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Short communication

Amlodipine, a Ca^{2+} channel antagonist, modifies cerebral blood flow autoregulation in hypertensive rats

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Abstract

We measured the cerebral blood flow at rest and during hemorrhagic hypotension in 7 rats of each group using laser-Doppler flowmetry. Simultaneously, the absolute baseline cerebral blood flow values in the parietal cortex were quantified with the hydrogen clearance method. Baseline mean arterial pressure was significantly lowered, by 29 mm Hg, in the amlodipine-treated group, while the baseline cerebral blood flow was $36 + 4$ ml/100 g/min (mean $\pm$ S.D.) which was almost the same as the 40 ± 5 in the control group. The lower limits of the cerebral blood flow autoregulation, defined as the mean arterial pressure at which the cerebral blood flow decreased by 10% of the baseline value, were shifted to a lower level of 107 ± 9 mm Hg in the treated group compared with 133 ± 5 mm Hg in the control ($P < 0.001$). The results demonstrated that, in hypertensive rats with amlodipine treatment, cerebral perfusion was preserved at a lower blood pressure level, which is advantageous under hypotensive conditions.

Keywords: Cerebral blood flow autoregulation; Amlodipine; Ca^{2+} channel antagonist; Hemorrhagic hypotension; Spontaneously hypertensive rat (SHR)

1. Introduction

Amlodipine, (±) 3-ethyl-5-methyl-2-(-2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate benzenesulfonate, related to the structure of nifedipine (Burge et al., 1985), is a Ca^{2+} channel antagonist with a half-elimination life of 30 h, which offers efficacy on once-daily dosing in man (Beresford et al., 1985). Recently, amlodipine has been used as an effective antihypertensive agent. Cerebral blood flow in hypertensive patients was increased after acute administration of nifedipine (Bertel et al., 1983). However, the effect of amlodipine on cerebral blood flow and cerebral autoregulation is not yet clarified, because there are only few reports on the influence of Ca^{2+} channel antagonists on cerebral blood flow autoregulation. Accordingly, there is general disagreement as to the effects of Ca^{2+} channel antagonists on cerebral blood flow and autoregulatory capacity (Florence et al., 1993). McCalden and Nath (1989) suggested that cerebral blood flow in baboons was increased and the autoregulatory capacity was unchanged by nimodipine, whilst in the study by Kummer et al. (1991), cerebral blood flow was unchanged and the autoregulatory capacity was impaired after administration of nimodipine to cats. Therefore, we investigated the changes in cerebral blood flow and the lower limit of cerebral blood flow autoregulation after the administration of amlodipine for 7 days to spontaneously hypertensive rats.

2. Materials and methods

Fourteen male spontaneously hypertensive rats (5–7 months of age) were separated into two groups. Seven rats were treated with amlodipine, and the other seven served as controls; mean body weights after 7 days treatment were 362 ± 13 and 365 ± 11 g, respectively. Amlodipine (3.0 mg/kg/day), which was dissolved in distilled water, was administered by gavage into the stomach once a day. The control rats were treated with the same amount of distilled water alone (3.0 ml/kg/day). The rats had free access to food and water prior to the cerebral blood flow study. The experiments were carried out on the 7th day about 3.5 h after the final treatment with amlodipine or distilled water.

Under amobarbital anesthesia (100 mg/kg body weight i.p.), both femoral arteries were cannulated, one for continuous recording of mean arterial blood pressure and for

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sampling blood, and the other for stepwise hypotension by controlled bleeding. Rectal temperature was maintained at 37°C with a heating pad. The rat was 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 by laser-Doppler flowmetry according to Dirnagl et al. (1989). Briefly, for laser-Doppler flowmetry, 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 by laser-Doppler flowmetry (CBF_{LDF}) were expressed as percentages of the baseline values. Baseline cerebral blood flow was also determined by the hydrogen clearance method (CBF_{H2}) (Aukland et al., 1964) 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 both laser-Doppler flowmetry and hydrogen clearance at intervals of 10 min. Arterial blood was then withdrawn from the femoral arterial catheter to decrease the systemic arterial pressure in a stepwise manner, by 10 mm Hg/steps. Arterial blood pressure was maintained at each level for at least 5 min during which the arbitrary unit of cerebral blood flow determined by laser-Doppler flowmetry was recorded. Arterial gases and pH were determined three times, i.e. under resting conditions (before hypotension), during moderate hypotension (Δmean arterial blood pressure about -50 mm Hg) and during severe hypotension (Δmean arterial blood pressure about -100 mm Hg). 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 blood 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 *t*-test.

3. Results

The mean values for physiological variables during the autoregulation study were as follows: $PaCO_2$ 32–47 and 36–46 mm Hg, PaO_2 81–113 and 89–106 mm Hg, pH 7.34–7.44 and 7.39–7.44, Hct 39–47 and 39–45% in the control and the amlodipine-treated groups, respectively. No significant differences were observed between the two groups. The cerebral blood flow values and pressure-flow relationship in the parietal cortex in the two groups are shown in Table 1 and Fig. 1. Resting values for mean arterial blood pressure were 173 ± 5 and 144 ± 6 mm Hg in the control and treated groups, respectively. Although baseline mean arterial blood pressure was significantly lowered, by 29 mm Hg, in the amlodipine-treated group, the resting cerebral blood flow in the parietal cortex determined by the hydrogen clearance method showed no difference between the two groups (40 ± 5 ml/100 g/min in the control group and 36 ± 4 ml/100 g/min in the treated one). Cerebral vascular resistance, calculated as the mean arterial blood pressure/cerebral blood flow ratio, was 4.05 ± 0.59 (mm Hg/ml)/100 g per min in the amlodipine-treated group, which was lowered by 8.4% than 4.42 ± 0.59 (mm Hg/ml)/100 g per min in the control group (not significant). The lower limit of cerebral blood flow autoregulation, defined as the mean arterial blood pressure at which cerebral blood flow decreased by 10% of the baseline value, was 107 ± 9 mm Hg in the treated group, which was significantly lower than the 133 ± 5 mm Hg in the control group ($P < 0.001$). The autoregulatory range for cerebral blood flow was well

Table 1
Mean arterial pressure, cerebral blood flow, vascular resistance at rest and lower limits of autoregulation in control and amlodipine-treated rats

	Control ($n = 7$)	Amlodipine ($n = 7$)
Baseline MAP (mm Hg)	173 ± 5	144 ± 6 [a]
Baseline CBF_{H2} (ml/100 g/min)	40 ± 5	36 ± 4
CVR [(mm Hg/ml)/100 g/min]	4.42 ± 0.59	4.05 ± 0.59
Lower limits of autoregulation (mm Hg CBF_{LDF} decreased by 10%)	133 ± 5	107 ± 9 [a]

Values are mean ± S.D. [a] $P < 0.001$, vs. control group.
MAP, mean arterial pressure. CVR, cerebral vascular resistance. CBF_{H2}, cerebral blood flow measured by hydrogen clearance method. CBF_{LDF}, cerebral blood flow measured by laser-Doppler flowmetry.

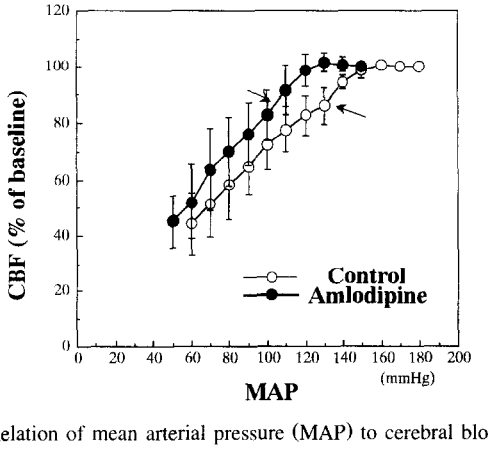

Fig. 1. Relation of mean arterial pressure (MAP) to cerebral blood flow (CBF) in the parietal cortex in spontaneously hypertensive rats treated with amlodipine (closed circles) or distilled water as a control (open circles). Arrows indicate lower limits of autoregulation defined as the mean arterial blood pressure at which cerebral blood flow decreased by 10% of the baseline value.

preserved, i.e. the range of mean arterial blood pressure between resting and the lower limit of cerebral blood flow was approximately 35–40 mm Hg in both groups.

4. Discussion

More than 20 years ago, the Ca^{2+} channel antagonists of the so-called first generation (verapamil, diltiazem and nifedipine) were introduced for the treatment of angina pectoris and later of essential hypertension. In the last decade, an increasing number of agents, structurally related to dihydropyridines, were developed for the treatment of hypertension and coronary heart disease. These new Ca^{2+} channel antagonists of the so-called second generation (amlodipine, felodipine, isradipine, lacidipine, nimodipine, nisoldipine and nitrendipine) tend to have a longer duration of action; substantial selectivity for a specific vascular bed or resistant vessels such as coronary, renal and cerebral vessels, and, in addition, potentially useful effects such as diuretic or anti-atherogenic activity (Rameis, 1993; Van Zwieten and Pfaffendorf, 1993).

The major antihypertensive mechanism of Ca^{2+} channel antagonists involves the decreasing of systemic vascular resistance. In contrast to other vasodilator agents, however, most Ca^{2+} channel antagonists have selective actions on vasoconstricted vessels and differential effects in different regional vascular beds, i.e. vasodilatation by Ca^{2+} channel antagonist favours blood flow to vital organs such as myocardium, brain and kidney, whereas flow to other regional vascular beds is not affected or even reduced (Hof et al., 1982; Kazda and Towart, 1981). Within the cerebral circulation, at least some Ca^{2+} channel antagonists increase cerebral blood flow in low-flow areas, while that in high-flow areas remains constant (Gaab et al., 1985). All these make such drugs as nifedipine, nitrendipine and verapamil first-line drugs in the treatment of patients at an elevated risk of cerebral hypoperfusion. In a previous study, it was demonstrated that amlodipine does not affect blood pressure in normotensive animals, but is a highly effective, long-acting antihypertensive agent in hypertensive dogs (Bertel et al., 1983). Those vascular beds with the highest vasoconstrictor tone and vasospastic vessels are mainly affected by amlodipine.

Under certain conditions, it is important to consider the differential effect of antihypertensive drugs on cerebral perfusion, apart from their antihypertensive effects. Although 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), the lower limit of cerebral blood flow autoregulation is shifted upward, being highly susceptible to hypotension during long-standing hypertension (Strandgaard, 1976). Thus, in acute treatment of chronic hypertension, the therapy itself introduces a risk of severe and permanent ischemic complications of the brain or the retina by reducing cerebral or retinal blood flow.

Because Ca^{2+} channel antagonists may be used under pathological conditions, such as ischemic stroke, it is important to know whether these drugs impair the cerebral blood flow autoregulation during acute hypotension. In contrast to our study, results of previous studies suggested that nimodipine impairs cerebral blood flow autoregulation (Harris et al., 1982; Kummer et al., 1991). This may have been because different species were used, and the drug was given as an intravenous bolus whereas we treated the rats for 7 days. The lower limit of cerebral blood flow autoregulation was significantly improved, namely shifted to the left by 26 mm Hg in the present study. However, there is an overlap of the S.D. bars at mean arterial blood pressure values of 110 mm Hg or lower, so the improvement of autoregulation by amlodipine treatment was not marked. In other words, amlodipine treatment can no longer attenuate cerebral blood flow under moderate to severe hypotension. In contrast, a certain dose of nimodipine, by which the blood pressure was not affected, increased cerebral blood flow while the cerebral blood flow autoregulation was kept unchanged (McCalden and Nath, 1989). The plausible explanation for such a discrepancy between results of the two studies would be that the dose used in the present study was enough to decrease resting blood pressure, and furthermore, was given for as long as 7 days. Another study found the greatest increase in cerebral blood flow with the lowest dose of nimodipine (1 μg/kg/min) (McCalden et al., 1984; Mohamed et al., 1984). However, cerebral blood flow was essentially unchanged when the mean arterial blood flow was decreased by the higher dose (10 μg/kg/min) (McCalden et al., 1984). Together, the results show that Ca^{2+} channel antagonists can increase cerebral blood flow and improve the lower limit of cerebral blood flow autoregulation when a sufficient perfusion pressure is maintained.

Several conclusions can be drawn from the present study. Mean arterial blood pressure is moderately decreased after 7 days administration of amlodipine to spontaneously hypertensive rats, while cerebral perfusion can be preserved. Under hypotensive conditions induced by controlled hemorrhage, the lower limit of cerebral blood flow autoregulation in amlodipine-treated spontaneously hypertensive rats shifts to a lower level.

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