Effects of Antihypertensive Drugs on Experimental Cerebral Ischemia in Spontaneously Hypertensive Rats

Satoru TANAKA, Shin-Ichiro ASHIDA and Akira AKASHI
Research Institute, Daiichi Pharmaceutical Co., Ltd.,
1-16-13 Kita-kasai, Edogawa-ku, Tokyo 134, Japan
Accepted May 24, 1990

Abstract—The effects of antihypertensive drugs on ischemic cerebral damage were investigated using the bilateral carotid artery occlusion (BCAO) model in SHR. Oral budralazine and nifedipine, at doses that increased cerebral blood flow (CBF) in SHR in our previous study (Tanaka, S. et al., Folia Pharmacol. Japon. 87, 1986), significantly improved cerebral energy failure after the BCAO, but prazosin which does not increase CBF had no effect on the energy failure. These results suggest that the amelioration by these antihypertensive drugs of the energy failure after the BCAO results from its CBF-increasing effects in SHR.

Fujishima et al. have reported that the bilateral occlusion of the common carotid artery causes cerebral ischemia in spontaneously hypertensive rats (SHR) (1-3). It has also been demonstrated that CBF in SHR is decreased more potently by the BCAO than that in normotensive rats, because the lower limit of cerebral blood flow autoregulation is significantly higher in SHR than in normotensive rats (4). Biochemical studies in rats have shown that increases in lactate and lactate/pyruvate ratio are accompanied with a decrease in adenosine triphosphate (ATP), indicating enhanced anaerobic metabolism in the brain (1-3).

We have shown previously that the phthalazin derivative budralazine and nifedipine produced a significant increase in CBF in SHR, but prazosin does not, at oral doses reducing arterial blood pressure to near normotensive levels, and that budralazine is more potent in increasing CBF than nifedipine (5). Our present study was designed to evaluate the effect of antihypertensive drugs on cerebral energy failure induced by the BCAO in SHR. The rats were treated with the same doses of the drugs in our previous study (5) to estimate the influence of CBF on cerebral ischemia.

Ninety-two male SHR, 6 months old and weighing 340-400 g, were divided randomly into 8 groups (12 or 10 rats per group). The rats were anesthetized with a gas mixture of 70% N2O-30% O2 containing 2% halothane, and the common carotid artery was bilaterally exposed. After the gas mixture was discontinued, the bilateral carotid artery was occluded with cotton thread to produce cerebral ischemia. After 4 hr following the BCAO, the rats were sacrificed with microwave irradiation (5 kw, 1.1 sec, Toshiba Electric). The brain was removed immediately after the irradiation and was homogenized with a 7-fold volume of 0.6 N perchloric acid. The tissue homogenate maintained at 0-4°C was centrifuged (3000 rpm, 15 min); and ATP, pyruvate and lactate concentrations in the neutralized supernatant fluid were determined by standard enzymatic fluorometric methods. Protein level in the sample was determined by Lowry’s method (6). Dead animals were excluded from the determination of cerebral energy metabolites. In Experiment 1, budralazine (20 or 40 mg/kg) or its vehicle (0.5% carboxymethylcellulose) was administered orally to SHR 3 hr before the BCAO. In Experiment 2, SHR received oral nifedipine (7 mg/kg), prazosin (6 mg/kg) or the vehicle for prazosin (0.5% Tween 80) at 1, 0.5 or 0.5 hr before the BCAO, respectively. The normal group of SHR without the surgical operation received nothing. The BCAO was...
performed at the times when the pressure reduced to near normotensive levels after administration of the drugs, as described in our previous study (5). Results were analyzed using ANOVA and Fisher's multiple comparison, and values of $P<0.05$ were regarded as significant.

Figures 1 and 2 show the levels of energy metabolites evaluated in Experiments 1 and 2, respectively. The BCAO produced a significant decrease in ATP and produced significant increases in lactate, pyruvate and lactate/pyruvate ratio in the control group compared to the normal group ($P<0.01$) in both experiments. Budralazine showed a dose-dependent improvement on the changes in ATP, lactate and lactate/pyruvate ratio; and at a dose of 40 mg/kg, it ameliorated these changes by $47.1$, $64.9$ and $81.8\%$, respectively. Nifedipine also improved the changes in ATP, lactate and lactate/pyruvate ratio by $54.4$, $82.5$ and $92.9\%$, respectively. Thus, budralazine and nifedipine had similar ameliorating effects on the energy deficit; the effect of nifedipine was, however, more potent than that of budralazine (a dose of 40 mg/kg). Prazosin was not significantly effective in improving energy deficit.

In Experiment 1, 5 of 12 control rats (42\%) had seizures for 4 hr during the BCAO and one rat died. The treatment with budralazine, at doses of 20 and 40 mg/kg, reduced the seizure
Our previous study (5) showed that budralazine (40 mg/kg) and nifedipine (7 mg/kg) cause a significant increase in CBF in the parietal cortex and caudate nucleus of SHR, but prazosin (6 mg/kg) does not, at each oral dose which produces equihypotensive responses. In addition, the CBF-increasing effect of budralazine is more potent than that of nifedipine (5). In the present study, budralazine and nifedipine, but not prazosin, improved cerebral energy failure induced by the BCAO in SHR at the same doses used in the previous study. Additionally, the improvement by budralazine was less than that by nifedipine. We also demonstrated that CBF (39–46 ml/100 g/min) in the budralazine (40 mg/kg) treated group is significantly higher than that (about 28 ml/100 g/min) in the control group, when mean arterial blood pressure is lowered to about 50 mmHg by controlled hemorrhage (7). It is well-known that calcium antagonists increase CBF without affecting cerebral energy metabolism (8–10). These findings suggest that the improving effects of the
antihypertensive drugs on the energy failure after BCAO result from their CBF-increasing effect rather than their direct effect on the cerebral metabolism in ischemic SHR. Thus, CBF may be an important factor to ameliorate the energy deficit induced by the BCAO in SHR. Nifedipine has a larger improving effect on energy failure rather than on CBF, as compared with budralazine. The explanation for this discrepancy remains speculative. Some investigators have reported that treatment with calcium channel blockers such as dihydropyridine derivatives decreases neural damage in ischemic brain (11–13). The improving effect may be due to the blocking of the increased calcium flux during ischemia and inhibition of detrimental calcium activated processes (11–13). It is therefore possible that nifedipine showed more potent amelioration of ischemic damage by inhibiting neural damage due to calcium overload in addition to increasing CBF.

In behavioral observations, seizure incidence was markedly lower in all groups treated with drugs tested here as compared with the control group. The diminution of seizure incidence after budralazine and nifedipine was due probably to the improvement of cerebral ischemia and subsequent amelioration of energy failure. The preventing effect on seizure was also observed with prazosin treatment which showed a tendency to improve energy deficits without affecting CBF. This result suggests that the onset of seizure is associated with the depletion of energy metabolites rather than decreased CBF resulting from the BCAO in SHR.

Acknowledgment: We thank Miss Wakako Endo for her excellent technical assistance.

References
1 Fujishima, M., Sugi, T., Morotomi, Y. and Omae, T.: Effect of bilateral carotid artery ligation in brain lactate and pyruvate concentration in normotensive and spontaneously hypertensive rats. Stroke 6, 62–66 (1975)
2 Fujishima, M. and Omae, T.: Mortality and cerebral metabolism after bilateral carotid artery ligation in normotensive and spontaneously hypertensive rats. J. Neurosurg. Psychiatry 39, 212–217 (1976)
3 Fujishima, M. and Omae, T.: Cerebral lactate, pyruvate and ATP concentrations, and arterial acid-base balance at various time intervals following bilateral carotid artery occlusion in normotensive and spontaneously hypertensive rats, Acta Neuroi. Scand. 54, 13–21 (1976)
4 Fujishima, M., Ishitsuka, T., Nakatomi, Y., Tamaki, K. and Omae, T.: Changes in local cerebral blood flow following bilateral carotid occlusion in spontaneously hypertensive and normotensive rats. Stroke 12, 874–876 (1981)
5 Tanaka, S., Tanaka, M. and Akashi, A.: Effect of an antihypertensive drug, budralazine, on the cerebral circulation in spontaneously hypertensive rats. Folia Pharmacol. Japon. 87, 655–663 (1986)
6 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)
7 Tanaka, S., Tanaka, M. and Akashi, A.: Influence of antihypertensive treatment with budralazine on autoregulation of cerebral blood flow in spontaneously hypertensive rats. Stroke 20, 1724–1729 (1989)
8 Harper, A.M., Craigen, L. and Kazda, S.: Effect of the calcium antagonist, nimodipine, on cerebral blood flow and metabolism in the primate. J. Cereb. Blood Flow Metab. 1, 349–356 (1981)
9 Pearce, W.J. and Bevan, J.A.: The cerebrovascular selectivity of diltiazem. J. Cereb. Blood Flow Metab. 3, Supp. 1, S546–S547 (1983)
10 Beck, T. and Kriegstein, J.: Local cerebral glucose utilization and local cerebral blood flow in conscious rats after administration of flunarizine. Naunyn Schmiedebergs Arch. Pharmacol. 335, 680–685 (1987)
11 Schanne, F.A.X., Kane, A.B., Young, E.E. and Farber, J.L.: Calcium dependence of toxic cell death: A final common pathway. Science 206, 700–702 (1979)
12 Van Reempts, J., Haseldonckx, M., Van Deuren, B., Wouters, L. and Borgers, M.: Structural damage of the ischemic brain: Involvement of calcium and effects of postsischemic treatment with calcium entry blockers. Drug Dev. Res. 8, 387–395 (1986)
13 Siesjo, B.K.: Calcium and ischemic brain damage. Eur. Neurol. 23, Supp. 1, 45–56 (1986)