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The α_1-adrenoceptor antagonist, doxazosin, modulates the lower limit of autoregulation of cerebral blood flow during hemorrhagic hypotension in anesthetized hypertensive rats

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Abstract

The objective of the present study was to examine the effects of administration of an α_1-adrenoceptor antagonist, doxazosin, for 7 days on cerebral blood flow and the autoregulatory response to hypotension in anesthetized spontaneously hypertensive rats. We determined cerebral blood flow at rest and during hemorrhagic hypotension in 14 rats of each group using laser-Doppler flowmetry, and at the same time, the absolute baseline cerebral blood flow values at the parietal cortex were also quantified in some of the rats with the hydrogen clearance method. Baseline mean arterial pressure was significantly lowered, by 41 mm Hg, in the doxazosin-treated group, while the baseline cerebral blood flow was 31 ± 4 ml/100 g/min (mean $\pm$ S.D.) which was almost the same as the 32 ± 5 ml/100 g/min in the control group. The lower limits of cerebral blood flow autoregulation were 139 ± 9 mm Hg in the control group and 96 ± 12 mm Hg in the treated group; the difference was significant ($P < 0.001$). The present results demonstrated that the lower limit of cerebral blood flow autoregulation shifts to a lower level after chronic treatment with doxazosin, an effect which is favorable for the maintenance of cerebral blood flow under hypotensive conditions.

Keywords: Cerebral blood flow autoregulation; Doxazosin; α-Adrenoceptor; Hemorrhagic hypotension; Spontaneously hypertensive rat (SHR)

1. Introduction

Hypertension is one of the major risk factors for the development of cerebrovascular diseases, and it is now apparent that prophylactic antihypertensive therapy contributes to reduce the incidence not only of hemorrhagic but also of ischemic cerebrovascular diseases (Ueda et al., 1981, 1988; Beard et al., 1992). The lower limit of cerebral blood flow autoregulation is shifted to a higher level in chronic, long-standing hypertension (Strandgaard et al., 1973; Fujishima and Omae, 1976) and thereby an acute excessive reduction of systemic arterial pressure may occasionally lead to a marked reduction in cerebral blood flow, and induce brain ischemia (Graham, 1975; Strandgaard et al., 1984). Therefore, the effects of antihypertensive drugs on cerebral blood flow autoregulation should be carefully investigated so as not to cause ischemic cerebrovascular events during antihypertensive therapy. For example, an α-adrenoceptor antagonist, phenoxybenzamine, attenuated, whereas a β-adrenoceptor antagonist, propranolol, aggravated the cerebral blood flow autoregulation of the lower limits (Shiokawa et al., 1989). The characteristic effects of each antihypertensive drug on cerebral microcirculation should thus be carefully examined.

Doxazosin mesylate (UK-33274, Pfizer UK) is an effective antihypertensive agent, the antihypertensive activity of which is a consequence of selective inhibition of postsynaptic α_1-adrenoceptors both in experimental animals (Karamat et al., 1980; Timmermans et al., 1980) and in humans (Singleton et al., 1980). The effective action of doxazosin to lower blood pressure (Donnelly et al., 1989; Mozzato et al., 1989; Marwood et al., 1992) and to potentially increase cerebral blood

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flow has been reported. However, it is not clear that doxazosin favorably modulates the lower limits of cerebral blood flow autoregulation either in the clinical or in the experimental setting. The main objective of the present study was to elucidate whether or not administration of doxazosin for 7 days attenuates the changes in cerebral circulation during doxazosin-induced and subsequent hemorrhagic hypotension in spontaneously hypertensive rats (SHRs).

2. Materials and methods

Twenty-eight male SHRs (6–7 months of age, 350–380 g) were separated into two groups. Fourteen rats were treated with doxazosin, and the other 14 rats were controls. Doxazosin (1.0 mg/kg/day), which was dissolved in distilled water, was administered by gavage into the stomach once a day. The rats for control were treated with the same amount of distilled water alone (1.6 ml/kg/day). The rats had free access to food and water prior to the experiment. The experiments were carried out, on the seventh day, about 3.5 h after the final treatment with doxazosin or distilled water.

Under amobarbital anesthesia (100 mg/kg body weight i.p.), both femoral arteries were cannulated, one for continuous recording of arterial blood pressure and for sampling blood, and the other for production of stepwise hypotension by controlled bleeding. Rectal temperature was maintained at 37°C with a heating pad. Rats were mounted on a stereotaxic head-holder in a sphinx position. One burr hole (5 mm in diameter) for cerebral blood flow measurement was made in the parietal bone with a high-speed drill under an operating microscope. Cerebral blood flow of the parietal cortex was continuously monitored with laser-Doppler flowmetry according to Dirnagl et al. (1989). Briefly, a laser-Doppler probe was placed on the dura mater approximately 4 mm posterior and 2 mm lateral to the bregma. This probe was connected to a perfusion monitor (Periflux PF3, Perimed, Sweden). Changes in cerebral blood flow determined with laser-Doppler flowmetry (CBF_{LDF}) were expressed as percentages of the baseline values. In seven rats of each group, baseline cerebral blood flow was also determined by the hydrogen clearance method (CBF_{H2}) as described in detail elsewhere (Fujishima et al., 1981). A Teflon-coated platinum electrode with platinum black on its tip was placed 1.5 mm deep from the brain surface and 2 mm away from the laser-Doppler flowmetry probe.

Twenty minutes after stabilization, at least three baseline values of cerebral blood flow were measured by laser-Doppler flowmetry and the hydrogen clearance method at intervals of 10 min. Then arterial blood was withdrawn from the femoral artery to decrease systemic arterial pressure in a stepwise manner, 10 mm Hg/step. Arterial blood pressure was maintained at each level for at least 5 min during which the arbitrary unit of CBF_{LDF} was recorded. Arterial gases and pH were determined 3 times, i.e., under resting conditions, at blood pressure levels of 120 mm Hg (in the control group) or 90 mm Hg (in the doxazosin group) as well as 60 mm Hg (control) or 40 mm Hg (doxazosin). The animals were killed by an injection of saturated KCl solution into the femoral artery at the end of the experiment.

The lower limit of cerebral blood flow autoregulation was defined as the mean arterial pressure at which cerebral blood flow decreased by 10% of the baseline value. The results were expressed as means ± S.D. Statistical analysis was performed with the two-tailed *t*-test.

3. Results

Physiological variables during the autoregulation study are summarized in Table 1. Mean values of $PaCO_2$, PaO_2, pH and hematocrit were not significantly different between the two groups. Mean arterial pressure at rest and lower limits of autoregulation are shown in Table 2. Resting values of mean arterial pressure were 171 ± 8 mm Hg and 130 ± 10 mm Hg in the control and doxazosin-treated groups, respectively. Even though baseline mean arterial pressure was significantly lowered, by 41 mm Hg, in the doxazosin-treated group, the resting cerebral blood flow (31 ± 4 ml/100 g/min), determined by the hydrogen clearance method, was almost the same as the 32 ± 5 ml/100

Table 1
Physiological variables

	Control ($n = 14$)	Doxazosin ($n = 14$)
$PaCO_2$, mm Hg		
Baseline	42 ± 3	43 ± 4
Moderate hypotension	40 ± 3	42 ± 4
Severe hypotension	36 ± 6	38 ± 5
PaO_2, mm Hg		
Baseline	88 ± 5	86 ± 8
Moderate hypotension	96 ± 8	90 ± 8
Severe hypotension	107 ± 15	102 ± 13
pH		
Baseline	7.40 ± 0.02	7.40 ± 0.03
Moderate hypotension	7.41 ± 0.02	7.41 ± 0.02
Severe hypotension	7.40 ± 0.05	7.42 ± 0.03
Hct, %		
Baseline	42 ± 3	39 ± 3
Moderate hypotension	41 ± 3	40 ± 3
Severe hypotension	39 ± 4	39 ± 3

Values are means ± S.D. Hct, hematocrit. Physiological variables were determined at rest (before hypotension), during moderate hypotension (ΔMAP about −50 mm Hg) and during severe hypotension (ΔMAP about −100 mm Hg).

Table 2
Mean arterial pressure and cerebral blood flow at rest and lower limits of autoregulation in control and doxazosin-treated rats

	Control $n=14$	Doxazosin $n=14$
Baseline MAP, mm Hg	171 ± 8 [a]	130 ± 10
Baseline CBF_{H2}, ml/100 g/min [b]	32 ± 5	31 ± 4
Lower limits of autoregulation, mm Hg		
CBF_{LDF} decreased by 10%	139 ± 9 [a]	96 ± 12
CBF_{LDF} decreased by 20%	123 ± 9 [a]	88 ± 12

Values are means ± S.D. MAP, mean arterial pressure; CBF, cerebral blood flow. [a] $P < 0.001$ vs. doxazosin-treated group. [b] Baseline values of CBF with the hydrogen clearance method (CBF_{H2}) were determined in seven rats in each group.

g/min in the control group. Fig. 1 demonstrates the relationship between mean arterial pressure and cerebral blood flow in the parietal cortex during hemorrhagic hypotension in the two groups. Although there are some overlaps in S.D. bars between the two groups at a mean arterial pressure range of 60–90 mm Hg, the differences between the two groups are clear on the basis of the average values. The lower limit of cerebral blood flow autoregulation, defined as the mean arterial pressure at which cerebral blood flow decreased by 10% of the baseline value, was 96 ± 12 mm Hg in the treated group, which was lower than the 139 ± 9 mm Hg in the control group ($P < 0.001$). In the treated group, the mean arterial pressure value at which cerebral blood flow decreased by 20% of the baseline value was also shifted to a level (88 ± 12 mm Hg) lower than the 123 ± 9 mm Hg in the control group ($P < 0.001$). The autoregulatory range for cerebral blood flow was well preserved in both groups.

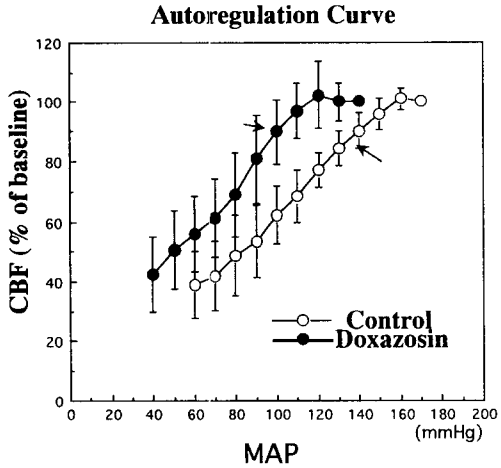

Fig. 1. Relation of mean arterial pressure (MAP) to cerebral blood flow (CBF) in the parietal cortex in spontaneously hypertensive rats (SHRs) treated with doxazosin (closed circles) or distilled water as a control (open circles). Arrows indicate lower limits of autoregulation defined as the MBP at which CBF decreased by 10% of the baseline value. Lower limit blood pressure level was 139 ± 9 mm Hg in the control and 96 ± 12 mm Hg in the treated groups.

4. Discussion

Studies on a quinazoline derivative, doxazosin, a selective blocker of α_1-adrenoceptors, established that doxazosin decreases total peripheral resistance and blood pressure and slightly increases both cardiac output and heart rate (Elliott et al., 1982; Frick et al., 1986). Drugs such as prazosin, bunazosin, terazosin, etc., antagonized α_1-adrenoceptors even in the central nervous system, and the side-chains bound to quinazoline and quinazolinedione residues may play an important role in the antagonistic potencies for α_1-adrenoceptors in the central nervous system as they do in the peripheral tissues (Tsuchihashi and Nagotomo, 1989). Doxazosin is currently used as an effective antihypertensive agent in a clinical setting. This drug has a slow rate of elimination from blood, therefore, a satisfactory antihypertensive effect can be obtained by once-daily administration. Moreover, doxazosin seems to induce fewer side-effects than former drugs of the same class (Mozzato et al., 1990); it has favorable lipid change characteristics; it reduces hyperinsulinemia and glucose intolerance and attenuates the adverse hemodynamic and hemostatic effects of smoking (Pool, 1994). Therefore, doxazosin is used especially for patients with concomitant metabolic disorders such as hyperlipidemia, diabetes mellitus, and metabolism-related coronary heart disease (Hitzenberger and Ganzinger, 1993).

In order to make clear the effects of hemorrhagic hypotension on cerebral circulation with or without doxazosin, the changes in cerebral blood flow and lower limit of cerebral blood flow autoregulation were examined in hypertensive rats. For this purpose, laser-Doppler flowmetry, a method allowing non-invasive measurement of continuous changes in microcirculatory blood flow in a small tissue sample (Oeberg et al., 1984), was used in the present study. Because laser-Doppler flowmetry does not provide absolute cerebral blood flow values, baseline cerebral blood flow was determined by the hydrogen clearance method in seven rats of each group, and the changes in cerebral blood flow during hemorrhagic hypotension (i.e., the lower limit of autoregulation) were then determined by laser-Doppler flowmetry.

Although mean arterial pressure was decreased from 171 ± 8 mm Hg in the control group to a level as low as 130 ± 10 mm Hg in the treated group after chronic administration of doxazosin for 7 days, the baseline values of cerebral blood flow measured by the hydrogen clearance method were essentially the same in the two groups, indicating that vascular resistance of brain vessels was attenuated by doxazosin treatment. Our study clearly demonstrated that the lower limit of cerebral blood flow autoregulation was 139 mm Hg in the control group and was significantly shifted to a lower level (96 mm Hg) by the treatment.

Cerebral blood flow is maintained at a constant level despite wide variations in systemic blood pressure or cerebral perfusion pressure, i.e., cerebral blood flow autoregulation. However, the lower limit as well as the upper limit of cerebral blood flow autoregulation are shifted to a higher blood pressure level during long-standing hypertension. This would be explained by structural changes of the cerebral vessels, such as hypertrophy of the interstitial connective tissue and smooth muscle cells, and hyaline degeneration of the intima and media (Amano, 1977). Although such a change in cerebral blood flow regulation is a kind of adaptation to chronic hypertension, protecting the brain against an abrupt increase in mean arterial pressure or in case of hemorrhagic stroke (Sadoshima et al., 1981), this upward shift of the autoregulatory lower limit may result in a marked decrease in cerebral blood flow or lead to cerebral ischemia by only a small decrease in mean arterial pressure. Correspondingly, SHRs are more susceptible to hypotension than normotensive rats. Therefore, antihypertensive agents which preserve cerebral blood flow in hypotension are desirable for chronic hypertensives. Several lines of evidence have suggested that the resting cerebral blood flow is unchanged and the lower limits of cerebral autoregulation could be shifted to a lower level in SHRs by administration of anti-hypertensive agents, such as calcium antagonists, angiotensin-converting enzyme inhibitors and α-adrenoceptor antagonists (Barry et al., 1984; Ooboshi et al., 1990; Sadoshima et al., 1994; Shiokawa et al., 1989).

Cerebral arteries have dense sympathetic nerve fibers. Under resting conditions, however, sympathetic activity exerts little or minimal effect on cerebral vessels and cerebral blood flow (Busija et al., 1982; Heistad et al., 1978). In contrast, during hypotension, sympathetic activity in the cerebral and in the systemic vascular bed is increased and constricts cerebral vessels, resulting in a reduction of cerebral blood flow. Either pretreatment with an α-adrenoceptor antagonist or acute cervical sympathectomy extends the lower limit of cerebral blood flow autoregulation in baboons, dogs and SHRs (Fitch et al., 1975; Pearce and D'Alecy, 1980; Shiokawa et al., 1989). Therefore, antihypertensive agents of the α-adrenoceptor antagonist class have a theoretical basis for their effect to maintain the cerebral circulation against a reduction in cerebral perfusion pressure. Previous reports have shown that α-adrenoceptor antagonists, phenoxybenzamine and prazosin, but not a β-adrenoceptor antagonist, propranolol, attenuate the constriction of the cerebral vessels caused by acute hemorrhagic hypotension (Barry et al., 1984; Shiokawa et al., 1989). The present study, in which cerebral blood flow was substantially maintained by doxazosin treatment during hypotension, would suggest that doxazosin is similar to prazosin, which could pass through the blood-brain barrier and bind with each adrenergic receptor, consequently increasing the capacity of cerebral vessels to dilate in SHRs.

In summary, doxazosin decreased cerebral vascular resistance and shifted the cerebral blood flow autoregulation curve to the left, both effects serving to protect against cerebral ischemia. It is concluded that doxazosin, an α_1-adrenoceptor antagonist, has the fairly favorable property of maintaining cerebral blood flow under hypotensive conditions.

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