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Indirect adrenergic effect of histamine in human cerebral arteries: influence of post-mortem period

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Histamine (10^{-4} M) increased the radioactivity released from human cerebral arteries obtained within 6 h of death and preincubated with [^{3}H]noradrenaline. In the presence of 10^{-6} M cocaine or if 7 or more hours had elapsed since death, 10^{-4} M histamine was unable to change basal levels of tritium outflow. The radioactivity retained by the tissue was higher when cerebral blood vessels were obtained within a post-mortem period of 6 h. These results suggest that histamine may release noradrenaline from the sympathetic innervation of human cerebral arteries and that the function of this innervation lasts only 6 h after death.

Histamine has been proposed to participate in some disorders affecting cerebral blood vessels such as cluster headache (Anthony et al 1978; Sanders et al 1980). The histamine responsible for the symptoms might be released from leucocytes (Sanders et al 1980) or mast cells which surround the cerebral blood vessels even in man (Edvinsson et al 1976; Edvinsson et al 1977). Hence, a better understanding of histamine's mechanism of action in the cerebral circulation will help to elucidate whether this amine plays a role in the normal physiology or in some pathological states involving this vascular bed.

The effects of histamine on cerebral blood vessels are either vasodilatation or vasoconstriction. The vasodilatation is attributed to the activation of H_2-receptors and the vasoconstriction to the interaction of histamine with H_1-receptors (Edvinsson & MacKenzie 1977; Kuschinsky & Wahl 1977; Urquilla et al 1975). In addition to this, an indirect adrenergic component has been reported to participate in the vasoconstriction induced by histamine in cat cerebral arteries (Marco et al 1980). This effect was predominant, if not exclusive, at medium and low concentrations of histamine. It appeared reduced in the presence of phentolamine or cocaine and after reserpine pretreatment or cervical ganglionectomy.

Histamine is also a potent constrictor agent of isolated human arteries (Shibata et al 1977), which could be related to the vasoconstriction occurring before headache attacks in migrainous neuralgia (Edmeads 1979). It seemed reasonable to assume that part of this contractile effect might be due to the liberation of noradrenaline from the sympathetic innervation as happens in cat cerebral arteries. In the present report we tested whether histamine has this property in human cerebral blood vessels. To achieve this, the effect of histamine on spontaneous tritium outflow from human cerebral arteries pre-incubated with [^{3}H]noradrenaline ([^{3}H]NA) was studied. Since the release of radioactivity would depend on the integrity of the sympathetic innervation, we also established the post-mortem period within which human cerebral arteries seem to retain their adrenergic innervation functionally active.

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MATERIALS AND METHODS

Branches of the middle cerebral artery were obtained from an 8 year old child and 11 adults of either sex aged between 43 and 82 years (65 ± 3·5 years). Autopsies were performed by the Pathology Service of La Paz Hospital (Madrid) 3 to 13 h after death. The cause of death was neither from cerebrovascular disease nor tumours affecting the central nervous system.

The release of [^{3}H]NA by histamine was studied according to Farnebo & Malmfors (1971). The branches of the middle cerebral arteries (9·8 ± 1·4 mg, n = 14) were cleaned to remove traces of blood and incubated for 1 h in Krebs–Henseleit solution containing [^{3}H]NA (2 μCi ml^{-1}, 1·3 × 10^{-7} M, specific activity 15 Ci mmol^{-1}). The solution 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 at 37 °C through which a pre-warmed Krebs-Henseleit solution was allowed to superfuse at a constant flow rate of 0·5 ml min^{-1} by means of a perfusion pump. A stream of oxygen with 5% CO_2 was passed at the same time through the chamber. Once the spontaneous tritium outflow had reached a steady level, samples of the effluent were collected every 3 min. 0·5 ml aliquots 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 started to deliver Krebs-Henseleit solution containing histamine into the superfusion chamber after 9 min of collection and remained on for 12 min. The flow rate was 0·05 ml min^{-1} and the final concentration of histamine in the chamber was 10^{-4} M.

To study the effect of cocaine on the tritium release evoked by histamine, after 9 min of collections the Krebs-Henseleit solution via the first pump was quickly replaced by another containing the drug and 9 min later the solution with histamine was pumped into the chamber. The solution with cocaine was allowed to flow at 0·5 ml min^{-1} for the rest of the experiment. The final concentration of cocaine in the chamber was 10^{-6} M.

The tritium released in the sample preceding the addition of histamine was considered as the control level.

At the end of the experiments the blood vessels were placed in 0·4 M $HClO_4$ and kept at −15 °C for assay of their residual radioactivity content. To achieve this, the tissues were solubilized with soluene 100 and tritium was measured in both 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; $MgSO_4.7H_2O$, 1·2; $NaHCO_3$, 25; glucose, 11·1. Ethylenediaminetetraacetic acid (EDTA, 3×10^{-5} M) was added to prevent oxidation of unstable substances.

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), cocaine hydrochloride (Abelló), and (−)-[7,8-^{3}H]noradrenaline hydrochloride (Amersham).

RESULTS

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

The presence of 10^{-4} M histamine in the superfusion chamber induced a 2·2-fold rise over basal levels in the radioactivity released from human cerebral arteries obtained within a post-mortem period of 6 h (Fig. 1). The predrug tritium outflow was of 89 ± 21 counts min^{-1} mg^{-1} (n = 4). When the arteries were from autopsies performed 7 to 13 h after death no peak of radioactivity could be brought about by 10^{-4} M histamine (Fig. 1), though basal radioactivity release remained unchanged (68 ± 15 counts min^{-1} mg^{-1}, n = 6). The radioactivity retained by the tissue at the end of the experiments was significantly higher when the arteries were obtained within 6 h after death (Table 1).

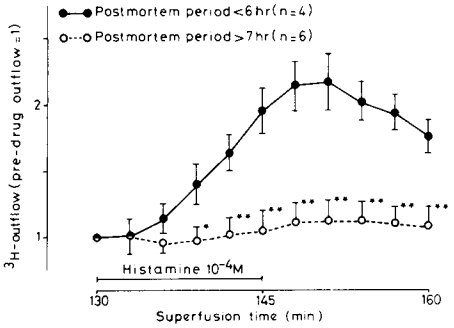

FIG. 1. Effect of post-mortem period on the tritium efflux evoked by 10^{-4} M histamine. Figures in parentheses indicate the number of experiments. Each point represents the mean ± s.e.m. $^*P < 0.05$. $^{**}P < 0.005$.

Table 1. Effects of post-mortem period on the radioactivity retained by human cerebral arteries.

Time after death	Counts min^{-1} mg^{-1} wet tissue
6 h or less (4)	34 204 ± 10 265
7 h or more (6)	7103 ± 2197*

* $P < 0.01$. Figures in parentheses indicate number of experiments.

The presence of 10^{-6} M cocaine in the superfusion chamber (Fig. 2) abolished the enhancement in tritium outflow evoked by 10^{-4} M histamine without altering the spontaneous radioactivity efflux (76 ± 15 counts min^{-1} mg^{-1}, n = 4). The human cerebral arteries used in these experiments were obtained within 6 h of death. The radioactivity retained by these vessels at the end of the test was not significantly different from control values (44 706 ± 14 169 counts min^{-1} mg^{-1}, n = 4).

DISCUSSION

Earlier work from this laboratory showed that hitamine possessed an indirect adrenergic mechanism in cat cerebral arteries (Marco et al 1980) that was reduced after treatments interfering with the function of the adrenergic system. The present

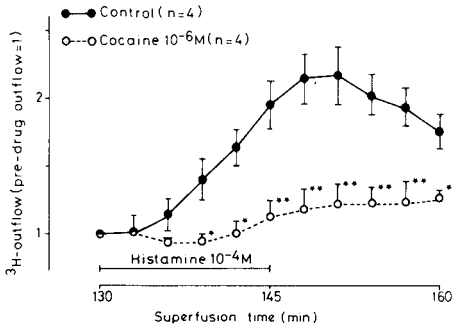

FIG. 2. Effect of 10^{-6} M cocaine on the tritium efflux evoked by 10^{-4} M histamine. Arteries were obtained within 6 h of death. Figures in parentheses indicate the number of experiments. Each point represents the mean ± s.e.m. $^{*}P < 0{\cdot}05$. $^{**}P < 0{\cdot}01$.

results indicate that histamine may also have the same effect in human cerebral arteries.

For obvious ethical reasons, and because of the scarcity of material, our approach had to be more restricted than with animal cerebral blood vessels. Therefore, the effect of histamine on the spontaneous tritium outflow from human cerebral arteries preincubated with [^{3}H]NA was chosen to indicate that histamine could release noradrenaline from adrenergic terminals. Histamine increased the tritium release from prelabelled human vessels indicating that part of the stored neurotransmitter was being liberated. The fact that the same kind of experiments carried out in the presence of cocaine showed a diminished release of radioactivity supports that the tritium liberated came from sympathetic nerve endings and that histamine needed to enter them to achieve its releasing effect. The same results were previously obtained in cat cerebral arteries (Marco et al 1980). These findings also suggest that the contractile responses of the human isolated cerebral arteries (Shibata et al 1977) may be due in part to the release of noradrenaline in the same way as in cat, although this needs further confirmation. It is also a matter of speculation whether histamine might participate in the cerebral vasoconstriction occurring during the prodromal phase of a migraine attack (Edmeads 1979) by means of this effect.

The time elapsed after the death of the tissue donor constituted an additional problem to this study. A failure of histamine to alter the spontaneous release of radioactivity could mean either a lack of indirect adrenergic component or an impairement of the sympathetic nerve endings function. Our results show that the latter possibility is more likely to be true. When the cerebral blood vessels were obtained within a post-mortem period of 6 h the radioactivity content of the tissue was much higher than when the elapsed time was 7 h or longer. Since most of the radioactivity is taken up by sympathetic nerve endings when tissues are incubated with [^{3}H]NA (Iversen 1975), this suggests that the adrenergic innervation of human cerebral arteries is degenerated or damaged 7 h after the death of the donor. On the other hand, histamine was able to bring about an increase in spontaneous tritium release only when the vessels were obtained within a post-mortem period of 6 h, supporting, firstly, that histamine needs the presence of functionally active sympathetic nerve endings and, secondly, that these last only 6 h after the donor's death. These results agree with observations made in human meninges (Iglesias et al 1981) which showed that the adrenergic innervation of cerebral blood vessels began to disappear gradually 3–4 h after death.

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