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

MICHAEL WAHL AND WOLFGANG KUSCHINSKY

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 10⁻⁷ M and were maximal at 10⁻⁵ and 10⁻⁴ M. The H₁ blocker, cimetidine, induced no vascular reaction over the whole concentration range tested (10⁻⁷ to 10⁻³ M). The H₂ blocker, mepyramine, was not vasoactive in the concentration range from 10⁻⁷ to 5 x 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
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 X 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%).

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^{-3} M cimetidine 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
The effect of increasing concentrations of cimetidine (10^{-6} to 10^{-4} M) on histamine (10^{-6} M) induced dilations. Means ± SEM; n = number of vessels tested.

following combinations on the dilation induced by 10^{-6} M histamine were tested: (1) 5 \times 10^{-6} M cimetidine alone, and together with 5 \times 10^{-6} M mepyramine; (2) 2.5 \times 10^{-6} M cimetidine alone, and together with 5 \times 10^{-6} M mepyramine.

Discussion

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., 1929; Raper et al., 1972), no vascular response has been found in the mouse (Rosenblum, 1978). 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 constricitions 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; Edvinsson et al., 1976). 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^{-5} M) could be reduced stepwise up to a complete blockade by the H2 blocker, cimetidine (Fig. 5). (3) The H1 blocker, mepyramine (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^{-5} 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...
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
Owen DAA: Histamine receptors in the cardiovascular system. Gen Pharmacol 8: 141-156, 1977
The dilating effect of histamine on pial arteries of cats and its mediation by H2 receptors.
M Wahl and W Kuschinsky

Circ Res. 1979;44:161-165
doi: 10.1161/01.RES.44.2.161

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1979 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/44/2/161

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/