Effects of long-term antihypertensive treatment on brain metabolism after bilateral carotid artery occlusion in spontaneously hypertensive rats

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SUMMARY The effects of antihypertensive treatment on brain metabolism after bilateral carotid occlusion were studied in spontaneously hypertensive rats. The results indicate that an increase in metabolites of ischaemic brain such as lactate and the lactate/pyruvate ratio after carotid occlusion in spontaneously hypertensive rats is apparently suppressed by treating hypertension. This suggests that hypertension may play an important role in susceptibility to cerebral ischaemia.

Fujishima *et al.* (1975) have reported that one hour after bilateral carotid artery occlusion both cerebral lactate and the lactate/pyruvate (L/P) ratio were markedly increased in spontaneously hypertensive rats (SHR), but only slightly increased in normotensive rats (NTR), indicating that SHR are susceptible to cerebral ischaemia. Such ischaemic damage to the brain has been confirmed pathologically in SHR (Ogata *et al.*, 1976). The haemodynamic differences in the collateral circulation between SHR and NTR seem to be responsible for susceptibility to cerebral ischaemia rather than the morphological difference of cerebral vasculature (Fujishima and Omae, 1976a, b).

In the present experiment the effects of bilateral carotid occlusion on brain metabolism were studied in spontaneously hypertensive rats whose blood pressure was lowered by long-term antihypertensive treatment.

Methods

Male SHR (Okamoto and Aoki, 1963) aged 10 weeks were used. They were divided at random into two groups, one treated and the other untreated. The animals were fed a regular diet and

tap water alone (untreated group), or with addition of both hydralazine (3.5 mg/dl) and guanethidine (15 mg/dl) for 10 weeks (treated group). Blood pressure was measured twice weekly by a tail-cuff method without anaesthesia. Seven Wistar normotensive rats (NTR) were used as a control group.

After 10 weeks of treatment, all animals were anaesthetised with intraperitoneally administered amylobarbitone (100 mg/kg body weight). In each rat, one femoral artery was cannulated for blood pressure recording with an electromanometer, and for anaerobic sampling of blood for pH, PCO₂, and PO₂ determinations (IL meter Model 113). Through ventral midline cervical incisions, both common carotid arteries were exposed and separated carefully from the vagosympathetic trunks. Before ligation of the carotid arteries, 0.5 ml of arterial blood was taken for gas analysis. After both carotid arteries were ligated simultaneously with double sutures, the animal was placed in a head-holder, and a plastic funnel was fitted into a skin incision over the skull bone for subsequent freezing of the brain tissue.

Immediately after the second arterial sample for gas analysis was taken, one hour after carotid occlusion, the head was frozen *in situ* by filling a plastic funnel with liquid nitrogen. The whole brain was chiselled out in the frozen state and separated grossly into the supra- and infratentorial portions. The supratentorial brain was weighed and ground in a rapid sequence, and homogenised

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after the addition of ice-cold perchloric acid. The tissue homogenate maintained at 0–4°C was centrifuged and neutralised with potassium hydroxide at a pH 4.5–5.0. Lactate, pyruvate, and adenosine triphosphate (ATP) concentrations were determined by the standard enzymatic-fluorometric methods (Bergmeyer, 1974).

During the experiment, the animals were breathing room air and their body temperature as measured in the rectum was maintained close to 37°C. Immediately after killing, the heart was removed and weighed for relative heart weight or percentile ratio of heart/body weight.

We discarded the results of those animals whose systolic blood pressure did not exceed 180 mmHg in untreated SHR or did not fall below that level in the treated SHR during an observation period of 10 weeks, or whose arterial acid-base parameters did not show the normal range of values during the experiment. Nine untreated and nine treated SHR were finally available for analysis in the present study, which was of five paired siblings.

Results

Average values for systolic blood pressure (SBP) at age 10 weeks before antihypertensive treatment were 177 ± 4 mmHg (SEM) in the untreated SHR group and 185 ± 6 mmHg in the treated group, and the difference was not significant. During the next 10 weeks, SBP in the untreated group rose progressively and reached a mean value of 222 ± 4 mmHg. In treated animals SBP fell markedly for the first two weeks, and then showed a gradual rise but below the initial level (174 ± 4 mmHg). The difference in the final SBP level between the

two groups was highly significant (Fig. 1). On the other hand, SBP in control normotensive rats was 130 ± 6 mmHg, significantly lower than in SHR.

Although there was no difference in body weight, average value for relative heart weight ratio was $0.44 \pm 0.01\%$ in untreated SHR and $0.41 \pm 0.01\%$ in treated ones, a statistically significant difference ($P < 0.05$). This indicates that antihypertensive treatment may prevent hypertension-related cardiac hypertrophy.

In the untreated SHR group, mean arterial blood pressure (MAP), directly measured through the cannulated femoral artery, averaged 189 mmHg before carotid occlusion, and it rose abruptly after occlusion, with a restoration to the initial level at one hour after occlusion. The MAP in the treated SHR group was also raised and remained above the initial level (153 mmHg) one hour after occlusion, while the control group showed no remarkable change in MAP (119 mmHg).

As shown in Table 1, arterial PCO_2 did not differ between untreated and treated SHR before carotid occlusion. After occlusion, there was a significant reduction of PCO_2 by 13.1 ± 1.6 mmHg in untreated and 4.6 ± 1.1 mmHg in treated SHR with a reciprocal rise in arterial pH in each group. On the other hand, arterial PCO_2 in NTR remained unchanged. Arterial PO_2 did not differ between untreated and treated SHR before and after carotid occlusion.

Mean values for supratentorial metabolites are summarised in Table 2. After carotid occlusion, cerebral lactate in treated SHR averaged 6.82 mmol/kg which was significantly lower ($P < 0.001$) than the level of 14.42 mmol/kg found in untreated animals (Fig. 2). Levels of ATP in treated SHR were slightly but not significantly higher than in untreated ones.

Figure 3 illustrates a linear correlation between MAP before and cerebral lactate after carotid occlusion in the individual animal. A similar correlation was also obtained between MAP and L/P ratio ($r = 0.63$, $P < 0.01$). In contrast, there was an inverse relationship between cerebral lactate and arterial PCO_2 . The animals with higher lactate levels tended to have a reduced PCO_2 after carotid occlusion, suggesting that cerebral ischaemia caused hyperventilation.

Discussion

It takes approximately two months to develop a sustained hypertension in spontaneously hypertensive rats (Okamoto and Aoki, 1963), and hypertension is usually associated with an increase in

Fig. 1 Systolic blood pressure in untreated (●) and treated (○) SHR. Vertical bar denotes SEM, * $P < 0.001$.

Table 1 Arterial acid-base balance before and one hour after bilateral carotid occlusion

Group	pH		PCO ₂ (mmHg)		PO ₂ (mmHg)	
	Before	After	Before	After	Before	After
Untreated SHR (N=9)	7.397±0.008	7.499±0.019	33.6±1.1	22.5±1.3	81.8±2.8	90.2±3.6
Treated SHR (N=9)	7.425±0.010	7.458±0.014	33.2±1.2	28.6±1.1	85.1±3.6	89.2±3.9
NTR (N=7)	7.392±0.011	7.431±0.017	40.9±1.5	38.6±1.6	103.0±2.9	95.9±3.8

Values are mean±SEM

Table 2 Supratentorial brain metabolites one hour after bilateral carotid occlusion in untreated SHR, treated SHR, and NTR

Group	Lactate (mmol/kg)	Significance	L/P ratio	Significance	ATP (mmol/kg)	Significance
Untreated SHR (N=9)	14.42±1.62	P<0.001	101.5±14.5	P<0.02	1.14±0.60	NS
Treated SHR (N=9)	6.82±0.93		51.3±11.6	NS	1.51±0.15	P<0.001
NTR (N=7)	3.21±0.21	P<0.01	25.1±2.4		2.88±0.10	

Values are mean±SEM

Fig. 2 Supratentorial lactate and lactate/pyruvate ratio one hour after bilateral carotid artery occlusion in untreated SHR, treated SHR, and NTR.

the peripheral vascular resistance (Finch and Haessler, 1974; Lais *et al.*, 1974; Iriuchijima *et al.*, 1975). Folkow *et al.* (1970a, b) have reported that the increased vascular resistance is caused by an adaptive hypertrophy of the vascular wall exposed to the increased strain of the intraluminal blood pressure.

Although the distribution of cerebral vasculature, particularly of the circle of Willis, is essentially the same, the diameter of cerebral arteries is somewhat smaller in SHR than NTR but the hypertension-related morphological

Fig. 3 Relationship between supratentorial lactate and mean arterial pressure (MAP) before ligation in untreated SHR, treated SHR, and NTR.

changes of vessel walls such as angioneurosis are not evident in SHR (Ogata *et al.*, 1976). On the contrary, Amano (1977) has observed the arterial changes designated as hyaline sclerosis in SHR over the age of 20 weeks, but not in either younger SHR or NTR.

By lowering the blood pressure in hypertension,

a high vascular resistance is reduced in either humans (Sivertsson and Hansson, 1976) or laboratory animals (Folkow *et al.*, 1971a, b; Weiss, 1974). Folkow (1971b) demonstrated that a rapid and considerable regression of the hypertrophic vascular adaptation to hypertension takes place when the pressure load is kept reduced to a normal level for only eight weeks or less. In the present study, antihypertensive treatment for 10 weeks achieved a significant reduction of the blood pressure but did not restore it to the normotensive level.

An increase in supratentorial lactate and L/P ratio after carotid occlusion was markedly suppressed in SHR by long-term treatment of hypertension. The differences in ischaemic metabolites after carotid occlusion among three groups—treated and untreated SHR and NTR—seem to be related to the level of blood pressure. Therefore, a moderate increase in both cerebral lactate and L/P ratio even in treated SHR is probably the result of an insufficient lowering of the blood pressure despite 10 weeks of treatment.

The lower limit of cerebral autoregulation is shifted to a higher level in SHR than NTR (Fujishima and Omae, 1976a). Meanwhile, carotid back pressure as an indicator of the cerebral perfusion pressure is greatly lowered after bilateral carotid occlusion in SHR but not in NTR (Fujishima and Omae, 1976b). These findings in SHR seem to be responsible for an increased vascular resistance of the brain, resulting in an insufficient perfusion through the collateral circulation after carotid occlusion. When a high blood pressure is reduced by antihypertensive treatment for a certain period of time, hypertrophic vascular changes might regress, with restoration of cerebral vascular resistance. Cerebral autoregulation is also returned towards or close to the normal level as demonstrated in hypertensive patients (Strandgaard, 1976), and then collateral channels might function more effectively. Therefore, cerebral perfusion might be kept reasonably good even after bilateral carotid occlusion in those rats with a long-term administration of antihypertensive agents, possibly lowering the susceptibility to cerebral ischaemia.

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