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α_2-Adrenoceptor-mediated inhibition of histamine release from rat cerebral cortical slices

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1 Depolarization of rat cerebral cortical slices, prelabelled with [^{3}H]-histidine, in high potassium (40 mM KCl) medium stimulated the release of [^{3}H]-histamine. The K^+-evoked release of [^{3}H]-histamine was attenuated by incubation in calcium-free medium and prevented by prior incubation of brain slices with the selective histidine decarboxylase inhibitor S-(α)-fluoromethylhistidine.

2 The K^+-evoked release of [^{3}H]-histamine was significantly ($P < 0.001$) reduced following stimulation of histamine H_3-receptors with R-(α)-methylhistamine (1 μM) and this effect was antagonized by the H_3-antagonist thioperamide (1 μM).

3 Noradrenaline and the α_2-selective adrenoceptor agonists clonidine and UK-14,304 inhibited the K^+-evoked release of [^{3}H]-histamine in a concentration-dependent manner yielding EC_{50} values of 2.5, 0.8 and 1.2 μM, respectively. However, the maximum response to clonidine was only 52 ± 8% of that obtained with noradrenaline.

4 The inhibitory effect of noradrenaline was antagonized by the non-selective α-antagonist phentolamine and by the selective α_2-antagonists yohimbine and idazoxan. However, the response to noradrenaline was not inhibited by the α_1-antagonist prazosin at concentrations up to 1 μM.

5 These results suggest that both histamine H_3-receptors and α_2-adrenoceptors are present on histamine-containing nerve terminals in rat cerebral cortex and can exert an inhibitory influence on neurotransmitter release.

Introduction

There is now strong evidence to suggest that histamine may have a role in the mammalian CNS as a neurotransmitter or neuromodulator (Schwartz *et al.*, 1986b). Immunohistochemical studies, using antibodies raised against both histamine and its synthetic enzyme histidine decarboxylase, have shown in rat brain that histamine-containing cell bodies are restricted to various magnocellular nuclei in the posterior hypothalamus and mammillary body but send diffuse projections to large areas of cerebral cortex, hippocampus, striatum and amygdala (Steinbusch *et al.*, 1986; Pollard & Schwartz, 1987). This pattern of a widespread ascending innervation from a localised cell body region is also observed with other monoamine neurotransmitters such as noradrenaline and 5-hydroxytryptamine (Cooper *et al.*, 1986). This distribution, together with the well established sedative actions of histamine H_1-receptor antagonists (Faingold, 1966), is suggestive of a role for histamine in arousal mechanisms (Kalivas, 1982).

Three different histamine-receptor subtypes have been identified in the mammalian CNS (Schwartz *et al.*, 1986a; Hill, 1987). Histamine H_1- and H_2-receptors are coupled to inositol phospholipid hydrolysis and adenylate cyclase activity respectively (Hegstrand *et al.*, 1976; Daum *et al.*, 1984; Hill, 1987), whilst a novel histamine H_3-receptor appears to act as an autoreceptor and controls the synthesis and release of histamine from histamine-containing nerve endings in rat cerebral cortex (Arrang *et al.*, 1983; 1987a,b; Van der Werf *et al.*, 1987). Recently, agonists and antagonists have been developed which are highly selective for the H_3-receptor controlling histamine release (Arrang *et al.*, 1987a). The present study was undertaken to investigate whether noradrenaline, which is also contained in fibres projecting to the cerebral cortex (Cooper *et al.*, 1986), can modulate the neuronal release of histamine. A preliminary account of this work has been presented to the British Pharmacological Society (Hill & Young, 1988).

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Methods

Measurement of [^{3}H]-*histamine release*

Cerebral cortical slices (300 × 300 μm) from two rats (Wistar males, 200–300 g) were prepared with a

McIlwain tissue slicer and washed three times with 50 ml of a modified Krebs-medium (composition in mM: NaCl 120, KCl 2, $CaCl_2$ 2.6, $MgSO_4$ 0.7, KH_2PO_4 1.2, $NaHCO_3$ 27.5, glucose 10; pH 7.5, gassed with 95% O_2/5% CO_2). Slices were then transferred to 25 ml of a depolarizing Krebs medium containing 50 mM KCl and incubated at 37°C under an atmosphere of 95% O_2/5% CO_2 for 3 periods of 5 min (medium changed after each incubation), in order to stimluate both the release of endogenous histamine and its synthesis from histidine. Following this preincubation period the slices were rapidly washed in Krebs medium containing 2 mM KCl and resuspended in 3 ml of Krebs medium containing 100 μCi [^{3}H]-histidine before incubation under an atmosphere of 95% O_2/5% CO_2 at 37°C. After 40 min the slices were washed three times with 25 ml Krebs medium and allowed to settle under gravity. Fifty μl aliquots of the gravity packed slice suspension were then added to 410 μl of Krebs medium, containing antagonist drug where appropriate, in 1.5 ml microfuge tubes. The tubes were gassed with 95% O_2/5% CO_2, capped and incubated at 37°C for 20 min. Agonist drugs were then added in 20 μl of medium and the incubation continued for a further 7 min before the addition of KCl (in 20 μl, final concentration 40 mM). Incubations were terminated 7 min later by rapid centrifugation at 12,000 g (30 s) in a Beckman microfuge 2.

Samples (400 μl) of the supernatant were diluted to 3 ml with 10 mM Tris buffer, pH 8.0, and [^{3}H]-histamine was separated from [^{3}H]-histidine by ion-exchange chromatography essentially as described by Arrang *et al.* (1983). Briefly, the sample was added to ECONO columns containing 0.5 ml Amberlite CG50 resin (applied as 1 ml of a 50:50 slurry in 10 mM Tris HCl, pH 8.0) which had been equilibrated with 10 ml of 10 mM Tris HCl pH 8.0. The columns were then washed sequentially with 40 ml 10 mM Tris HCl pH 8.0 and 20 ml 0.02 M HCl to remove [^{3}H]-histidine. [^{3}H]-histamine was finally eluted in 3 ml of 1 M HCl and tritium determined by liquid scintillation counting. Recovery of [^{3}H]-histamine was 50–70% in these assays.

In some experiments the pellets remaining in the microfuge tubes were homogenised in 0.02 M HCl for the determination of the [^{3}H]-histamine content of the slices. The microfuge tubes were then centrifuged at 12,000 g for 5 min and 200 μl aliquots of the supernatant were diluted to 3 ml with 10 mM Tris HCl pH 8.0 before separation of [^{3}H]-histamine by ion-exchange chromatography as described above.

Preincubation with S(α)-fluoromethylhistidine

In some experiments the slice suspension was divided into two portions immediately after the preincubation in depolarizing medium. Slices were then incubated in the presence or absence of 20 μM S(α)-fluoromethylhistidine for 20 min before the addition of [^{3}H]-histidine and the experiment continued as described above. S(α)-fluoromethylhistidine was also included in the final incubations of those slices which had been exposed to the histidine decarboxylase inhibitor during the labelling period with [^{3}H]-histidine.

Authentication of [^{3}H]-histamine

The tritiated material released from rat cerebral cortical slices in the presence of 40 mM KCl was identified as [^{3}H]-histamine by enzymatic transformation to chloroform-extractable N^τ-methylhistamine under the action of histamine-N-methyltransferase prepared from rat kidney as described by Shaff & Beavan (1979). Aliquots (450 μl) of the supernatant samples were incubated with 100 μl of the diluted rat kidney histamine-N-methyltransferase enzyme (1: 2 v/v in 100 mM sodium phosphate buffer pH 7.8) in the presence of 8 μM S-adenosyl-methionine for 60 min at 37°C. The reaction was stopped with 200 μl 10 M NaOH and N^τ-methylhistamine extracted with 3 ml chloroform. After brief centrifugation 600 μl of the aqueous supernatant was collected and buffered to pH 8 before separation of [^{3}H]-histamine by ion-exchange chromatography on Aberlite CG50 resin. Authentic [^{3}H]-histamine (34,000 d.p.m.) was included in parallel incubations for comparative purposes. Under these conditions 87% of the authentic [^{3}H]-histamine and 83% of the released [^{3}H]-histamine was removed by this procedure. No significant difference was observed in the recoveries of authentic and released [^{3}H]-histamine in samples which were extracted with chloroform in the presence of 200 μl 10 M NaOH but not incubated with histamine-N-methyltransferase.

Data analysis

The concentration of agonist (EC_{50}) producing a half-maximal effect on [^{3}H]-histamine release was obtained from visual inspection of dose-response curves. Dissociation constants of antagonists were determined from parallel shifts of the dose-response curve for noradrenaline using the relationship K_D = A/(Dose-ratio − 1) where A is the antagonist concentration and the dose-ratio is the ratio of the concentration of noradrenaline necessary to give a specified response in the presence of antagonist to the concentration of agonist required for the same response in the absence of antagonist.

Statistical analysis of the data was performed by one-way analysis of variance. Each experiment was

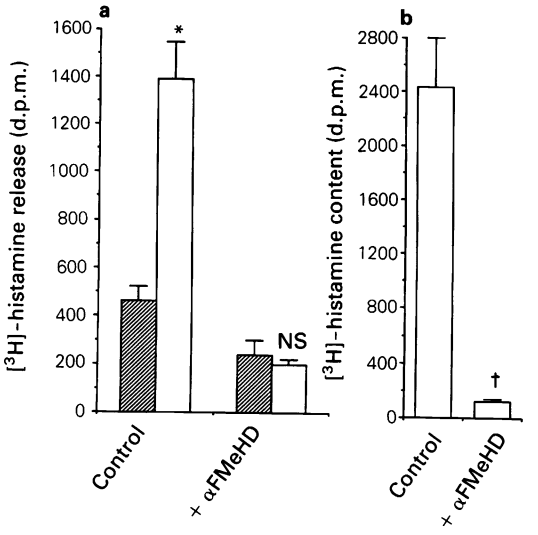

Figure 1 The effect of S(α)-fluoromethylhistidine (αFMeHD, 20 μM) on (a) the release and (b) the slice content of [^{3}H]-histamine. In (a) the [^{3}H]-histamine released into the medium was determined in the presence (open columns) and absence (hatched columns) of 40 mM KCl. Values represent mean and bars s.e.mean of quadruplicate determinations in a single experiment. * $P < 0.001$ or not significant (NS) compared to the release of [^{3}H]-histamine in the absence of 40 mM KCl. † The slice [^{3}H]-histamine content was significantly reduced in the presence of 20 μM αFMeHD ($P < 0.001$). Similar results were obtained in a second experiment.

performed at least three times. Values are expressed as mean ± s.e.mean and n in the text refers to the number of separate experiments.

Chemicals

L-[2,5-^{3}H]-histidine (60 Ci mmol^{-1}) and 2,5-[^{3}H]-histamine dihydrochloride (10 Ci mmol^{-1}) were purchased from Amersham International. Immediately before use [^{3}H]-histidine was passed through a small column (0.2 ml) of Amberlite CG50 resin in order to remove any [^{3}H]-histamine or [^{3}H]-N$^\tau$-methylhistamine contaminents. (−)-Noradrenaline bitartrate, (±)-propranolol hydrochloride, phentolamine hydrochloride, clonidine hydrochloride and yohimbine hydrochloride were obtained from Sigma. Gifts of idazoxan (Reckitt & Colman), prazosin, UK-14, 304 tartrate (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline) (both from Pfizer), S(α)-fluoromethylhistidine (Merck, Sharp & Dohme), R(α)-methylhistamine dihydrochloride and thioperamide (Prof. J.-C. Schwartz, Paris) are gratefully acknowledged.

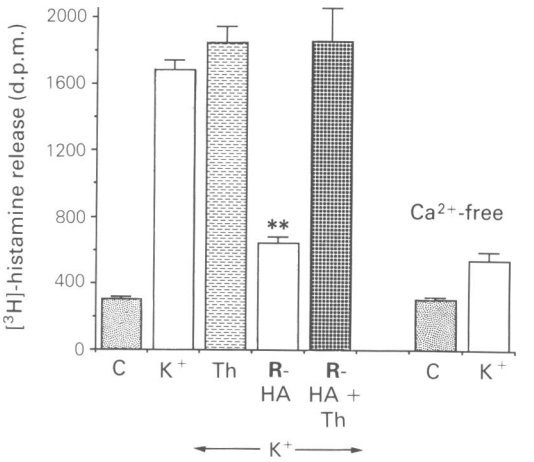

Figure 2 Effect of R-α-methylhistamine (R-HA, 1 μM) and thioperamide (Th, 1 μM) on the K$^+$-evoked release of [^{3}H]-histamine from slices of rat cerebral cortex. In two conditions the basal (C) and K$^+$-evoked release of [^{3}H]-histamine was measured in calcium-free Krebs medium containing 0.5 mM EGTA. Values represent mean and bars s.e.mean of quadruplicate determinations. ** $P < 0.001$ compared to the data obtained in the presence of 40 mM KCl alone. Similar results were obtained in two further experiments.

Results

K$^+$-evoked release of [^{3}H]-histamine

Depolarization of rat cerebral cortical slices with 40 mM KCl produced a 4.6 ± 0.2 fold ($n = 52$) increase in the release of [^{3}H]-histamine into the medium (Figure 1). Preincubation of slices with S(α)-fluoromethylhistidine (20 μM), a selective inhibitor of histidine decarboxylase (Kollonitsch *et al.*, 1978), significantly reduced ($P < 0.001$) the slice content of [^{3}H]-histamine and completely prevented the K$^+$-evoked release of [^{3}H]-histamine from slices of rat cerebral cortex (Figure 1). Incubation of slices in calcium-free medium containing 0.5 mM EGTA markedly reduced the K$^+$-evoked release of [^{3}H]-histamine by 81 ± 3% ($n = 3$) (Figure 2). EGTA was included in the incubation buffer in order to chelate the calcium that was present in the 50 μl slice aliquot. The selective H_3-receptor agonist R(α)-methylhistamine (Arrang *et al.*, 1987a) significantly inhibited ($P < 0.001$) the efflux of [^{3}H]-histamine produced by 40 mM KCl (Figure 2). The mean percentage inhibition produced by 1 μM R(α)-methylhistamine in four experiments was 74 ± 1%. This response to R(α)-methylhistamine was completely prevented by preincubation of brain slices in the presence of the selective H_3-antagonist thioperamide (Arrang *et al.*, 1987a) (Figure 2).

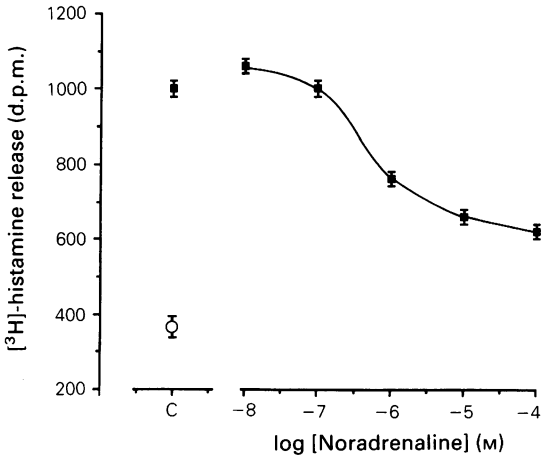

Figure 3 Inhibition of the K^+-evoked release of [^{3}H]-histamine by noradrenaline. (○) The basal release of [^{3}H]-histamine; (■) K^+-evoked (40 mM KCl) release of [^{3}H]-histamine in the absence (C) or presence of increasing concentrations of noradrenaline. Values represent mean of quandruplicate determinations; vertical lines indicate s.e.mean. Similar results were obtained in eighteen other experiments (Table 1).

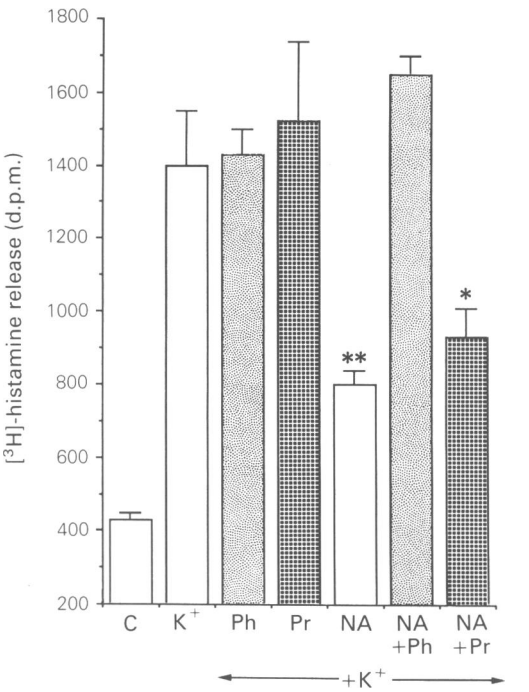

Figure 4 Effect of phentolamine and propranolol on the response to noradrenaline. Data were obtained in the presence of 40 mM KCl and: 2 μM phentolamine (Ph); 2 μM propranolol (Pr); 20 μM noradrenaline (NA); 2 μM phentolamine + 20 μM noradrenaline (NA + Ph) or 2 μM propranolol + 20 μM noradrenaline (NA + Pr). The basal response in the absence of 40 mM KCl is shown by column C. Values represent mean of quadruplicate determinations in a single experiment; bars show s.e.mean. ** $P < 0.001$ or * $P < 0.01$ with respect to the values obtained in the presence of 40 mM KCl alone. Similar results were obtained in two further experiments.

Inhibition of [^{3}H]-histamine release by noradrenaline

The K^+-evoked release of [^{3}H]-histamine was inhibited by increasing concentrations of noradrenaline (Figure 3). The EC_{50} for noradrenaline was 2.6 ± 0.4 μM ($n = 19$) and the maximal inhibition produced by noradrenaline was $69.4 \pm 2.2\%$ ($n = 19$) of the K^+-stimulated release of [^{3}H]-histamine. The response to noradrenaline (20 μM) was antagonized by the α-adrenoceptor antagonist phentolamine (2 μM) but not by the β-adrenoceptor antagonist propranolol (2 μM; Figure 4). The response to 40 μM noradrenaline was also completely abolished by 2 μM yohimbine (data not shown, $n = 3$) suggesting the involvement of α_2-adrenoceptors.

Characterization of the α_2-adrenoceptor response

The α_2-selective agonists clonidine and UK-14,304 (Cambridge, 1981) also inhibited the K^+-evoked release of [^{3}H]-histamine from slices of rat cerebral cortex (Table 1, Figure 5). Both compounds were effective over the same concentration range, yielding EC_{50} values similar to that obtained with noradrenaline (Table 1). However, a striking feature of the data obtained with these two agonists was that they appeared to have very different agonist efficacies, as judged by the maximal inhibitory responses (Table 1) obtained with each agonist rela-

Table 1 Dose-response parameters for the inhibition of [^{3}H]-histamine release by α_2-adrenoceptor-agonists

Agonist	*EC_{50} value* (μM)	B_{max} (%)*	(n)
Noradrenaline	2.5 ± 0.4	100	(19)
Clonidine	0.8 ± 0.4	52 ± 8	(5)
UK-14,304	1.2 ± 0.5	113 ± 9	(4)

Values represent means ± s.e.mean. *The maximum response obtained with a given agonist is expressed as a percentage of the response to 0.1 mM noradrenaline which was measured in each experiment. The number of individual experiments is given in parentheses.

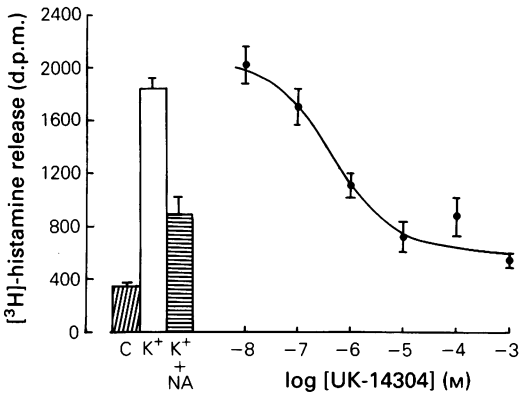

Figure 5 Concentration-response curve for UK-14,304 (●) obtained from inhibition of the K^+-evoked release of histamine. The columns show the basal (C) and K^+-evoked release of [^{3}H]-histamine obtained in the absence (K^+) and presence of 0.1 mM noradrenaline (K^+ + NA). Measurements of the effect of UK-14,304 were obtained in the presence of 40 mM KCl as described under Methods. Values represent mean of five replicate determinations; vertical lines indicate s.e.mean. Similar results were obtained in three other experiments (Table 1).

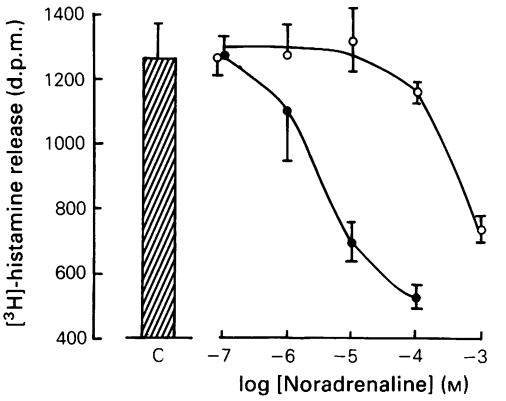

Figure 6 The effect of idazoxan on the concentration-response curve for noradrenaline. Data were obtained in the presence (○) or absence (●) of 1 μM idazoxan. All incubations contained 40 mM KCl during the final 7 min incubation. The column (C) shows the release of [^{3}H]-histamine obtained in the presence of 40 mM KCl alone. Values represent mean of quadruplicate determinations; vertical lines indicate s.e.mean.

tive to the response to 0.1 mM noradrenaline which was measured in every experiment (Figure 5).

Table 2 shows the equilibrium dissociation constants obtained for four representative α-adrenoceptor antagonists from inhibition of the response to noradrenaline. It can be seen that phentolamine and the two α_2-selective antagonists, yohimbine and idazoxan, are potent inhibitors of the effect of noradrenaline on [^{3}H]-histamine release. The effect of idazoxan (1 μM) on the dose-response curve for noradrenaline is illustrated in Figure 6. In contrast, the α_1-selective antagonist prazosin produced no significant dextral displacement of the dose-response curve for noradrenaline at concentrations up to 1 μM (Table 2).

Discussion

The results presented here confirm the earlier observations of Arrang *et al.* (1983, 1987a) that depolarization in high potassium medium can elicit a calcium-dependent release of [^{3}H]-histamine from slices of rat cerebral cortex, which have been prelabelled with [^{3}H]-histidine. Previous studies have shown that histamine is localised in both neuronal and non-neuronal (probably mast cells) pools in rat brain (Garbarg *et al.*, 1976). This raises the possibility that the observed release of histamine may be from non-neuronal cells. However, histidine decarboxylase activity is almost exclusively localised in histamine-containing neurones (Baudry *et al.*, 1973) and inhibition of histidine decarboxylase has been shown to deplete only the neuronal histamine pool

Table 2 Equilibrium dissociation constants for α_2-adrenoceptor antagonists obtained from inhibition of the effect of noradrenaline on [^{3}H]-histamine release in rat cerebral cortex

Antagonist	[^{3}H]-*histamine release* K_D (μM)	(n)	[^{3}H]-*idazoxan binding* K_D (μM)[a]
Phentolamine	0.017 ± 0.006	(3)	0.040
Yohimbine	0.024 ± 0.009	(4)	0.046
Idazoxan	0.033 ± 0.015	(5)	0.012
Prazosin	> 1.0*	(4)	1.6

Values represent mean ± s.e.mean. The number of separate experiments is given in parentheses. * No significant dextral displacement of the dose-response curve for noradrenaline was obtained with concentrations up to 1 μM. [a] Values taken from Nasseri & Minneman (1987).

without any effect on the non-neuronal compartments of histamine (Taylor & Snyder, 1972; Verdiere *et al.*, 1977; Garbarg *et al.*, 1980). Thus, the fact that S(α)-fluoromethylhistidine, which is a specific and irreversible inhibitor of histidine decarboxylase (Kollonitsch *et al.*, 1978; Garbarg *et al.*, 1980), can completely attenuate the synthesis and release of [^{3}H]-histamine from cerebral cortical slices provides strong evidence that neuronal histamine release is being measured.

The presence of histamine H_3-autoreceptors on histaminergic nerve terminals in rat cerebral cortex was confirmed with the use of the selective H_3-receptor ligands thioperamide and R(α)-methylhistamine (Arrang *et al.*, 1987a). However, in addition the results presented in this paper provide strong evidence that histamine release can be regulated by stimulation of receptors for other cerebral cortical neurotransmitters. Thus, noradrenaline, which is contained in neurones projecting from the locus coeruleus (Cooper *et al.*, 1986), is able to inhibit potently the efflux of [^{3}H]-histamine from rat cortical slices. This effect of noradrenaline was attenuated by the α-antagonist phentolamine but not by the β-antagonist propranolol indicating the involvement of α-adrenoceptors.

Studies with selective ligands for the two α-adrenoceptor subtypes provide strong evidence that the inhibitory control of [^{3}H]-histamine release is mediated by α_2-adrenoceptors. Thus, the α_2-selective antagonists yohimbine and idazoxan (Chapleo *et al.*, 1981; Nasseri & Minneman, 1987) are potent inhibitors of the noradrenaline response while the α_1-selective antagonist prazosin is ineffective at concentrations up to 1 μM. The dissociation constants obtained for the α-antagonists for inhibition of the effect of noradrenaline on [^{3}H]-histamine release were similar to those obtained from inhibition of [^{3}H]-idazoxan binding or from studies of the α_2-mediated inhibition of [^{3}H]-noradrenaline release in rat cerebral cortex (Nasseri & Minneman, 1987).

The effect of noradrenaline on [^{3}H]-histamine release was mimicked by the selective α_2-agonists clonidine and UK-14,304 (Cambridge, 1981). However, it was notable that, whereas UK-14,304 appeared to be a full agonist in this system (maximum inhibition = 113 ± 9% of response to 0.1 mM noradrenaline), clonidine produced a maximum response which was only 52 ± 8% of that produced by noradrenaline. These data suggest that clonidine is a low efficacy agonist at presynaptic α_2-receptors on histamine-containing nerve terminals in rat cerebral cortex. A similar low efficacy of clonidine was observed in studies of [^{3}H]-noradrenaline release from noradrenergic nerve terminals in rat cerebral cortical slices (Nasseri & Minneman, 1987).

One of the most common behavioural effects of drugs such as clonidine is sedation (Drew *et al.*, 1979). Studies with selective α_1- and α_2-adrenoceptor agonists and antagonists suggest that the sedation produced by clonidine is mediated by stimulation of central α_2-adrenoceptors (Drew *et al.*, 1979). In this respect it is interesting that neuronal histamine has been implicated in the control of arousal mechanisms (Schwartz, 1977; Kallivas, 1982). The results of the present study therefore raise the intriguing possibility that the inhibitory effect of α_2-agonists on the neuronal release of histamine in rat cerebral cortex may contribute to the sedative effect of these compounds.

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