Heterogeneous responses of canine basilar and middle cerebral arteries to serotonin at normal and high CO_2 tension

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Received 25 November 1991; accepted 30 July 1992

Abstract. The responses of basilar arteries (BAs) to serotonin were attenuated by high P_{CO_2} (86 ± 1 mm Hg) and the pH matched acidotic solution (P_{CO_2} 37 ± 1 mm Hg), whereas the responses of middle cerebral arteries (MCAs) were not. High P_{CO_2} decreased the basal tone of both arteries, and the changes in basal tone due to high P_{CO_2} were not influenced by 3×10^{-7} M imipramine, 10^{-5} M pargyline or 10^{-4} M aspirin. The responses of BAs to serotonin were attenuated by high P_{CO_2} in the presence of imipramine, pargyline and aspirin. The responses of MCAs to serotonin were not influenced by high P_{CO_2} in the presence of pargyline and aspirin, but attenuated by high P_{CO_2} in the presence of imipramine.
Key words. Carbon dioxide; serotonin; canine cerebral artery; basilar artery; middle cerebral artery.

Mammalian cerebral arteries are highly sensitive to serotonin which elicits strong vasoconstriction[1-5]. Serotonin stored in platelets is released when platelets aggregate, for example in subarachnoid hemorrhage. Exaggerated liberation of serotonin from aggregating platelets affects cerebrovascular tone[1]. Furthermore, serotonin can be taken up into sympathetic nerves innervating cerebral arteries and can be released to act as an alternative transmitter[2]. Patients with cerebral hemorrhage sometimes become hypercapnic because of airway obstruction due to loss of consciousness. Thus, in such pathological conditions it is important to know how high P_{CO_2} affects the responses of cerebral arteries to serotonin. Large cerebral arteries such as the basilar (BAs) and middle cerebral arteries (MCAs) are thought to be major resistance vessels[3]. In addition, there is heterogeneity of vascular response to agonists (including serotonin) among anatomically distinct cerebral vessels[4-6]. However, little is known about the effects of high P_{CO_2} on the responses to serotonin in the cerebral vessels. Therefore, we investigated the responses of basilar and middle cerebral arteries to serotonin at normal and high P_{CO_2}, and found different effects. To investigate the different effects of high P_{CO_2} on these two cerebral arteries, we also examined their serotonin responses in the presence of the serotonin uptake inhibitor, imipramine, the MAO inhibitor, pargyline and the cyclooxygenase inhibitor, aspirin.

Materials and methods

This study was approved by the Animal Research Committee of Niigata University School of Medicine. Mongrel dogs of either sex, weighing 9–13 kg, were anesthetized with an intravenous injection of sodium thiopental (30 mg/kg) and killed by bleeding from the common carotid arteries. The methods used in the present study were almost the same as those used previously[7]. The BAs and MCAs (0.6–0.9 mm diameter) were isolated and ring preparations (3 mm length) were made. The specimens were fixed vertically between hooks at a resting tension of 1.5 g in a muscle bath (20 ml capacity) containing modified Krebs solution. The composition of the nutrient solution was as follows (mM): Na^+ 143.0; K^+ 5.9; Ca^{2+} 2.5; Mg^{2+} 1.2; Cl^- 153.9; HCO_3^- 25.0; SO_4^{2-} 1.2; H_2PO_4 1.2; dextrose 10.0. Hooks anchoring the upper ends of the strips were connected to the lever of a force-displacement transducer (TB-612T, Nihon Koden Kogyo Co. Tokyo, Japan). The solution was maintained at 37 °C and aerated with a mixture of 95% O_2 and 5% CO_2.

After a 1-h equilibration period, serotonin was added cumulatively to concentrations ranging from 10^{-9} to 3×10^{-6} M. After washing with fresh Krebs solution and stabilizing the basal tone, cumulative doses of serotonin were administered again. To test the effects of high P_{CO_2} on serotonin dose-response curves, the solutions were aerated with 88.0% O_2 and 12.0% CO_2 (high P_{CO_2}), 10 min before obtaining the second dose response curve, and changes in basal tone were observed. The O_2 tension (P_{O_2}), CO_2 tension (P_{CO_2}), and pH were measured at 37 °C using ABL-2 ®blood gas analyzer (Radiometer, Copenhagen, Denmark). After the second serotonin dose-response curves were completed, the bath fluid was collected, and its pH, P_{CO_2} and P_{O_2} were immediately measured with the blood gas analyzer.

In the second set of experiments, the effects of hydrogen ion (H^+ ion) on the responses of BAs and MCAs to serotonin were studied at normal P_{CO_2}. The acidotic solution (approximately pH 7.00) was obtained by lowering the HCO_3^- concentration of the Krebs solution at normal P_{CO_2}. To make a solution of approximately pH 7.00 at normal P_{CO_2} (P_{CO_2} 40 mm Hg) the $NaHCO_3$ concentration was decreased from 25.0 mEq/l to 9.9 mEq/l and the concentration of NaCl was increased from 118 mEq/l to 133 mEq/l to adjust isotonicity.

In the third set of experiments, 3×10^{-7} M imipramine (which inhibits serotonin uptake into sympathetic nerves) or 10^{-5} M pargyline (MAO inhibitor which inhibits degradation of serotonin) or 10^{-4} M aspirin (cyclooxygenase inhibitor) was added 30 min before the administrations of normal or high concentrations of carbon

dioxide. The effects of the pre-administered drugs on dose response to serotonin were then observed. The values presented in the text and figures are means ± SEM. Two means were compared using Student's t-test for unpaired data. When more than two means were compared, one-way analysis of variance was used followed by least significant different test (LSD) for multiple comparisons. Statistical significance was established when the probability value was less than 0.05.

Results

The values of pH and P_{CO_2} were 7.39 ± 0.00 and 37 ± 1 mm Hg at normal P_{CO_2} (n = 59), and 7.01 ± 0.01 and 86 ± 1 mm Hg at high P_{CO_2} (n = 54). The values of pH and P_{CO_2} in each group were 7.39 ± 0.00 and 37 ± 1 mm Hg (control: normal pH and normal P_{CO_2}: n = 59), 6.99 ± 0.00 and 91 ± 1 mm Hg (low pH and high P_{CO_2}: n = 54), and 7.01 ± 0.01, 36 ± 1 mm Hg (low pH and normal P_{CO_2}: n = 14), respectively.

High P_{CO_2} decreased basal tone by 107 ± 30 mg in non-treated BAs (n = 7) and by 122 ± 19 mg in non-treated MCAs (n = 6). The acidotic solution with normal P_{CO_2} also decreased basal tone by 110 ± 24 mg in non-treated BAs (n = 7) and by 97 ± 19 mg in non-treated MCAs (n = 7). Changes in basal tone due to high P_{CO_2} were not influenced by 3×10^{-7} M imipramine, 10^{-5} M pargyline or 10^{-4} M aspirin. The decrease in resting tension of 100 mg did not influence the response to serotonin in either artery (data not shown).

In non-treated BAs, the responses to 10^{-9}–3×10^{-6} M serotonin were attenuated by high P_{CO_2} (n = 7) and the pH matched acidotic solution with normal P_{CO_2} (n = 7) compared with normal P_{CO_2} (n = 14) (fig. 1, left panel).

In non-treated MCAs, the responses to serotonin were not significantly influenced by high P_{CO_2} (n = 6) nor the pH matched acidotic solution (n = 7) compared with normal P_{CO_2} (n = 13) (fig. 1, right panel).

In imipramine-treated BAs, the responses to serotonin were attenuated by high P_{CO_2} (n = 8) compared with normal P_{CO_2} (n = 8) (fig. 2, left panel). In imipramine-treated MCAs, the responses to serotonin were attenuated by high P_{CO_2} (n = 8) compared with normal P_{CO_2} (n = 7) (fig. 2, right panel).

In pargyline-treated BAs, high P_{CO_2} attenuated the responses to serotonin (n = 7) compared with normal P_{CO_2} (n = 7) (fig. 3, left panel), but in pargyline-treated MCAs high P_{CO_2} did not attenuate the responses to serotonin (n = 8) compared with normal P_{CO_2} (n = 7) (fig. 3, right panel).

In aspirin-treated BAs, high P_{CO_2} attenuated the responses to serotonin (n = 8) compared with normal P_{CO_2} (n = 6) (fig. 4, left panel), but in pargyline-treated MCAs high P_{CO_2} did not attenuate the responses to serotonin (n = 7) compared with normal P_{CO_2} (n = 7) (fig. 4, right panel).

Discussion

The present study reveals that the effects of high P_{CO_2} on the responses to serotonin differ between canine BAs and MCAs. Our previous study demonstrated the direct inhibitory action of high P_{CO_2} on serotonin-induced contractions in rat aortic smooth muscle cells[8]. High P_{CO_2} and the pH-matched acidotic solution attenuated the responses to serotonin in BAs to a similar extent. The finding suggests that the attenuation by high P_{CO_2} of the response to serotonin in BAs may be due to the changes in pH. In the present study, we expected the contractile

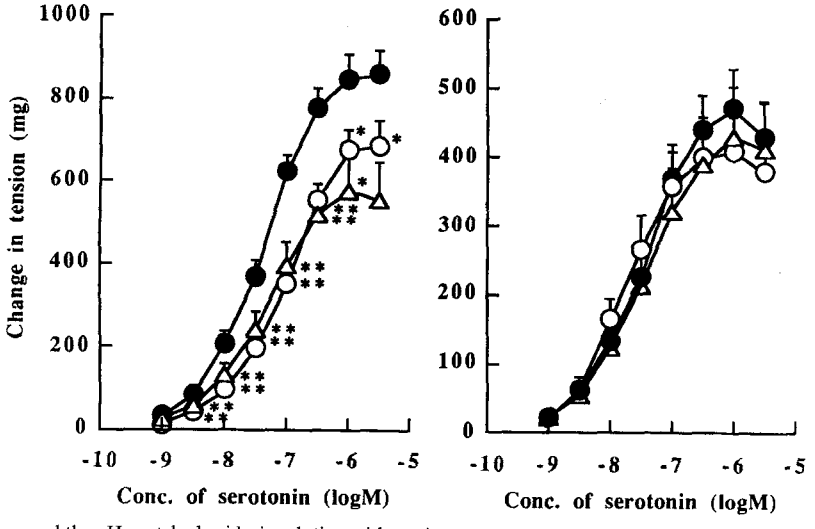

Figure 1. Effects of high P_{CO_2} and the pH-matched acidotic solution with normal P_{CO_2} on the responses to serotonin in non-treated BAs (left panel) and MCAs (right panel). Closed circles represent the group with normal pH and P_{CO_2}, open circles represent the group with low pH and high P_{CO_2}, and open triangles represent the group with low pH and normal P_{CO_2}.

* $p < 0.05$, ** $p < 0.01$; significantly different from the contractions at normal P_{CO_2}. In BAs, high P_{CO_2} and the pH-matched acidotic solution significantly attenuated the responses to serotonin to a similar extent (left panel), whereas no similar attenuation was seen in MCAs (right panel).

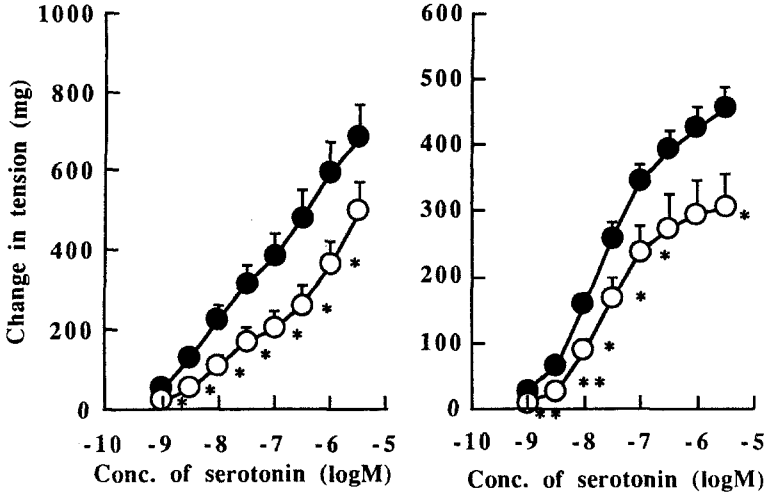

Figure 2. Effects of high P_{CO_2} on serotonin-induced responses in 3×10^{-7} M imipramine-treated BAs (left panel) and MCAs (right panel). Closed circles represent the group with normal P_{CO_2} and open circles represent the group with high P_{CO_2}. * $p < 0.05$, significantly different from the contractions at normal P_{CO_2}. In the presence of imipramine, high P_{CO_2} attenuated the responses to serotonin in the BAs (left panel) and MCAs (right panel).

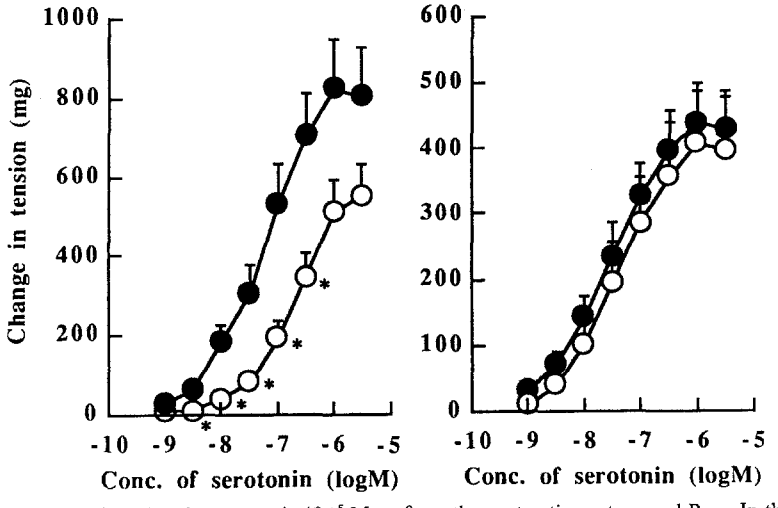

Figure 3. Effects of high P_{CO_2} on serotonin-induced responses in 10^{-5} M pargyline-treated BAs (left panel) and MCAs (right panel). Closed circles represent the group with normal P_{CO_2} and open circles represent the group with high P_{CO_2}. * $p < 0.05$, ** $p < 0.01$; significantly different from the contractions at normal P_{CO_2}. In the presence of pargyline, the responses to serotonin were attenuated by high P_{CO_2} in the BAs (left panel), but not in MCAs (right panel).

responses to serotonin to be attenuated by high P_{CO_2} in both the BAs and MCAs. However, the responses to serotonin were not altered by high P_{CO_2} in the MCAs, whereas those to serotonin were attenuated by high P_{CO_2} in BAs as expected. One possible explanation for the lack of effects of high P_{CO_2} in MCAs is that a factor counteracting the inhibitory action of CO_2 on smooth muscle cells exists in the MCAs. Another is that pH- or high P_{CO_2}-dependency of serotonin tone is present in BAs, but not in MCAs. If the latter hypothesis is correct, high P_{CO_2} would not attenuate the response of MCAs to serotonin in any conditions. However, in the present study, high P_{CO_2} attenuated the response of MCAs to serotonin in the presence of imipramine. It has been reported that the MCAs are more densely innervated by sympathetic nerves than the BAs[9]. It has also been recognized that serotonin is taken up into sympathetic nerve terminals of cerebral arteries as a 'false transmitter', but not into smooth muscle cells and endothelial cells[2,9,10], and that uptake of the amines into sympathetic nerve terminals is inhibited by high P_{CO_2}[11]. Thus, in non-treated MCAs, surplus serotonin in the sympathetic nerve cleft by high P_{CO_2} may counteract the attenuated response to serotonin in vascular smooth muscle cells. High P_{CO_2} attenuated the responses to serotonin in imipramine-treated MCAs, but not in pargyline-treated ones. Imipramine and CO_2 share the similar inhibitory action on amine uptake at sympathetic nerve terminals[2,10]. Thus, in imipramine-treated MCAs the action of CO_2 at sympathetic nerve terminals may be masked by imipramine,

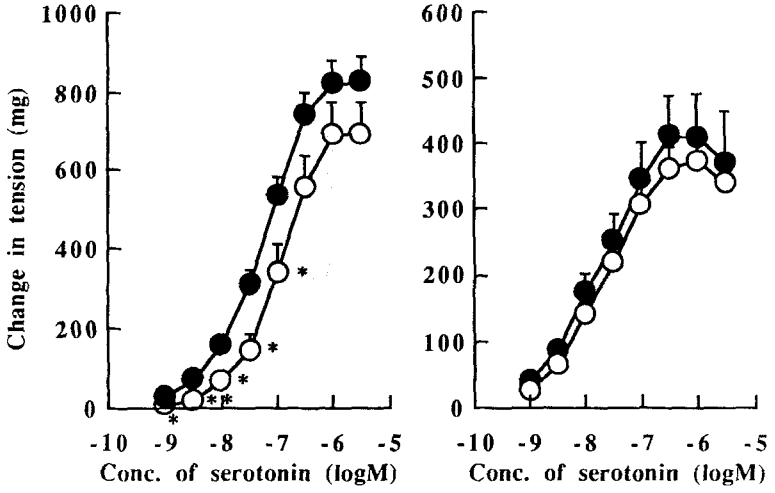

Figure 4. Effects of high P_{CO_2} on serotonin-induced responses in 10^{-4} M aspirin-treated BAs (left panel) and MCAs (right panel). Closed circles represent the group with normal P_{CO_2} and open circles represent the group with high P_{CO_2}. * $p < 0.05$; ** $p < 0.01$; significantly different from the contractions at normal P_{CO_2}. In the presence of aspirin, high P_{CO_2} also attenuated the responses to serotonin in BAs (left panel), but not in MCAs (right panel).

and the depressant action of CO_2 on smooth muscle cells may become apparent. In contrast, in pargyline-treated preparations, the action of CO_2 at sympathetic nerve terminals may oppose the inhibitory action of CO_2 at smooth muscle cells.

The contribution of intrinsic eicosanoids to CO_2-induced cerebral vasodilation is controversial. Inhibition of cyclooxygenase attenuates the increase in total cerebral blood flow during hypercapnia in rat, cat, baboon and human[12,13]. In contrast, there are reports demonstrating that prostaglandins do not participate in hypercapnia-induced cerebral vasodilation in cat and dog[14,15]. These studies are concerned with total cerebral blood flow or pial arterial dilatation but not with large arteries which are responsible for most of the resistance to cerebral blood flow[3]. In the present study, aspirin did not influence the change in basal tone due to high P_{CO_2} in both the BAs and MCAs. Thus, cyclooxygenase related eicosanoids may not be involved in vasodilation induced by high P_{CO_2} in both these large cerebral arteries.

In the context of the control of the cerebral blood flow, the reduced responses to serotonin of BAs, which mainly perfuse the brain stem, during hypercapnia might be an intrinsic protective property in conditions like cerebral hemorrhage.

In summary, high P_{CO_2} has heterogeneous effects on the serotonin contraction in the two anatomically distinct cerebral vessels. The resistance to high P_{CO_2} of serotonin in non-treated MCAs may be related to the innervation of the sympathetic nerves.

Acknowledgments. This work was supported in part by the Grant-in-Aid 03454371 from the Japanese Ministry of Education, Science and Culture.

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