J. Pharm. Pharmacol. 1984, 36: 248–252
Received August 2, 1983

Mechanism of the indirect adrenergic effect of histamine in cat cerebral arteries

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KCl (50 mM), tyramine (10^{-7} M), and histamine (10^{-4} M) induced an increase in tritium release from cat cerebral arteries preincubated with [^{3}H]noradrenaline, this increase being due in part to noradrenaline. When calcium was absent from the superfusion medium, only tyramine (10^{-7} M) enhanced the tritium outflow. Colchicine (10^{-3} M) partially inhibited the increase in radioactivity brought about by 10^{-4} M histamine. KCl (50 mM) also evoked release of radioactivity from cerebral arteries preloaded with [^{3}H]histamine; this release was unafffected by reserpine pretreatment or removal of both superior cervical sympathetic ganglia. Neither tyramine (10^{-7} M) nor compound 48/80 (300 μg ml^{-1}) altered the spontaneous tritium outflow from cerebral blood vessels preincubated with [^{3}H]histamine. These results suggest that histamine is not accumulated by sympathetic nerve endings and elicits its noradrenaline-releasing effect by means of an exocytotic process.

Histamine has been suggested to participate in some pathological states involving cerebral blood vessels such as cluster headache or migraine (Anthony et al 1978). The origin of the histamine responsible for the symptoms might be the leucocytes (Sanders et al 1980) or the mast cells surrounding the cerebral vascular bed (Edvinsson et al 1976). Hence, a better understanding of histamine's mechanism of action in these vessels should help to assess whether it plays a role in those pathological states or in the normal physiology of the cerebral circulation.

The effects of histamine on cerebral arteries seem to be mediated through the activation of different types of histamine receptors. The vasoconstriction is attributed to the activation of H_1-receptors, whereas the vasodilation seems to be due to the interaction of histamine with H_2-receptors (Edvinsson et al 1976; Kuschinsky & Wahl 1977; Urquilla et al 1975). However, earlier work from this laboratory (Marco et al 1980) showed that the vasoconstriction of cat isolated cerebral arteries elicited by histamine was also due to an indirect adrenergic component, though no direct evidence of noradrenaline release by histamine was given. This adrenergic component was predominant, if not exclusive, at low and medium doses of histamine.

The data available to explain the intimate mechanism through which histamine released the neurotransmitter were insufficient. Since cocaine was able to reduce the contractile response to histamine from cat cerebral arteries preloaded with [^{3}H]noradrenaline, it was assumed that it had a tyramine-like effect. The same explanation had been given previously for the rabbit pulmonary artery (Starke & Weitzell 1978).

The present work was undertaken to show that histamine actually releases noradrenaline from cat cerebral arteries as well as to get a better insight into the mechanism underlying this process. The presence of noradrenaline was analysed as the radioactivity released by histamine from cerebral blood vessels preincubated with [^{3}H]noradrenaline. The way histamine can release noradrenaline was studied by a double approach. On the one hand, we investigated whether histamine could be taken up and stored by the synaptic vesicles of the sympathetic nerve endings. On the other, we also studied the effect of external calcium removal and colchicine on the release of noradrenaline from cerebral arteries evoked by histamine, since a neurotransmitter can be released either by means of a calcium-dependent exocytotic process (García & Kirpekar 1975; Thoa et al 1975) or by a stoichiometric displacement from its stores, which is independent of external calcium (Thoenen et al 1969; Chubb et al 1972; Thoa et al 1975; Marín & Sánchez 1980). In its turn, colchicine impairs exocytosis but does not affect a stoichiometric displacement (Sorimachi et al 1973).

MATERIALS AND METHODS

Cats of either sex, 1·5–4 kg, were anaesthetized with sodium pentobarbitone (35 mg kg^{-1} i.p.) and killed by bleeding. The brain was removed and the circle of Willis with its branches was dissected out. The vessels were cleaned to remove traces of blood and brain tissue and incubated for 1 h in Krebs–Henseleit

* Correspondence.

solution containing [^{3}H]noradrenaline (2 μCi ml^{-1}, spec. act. 10·5 Ci mmol^{-1}) or [^{3}H]histamine (2 μCi ml^{-1}, spec. act. 43 Ci mmol^{-1}), which was continuously bubbled with a 95% O_2 – 5% CO_2 mixture and kept at 37 °C. After this incubation period the arteries were transferred into a superfusion chamber to study the release of radioactivity according to Farnebo & Malmfors (1971) as described by Marco et al (1980). The chamber was superfused with a prewarmed Krebs–Henseleit solution at a constant flow rate of 0·5 ml min^{-1} by means of a perfusion pump and aerated with 95% O_2 – 5% CO_2. Once the spontaneous tritium outflow had reached a steady level, samples of the effluent were collected every 3 min. Aliquots, 0·5 ml, of the samples were added to vials containing 10 ml of Bray's solution and the radioactivity measured in a scintillation counter (Isocap/3000, Nuclear Chicago, USA).

A second perfusion pump delivered Krebs–Henseleit solution containing KCl, tyramine, histamine and compound 48/80 after 9 min of collection and remained on for 15 min at a flow rate of 0·05 ml min^{-1}.

In the experiments undertaken in the absence of calcium, the normal Krebs–Henseleit solution of the first pump was substituted for calcium-free solution 48 min before the infusions of the drugs which were also prepared in 0-calcium solution.

To study the effect of colchicine on the tritium release evoked by histamine, after 9 min of collection and 15 min before the superfusion of histamine the Krebs–Henseleit solution via the first pump was quickly replaced by another containing colchicine which remained in the chamber throughout the rest of the experiments.

Noradrenaline and its metabolites were analysed according to Graefe et al (1973) in the effluxes collected during 12 min just before the addition of histamine, tyramine, and KCl as well as during the time these compounds were superfused.

At the end of the experiments the blood vessels were placed in 0·4 M $HClO_4$ and kept at −15 °C for a subsequent assay of their radioactivity content. To achieve this, the tissues were solubilized with soluene 100 and tritium was measured in both the perchloric supernatants and digested arteries.

The composition of the Krebs–Henseleit solution was (mM): NaCl, 115; KCl, 4·6; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $MgSO_4 \cdot 7H_2O$, 1·2; $NaHCO_3$, 25; glucose, 11·1; EDTA (ethylene-diaminetetraacetic acid) 0·03. In the calcium-free experiments $CaCl_2$ was omitted and EDTA substituted for 10^{-3} M EGTA (ethyleneglycoldiaminetetraacetic acid).

Statistical analysis was by means of Student's *t*-test; a probability value of less than 5% was considered significant.

The drugs used were: histamine dihydrochloride (Sigma), diphenhydramine (Sigma), tyramine hydrochloride (Sigma), cocaine hydrochloride (Abelló), sodium pentobarbitone (Abbott), KCl (Merck), (−)-[7,8-^{3}H]noradrenaline hydrochloride (Amersham), [2,5-^{3}H]histamine dihydrochloride (Amersham), and colchicine (Sigma).

RESULTS

Outflow of noradrenaline and its metabolites from cat cerebral arteries

DOPEG (3,4-dihydroxyphenylglycol), NMN (normetanephrine), MOPEG (3-methoxy-4-hydroxyphenylglycol) + VMA (3-methoxy-4-hydroxymandelic acid), DOMA (3,4-dihydroxymandelic acid) and NA (noradrenaline) were analysed in the spontaneous ^{3}H (tritium) release from cerebral blood vessels prelabelled with [^{3}H]NA. The fraction corresponding to NA accounted for the 12% of the total radioactivity released (Table 1).

When KCl (50 mM), tyramine (10^{-7} M), and histamine (10^{-4} M) were present in the superfusion medium, the tritium outflow was increased to 545,

Table 1. Effect of histamine (10^{-4} M), tyramine (10^{-7} M), and KCl (50 mM) on the release of NA and its metabolites from cat cerebral arteries preincubated with [^{3}H]NA.

		counts min^{-1} mg^{-1} in 12 min					
	n	MOPEG+VMA	NMN	DOPEG	NA	DOMA	^{3}H
Pre-drug levels	10	449 ± 63	34 ± 6	136 ± 16	111 ± 14	53 ± 6	944 ± 79
Histamine	4	741 ± 191	74 ± 22**	489 ± 151***	530 ± 136***	248 ± 180	2293 ± 385***
Tyramine	4	495 ± 56	90 ± 43*	320 ± 90**	359 ± 58***	63 ± 6	1468 ± 206***
KCl	4	1118 ± 196***	181 ± 37***	524 ± 81*	3033 ± 109***	160 ± 23***	5147 ± 407***

n = number of experiments, *$P < 0·05$, **$P < 0·025$, ***$P < 0·001$ when compared to basal conditions.

155, and 243% of the basal level values, respectively. KCl (50 mM) enhanced all the fractions corresponding to NA and its metabolites while the superfusion of 10^{-7} M tyramine and 10^{-4} M histamine only significantly increased those of NMN, DOPEG, and NA (Table 1).

NA constituted the 59, 24, and 23% of the total radioactivity released by these concentrations of KCl, tyramine, and histamine, respectively. In the case of histamine (10^{-4} M) NA had a 5-fold increase over basal levels whereas the tritium efflux increased only 2·4 times.

Effect of calcium removal on the tritium release evoked by histamine, tyramine, and KCl from brain vessels preincubated with [^{3}H]NA

The spontaneous tritium efflux from arteries preloaded with [^{3}H]NA showed a sharp decrease during the first 3 min followed by a progressively slower decline until it reached a steady level after 90–100 min of washing.

The addition of 10^{-4} M histamine to the superfusion chamber induced a 2·2-fold rise over the basal level in the radioactivity released from cat cerebral arteries that was absent when the experiment was performed in a calcium-free medium (Fig. 1). The removal of calcium from the superfusion medium brought about a significant increase ($P < 0·05$) in the spontaneous tritium outflow from 89 ± 6 counts min^{-1} mg^{-1} (16 determinations) to 141 ± 15 counts min^{-1} mg^{-1} (15 determinations).

A similar result was obtained when 50 mM KCl was used (Fig. 2). The 5·2-fold increase in the radioactivity released by this agent was eliminated when calcium was suppressed from the superfusion medium.

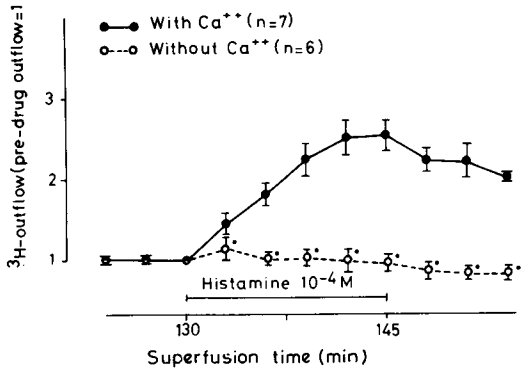

FIG. 1. Effect of external calcium removal on the tritium efflux induced by 10^{-4} M histamine from cat cerebral arteries. Figures in parentheses indicate the number of circles of Willis used. Each point represents the mean ± s.e.m. * denote statistically significant differences.

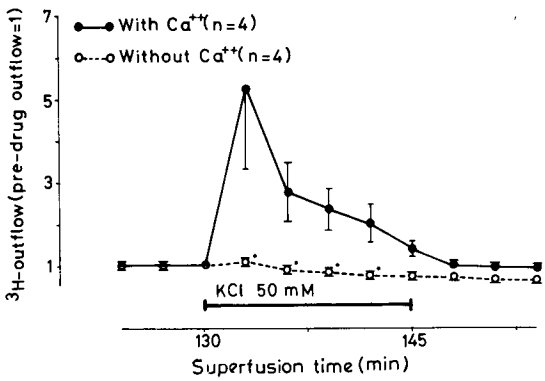

FIG. 2. Effect of external calcium removal on the tritium efflux induced by 50 mM KCl from cat cerebral arteries. Figures in parentheses indicate the number of circles of Willis used. Each point represents the mean ± s.e.m. * denote statistically significant differences.

However, the 3·5-fold enhancement in the tritium outflow evoked by tyramine (10^{-7} M) was not significantly affected in a calcium-free superfusion medium (Fig. 3).

Effect of colchicine on the tritium release evoked by histamine

When colchicine at a final concentration of 10^{-3} M was present in the superfusion chamber 15 min before the superfusion of the cerebral blood vessels with 10^{-4} M histamine, there was a significant inhibition ($P < 0·05$) of the radioactivity release induced by this amine (Fig. 4). The spontaneous tritium outflow remained unchanged after the addition of colchicine to the bath medium.

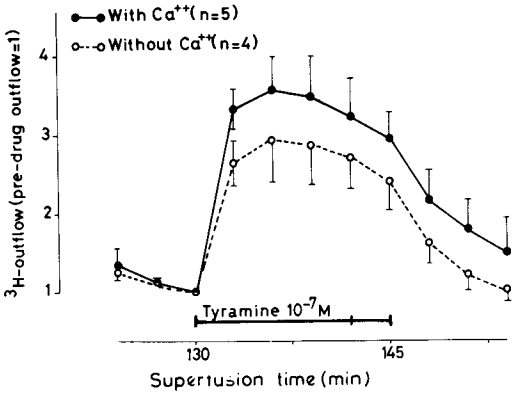

FIG. 3. Effect of external calcium removal on the tritium efflux evoked by 10^{-7} M tyramine from cat cerebral arteries. Figures in parentheses indicate the number of circles of Willis used. Each point represents the mean ± s.e.m. * denote statistically significant differences.

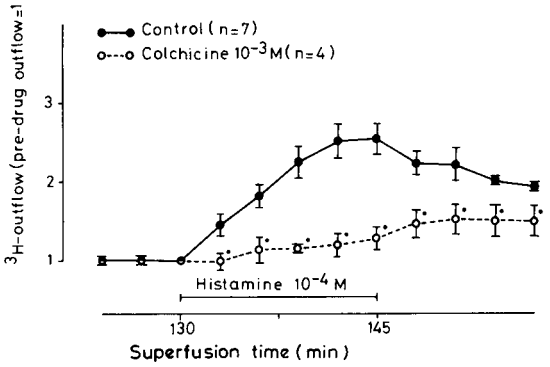

FIG. 4. Effect of 10^{-3} M colchicine on the tritium efflux evoked by 10^{-4} M histamine from cat cerebral arteries. Figures in parentheses indicate the number of circles of Willis used. Each point represents the mean ± s.e.m. * denote statistically significant differences.

Effect of KCl, tyramine, and compound 48/80 on spontaneous tritium release from brain vessels preincubated with [^{3}H]histamine

KCl (50 mM) induced a 57% increase over basal levels in tritium outflow from cerebral arteries prelabelled with [^{3}H]histamine (Fig. 5). Neither pretreatment of the animals with reserpine nor removal of both superior cervical sympathetic ganglia 15 days before the experiments changed the increase obtained with 50 mM KCl (Fig. 5). These treatments were also unable to affect the tritium outflow previous to the superfusion with KCl. The values for predrug tritium outflow were 59 ± 10 counts min^{-1} mg^{-1} (10 determinations) under control conditions, 66 ± 11 counts min^{-1} mg^{-1} (5 determinations) after reserpine pretreatment, and 50 ± 10 counts min^{-1} mg^{-1} after cervical sympathectomy (6 determinations). No change was found either in the radioactivity content of these tissues after studying the effect of 50 mM KCl (Table 2).

The presence of 10^{-7} M tyramine or compound 48/80 (300 μg ml^{-1}) in the superfusion chamber did not alter the radioactivity levels released by cerebral arteries preloaded with [^{3}H]histamine (Fig. 5).

Table 2. Radioactivity retained by cat cerebral arteries preincubated with [^{3}H]histamine or [^{3}H]NA after KCl effect under several treatments.

	counts min^{-1} mg^{-1} wet tissue ± s.e.m.	
Treatment	[^{3}H]histamine	[^{3}H]NA
None	4918 ± 1124 (4)	33 541 ± 6945 (5)
Reserpine	5318 ± 1344 (4)	—
Cervical sympathectomy	3124 ± 481 (5)	—

Numbers in parentheses are the number of determinations.

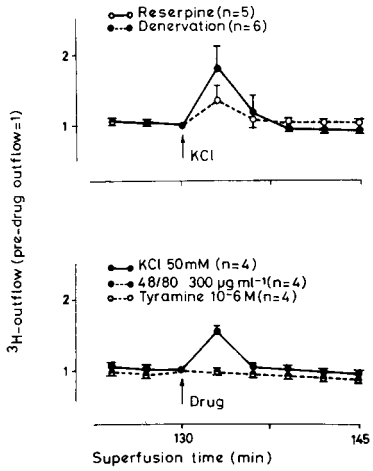

FIG. 5. Upper plot: effect of reserpine pretreatment or cervical sympathectomy on tritium release evoked by 50 mM KCl from cat cerebral arteries preincubated with [^{3}H]histamine. Lower plot: effect of 50 mM KCl, 10^{-7} M tyramine and compound 48/80 (300 μg ml^{-1}) on spontaneous tritium release from cat cerebral arteries preincubated with [^{3}H]NA. Figures in parentheses indicate the number of circles of Willis used. Each point represents the mean ± s.e.m.

DISCUSSION

Previous work has shown that histamine has an indirect adrenergic component in its contractile effect on cat cerebral arteries (Marco et al 1980). Both the contractile response of these vessels to histamine and the release of radioactivity elicited by histamine from cerebral arteries preloaded with [^{3}H]NA were reduced after treatments that interfered with the sympathetic innervation, indicating that histamine should release noradrenaline from sympathetic nerve endings present in the walls of brain blood vessels. However, direct evidence of the ability of histamine to release noradrenaline also requires an understanding of the mechanism of the release.

The results presented here reveal the presence of noradrenaline in the bulk of radioactivity released by histamine from cat cerebral arteries preincubated with [^{3}H]NA. Since this radioactivity comes from sympathetic nerve endings (Marco et al 1980), this confirms the ability of histamine to release noradrenaline from the adrenergic innervation of cerebral blood vessels. Similar results have been reported for the rabbit pulmonary artery (Starke & Weitzell 1978).

The best known mechanisms by which noradrenaline can be released are stoichiometric displacement and exocytosis (Vanhoutte et al 1981). If histamine released noradrenaline by means of the first process,

it should enter the sympathetic nerve endings via a cocaine-sensitive transport system and, then, induce the displacement of noradrenaline from synaptic vesicles into the neuroplasm and the synaptic cleft. According to our results, histamine does not accumulate in adrenergic terminals in spite of its need to enter them in order to have its effect (Marco et al 1980). Tyramine is not able to evoke a release of radioactivity from cerebral arteries preloaded with [^{3}H]histamine, indicating that histamine is not taken up by the synaptic vesicles. This is also supported by the fact that the tritium release induced by KCl from the same kind of preparation was not affected by reserpine pretreatment. Nor does histamine accumulate in the neuroplasm, since removal of both superior cervical sympathetic ganglia had no effect on the tritium outflow elicited by KCl from cat cerebral arteries prelabelled with [^{3}H]histamine. The findings that the radioactivity levels corresponding to [^{3}H]histamine retained by the tissue remained unchanged after pretreatment with reserpine or cervical sympathectomy also add further support. Furthermore, the radioactivity content under these circumstances was much lower than when the vessels were incubated with [^{3}H]NA, which is preferentially taken up by sympathetic nerve endings (Iversen 1975).

Regarding the origin of the peak of radioactivity brought about by KCl in cerebral arteries preincubated with [^{3}H]histamine, that it comes from mast cells is also ruled out as the superfusion of the vessels with compound 48/80 at a degranulating dose (Gristwood et al 1981) was devoid of any effect on spontaneous tritium release.

With a stoichiometric displacement being set aside, histamine may have its indirect adrenergic effect through an exocytotic process. Our findings show that histamine depends on external calcium to release NA as does potassium. Since potassium releases NA by means of a calcium-dependent exocytotic process (García & Kirpekar 1975; Thoa et al 1975), it can be assumed that the indirect adrenergic effect of histamine may also be exocytotic. The need of histamine for calcium to accomplish its catecholamine releasing effect has been also described in cat and cow adrenal medulla (Poisner & Douglas 1966; Göthert et al 1976). This interpretation of the mechanism of action of histamine appears strengthened by the fact that colchicine, a substance known to interfere exocytosis (Sorimachi et al 1973), also reduced the effect of histamine.

From all these results taken together, the following mechanism may be proposed for the indirect adrenergic effect of histamine in cat cerebral arteries. Histamine enters the sympathetic nerve ending probably in minute amounts through the amine uptake system. Once inside, it releases noradrenaline either by promoting the influx of external calcium, which will trigger exocytosis, or by interacting with some intraneuronal structure(s) giving exocytosis as the final result.

Acknowledgements

The authors are indebted to Dr S. Lluch for his critical review of the manuscript as well as to Miss Esther Martínez for her clerical assistance. Work supported in part by fundings from Comisión Asesora de Investigación and Fondo de Investigaciones Sanitarias.

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