Mulder AH, Smits RPJM and Steinbusch HWM, Neuronal uptake and release of histamine in the central nervous system. In: *Advances in the Biosciences* Vol. 15: *Frontiers in Histamine Research* (Eds Ganellin CR and Schwartz JC), pp. 155–168. Pergamon Press, Oxford, 1985.
36. Collard KJ and Evans SM, The effect of monoamine oxidase inhibitors on the calcium-dependency of 5-HT release from rat brain synaptosomes. *Br J Pharmacol* **90**: 86P, 1987.
37. Mulder AH, Van Den Berg WR and Stoof JC, Calcium-dependent release of radiolabelled catecholamines and serotonin from rat brain synaptosomes in a superfusion system. *Brain Res* **99**: 419–424, 1975.
38. Rang HP and Dale MM, *Pharmacology*. Churchill Livingston, Edinburgh, 1987.
39. Ibrahim MZM, Al-Wirr ME and Bahuth N, The mast cells of the mammalian central nervous system. III Ultra structural characteristics in the adult rat brain. *Acta Anat* **104**: 134–154, 1979.
40. Sturrock RR, A morphological life span study of neurolipomastocytes in various brain regions of the mouse forebrain. *Neuropath Appl Neurobiol* **6**: 211–219, 1980.
41. Bristow L and Bennett GW, Micro injection of histamine into the nucleus accumbens alters behavioural activity in rats. *Br J Pharmacol* **91**: 343P, 1987.
42. Pilc A and Novak JZ, A possible serotinergic link in the hypothermic action of histamine. *Agents Actions* **9**: 87–88, 1979.