The Dilating Effect of Histamine on Pial Arteries of Cats and Its Mediation by H₂ Receptors

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SUMMARY We studied the effect of histamine and H₁ or H₂ blockers on the diameter of pial arteries (39-227 μm) using microapplication into the perivascular space. Concentration-response curves for histamine showed dilations which started at 10⁻³ M and were maximal at 10⁻² and 10⁻¹ M. The H₂ blocker, cimetidine, induced no vascular reaction over the whole concentration range tested (10⁻² to 10⁻⁴ M). The H₁ blocker, mepyramine, was not vasoactive in the concentration range from 10⁻³ to 5 × 10⁻⁵ M and evoked dilations at higher concentrations. The concentration-response curve for histamine was only slightly displaced by 10⁻⁷ M mepyramine but was significantly shifted to the right by 10⁻⁷ M cimetidine. The dilating effect of histamine could be reduced in a stepwise manner by increasing concentrations of cimetidine. These findings are in accordance with a selective antagonism between histamine and cimetidine at the H₂ receptors of smooth muscle cells of pial arteries. The insignificant role of H₁ receptors in histamine-induced dilations is supported by the finding that a combination of H₁ and H₂ blockers resulted in the same reduction of histamine-induced dilation as did the application of the H₂ blocker. Circ Res 44: 161-165, 1979

THE histamine located in the brain seems to serve more than one function. First, it may act as a neurotransmitter for synaptic transmission between central neurons (for references see Schwartz, 1977). Second, it also may be involved in the regulation of cerebrovascular resistance (Edvinsson et al., 1976; Schwartz, 1977), as can be deduced from the considerable amount of mast cells distributed throughout the whole brain (Ibrahim, 1974), predominantly in the perivascular region (Edvinsson et al., 1976; Ibrahim, 1974; Kruger, 1974; Rönnberg et al., 1973), which have been shown histochemically to contain histamine (Edvinsson et al., 1976; Rönnberg, 1973). Since the histamine-containing mast cells are especially concentrated in the leptomeninges (Rönnberg et al., 1973; Schwartz, 1977), the question arises whether the resistance of pial arteries that run within this tissue can be influenced by histamine. The aim of the present study was to investigate the effect of histamine on pial arteries, when applied locally into the perivascular space using the microapplication technique, which mimics the normal route of penetration of histamine from the perivascular side to the vascular smooth muscles of the brain vessels.

Methods

Experiments were performed on 35 cats of both sexes, which were anesthetized with α-chloralose (40-50 mg/kg, iv) and immobilized with gallamine (15 mg/kg per hour, iv). The cats were ventilated artificially by means of a Bird Mark 8 respirator. Arterial blood pH, PCO₂, and PO₂ were measured at 38°C using Radiometer BEUi and BMS equipment. The pH was 7.35 ± 0.037 (SD), PCO₂ was 29.5 ± 2.01 (SD) mm Hg, and PO₂ was 136.1 ± 22.0 (SD) mm Hg. The value of arterial PCO₂ was close to that obtained in conscious cats (Herbert and Mitchell, 1971). Endtidal CO₂ was 4.36 ± 0.25 (SD) vol%.

Arterial blood pressure was recorded continuously, and only cats with a mean pressure of more than 100 mm Hg were used for experiments. Body temperature was maintained between 37°C and 38°C. Tyrode's solution (2.5 ml/kg per hour) was infused iv. The brain surface (part of parietal lobe) was bathed with mineral oil heated to between 37°C and 38°C. Glass micropipettes with sharpened tips (8-10 μm o.d.) were filled with test solutions and sealed between oil, as described elsewhere (Wahl et al., 1973). The tip of a micropipette was positioned by a micromanipulator in the immediate vicinity of a superficial artery or arteriole. By applying pressure to a syringe attached to the micropipette, 1-3 μl of fluid was injected into the perivascular space. A Bausch and Lomb stereozoom microscope was used at a magnification of 70x. Vascular diameter was measured with the image-splitting method, using a 625 line Grundig TV camera (equipped with a multidiodal vidicon) and a Watanabe multicorder. The reproducibility of the method and the error due to defocussing have been discussed previously (Wahl et al., 1973). The data were obtained from 40-sec injection periods, during which measurements were made at 20 and 40 sec. The mean values of these two measurements were taken for the
calculations of the average curves. Test substances (histamine, mepyramine,† cimetidine‡) were dissolved in an inert mock spinal fluid of the following composition (in mM): Na⁺, 156; K⁺, 3; Ca²⁺, 1.5; Cl⁻, 151; HCO₃⁻, 11. The pH (38°C) was 7.16, and osmolarity, 305 mOsm/liter. The solution was gassed with a mixture of 5% CO₂ and 95% O₂ equilibrated with water. The composition of the mock spinal fluid was determined as described in a previous paper (Wahl et al., 1973). The electrolyte concentrations of this mock spinal fluid are similar to those of cisternal fluid of normal cats with the exception of the bicarbonate concentration. There appears to exist a gradient for bicarbonate from the cisternal fluid to the subarachnoidal and perivascular fluid, as has been discussed in two earlier papers (Kuschinsky et al., 1972; Kuschinsky and Wahl, 1975). Since it was not tested whether varying the composition of the mock spinal fluid would influence the histamine effect, the possibility of a modified histamine effect under other experimental conditions cannot be excluded.

First, the reactivity of each vessel under investigation to alteration in pH was tested (Kuschinsky et al., 1972). Then the effect of the inert mock spinal fluid (composition see above) was measured. The mean reaction to this solvent fluid was a constriction of 0.05%. Following this, the test substances dissolved in this inert mock spinal fluid were applied. Subtraction of the respective solvent effect yielded the data shown in the results. The statistical analysis of the data was performed by analysis of variance.

Results

We investigated the effect of histamine on pial arteries and arterioles with control diameters ranging from 39 to 227 μm. Histamine was applied in ascending concentrations. The average curve shown in Figure 1 demonstrates concentration-dependent dilations. Constrictions were never observed. Statistical analysis of the results revealed that the dilations at 10⁻⁷ M histamine and at higher concentrations were significantly (P < 0.01) different from the reactions to the solvent itself, which were taken as control values. The reactions to each histamine concentration were significantly (P < 0.01) different from each other, with the exception of the reactions to the highest concentrations of histamine (10⁻⁵ and 10⁻⁴ M) when maximal dilations due to histamine were observed. The degree of vascular dilation during application of histamine was not dependent on the initial vessel diameter.

The effects of the H₁ receptor blocker mepyramine and of the H₂ receptor blocker cimetidine on pial arterial diameter are depicted in Figure 2. Whereas the H₂ blocker, cimetidine, induced no vascular reaction over the whole concentration range tested, the H₁ blocker, mepyramine, induced significant (P < 0.01) dilations at concentrations higher than 5 × 10⁻⁵ M. These dilations are considered as nonspecific and are due to the well-known local anesthetic effects of H₁ blockers.

* Courtesy of E. Scheurich GmbH, Appenweier, FRG.
† Courtesy of Smith, Kline and French Laboratories, Welwyn Garden City, England.
To test which receptors mediate the histamine-induced dilations, the effects of these blocking agents on the histamine-induced dilations were investigated. Two concentration-response curves were determined for the same vessel, the first with histamine alone and the second during simultaneous application of histamine and the blocking agent. This procedure was deemed valid because repetition of the concentration-response curve for histamine with the same pial artery revealed exactly the same values for the first and second curve.

Figure 3 shows the effect of the H₁ blocker mepyramine on the concentration-response curve for histamine. Mepyramine (10⁻⁵ M) induced a minute shift of the concentration-response curve for histamine to the right. When both curves were compared by analysis of variance, the values of the reactions to histamine alone and histamine together with mepyramine were not significantly different from each other, with the exception of the reactions to 10⁻⁶ M histamine, which were reduced by mepyramine (P < 0.05). To test whether this effect is due to non-steady state conditions during the 40-second application period, the following experiments were performed to see whether the reduction in the histamine effect became more evident during a longer exposure to mepyramine. After testing the dilation due to 10⁻⁶ M histamine, a continuous perivascular perfusion of 10⁻⁵ M mepyramine was performed at the same site for 5-9 minutes. During the last 40 seconds of the perfusion of mepyramine, histamine was applied simultaneously, using a second micropipette for injection. The dilations due to 10⁻⁶ M histamine were exactly the same when histamine was applied during the control period (+26%) and during the prolonged infusion of mepyramine (+26%).

![Figure 3](http://circres.ahajournals.org/)

**Figure 3** Influence of mepyramine (10⁻⁵ M) on the histamine concentration-response curve. Mepyramine was applied simultaneously with histamine after the histamine curve had been determined for the same vessel. Means ± SEM; n = number of vessels tested.

Figure 4 shows the effect of the H₂ blocker, cimetidine, on the concentration-response curve for histamine. It is evident that 10⁻⁵ M cimetidine induced a shift of the concentration response curve for histamine to the right. The maximal dilating effect of histamine was not reduced by cimetidine, but higher histamine concentrations were necessary to obtain the same effect during H₂ receptor blockade. When both curves were compared by analysis of variance, the values of the reactions to histamine alone and to histamine together with cimetidine were significantly (P < 0.01) different at the histamine concentrations of 10⁻⁶ and 10⁻⁵ M.

The blocking action of cimetidine was also established in experiments investigating the effect of increasing cimetidine concentrations on histamine-induced dilations. The results of these experiments are shown in Figure 5. It is evident that the dilations induced by 10⁻⁵ M histamine could be reduced by increasing the concentration of cimetidine. The statistical analysis revealed that the reduction of the histamine-induced dilation by cimetidine was significant (P < 0.01) at 10⁻⁵ and 10⁻⁴ M cimetidine.

In additional experiments, the effect of simultaneous application of the H₁ blocker, mepyramine, and the H₂ blocker, cimetidine, on the histamine-induced dilation was tested. The results are depicted in Figure 6. It is apparent that the reduction in the histamine (10⁻⁶ M) induced dilation due to cimetidine (2.5 × 10⁻⁶ M) could not be enhanced by the additional application of mepyramine (10⁻⁵ M). The reactions due to the blockers were both significantly (P < 0.01) different from the effect due to histamine alone but were not different from each other.

Corresponding results were obtained using other combinations of the blockers. The effects of the
Effects of cimetidine (2.5 × 10⁻⁶ M) and cimetidine (2.5 × 10⁻⁷ M) together with mepyramine (2 × 10⁻⁶ M) on histamine (10⁻⁴ M) induced dilations. The drugs were applied simultaneously to the same vessel. Means ± SEM; n = number of vessels tested.

**FIGURE 5** The effect of increasing concentrations of cimetidine (10⁻⁶ to 10⁻⁴ M) on histamine (10⁻⁴ M) induced dilations. Means ± SEM; n = number of vessels tested.

The data presented here clearly demonstrate a dilating effect of histamine on pial arteries under in vivo conditions, when applied locally from the perivascular side. The results of previous in vivo studies using superfusion of the brain surface were divergent. Whereas dilations of pial arteries by histamine have been reported in the cat (Forbes et al., 1951; Raper et al., 1972), no vascular response has been found in the mouse (Rosenblum, 1979). Inconsistent results also have been found in studies using intravascular application of histamine. An increase (Shenkin, 1951; Carpi et al., 1972; Tindall and Greenfield, 1973; Muravchick and Bergofsky, 1976), as well as no change of cerebral blood flow (Shenkin, 1951; Alman et al., 1952; Olesen, 1974), has been reported after intravenous (Shenkin, 1951; Alman et al., 1952; Carpi et al., 1972) or intraarterial (Tindall and Greenfield, 1973; Olesen, 1974; Muravchick and Bergofsky, 1976) application. The sensitivity of cerebral arteries to histamine cannot be quantified from these data, since a blood-brain barrier for histamine (Oldendorf, 1971; for further references see Calcutt, 1976) can modify the effect of intravascular histamine.

The present results are not in accord with most of the in vitro studies, in which constrictions of cerebral arteries have been found (Nielsen and Owman, 1971; Allen et al., 1974; Bevan et al., 1975; Edvinsson and Owman, 1975; Urquilla et al., 1975; Shibata et al., 1977). These constrictions could be inhibited by H₁ blockers in a nonspecific way (Edvinsson and Owman, 1975). Dilations of isolated cerebral arteries could be observed only when the vessels were precontracted with serotonin (Edvinsson and Owman, 1975). These dilations could be blocked by the H₂ blocker, burimamide, in a competitive manner (Edvinsson and Owman, 1975; Edvinsson et al., 1976). In contrast to these in vitro investigations, in the present in vivo microapplication study, the dilating effect of histamine was obvious without any pretreatment of the vessels. These dilations are mediated mainly by H₂ receptors. This conclusion is based on the following: (1) The H₂ blocker, cimetidine, induced a significant shift of the concentration-response curve for histamine to the right (Fig. 4). The reversible parallel displacement of the concentration response curve without reduction of the maximal effect indicates an antagonism between histamine and cimetidine at pial arteries. (2) The maximal dilation induced by histamine (10⁻⁶ M) could be reduced stepwise up to a complete blockade by the H₂ blocker, cimetidine (Fig. 5). (3) The H₁ blocker, mepyramine (2 × 10⁻⁶ M), induced only a minute shift of the concentration-response curve for histamine, which was significant (P < 0.05) for only one concentration of histamine (10⁻⁴ M). However, with the same concentrations, a blocking action could not be verified when mepyramine was applied over a prolonged period, thus assuring steady state conditions. (4) Simultaneous application of H₁ and H₂ blockers induced the same reduction of the histamine-induced dilations as did the

**FIGURE 6** Effects of cimetidine (2.5 × 10⁻⁶ M) and cimetidine (2.5 × 10⁻⁷ M) together with mepyramine (2 × 10⁻⁶ M) on histamine (10⁻⁴ M) induced dilations. The drugs were applied simultaneously to the same vessel. Means ± SEM; n = number of vessels tested.
H2 blocker alone (Fig. 6). These results from pial arteries under in vivo conditions demonstrate a difference from most other vascular beds, in which a combination of both H1 and H2 blockers is most effective, as has been recently reviewed by Owen (1977).

References
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