Brain Metabolism and Arterial Acid-Base Balance Following Bilateral Carotid Occlusion in Normotensive and Experimental Hypertensive Rats

M. FUJISHIMA, Y. MOROTOMI, K. TAMAKI, Y. NAKATOMI, J. OGATA, S. TAKISHITA, K. KUMAMOTO, K. FUKIYAMA AND T. OMAE

SUMMARY: *The effects of bilateral common carotid artery occlusion on brain metabolism and arterial acid-base balance were studied in normotensive and experimental renovascular hypertensive rats.*

One hour after carotid occlusion in hypertensive rats, supratentorial lactate increased to 383% and lactate-pyruvate ratio to 280% of the controls, while adenosine triphosphate (ATP) decreased to 69%. These metabolic changes were thought to be due to cerebral ischemia. Arterial pCO2 was low- *ered and the pH was raised in the hypertensive animals due to cerebral ischemia induced hyperventilation. In the normotensive rats, carotid occlusion had minimal effects on cerebral metabolism and arterial acid-base balance.*

These results suggest that hypertensive rats are more susceptible to cerebral ischemia caused by carotid occlusion than normotensive rats. Increased cerebrovascular resistance in hypertension is discussed as a casual factor in cerebral ischemia.

RÉSUMÉ: *Nous avons étudié l'effet de l'occlusion bilatérale de la carotide commune sur le métabolisme du cerveau et l'équilibre acido-basique chex des rats normotensifs ainsi que des rats avec hypertension rénovascularie expérimentale.*

Une heure après l'occlusion de la carotide chez les rats hypertendus la concentration du lactate supratentoriel augmentait à 383% de sa valeur de base, le rapport lactate/pyruvate à 280% de la valeur contrôle tandis que l'ATP diminuait à 69%. Ces modifications sont attribuables à l'ischémie cérébrale. Le pCO2 artériel était diminué et le pH *augmenté chez ces animaux, indiquant que c'est l'ischémie cérébrale qui causait l'hyperventilation. Cependant chez les rats normotendus, l'occlusion de la cartotide eut peu d'effet sur le métabolisme cérébral ou sur l'équilibre acido-basique.*

Les présent résultats suggèrent donc que l'animal hypertendu est plus susceptible à l'ischémie cérébrale par occlusion de la carotide que le rat normotendu. La résistance cérébrovasculaire augmentée dans l'hypertension peut être un facteur causal important de l'ischémie cérébrale.

INTRODUCTION

Bilateral occlusion of the common carotid arteries causes severe cerebral ischemia in spontaneously hypertensive rats (SHR) but not in normotensive rats (NTR). This has been confirmed biochemically (Fujishima et al., 1975; 1976; Fujishima and Omae, 1976a) and pathologically (Ogata et al., 1976). The hemodynamic differences in the collateral circulation between SHR and NTR are probably responsible for the susceptibility to cerebral ischemia following bilateral carotid artery occlusion (Fujishima and Omae, 1976b; 1976c) rather than the morphological differences of cerebral vasculature. The hypertensive vascular changes such as fibrinoid necrosis or thickening of the wall are not evident in SHR (Ogata et al., 1976).

This study was designed to determine if bilateral carotid artery occlusion would cause metabolic cerebral changes in experimentally induced renovascular hypertensive rats in which the hypertension was of shorter duration than that in SHR previously studied.

METHODS:

Goldblatt Operation

Male Wister rats weighing approximately 170 grams were anesthetized with intraperitoneal amobarbital (10 mg/100 g of body weight). The left renal artery was exposed by a dorsal incision and constricted by a silver clip to 0.2 mm in diameter. The contralateral kidney remained intact. After completing the operation, the animals were returned to their cages, and received a conventional rat diet and tap water. Arterial blood

From the Second Department of Internal Medicine, The Faculty of Medicine, Kyushu University, Fukuoka, Japan.

Reprint requests to: Dr. Masatoshi Fujishima, The Second Department of Internal Medicine, The Faculty of Medicine, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka City 812, Japan.

pressure was measured by a tail-cuff method without anesthesia at weekly intervals.

Systolic blood pressure started to rise within one to two weeks and hypertension was fully developed approximately four weeks after the operation. Forty rats, in which systolic blood pressure was higher than 150 mm Hg on more than one occasion during a six-week period, were defined as renovascular hypertensive rats (RHR) and used for this study.

Brain Metabolite Determination

All of the 40 RHR and 17 control normotensive rats (NTR) of the same age were anesthetized with intraperitoneal amobarbital (10 mg/100 g of body weight). One femoral artery was cannulated for blood pressure recording with an electromanometer and for anaerobic blood gas analysis by an IL meter model 113. When $paCO_2$ was between 32 and 45 mmHg and paO_2 above 60 mmHg while the animal was spontaneously breathing room air, the experiment was continued. In 25 RHR and 8 NTR, both common carotid arteries, were exposed through a ventral midline cervical incision, separated from the vagosympathetic trunks, and doubly ligated by silk sutures at the same time. Thirty min. after ligation the animal was placed in a head holder and a plastic funnel was fitted into a skin incision over the skull for subsequent freezing of the brain in situ. The body temperature, as measured in the recturm, was maintained close to 37°C.

The second arterial blood gas sample was obtained 60 min after carotid occlusion in 33 occluded animals; 25 RHR and 8 NTR. In the 24 control animals, 15 RHR and 9 NTR, without carotid occlusion, the second arterial blood gas sample was obtained 60 min. after the first sample. Then the heads of both occluded and non-occluded animals were frozen in situ by filling the plastic funnel with liquid nitrogen. The whole brain was chiselled out in the frozen state, and separated grossly into the supra- and infra-

tentorial portions. Each part was weighed and ground in rapid sequence, and homogenized after the addition of cold perchloric acid. The tissue homogenate, maintained at 0° to 4°C, was centrifuged and neutralized with potassium hydroxide at pH close to 5.0. Lactate, pyruvate and adenosine triphosphate (ATP) concentrations were determined by standard enzymatic methods. Wet weights of the heart and both kidneys were measured immediately after the brain was removed.

Two RHR were discarded from the study. One became hypotensive below 50 mmHg of mean arterial pressure and the other became hypoxemic at less than 50 mmHg of paO_2 after carotid occlusion.

RESULTS

The 38 RHR were divided into two groups related to their mean arterial blood pressure (MAP). Eighteen had MAP of 150 mmHg or higher and were called RHR-high (RHR-H), and 20 had MAP below 150 mmHg and were called RHR-low (RHR-L). The relative heart weight was 0.47 ± 0.02% (mean ± SEM) in 11 RHR-H, 0.31 ± 0.01% in 9 RHR-L, and 0.31 ± 0.01% in 7 NTR, respectively. The former value was significantly higher than the latter two values (p < 0.001), suggesting that the sustained hypertension had caused cardiac hypertrophy in RHR-H.

Mean values for arterial pH, pCO_2, pO_2 and MAP before and after
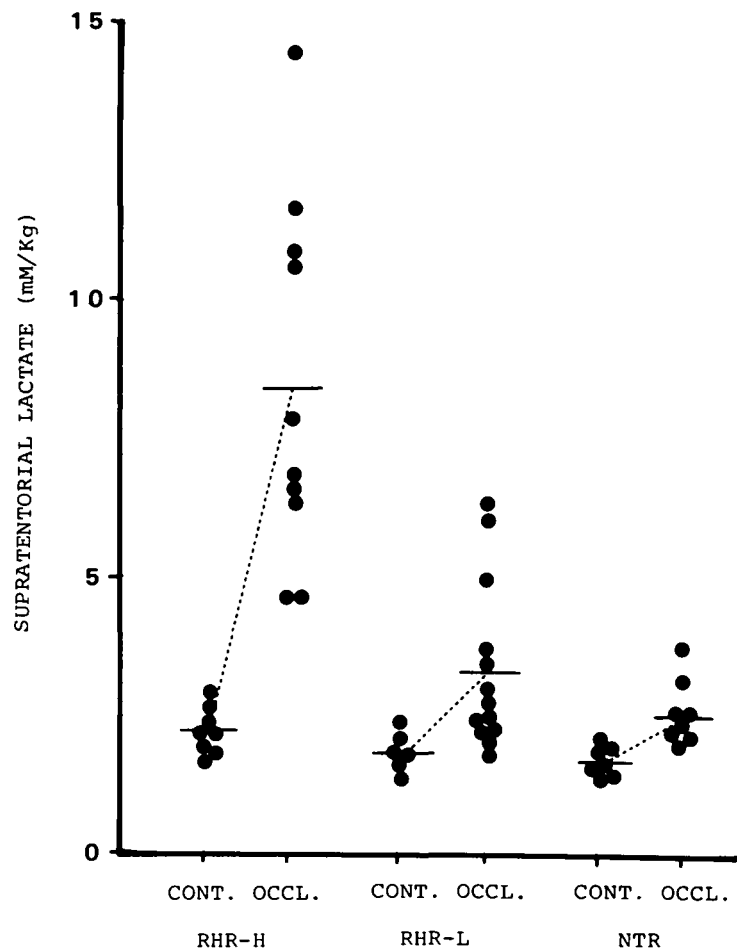

Figure 1—Supratentorial lactate before (C) and one hour after bilateral carotid artery occlusion (O) in renovascular hypertensive (RHR) and normotensive rats (NTR). RHR-H indicates that directly measured mean arterial pressure (MAP) is above 150 mmHg under anaesthesia. RHR-L indicates less than 150 mmHg of MAP.

TABLE 1

*Arterial Acid-Base Balance and Mean Arterial Pressure (Map) before and one hour after
Bilateral Carotid Occlusion in Renovascular Hypertensive and Normotensive Rats*

	RHR-H		RHR-L		NTR	
	Control	Occlusion	Control	Occlusion	Control	Occlusion
pH	7.453	7,586***	7.411	7.488	7.396	7.462
	±0.024 (8)	±0.025 (10)	±0.015 (7)	±0.014 (13)	±0.017 (9)	±0.010 (7)
pCO_2 (mmHg)	37.5	24.6****	41.8	32.9***	40.7	31.9****
	±1.3 (8)	±2.2 (10)	±1.6 (7)	±1.5 (13)	±0.9 (9)	±0.6 (7)
pO_2 (mmHg)	81.4	86.2	82.4	82.5	79.8	75.0
	±5.1 (8)	±7.0 (10)	±4.5 (7)	±3.8 (13)	±4.9 (8)	±4.1 (7)
MAP (mmHg)	176	191	128	135	123	141
	±7 (8)	±14 (10)	±4 (7)	±7 (13)	±4 (9)	±9 (8)

RHR-H, -L: renovascular hypertensive rats of which MAP was 150 mmHg or higher (-H), or lower (-L) than 150 mmHg under amobarbital anesthesia, NTR: normotensive rats, *** $p < 0.005$, **** $p < 0.001$. The numbers in parentheses denote number of rats. Values are mean ± SEM.

carotid occlusion in RHR and NTR are summarized in Table 1. Arterial pCO_2 significantly decreased after carotid occlusion in each group, whereas pH rose in RHR-H only. Arterial pO_2 remained unchanged and MAP tended to rise following occlusion in each animal but, this was not statistically significant.

Mean values for lactate, pyruvate, lactate-pyruvate (L/P) ratio and ATP of the supra- and infratentorial portions before and after carotid occlusion are summarized in Table 2. Supratentorial lactate in RHR-H increased from 2.19 mM/Kg of the control value to 8.39 mM/Kg (383% of control, $p < 0.001$) after carotid

occlusion. The increases for RHR-L (184%, $p < 0.05$) and NTR (152%, $p < 0.005$) are shown in Figure 1. Similarly, L/P ratio increased to 280% of control in RHR-H ($p < 0.005$), 178% in RHR-L ($p < 0.05$) and 147% in NTR ($p < 0.005$), respectively. Supratentorial ATP decreased from 2.09

TABLE 2

*Brain Metabolites before and one hour after Bilateral Carotid Occlusion in Renovascular
Hypertensive and Normotensive Rats*

	RHR-H		RHR-L		NTR	
	Control	Occlusion	Control	Occlusion	Control	Occlusion
Supratentorium						
Lactate (mM/Kg)	2.19	8.39****	1.77	3.25*	1.65	2.51***
	±0.13 (8)	±1.04 (10)	±0.12 (6)	±0.42 (13)	±0.07 (9)	±0.20 (8)
Pyruvate (mM/Kg)	0.156	0.218***	0.154	0.161	0.151	0.126
	±0.099 (8)	±0.012 (10)	±0.011 (6)	±0.012 (13)	±0.010 (9)	±0.028
L/P Ratio	14.6	40.9***	11.7	20.8*	11.7	17.2***
	±1.4 (8)	±6.6 (10)	±0.9 (6)	±2.4 (13)	±1.1 (9)	±1.5 (8)
ATP (mM/Kg)	2.09	1.45*	2.03	1.76	2.29	1.84
	±0.11 (8)	±0.18 (10)	±0.11 (6)	±0.11 (13)	±0.12 (6)	±0.20 (7)
Infratentorium						
Lactate (mM/Kg)	2.48	3.91***	2.32	2.68	2.41	2.61
	±0.12 (8)	±0.35 (9)	±0.14 (7)	±0.14 (10)	±0.22 (5)	±0.16 (8)
Pyruvate (mM/Kg)	0.159	0.219	0.139	0.189	0.176	0.157
	±0.015 (7)	±0.027 (7)	±0.015 (7)	±0.025 (10)	±0.017 (5)	+0.017 (8)
L/P Ratio	16.2	22.7	17.7	15.4	14.1	18.9
	±1.5 (7)	±3.7 (7)	±2.0 (7)	±1.1 (10)	±1.7 (5)	±3.1 (8)
ATP (mM/Kg)	1.80	1.70	1.95	1,80	2.19	1.78
	±0.11 (7)	±0.18 (9)	±0.06 (7)	±0.13 (10)	±0.22 (5)	±0.20 (7)

L/P Ratio: lactate/pyruvate ratio, * $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$ **** $p < 0.001$.

mM/Kg to 1.45 mM/Kg (69%, p < 0.05) after occlusion in RHR-H, whereas it did not change significantly in either RHR-L (87%) or NTR (80%).

On the other hand, none of the infratentorial metabolites changed after carotid occlusion except the lactate in RHR-H increased to 158% of control (p < 0.005).

Figure 2 reveals an inverse relationship betwen lactate and ATP levels of the supratentorial portion in individual animals, indicating an increase in lactate was accompanied by a decrease in ATP. There was a similar correlation between supratentorial lactate and paCO$_2$ as shown in Figure 3. The animals with the greatest increase in lactate after carotid occlusion hyperventilated

resulting in further reduction of paCO$_2$.

DISCUSSION

This study showed, one hour after bilateral carotid. occlusion in hypertensive rats, cerebral lactate and L/P ratio increased to approximately four times and three times the control values respectively. In normotensive animals, the changes were minimal. These metabolic changes were more marked in hypertensive rats with increased heart weight than in those without cardiac hypertrophy. In the latter, the metabolic derangement was greater than that in the normotensive control rats.

These results suggest that rats with sustained hypertension and

cardiac hypertrophy are more susceptible to cerebral ischemia following bilateral carotid occlusion.

Probably several causal factors are operative in the mechanism of cerebral ischemia in the hypertensive animals. As demonstrated in hypertensive humans (Kety et al., 1948) and experimentally or spontaneously hypertensive animals (Flohr et al., 1971/2, Choki et al., 1976), cerebral blood flow remains normal in the resting state. A persistent rise in blood pressure causes cerebral vasoconstriction and increased cerebrovascular resistance paralled to the changes in total peripheral resistance (Flohr et al., 1976). Furthermore, in hypertensive humans (Strandgaard et al., 1973) as well as animals (Jones et al., 1976; Fujishima and Omae, 1976b), the lower limit of cerebral autoregulation is shifted to a higher level below which cerebral blood flow decreases in relation to changes in arterial pressure.

An increased vascular resistance of the brain and a shifted lower limit of cerebral autoregulation in hypertension might cause cerebral hypoperfusion when cerebral perfusion pressure is lowered for any reason. Indirect measurements of cerebral perfusion pressure have been made by the method of Chungcharoen et al. (1952), using a unilateral cannulated carotid artery. In sustained renovascular hypertensive rats carotid pressure fell from 130mmHg to 25mmHg (19% of control) following contialateral carotid occlusion. It fell to only 75% of control in normotensive rats (unpublished data). This indicates that after occlusion of the carotid artery perfusion pressure to the brain is far below the autoregulatory range in hypertensive rats but remains within its range in normotensive ones. A marked reduction of cerebral perfusion pressure in hypertensive animals with bilateral carotid occlusion is due to the increased cerebral vascular resistance of the collateral circulation, mainly through the posterior communicating arteries. Finnerty et al., (1954) have observed that hypertensive patients are less tolerant of the
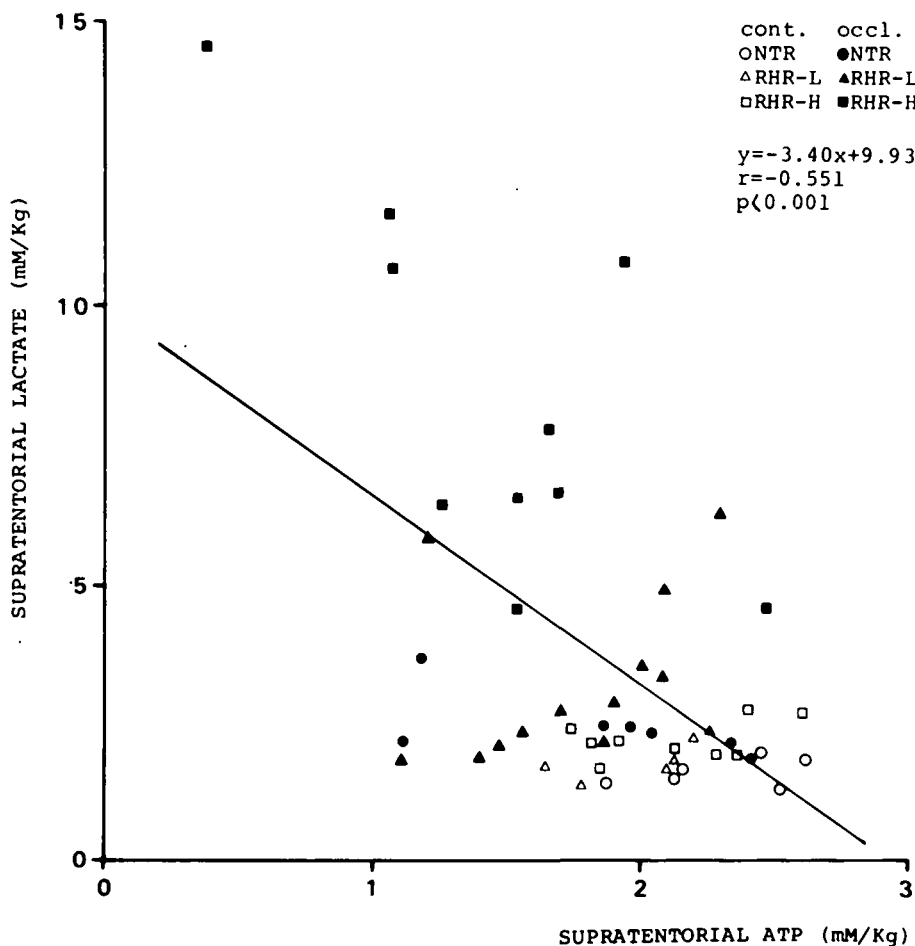

Figure 2—Inverse relation between lactate and ATP of the supratentorial compartment before and after carotid occlusion in RHR and NTR.
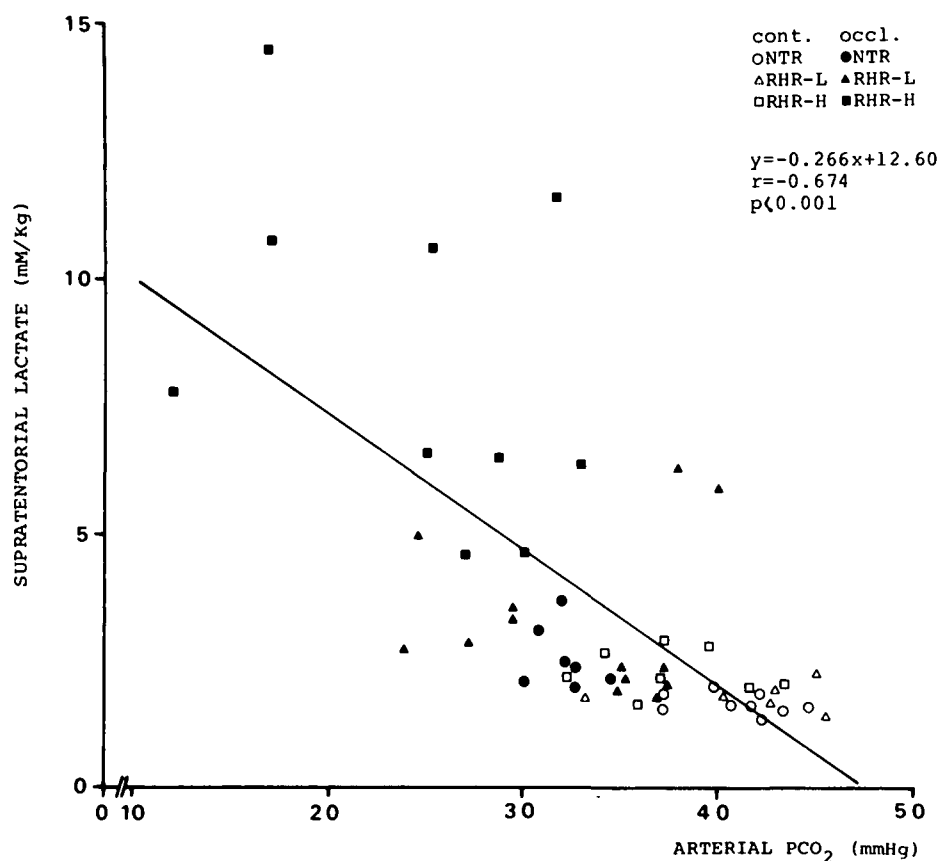An increase in supratentorial lactate is accompanied by a decrease in ATP.

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Figure 3—Relationship between supratentorial lactate and arterial pCO_2 before and after carotid occlusion in RHR and NTR.
$PaCO_2$ tends to be lowered in RHR-H which had the increased lactate, indicating that brain ischemia causes hyperventilation, resulting in hypocapnia.

abrupt fall in blood pressure from posture changes or drug-induced hypotension, resulting in symptoms of cerebral ischemia. Similarly, hypertensive animals are susceptible to cerebral ischemia by carotid occlusion which may lower perfusion pressure to the brain.

In SHR studied previously (Fujishima et al., 1975), metabolic changes in the brain one hour after carotid occlusion were twice as severe as those in RHR-H. Although their blood pressure levels were identical, neither the duration nor etiology of the hypertension was the same. These factors were considered to affect the severity of metabolic changes after carotid occlusion.

In SHR it takes approximately two months to develop a sustained hypertension. In RHR it requires only two weeks.

In two-kidney renovascular hypertension preparations, the renin-angiotensin system is operative in the development and the maintenance of hypertension, while it is less important in SHR (Koletsky et al., 1970). Recently, Brunner et al. (1972) have reported a greater incidence of vascular complications such as strokes and myocardial infarct in hypertensive subjects with a high plasma renin activity than in those with a normal or subnormal renin level. From the present results, however, it cannot be determined if renin is responsible for the suceptibility to cerebral ischemia.

It is concluded that following bilateral carotid occlusion hypertensive animals were more vulnerable to cerebral ischemia than normotensive animals. The severity of the changes appears related to the grade and duration of the hypertension.

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