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H_2 receptor-mediated facilitation and H_3 receptor-mediated inhibition of noradrenaline release in the guinea-pig brain

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Abstract The effect of histamine and related drugs on the tritium overflow evoked electrically (0.3 Hz) or by introduction of Ca^{2+} ions into Ca^{2+}-free K^+-rich (25 mmol/l) medium containing tetrodotoxin was studied in superfused guinea-pig brain cortex, cerebellum, hippocampus or hypothalamus slices and in mouse brain cortex slices preincubated with ^{3}H-noradrenaline.

The electrically evoked tritium overflow in *guinea-pig* cortex slices was inhibited by histamine; the H_3 receptor antagonist clobenpropit reversed the effect of histamine to a slight facilitation. The facilitatory effect of histamine (obtained in the presence of clobenpropit) was not affected by the H_1 receptor antagonist mepyramine but abolished by the H_2 receptor antagonist ranitidine. In the absence of clobenpropit, ranitidine augmented the inhibitory effect of histamine. In slices superfused in the presence of ranitidine, the evoked overflow was inhibited by histamine and, more potently, by the H_3 receptor agonist R-α-methylhistamine in a concentration-dependent manner (maximum inhibitory effect obtained for both agonists 30–35%). The concentration-response curve of histamine was shifted to the right by the H_3 receptor antagonist thioperamide. R-α-Methylhistamine inhibited the electrically evoked tritium overflow also in guinea-pig cerebellar, hippocampal and hypothalamic slices. In cortex slices superfused in the presence of clobenpropit, the H_2 receptor agonists impromidine and, less potently, R-sopromidine facilitated the evoked overflow in a concentration-dependent manner. S-Sopromidine only tended to increase the evoked overflow. The effect of impromidine was counteracted by the H_2 receptor antagonists ranitidine and cimetidine. The extent of the maximum facilitatory effect of impromidine (by 15–20%) was about the same when (i) the Ca^{2+} concentration in the medium was reduced from 1.3 to 0.98 mmol/l, (ii) the time of exposure to impromidine was reduced from 28 to 8 min or (iii) cerebellar, hippocampal or hypothalamic slices were used instead of cortical slices. The Ca^{2+}-induced tritium overflow in guinea-pig cortex slices was inhibited by histamine (in the presence of ranitidine); this effect was abolished by clobenpropit. In slices superfused in the presence of clobenpropit, impromidine failed to facilitate the Ca^{2+}-evoked tritium overflow. The electrically evoked tritium overflow in *mouse* brain cortex slices was inhibited by histamine by about 60% (both in the absence or presence of ranitidine). The inhibitory effect of histamine was abolished (but not reversed) by clobenpropit.

In conclusion, noradrenaline release in the guinea-pig brain cortex is inhibited via presynaptic H_3 receptors and facilitated via H_2 receptors not located presynaptically. In the mouse brain cortex, only inhibitory H_3 receptors occur. The extent of the H_3 receptor-mediated effect is more marked in the mouse than in the guinea-pig brain cortex.

Key words H_2 and H_3 receptors · Noradrenaline release · Guinea-pig and mouse brain slices · Presynaptic receptors · Impromidine · Sopromidine · Ranitidine · Clobenpropit

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Introduction

A third type of histamine receptor, termed H_3, was described by Arrang et al. (1983); this receptor is involved in the inhibitory effect of histamine on its own release in rat brain cortex slices (Arrang et al. 1983). Subsequent studies revealed that in the CNS also the release of noradrenaline, serotonin, dopamine, acetylcholine (for review, see Arrang et al. 1992; Schlicker et al. 1994; Leurs et al. 1995; Stark et al. 1996) and probably also that of GABA (Garcia et al. 1997) and of glutamate (Brown and Reymann 1996) is inhibited via H_3 receptors. All in vivo studies related to the H_3 receptor-mediated modulation of

transmitter release were carried out on rats; the in vitro studies were performed in isolated CNS preparations from rats and, less frequently, from humans and mice (for review, see Arrang et al. 1992; Schlicker et al. 1994; Leurs et al. 1995; Stark et al. 1996).

H_3 receptor-mediated modulation of transmitter release has so far not been described for the guinea-pig CNS in vitro or in vivo; this is surprising since the guinea-pig is frequently used to determine physiological and pathophysiological effects of histamine. This is also reflected by the fact that (peripheral) preparations from the guinea-pig are used as the typical screening models for H_1 and H_2 receptors (contraction in the ileum and positive chronotropic effect in the atrium, respectively; Arrang et al. 1983) and even for H_3 receptors (inhibition of the neurogenic contraction in the ileum; Vollinga et al. 1992).

We have found an inhibitory effect of histamine on the electrically evoked ^{3}H-noradrenaline release also in superfused guinea-pig brain cortex slices; unexpectedly, this effect was not only abolished but rather reversed to a facilitation by an H_3 receptor antagonist. It was the aim of the present study to examine the pharmacological properties of the inhibitory and facilitatory histamine receptors in this tissue and to determine their location with respect to the noradrenergic neurones. For the sake of comparison, some experiments were also done on superfused *mouse* brain cortex slices in which H_3 receptor-mediated inhibition of noradrenaline release has been described previously and modulatory effects of histamine on noradrenaline release via H_1 and H_2 receptors have been excluded (Schlicker et al. 1992 a).

Materials and methods

Slices (0.3 mm thick, diameter 3 mm) were prepared from the cerebral cortex, hippocampus, hypothalamus and cerebellum of male Dunkin-Hartley guinea-pigs (weighing 200–580 g) and from the cerebral cortex of male NMRI mice (weighing 35–45 g). The slices were incubated for 60 min with physiological salt solution (PSS) containing ^{3}H-noradrenaline 25 nmol/l (specific activity 42.0–70.2 Ci/mmol) and superfused with PSS (37° C). The PSS was composed as follows (unless stated otherwise; mmol/l): NaCl 118, $NaHCO_3$ 25, KCl 4.8, $CaCl_2$ 1.3, KH_2PO_4 1.2, $MgSO_4$ 1.2, ascorbic acid 0.06, disodium EDTA 0.03, glucose 10; it was gassed with 95% O_2 and 5% CO_2. The superfusate was collected in 5-min samples.

Tritium overflow was evoked twice (S_1 and S_2), either electrically or by introduction of Ca^{2+} into the superfusion medium.

Electrical stimulation. Two 2-min periods of stimulation (rectangular pulses of 50 mA and 2 ms; 0.3 Hz) were administered to the slices after 40 and 90 min of superfusion (experiments lasted for 110 min).

Stimulation by introduction of Ca^{2+} ions. The slices were superfused with Ca^{2+}-free PSS containing 25 mmol/l K^+ (the concentration of Na^+ was reduced accordingly) and tetrodotoxin 1 μmol/l. Tritium overflow was evoked by introducing Ca^{2+} 1.3 mmol/l into the medium for 2 min after 60 and 110 min of superfusion (experiments lasted for 130 min).

Regardless of the type of stimulation, the histamine receptor agonist under study (histamine, impromidine, R-, S-sopromidine, R-α-methylhistamine or imetit) was added to the PSS from 28 min before S_2 onward (unless stated otherwise) whereas the histamine receptor antagonist(s) under study (mepyramine, ranitidine, cimetidine, clobenpropit or thioperamide) as well as the auxiliary drugs (desipramine and rauwolscine) were present throughout superfusion.

Calculations. Tritium efflux was calculated as a fraction of the tritium content of the tissue at the onset of the respective collection period (fractional rate of tritium efflux). In order to quantify the effects of drugs on the basal tritium efflux, the ratio of the fractional rate of tritium efflux in the 5-min period prior to S_2 (t_2) over that in the 5-min period 15–20 min after the onset of S_1 (t_1) was determined (t_2/t_1). The stimulation-evoked tritium overflow was calculated as the amount of tritium in excess of the basal tritium efflux (the latter was assumed to decline linearly from the 5-min period before to that 15–20 min after onset of stimulation). To quantify drug-induced effects on the stimulated tritium overflow, the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1) or the overflow evoked by S_1 obtained in the presence of a given drug was compared to the S_1 value obtained in its absence. The apparent pA_2 value for thioperamide against histamine was determined according to equation 4 in the article by Furchgott (1972).

Statistics. Results are given as means ± SEM of *n* experiments. *n* refers to the number of animals; the t_2/t_1 and S_2/S_1 values are based on one slice per animal whereas the t_1 and S_1 values represent the mean of several slices per animal. For comparison of mean values, Student's *t*-test was used; if more than one experimental series was compared to the same control, the Bonferroni correction was applied.

Drugs used. R-(–)-[ring-2,5,6-^{3}H]-Noradrenaline base (NEN, Dreieich, Germany); cimetidine, impromidine trihydrochloride (Smith Kline Beecham, Harlow, England); clobenpropit dihydrobromide, imetit dihydrobromide (Professor H. Timmerman, Vrije Universiteit, Amsterdam, The Netherlands); desipramine hydrochloride (Ciba-Geigy, Wehr, Germany); histamine dihydrochloride, ranitidine hydrochloride (Sigma, München, Germany); mepyramine (pyrilamine) maleate (Asta, Frankfurt am Main, Germany); R-α-methylhistamine dihydrogen maleate, R- and S-sopromidine dimesotartrate (synthesized at the Institut für Pharmazie I, Freie Universität, Berlin, Germany); rauwolscine hydrochloride (Roth, Karlsruhe, Germany); tetrodotoxin (Sigma, München, Germany or Biotrend, Köln, Germany or ICN, Eschwege, Germany); thioperamide maleate (Tocris-Cookson, Bristol, England). Stock solutions of the drugs were prepared in DMSO (thioperamide) or water (other drugs) and diluted with PSS to the concentration required.

Results

Basal tritium efflux

Basal tritium efflux (t_2/t_1) in guinea-pig and mouse brain cortex slices superfused with PSS containing desipramine 1 μmol/l, rauwolscine 1 μmol/l plus clobenpropit 0.1 μmol/l was 0.77 ± 0.04 and 0.71 ± 0.03, respectively, in control experiments (i.e. no agonist present; n = 7–9). t_1 (expressed as fraction of the tritium content of the slice) was 0.0012 ± 0.0001 min^{-1} and 0.0018 ± 0.0001 min^{-1} in these two experimental series. Similar values of t_2/t_1 and t_1 were obtained in the control series on superfused guinea-pig cerebellar, hippocampal and hypothalamic slices. Basal efflux was not affected by the drugs under study (results not shown), with one exception. In guinea-pig brain cortex slices superfused in the absence of desipramine plus rauwolscine, basal tritium efflux (t_2/t_1), which was 0.87 ± 0.04 in controls, was increased ($P < 0.002$) by histamine 10 and 100 μmol/l (added to the PSS before and during S_2) by 51 ± 7% and 366 ± 50%, respectively (n = 5, each).

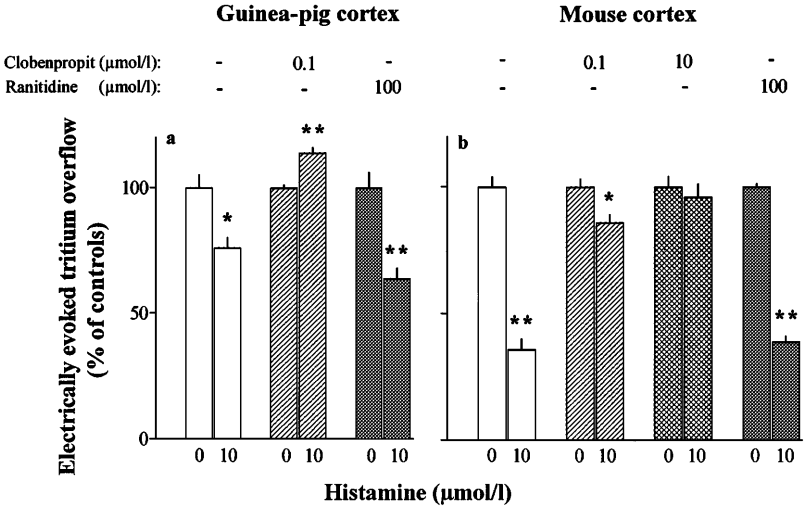

Fig. 1 Effect of histamine on the electrically evoked tritium overflow from superfused guinea-pig (**a**) and mouse brain cortex slices (**b**) preincubated with ^{3}H-noradrenaline, and interaction with clobenpropit and ranitidine. The slices were superfused with medium containing desipramine 1 μmol/l plus rauwolscine 1 μmol/l and, when studied, clobenpropit or ranitidine throughout superfusion and histamine from 62 min of superfusion onward. Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S_1, S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was formed. Results are expressed as percentages of the S_2/S_1 values in the corresponding control experiments; S_2/S_1 values in controls ranged from 1.02 (± 0.01) to 1.10 (± 0.07) (panel **a**) and from 1.12 (± 0.03) to 1.18 (± 0.03) (panel **b**). In slices not exposed to clobenpropit or ranitidine, S_1 (expressed as percent of tissue tritium) was 4.76 ± 0.27 (panel **a**) and 8.53 ± 0.69 (panel **b**). Means ± SEM of 5–11 experiments. * $P < 0.01$, ** $P < 0.001$

Electrically evoked tritium overflow

In the first series of experiments, guinea-pig or mouse brain cortex slices were superfused with PSS containing desipramine 1 μmol/l plus rauwolscine 1 μmol/l. The electrically evoked overflow (S_2/S_1; for absolute values, see legend to Fig. 1) in guinea-pig brain cortex slices was inhibited ($P < 0.001$) by tetrodotoxin 1 μmol/l (added before and during S_2) or omission of Ca^{2+} ions (omitted before and during S_2) by 88 ± 1% and 99 ± 1%, respectively (n = 4–8). Histamine 10 μmol/l inhibited the evoked overflow in guinea-pig brain cortex slices (Fig. 1 a) and to a more marked extent in mouse brain cortex slices (Fig. 1 b). The inhibitory effect of histamine was reversed to a slight facilitatory effect (by 12–14%) by the H_3 receptor antagonist clobenpropit 0.1 μmol/l in guinea-pig brain cortex slices (Fig. 1 a, Table 1). In mouse brain cortex slices, however, clobenpropit only attenuated (at 0.1 and 1 μmol/l) or abolished (at 10 μmol/l) the inhibitory effect of histamine (Fig. 1b or not shown). The electrically evoked tritium overflow in guinea-pig brain cortex slices was also inhibited by the H_3 receptor agonists R-α-methylhistamine and imetit; the inhibitory effect of both drugs was abolished (but not reversed to a facilitation) by clobenpropit 0.1 μmol/l (Table 1).

The facilitatory effect of histamine in guinea-pig brain cortex slices obtained in the presence of clobenpropit was not affected by the H_1 receptor antagonist mepyramine

Table 1 Effects of histamine and H_3 receptor agonists on the electrically evoked tritium overflow from superfused guinea-pig brain cortex slices preincubated with ^{3}H-noradrenaline, and interaction with H_1, H_2 and H_3 receptor antagonists. The superfusion medium contained desipramine 1 μmol/l plus rauwolscine 1 μmol/l in all experiments; further drugs were added to the medium as indicated below. Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S_1 and S_2), and the ratio of tritium overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1). Means ± SEM of 4–11 experiments

Antagonists (μmol/l); present in the medium throughout superfusion	S_2/S_1					
	Agonists (μmol/l); added to the medium from 28 min before S_2 onward					
	Histamine		R-α-Methylhistamine		Imetit	
	0	10	0	1	0	0.1
–	1.04 ± 0.05	0.79 ± 0.04* (76[a])	0.96 ± 0.02	0.64 ± 0.02* (67)	0.95 ± 0.01	0.72 ± 0.03* (76)
Clobenpropit (Clob.) 0.1	0.97 ± 0.02	1.09 ± 0.03* (112)	0.93 ± 0.06	0.92 ± 0.03 (99)	1.04 ± 0.04	0.99 ± 0.02 (95)
Clob. 0.1 + mepyramine 1	0.94 ± 0.01	1.05 ± 0.02* (112)	–	–	–	–
Clob. 0.1 + ranitidine 10	1.05 ± 0.03	0.98 ± 0.02 (93)	–	–	–	–

[a] Mean value, given as percentage of the corresponding agonist-free control
* $P < 0.005$, compared to the corresponding agonist-free control

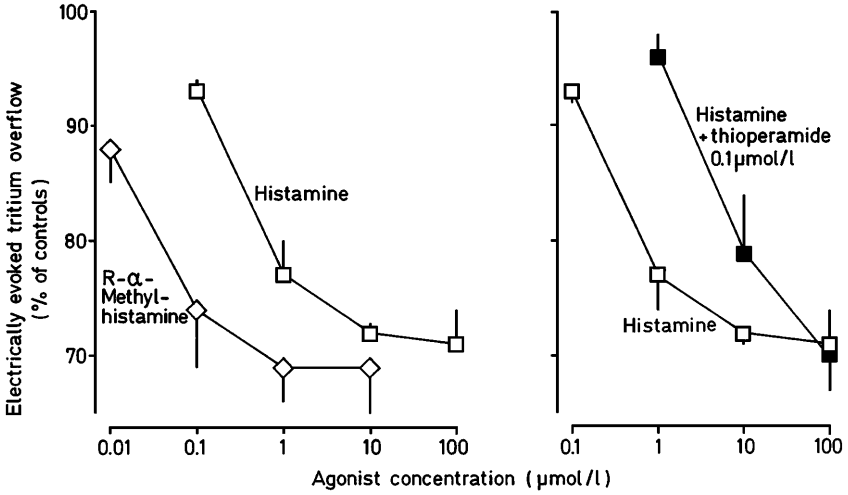

Fig. 2 Effects of histamine and R-α-methylhistamine on the electrically evoked tritium overflow from superfused guinea-pig brain cortex slices preincubated with ^{3}H-noradrenaline, and interaction of histamine with thioperamide. The slices were superfused with medium containing desipramine 1 μmol/l, rauwolscine 1 μmol/l plus ranitidine 100 μmol/l. Histamine (or R-α-methylhistamine) was added to the medium from 62 min of superfusion onward whereas thioperamide was present throughout superfusion. Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S_1, S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was formed. Results are given as percent of the S_2/S_1 values in the corresponding control experiments; S_2/S_1 values in controls ranged from 0.91 (± 0.02) to 0.98 (± 0.08). In slices not exposed to thioperamide, S_1 (expressed as percent of tissue tritium) was 6.06 ± 0.38. Means ± SEM of 4–6 experiments

but abolished by the H_2 receptor antagonist ranitidine (Table 1). The inhibitory effect of histamine (obtained in the absence of clobenpropit) was slightly but significantly ($P < 0.01$) increased by ranitidine 100 μmol/l in guinea-pig brain cortex slices (Fig. 1 a) but was not affected in mouse brain cortex slices (Fig. 1 b).

The antagonists mepyramine 0.1 μmol/l, ranitidine 10 and 100 μmol/l and clobenpropit 0.1 and 1 μmol/l, given alone or in combination, did not affect the electrically evoked tritium overflow (S_1) in guinea-pig and mouse brain cortex slices (not shown; for the absolute value of S_1 in the antagonist-free control, see legend to Fig. 1). Clobenpropit 10 μmol/l reduced S_1 in mouse brain cortex slices by 25 ± 6% ($P < 0.05$; $n = 7$).

The second series of experiments was performed in slices from various brain regions of the guinea-pig with PSS containing ranitidine 100 μmol/l *(to isolate the H_3 receptor)* in addition to desipramine plus rauwolscine. The electrically evoked tritium overflow (S_2/S_1) from cortex slices was inhibited by histamine and, more potently, by R-α-methylhistamine in a concentration-dependent manner (Fig. 2). The maximum inhibitory effect of both agonists amounted to about 30%; the pEC_{50} values (expressed as the $-\log_{10}$ values of the concentrations causing an inhibition by 15%) were 6.50 and 7.80, respectively. The concentration-response curve of histamine was shifted to the right by the H_3 receptor antagonist thioperamide 0.1 μmol/l (Fig. 2), yielding an apparent pA_2 value of 8.12. Thioperamide 0.1 μmol/l did not affect the evoked tritium overflow (S_1) by itself (not shown; for the absolute value of S_1 in the thioperamide-free control, see legend to Fig. 2). R-α-Methylhistamine 1 μmol/l inhibited the electrically evoked tritium overflow (S_2/S_1) also in cerebellar, hippocampal and hypothalamic slices; the extent of inhibition in cerebellar slices was smaller ($P < 0.05$) than that in cortical slices (Table 2). Finally, the evoked overflow (S_1) in hypothalamic slices was less pronounced than that in cortical slices (Table 2).

In the third series, guinea-pig brain cortex slices were superfused with PSS containing clobenpropit 0.1 μmol/l *(to isolate H_2 receptors)* in addition to desipramine plus rauwolscine. The H_2 receptor agonist impromidine facilitated the electrically evoked tritium overflow (S_2/S_1) in a concentration-dependent manner (Fig. 3). The maximum facilitatory effect, obtained at 10–100 μmol/l, amounted

Table 2 Effect of R-α-methylhistamine on the electrically evoked tritium overflow from superfused guinea-pig cortical, cerebellar, hippocampal and hypothalamic slices preincubated with ^{3}H-noradrenaline. Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S_1 and S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1). The superfusion medium contained desipramine 1 μmol/l, rauwolscine 1 μmol/l plus ranitidine 100 μmol/l throughout superfusion and R-α-methylhistamine (if necessary) from 28 min before S_2 on. Means ± SEM of 4–5 experiments

Brain region	S_1 (% of tissue tritium)	S_2/S_1 R-α-Methylhistamine (μmol/l)		
		0	1	
Cortex	5.76 ± 0.34	0.98 ± 0.01	0.70 ± 0.01**	(71[a])
Cerebellum	5.24 ± 0.52	0.94 ± 0.03	0.76 ± 0.03*	(81)
Hippocampus	5.50 ± 0.40	0.96 ± 0.02	0.70 ± 0.02**	(73)
Hypothalamus	2.47 ± 0.25***	1.01 ± 0.03	0.68 ± 0.02**	(67)

[a] Mean value, given as percentage of the corresponding R-α-methylhistamine-free control

* $P < 0.005$, ** $P < 0.001$, compared to the corresponding R-α-methylhistamine-free control

*** $P < 0.001$, compared to the S_1 value in cortex slices

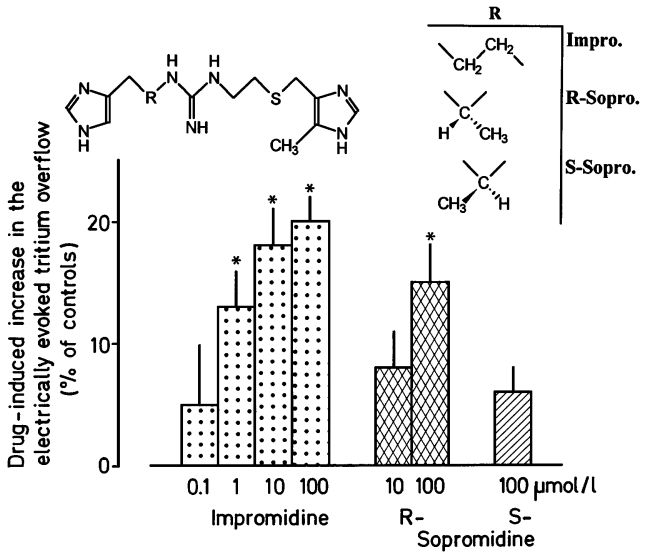

Fig. 3 Effect of impromidine (Impro.), R-sopromidine (R-Sopro.) and S-sopromidine (S-Sopro.) on the electrically evoked tritium overflow from superfused guinea-pig brain cortex slices preincubated with ^{3}H-noradrenaline. The slices were superfused with medium containing desipramine 1 µmol/l, rauwolscine 1 µmol/l plus clobenpropit 0.1 µmol/l. The drug under study was added to the medium from 62 min of superfusion onward. Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S_1, S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was formed. Results are given as percent of the S_2/S_1 values in the corresponding control (0.88 ± 0.02); note that only the increase beyond 100% is depicted. The chemical structures of the drugs under study are given *in the upper part* of the figure. Means ± SEM of 5–7 experiments

to 15–20%. Another H_2 receptor agonist, R-sopromidine, also facilitated the electrically evoked tritium overflow but was less potent in this respect (Fig. 3). The enantiomer of the latter, S-sopromidine, only tended to increase the evoked overflow at 100 µmol/l (Fig. 3). The facilitatory effect of impromidine was counteracted by the H_2 receptor antagonists ranitidine 1 µmol/l or cimetidine 10 µmol/l (Table 3), which, by themselves, did not affect the electrically evoked tritium overflow (S_1) (results not shown; for the absolute value of S_1 in the H_2 receptor antagonist-free control, see Table 4).

In the fourth experimental series, the same auxiliary drugs (desipramine, rauwolscine, clobenpropit) were used as in the third one; the effect of impromidine 10 µmol/l on the electrically evoked tritium overflow (S_2/S_1) was studied under a variety of experimental conditions. Compared to the standard experimental condition (Table 4, first line), the following modifications of the experimental procedure did not modify the extent of the facilitatory effect of impromidine on the evoked overflow: (1) reduction of the Ca^{2+} concentration in the superfusion medium by 25% (0.98 vs. 1.3 mmol/l); (2) reduction of the exposure time of the guinea-pig brain cortex slices to impromidine from 28 to 8 min and (3) use of cerebellar, hippocampal and hypothalamic slices instead of cortical slices from the guinea-pig (Table 4). In *mouse* brain cortex slices, impromidine failed to affect the evoked tritium overflow (Table 4). Compared to the standard experimental condition (Table 4, first line), the electrically evoked tritium overflow (S_1) was altered by some of the modified experimental conditions, i.e. by reduction of Ca^{2+} and by the use of guinea-pig hypothalamic and mouse brain cortex slices (Table 4).

Ca^{2+}-evoked tritium overflow

In these experiments, guinea-pig brain cortex slices were superfused with Ca^{2+}-free PSS containing K^+ 25 mmol/l and, in addition, desipramine 1 µmol/l, rauwolscine 1 µmol/l plus tetrodotoxin 1 µmol/l. Tritium overflow was evoked by introducing Ca^{2+} 1.3 mmol/l into the PSS for two 2-min periods (S_1, S_2).

In the first series of experiments, the PSS in addition contained ranitidine 100 µmol/l. Histamine 10 µmol/l (present in the PSS before and during S_2) reduced the evoked overflow (S_2/S_1), which was 0.93 ± 0.10 in 20 controls, to 0.68 ± 0.06 (n = 18; $P < 0.05$). The inhibitory

Table 3 Effect of impromidine on the electrically evoked tritium overflow from superfused guinea-pig brain cortex slices preincubated with ^{3}H-noradrenaline, and interaction with ranitidine and cimetidine. The superfusion medium contained desipramine 1 µmol/l, rauwolscine 1 µmol/l plus clobenpropit 0.1 µmol/l in all experiments; further drugs were added to the medium as indicated below. Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S_1 and S_2), and the ratio of tritium overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1). Note that the results obtained with impromidine are given as percentages of the impromidine-free controls to facilitate comparisons between experiments in which the H_2 receptor antagonist under study was absent and present. Means ± SEM of 4–11 experiments

Antagonists (µmol/l); present in the medium throughout superfusion	Impromidine (µmol/l); added from 28 min before S_2 onward			
	0 S_2/S_1 (absolute value)	0.1	1 S_2/S_1 (given as percent of the S_2/S_1 in controls)	10
–	0.91 ± 0.02	109 ± 2	110 ± 1	116 ± 3
Ranitidine 1	0.90 ± 0.02	101 ± 3	101 ± 4*	108 ± 3*
–	0.96 ± 0.03	106 ± 6	117 ± 4	116 ± 4
Cimetidine 10	0.98 ± 0.03	96 ± 6	105 ± 3*	107 ± 3

* $P < 0.05$, compared to the corresponding value without ranitidine or cimetidine

Table 4 Effect of impromidine on the electrically evoked tritium overflow from superfused guinea-pig and mouse brain slices preincubated with ^{3}H-noradrenaline under a variety of experimental conditions. Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S_1 and S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1). The superfusion medium contained desipramine 1 μmol/l, rauwolscine 1 μmol/l plus clobenpropit 0.1 μmol/l throughout superfusion and impromidine (if necessary) from 28 min before S_2 on (unless stated otherwise). Means ± SEM of 4–15 experiments

Species	Ca^{2+} concentration (mmol/l)	Time of exposure to impromidine before S_2 (min)	Brain region	S_1 (% of tissue tritium)	S_2/S_1 Impromidine (μmol/l)		
					0	10	
Guinea-pig	1.30	28	Cortex	4.93 ± 0.31	0.95 ± 0.01	1.10 ± 0.03**	(116[a])
"	0.98	28	Cortex	3.33 ± 0.44[o]	0.93 ± 0.03	1.09 ± 0.04*	(117)
"	1.30	8	Cortex	See first line[b]	0.93 ± 0.03	1.04 ± 0.03*	(112)
"	1.30	28	Cerebellum	4.15 ± 0.85	0.93 ± 0.02	1.14 ± 0.02**	(123)
"	1.30	28	Hippocampus	3.77 ± 0.66	1.01 ± 0.01	1.18 ± 0.02**	(117)
"	1.30	28	Hypothalamus	1.92 ± 0.13[oo]	1.04 ± 0.02	1.25 ± 0.02**	(120)
Mouse	1.30	28	Cortex	7.90 ± 0.69[oo]	1.28 ± 0.05	1.20 ± 0.05	(94)

[a] Mean value, given as percentage of the corresponding impromidine-free control

[b] Note that during S_1 the experimental conditions did not differ from those of the experiments shown in the first line

* $P < 0.025$, ** $P < 0.005$, compared to the corresponding impromidine-free control

[o] $P < 0.02$, [oo] $P < 0.005$, compared to the S_1 value given in the first line

effect of histamine was abolished by clobenpropit 0.1 μmol/l (present in the PSS throughout superfusion); the S_2/S_1 values were 0.94 ± 0.03 and 0.96 ± 0.05 in the absence and presence of histamine (n = 11–12). Clobenpropit 0.1 μmol/l, by itself, did not affect the evoked overflow, expressed as S_1, which amounted to 2.38 ± 0.23% of tissue tritium in the clobenpropit-free control. In the second series, the PSS contained clobenpropit 0.1 μmol/l (in addition to desipramine, rauwolscine plus tetrodotoxin). Impromidine 10 μmol/l (added before and during S_2) did not affect the evoked overflow; the S_2/S_1 values were 1.01 ± 0.03 and 0.98 ± 0.04 in its absence and presence (n = 16, each).

Discussion

The present study was carried out to examine the pharmacological properties and the location of the receptors involved in the modulatory effect of histamine on noradrenaline release in the guinea-pig brain cortex. The electrically evoked tritium overflow in slices from this brain region as well as in mouse brain cortex slices was shown to be tetrodotoxin-sensitive and Ca^{2+}-dependent (present study; Schlicker et al. 1992b); the electrically (and/or the Ca^{2+}-) evoked tritium overflow from these two tissues as well as that from guinea-pig cerebellar, hippocampal and hypothalamic slices may be assumed to represent quasi-physiological noradrenaline release. Desipramine was routinely added to the superfusion medium to inhibit the neuronal noradrenaline transporter; it has been shown in cortex slices from the guinea-pig (present study) and the rat (Taube et al. 1977) that high concentrations of histamine (which is a substrate of the neuronal noradrenaline transporter) displace tritium from the noradrenergic nerve endings and that this effect can be blocked by an inhibitor of the neuronal noradrenaline transporter. Desipramine and, in addition, the α_2-adrenoceptor antagonist rauwolscine were used to increase the amount of tritium overflow. A second reason for the addition of rauwolscine was that the H_3 receptor-mediated effect in slice preparations is increased or only detectable when the presynaptic α_2-adrenoceptors are blocked simultaneously (Schlicker et al. 1992b; Celuch 1995). Finally, rauwolscine was used to block the α_2-adrenoceptors in the tissue before addition of impromidine, which is also a weak α_2-adrenoceptor antagonist (Schlicker et al. 1989). Note that both blockade of α_2-adrenoceptors (for review, see Starke 1977) and activation of H_2 receptors (see below) causes facilitation of noradrenaline release in guinea-pig brain slices.

In guinea-pig brain cortex slices, a facilitatory effect of histamine on noradrenaline release was unmasked by the H_3 receptor antagonist clobenpropit; by contrast, clobenpropit only abolished (but did not reverse) the inhibitory effect of the selective H_3 receptor agonists R-α-methylhistamine and imetit (which are 10 and 100 times more potent than histamine (Kathmann et al. 1993) and were, therefore, studied at a 10- and 100-fold lower concentration). The facilitatory effect of histamine is mediated via H_2 receptors since it was abolished by the H_2 receptor antagonist ranitidine but not affected by the H_1 receptor antagonist mepyramine. The H_2 receptor-mediated effect of histamine could also be revealed in those experiments (carried out in the absence of clobenpropit) in which the inhibitory effect of histamine was increased by ranitidine.

The inhibitory histamine receptor was characterized pharmacologically in experiments in which H_2 receptors were blocked by ranitidine at a concentration (100 μmol/l) which is about 1000-fold higher than its dissociation constant at H_2 receptors (0.06 μmol/l; Alexander and Peters 1997) but still does not block H_3 receptors (Schlicker et al. 1989). Two findings demonstrate that the inhibitory effect of histamine is mediated via H_3 receptors. First, the concentration-response curve of histamine was shifted to the right by the H_3 receptor antagonist thioperamide,

yielding an apparent pA_2 value of 8.12 which is close to the value of 8.4 reported in the literature (Alexander and Peters 1997). Second, the effect of histamine was potently mimicked by R-α-methylhistamine, which, like in other H_3 receptor models, was about tenfold more potent than histamine itself (Arrang et al. 1987; Kathmann et al. 1993). Note that R-α-methylhistamine inhibited noradrenaline release also in guinea-pig cerebellar, hippocampal and hypothalamic slices, suggesting that release-modulating H_3 receptors also occur in these brain regions.

The facilitatory histamine receptor was characterized pharmacologically in experiments in which H_3 receptors were blocked by clobenpropit at a concentration (100 nmol/l) which is about 1000-fold higher than its dissociation constant at H_3 receptors (0.13 nmol/l; Alexander and Peters 1997) but still does not block H_2 receptors (own unpublished results). Since the H_2 receptor agonist impromidine exhibited a more marked facilitatory effect than histamine itself, this drug was preferred over histamine. Impromidine and, in addition, R- and S-sopromidine, which were studied as well, are also potent H_3 receptor antagonists (Arrang et al. 1983, 1985) and this property was an additional reason for using clobenpropit. Two findings demonstrate that the facilitatory effect of impromidine is mediated via H_2 receptors. First, this effect was counteracted by the H_2 receptor antagonists ranitidine and cimetidine. Note that determination of pA_2 values was not possible since the facilitatory effect of impromidine was very small (maximum 15–20%); however, the data in Table 3 are very similar to those in the literature inasmuch as ranitidine was about 10-fold more potent as an H_2 receptor antagonist than cimetidine (Arrang et al. 1983). Second, the effect of impromidine was mimicked by R-sopromidine, which, like in a previous study (Elz et al. 1989), was less potent in this respect than impromidine. The effect of sopromidine was stereoselective; thus, the S-enantiomer did not cause a statistically significant facilitation at a concentration as high as 100 μmol/l. The data do not allow a definite conclusion as to whether S-sopromidine is devoid of agonistic potency at H_2 receptors (and acts as an H_2 receptor antagonist) (as in the study of Elz et al. 1989) or whether it is a partial agonist.

Three attempts were made to increase the extent of the H_2 receptor-mediated effect. First, the Ca^{2+} concentration in the superfusion medium was decreased by 25% since the extent of effects mediated via presynaptic receptors is usually increased as the Ca^{2+} concentration decreases (see Starke 1977 for review). In another series of experiments, the time of exposure of the tissue to impromidine was reduced from 28 to 8 min since there are examples of desensitizing H_2 receptors in cell lines (see Smit et al. 1994 for references). Finally, the effect of impromidine on noradrenaline release was also studied in cerebellar, hippocampal and hypothalamic slices from the guinea-pig since the extent of effect mediated via certain types of presynaptic receptors markedly varies in different brain regions (see e.g. Taube et al. 1977). However, none of the three approaches led to a significantly stronger facilitatory effect of impromidine.

In order to study whether the two histamine receptors in the guinea-pig brain cortex are located presynaptically on the noradrenergic neurones themselves, experiments were carried out in which propagation of action potentials along the axons was blocked by tetrodotoxin and tritium overflow was evoked by introduction of Ca^{2+} ions into Ca^{2+}-free K^+-rich medium (for review, see Starke 1977; Starke et al. 1989). Histamine (studied in the presence of ranitidine) still inhibited the evoked overflow, in a manner sensitive to antagonism by clobenpropit, suggesting that the H_3 receptor is located presynaptically. Impromidine (studied in the presence of clobenpropit) lost its facilitatory effect, suggesting that the H_2 receptor is not located on the axon terminals of the noradrenergic neurones. The question whether the two histamine receptors may be activated also by *endogenous* histamine was not examined systematically in the present study. The fact that the selective H_3 and H_2 receptor antagonists did not cause effects on noradrenaline release opposite to those of the H_3 and H_2 receptor agonists is in harmony with the view that an endogenous tone does not develop at either receptor.

The experiments on mouse brain cortex slices, carried out for the sake of comparison, show again that facilitatory H_2 receptors modulating noradrenaline release are not detectable in this experimental model (Schlicker et al. 1992a). This is suggested by the failure (i) of impromidine to affect noradrenaline release; (ii) of clobenpropit to reverse the inhibitory effect of histamine to a facilitation and (iii) of ranitidine to increase the inhibitory effect of histamine. There is, however, also a quantitative difference between guinea-pig and mouse brain cortex slices with respect to the modulation of noradrenaline release by histamine. Thus, the H_3 receptor-mediated effect in mouse brain cortex slices (determined in the presence or absence of ranitidine; inhibition by about 60%) is almost double as high as that in guinea-pig brain cortex slices (determined in the presence of ranitidine; inhibition by 30–35%).

In conclusion, noradrenaline release in the guinea-pig brain cortex in vitro is inhibited by histamine via H_3 receptors and facilitated via H_2 receptors. The H_3 receptors are located presynaptically on the noradrenergic neurones whereas the H_2 receptors are not. The extent of the H_3 receptor-mediated inhibition of noradrenaline release is much more marked in the mouse than in the guinea-pig brain cortex. H_3 receptors causing inhibition of noradrenaline release in the CNS appear to be a common phenomenon; they have been identified in vitro in various brain regions of humans, rats, mice (for review, see Arrang et al. 1992; Schlicker et al. 1994; Leurs et al. 1995; Stark et al. 1996) and guinea-pigs (present study). On the other hand, H_2 receptors causing facilitation of noradrenaline release could not be shown in the mouse and rat brain cortex in vitro (Schlicker et al. 1989, 1992a) but have been identified in the rat hypothalamus in vitro (Blandina et al. 1989) and in the cat posterior hypothalamus in vivo (Philippu et al. 1984).

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