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Cerebral blood flow and histopathological changes following permanent bilateral carotid artery ligation in Wistar rats

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Summary. Cerebral blood flow and histopathological changes after bilateral carotid artery ligation (BCAL) in Wistar rats were studied. Eight of the 38 rats (21%) died within one week. In the 30 survivors, the incidence of histopathological change was 90% in the caudate nucleus, 23% in the cortex, 30% in the hippocampus, and 0% in the other structures. Local cerebral blood flow (LCBF) was measured using the quantitative autoradiographic ^{14}C-iodoantipyrine technique in 24 anatomically discrete regions of the brain. BCAL induced ischemia in the entire forebrain. The percent reduction of LCBF was between 25–94% of the control at 2.5 h after BCAL. LCBF tended to recover 1 week after BCAL except for the regions of neuronal damage. These results suggest that neuronal damage does not correlate with the flow rate. In the present study, selective neuronal damage was also observed in rats with chronic cerebral ischemia.

Key words: Autoradiography – Cerebral blood flow – Cerebral ischemia – Iodoantipyrine – Rat

Introduction

Previous studies suggested that the effects of bilateral carotid artery ligation (BCAL) in rats are too mild in ischemic degree to show the constant histopathological changes (Payan et al. 1965; Eklöf and Siesjö 1972, 1973; Fujishima et al. 1976, 1981; Choki et al. 1977; Umemura et al. 1982). These investigators also observed that there was a moderate reduction in local cerebral blood flow (LCBF) or a mild deterioration in the energy state, but that there was little or no development of irreversible brain lesions. However, all these studies were done on anesthetized animals.

In preliminary experiments we noted a high incidence of neuronal damage in the caudate nucleus after BCAL in Wistar rats. In the present study, we investigated the histopathological changes after BCAL in relation to residual blood flow, as measured by ^{14}C-iodoantipyrine (IAP) autoradiography (Sakurada et al. 1978).

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Material and methods

Experimental procedure

Male Wistar rats (150–270 g, Clea, Japan, Inc., Tokyo, Japan), fasted except for water for 16–20 h were used in all experiments. These rats were anesthetized with 1.5–2.0% halothane during cannulation of the right femoral artery and vein, and ligation of the bilateral carotid arteries. Both common carotid arteries were exposed through a ventral midline cervical incision, separated from the vagosympathetic trunks, and doubly ligated with 4–0 silk sutures. Before measuring the LCBF, these rats were allowed to recover from anesthesia. The lower half of the body was immobilized by making use of a loose-fitting plaster cast on a lead block. Body temperature was kept at around 37° C with a heating lamp. Prior to the LCBF study, mean arterial blood pressure (MABP) and blood gases were recorded. Determination of LCBF, was made 2.5 h and 1 week after BCAL. LCBF, was also measured in rats 2.5 h after a sham operation, and these values were used as the controls.

Mortality and histopathology

Mortality was recorded daily for one week after BCAL. The rats were then perfusion-fixed with 10% formalin, and the brains removed and stored at room temperature in 10% formalin until embedding in paraffin. Coronal sections (5 μm) were taken at the level of the anterior commissure, anterior hippocampus and cerebellum-brainstem. Brain sections were stained with Klüver–Barrera, and hematoxylin and eosin. Brain sections used for LCBF measurement were also stained for histological examination. At the coronal levels of the anterior commissure (caudate nucleus, frontal cortex) and of the dorsal hippocampus (thalamus, parietal cortex), the severity of the ischemic cell change was evaluated on a semiquantitative scale: 0 = normal; 1 = few affected neurons; 2 = many affected neurons; and 3 = most neurons affected. Mortality rate was calculated as the ratio of the number of dead rats to the total number of rats originally scheduled for longer study.

LCBF

LCBF was measured using the quantitative autoradiographic technique with ^{14}C-IAP as the tracer (Sakurada et al. 1978). Briefly, 25 μCi of ^{14}C-IAP in 1 ml of physiological saline was injected intravenously over a period of 1 min. During administration of the tracer, timed blood samples of 20 μl were collected through the arterial catheter into scintillation vials containing 1 ml of distilled water. ten ml of liquid scintillant (Clear-sol, Nacarai, Kyoto, Japan) were added to the vials. ^{14}C-IAP concentrations in whole blood were measured using a liquid scintillation counter (Beckman Instruments Inc., Fullerton, CA, U.S.A.). At the end of infusion of the tracer, the animals were decapitated, the brains were quickly removed and frozen in Freon (−40° C), and then coronally sectioned (20 μm thick) in a cryo-stat (−25° C). Autoradiographs were prepared by exposing the brain sections with precalibrated methacrylic standards (concentration range 96–1675 nCi/g) to X-ray films (Kodak SB–5) in X-ray cassettes, for one week. The optical densities of the autoradiographs were measured using a computer-based densitometer with an epidiascope and a video display (Unigraphy UHG–101; Unique Medical Co., Tokyo, Japan), and were converted to the LCBF using the equation described by Sakurada et al. (1978). The cerebral tissue blood partition coefficient used for IAP was 0.8 (Sakurada et al. 1978). Brain sections used for the measurement of LCBF were stained to examine the histopathological changes. LCBF was measured in 24 anatomically discrete regions of the right and left hemispheres and the mean LCBF of both hemispheres was calculated. LCBF in the area of neuronal damage was measured separately. LCBF of the BCAL groups was compared with values in the sham-operated control group.

Statistical analysis

All data are presented as means ± SD. of 5–6 rats. For comparison among control and BCAL groups, Duncan's multiple range test was used. A p value of <0.05 was considered to have a statistical significance.

Results

Mortality and histopathology

BCAL was performed on a total of 43 rats, of which 5 were used for LCBF measurement 2.5 h after ischemia. Eight rats died between 8 and 48 h after BCAL, the mortality rate being 21%. Prior to death, they became obtunded, comatose or had convulsive seizures and lay motionless. The histopathology of these rats showed a diffuse cerebral edema. Pyknotic changes of the nucleus were also observed in the caudate nucleus, cortex and hippocampus (Fig. 1). These data were not counted in the incidence of histological changes. Among the 30 survivors, ischemic brain lesions were observed in 27 rats. The incidence of neuronal damage was 27/30 (90%) in the caudate nucleus, 7/30 (23%) in the cortex, and 9/30 (30%) in the hippocampus (Fig. 2). Neuronal damage was usu-

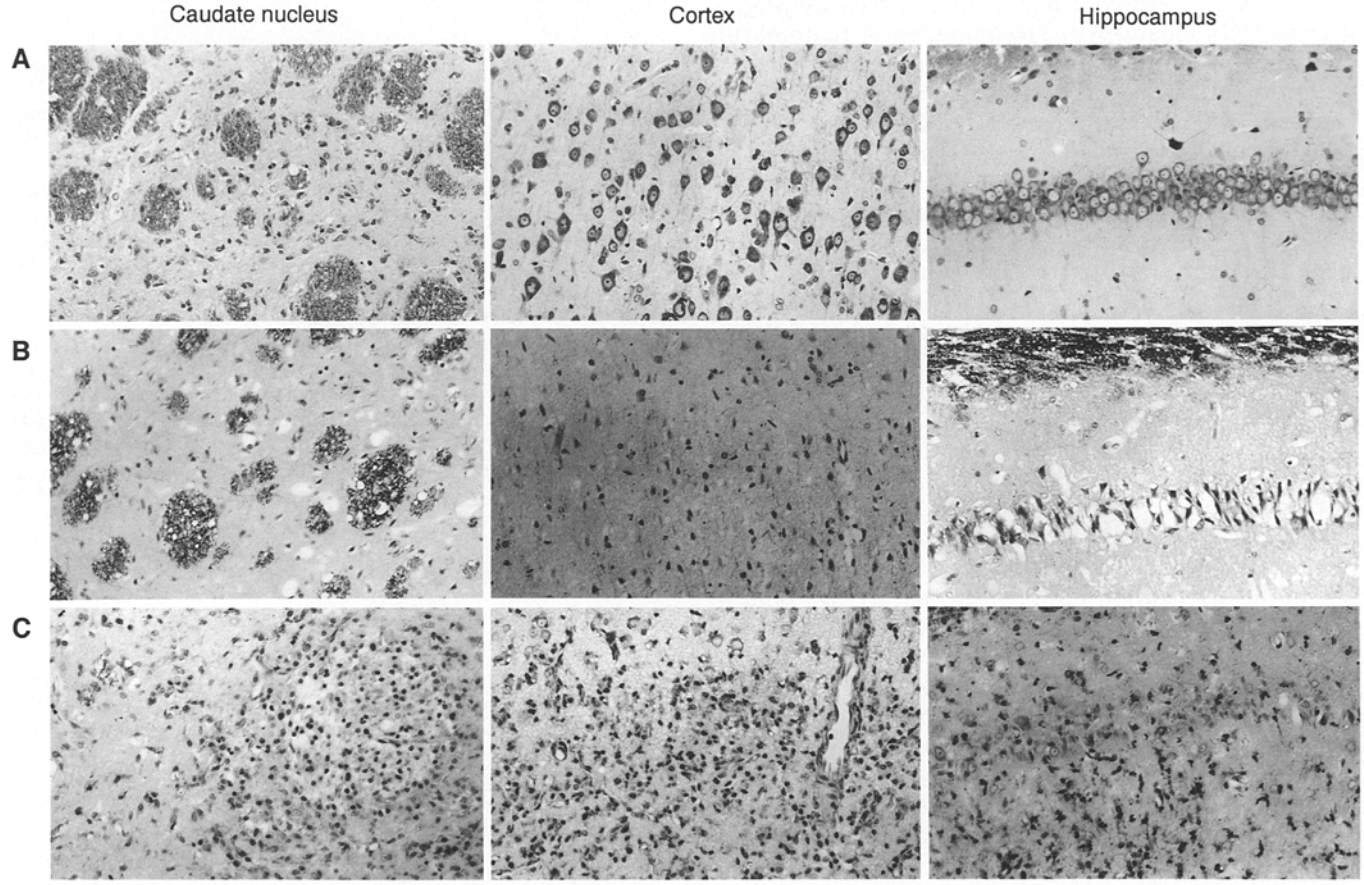

Fig. 1A–C. Histopathological changes in the caudate nucleus, cortex and hippocampus. **A** Control rat with a sham operation, **B** a rat which died within 48 h after BCAL, **C** a rat with BCAL for 1 week. Klüver–Barrera; ×100

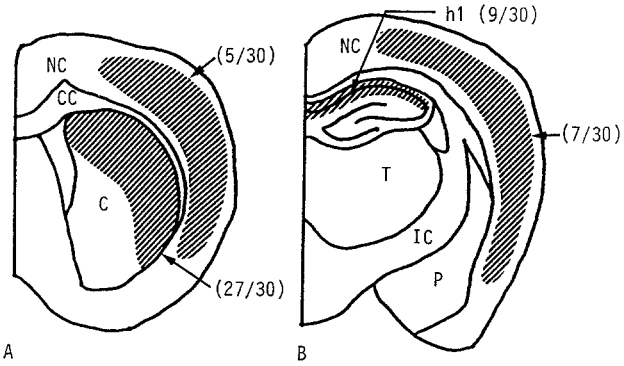

Fig. 2A, B. The incidence and extent of neuronal damage 1 week after BCAL. **A** Coronal level through the caudate nucleus, **B** coronal level through the anterior hippocampus. NC = neocortex; C = caudate nucleus; P = putamen; T = thalamus; h1 = zone of hippocampus; IC = internal capsule; CC = corpus callosum. Hatched areas represent regions with neuronal death. Figures within parenthesis indicate the incidence of neuronal death

Table 1. Distribution and severity of ischemic damage 1 week after BCAL

Grade of damage[b]	% Hemispheres[a] with grade 0–3 damage					
	Neocortex		Hippocampus		Caudate nucleus	Thalamus
	Frontal	Parietal	h1	Others		
0	92	88	82	95	33	100
1	2	0	3	3	2	0
2	3	3	8	0	37	0
3	3	8	7	2	28	0

[a] Thirty rats × (left and right) = 60 hemispheres
[b] Grade of ischemic injury; 0 = intact neurons, 1 = some neurons affected, 2 = many neurons affected, and 3 = the majority of neurons affected

ally seen in the dorsolateral crescent of the caudate nucleus, in zone h1 of the hippocampus, and in the layers 3–6 in the neocortex (Table 1, Fig. 2), but the extent of ischemic damage varied. The dorsolateral neurons of the caudate nucleus resulted in grade 2 to 3 damage in these areas in 65% of the cerebral hemispheres, while the vulnerability of h1 hippocampus and neocortex accounted for less than 20% (Table 1). The other structures were free of neuronal damage. Glial nodules or neuronophagia as shown in Fig. 1 were present in most regions with neuronal damage. A total of 41 small or large focal infarcts were found in 24 of 30 rats; they were located unilaterally in the caudate nucleus of 13 animals, unilaterally in the neocortex of 7, unilaterally in the hippocampus of 1, and bilaterally in the caudate nucleus of 10.

Physiological parameters

Table 2 shows the physiological parameters in control and BCAL groups. At 2.5 h after BCAL, $PaCO_2$ decreased from 31.3 to 25.4 mmHg ($P<0.05$), and PaO_2 increased from 84.0 to 98.8 ($P<0.01$). There was a slight but not statistical increase in plasma pH 2.5 h after BCAL. After one week, PaO_2 increased from 84.0 to 95.2 ($P<0.01$) but the $PaCO_2$ did not differ from that of the control. There were no differences in MABP, hematocrit and arterial pH among control and BCAL groups.

LCBF

Figure 3 shows representative ^{14}C-IAP autoradiograms after BCAL. Table 2 summarizes mean LCBF values in the control and BCAL groups. At 2.5 h after BCAL, the LCBF significantly decreased to 40% of the control in the caudate nucleus, 25–39% in the cortical regions, 52% in the hippocampus, 39% in the lateral geniculate body, and 45–85% in the thalamic regions, medial geniculate body, hypothalamus, amygdala, septal nucleus, nucleus accumbens, globus pallidus, substantia nigra, superior colliculus, corpus callosum and internal capsule. One week after BCAL, the LCBF significantly increased in all cortical and subcortical regions compared with the LCBF 2.5 h after BCAL. However, in the caudate nucleus, auditory cortex and lateral geniculate body, the LCBF one week after BCAL remained depressed statistically, compared to the control. With the exception of the caudate nucleus, the LCBF values of focal ischemic brain lesions in 6 rats were derived from a single hemisphere, in most instances. Therefore, only in case of the caudate nucleus was a mean value obtained, this being 51 ± 16 (Mean $\pm$ SD of 9 hemispheres) ml/100 g/min.

Discussion

Other workers found that the mortality rate was between 10–21% after BCAL in Wistar rats (Payan et al. 1965; Fujishima et al. 1976) and the present study showed a good agreement. However, a very low incidence of his-

Table 2. Effect of BCAL on physiological parameters

	Control ($n=5$)	BCAL (2.5 h) ($n=5$)	BCAL (1 week) ($n=6$)
MABP (mmHg)	124.4 ±7.3	127.8 ±5.9	126.2 ±2.8
PaO_2 (mmHg)	84.0 ±2.9	98.8 ±5.2[b]	95.2 ±3.3[b]
$PaCO_2$ (mmHg)	31.3 ±1.9	25.4 ±3.5[a]	32.6 ±4.2
Hematocrit (%)	51.6 ±3.2	47.7 ±4.6	51.8 ±1.5
pH	7.398 ± 0.029	7.433 ± 0.027	7.381 ± 0.024

Values are the mean ± SD of 5–6 rats
[a] $p<0.05$, [b] $p<0.01$ vs control group

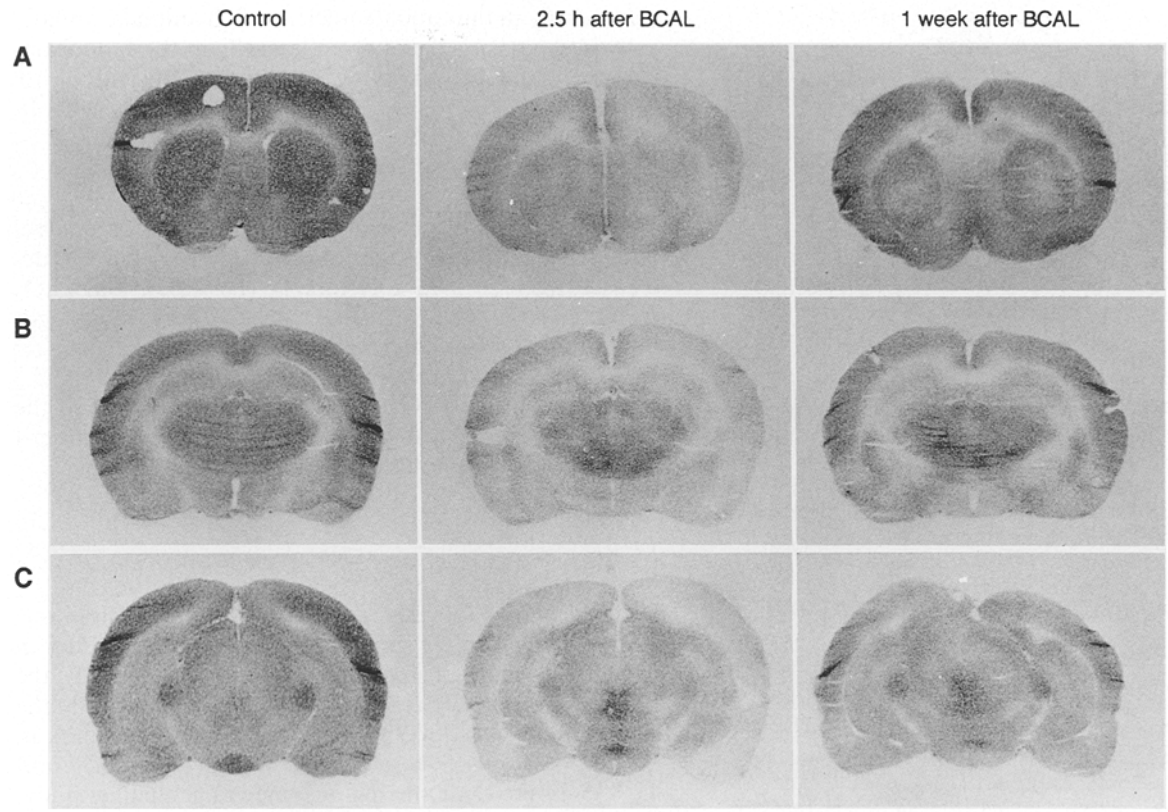

Fig. 3A–C. ^{14}C-IAP autoradiograms after BCAL. **A** Section of sensorimotor cortex, **B** parietal cortex, **C** a section of the medial geniculate body. Note the remarkable decrease in optical density over the whole cerebral hemisphere at 2.5 h after BCAL compared to the control. At one week after BCAL, optical density recovered close to the control except for the dorsolateral of caudate nucleus

tological changes after BCAL has been reported. Payan et al. (1965) noted that 4/9 (44%) female Wistar rats had focal ischemic brain lesions 5 days after BCAL. Umemura et al. (1982) observed shrinkage and dark staining of the cytoplasm in the cerebral cortex one day after BCAL and these changes in Wistar rats disappeared in time. In contrast, we found a higher incidence of lesions one week after BCAL. We used a heating lamp to maintain body temperature of our rats at around 37° C during surgical procedures and the autoradiography. When we did not use a heating lamp, the body temperature decreased to around 33° C during halothane anesthesia (unpublished data). Hypothermia (Busto et al. 1987) and long-acting anesthetics (Kirino et al. 1986) protect against brain damage following transient cerebral ischemia, but it remains to be seen whether protection is ensured in cases where they are only used during the initial several minutes or hours of permanent vascular occlusion. Therefore, the more likely explanation is that the strain of Wistar rats used in the present investigation produces a more uniform and severe ischemia.

LCBF was also measured in studies done on anesthetized animals and the percent reduction of LCBF was between 34–60% of the control (Choki et al. 1977; Eklöf and Siesjö 1972, 1973; Nördstrom and Rehncrona 1977; Fujishima et al. 1981; Umemura et al. 1982). In our experiments, the percent reduction of LCBF was 60% in the caudate nucleus, 61–75% in the cortical regions, 48% in the hippocampus, 42–51% in the thalamic regions and 61% in the lateral geniculate body 2.5 h after BCAL. Thus, we found a more severe reduction of LCBF after BCAL in rats. The disparity noted among the previous LCBF studies and the present one after BCAL is attributed to differences in the method used for LCBF measurements and use or non-use of anesthesia.

Our rats, which were allowed to breathe spontaneously, tended to hyperventilate 2.5 h after BCAL. This finding was similar to that reported by Fujishima et al. (1981) who considered that this hypocapnia appeared to be the cause of the additional reduction of LCBF. However as the effect of hypocapnia on LCBF in ischemic area is thought to be minimal (Waltz 1970), the decrease of $PaCO_2$, though not negligible, would be slight, if any.

LCBF which was remarkably reduced 2.5 h after BCAL tended to recover within one week, except for regions of neuronal death. Thus, collaterals may have formed in the ischemic regions.

In several brain structures there was little or no evidence of neuronal death even though the decrease of blood flow was similar to that in the caudate nucleus. Therefore, selective neuronal damage in the caudate nucleus does not correlate with the blood flow rate. There is evidence that excessive release of excitatory amino acids, e.g., glutamate plays a key role in ischemic brain damage (Rothman and Olney 1986). In addition, there have been many reports concerning selective neuronal

Table 3. Effect of BCAL on LCBF in conscious rats

Structure	LCBF (ml/100 g/min)		
	Control ($n=5$)	BCAL (2.5 h) ($n=5$)	BCAL (1 week) ($n=6$)
Cortical regions			
Visual	147±18	57±17[b]	97±33[b, d]
Auditory	216±15	53±13[b]	123±33[b, d]
Parietal	210±23	64± 9[b]	132±28[b, d]
Sensorimotor	193±18	75±14[b]	126±33[b, d]
Olfactory	176±17	63±11[b]	118±33[b, d]
Frontal	194±19	70±10[b]	127±35[b, d]
Subcortical regions			
Thalamus			
Lateral nucleus	153±12	75±10[b]	118±20[b, d]
Medial nucleus	168±21	83± 8[b]	134±18[b, d]
Ventral nucleus	146±15	85±11[b]	119±13[b, d]
Habenula	177±27	101± 6[b]	143±18[a, d]
Medial geniculate body	190±28	101± 6[b]	164±25[d]
Lateral geniculate body	156±12	61± 7[b]	77± 9[b, c]
Hypothalamus	83± 6	50± 4[b]	73± 5[b, d]
Mammillary body	209±35	196±34	198±42
Hippocampus			
Ammon's horn	121±18	62± 8[b]	89±15[b, c]
Dentate gyrus	110±16	58± 6[b]	83±15[b, d]
Amygdala	82± 2	43± 5[b]	65±10[b, d]
Septal nucleus	84± 7	51± 6[b]	65±12[b, c]
Caudate nucleus	149±10	60±10[b]	87±30[b, c]
Nucleus accumbens	170±32	77±10[b]	123±25[b, d]
Globus pallidus	59± 5	42± 4[b]	54± 5[d]
Substantia nigra	79± 3	67± 8[a]	76± 5[c]
White matter			
Corpus callosum	43± 4	21± 3[b]	31± 4[b, d]
Internal capsule	38± 3	23± 4[b]	31± 5[a, d]

Values are the mean ± SD of 5–6 rats
[a] $p<0.05$, [b] $p<0.01$ vs control group; [c] $p<0.05$ [d] $p<0.01$ vs BCAL (2.5 h) group

damage after transient global ischemia (Ito et al. 1975; Kirino 1982; Pulsinelli et al. 1982; Smith et al. 1984; Tomida et al. 1987), and glutamate may explain the development of selective vulnerability (Jorgensen and Diemer, 1982). While it is impossible to determine from the present data whether glutamate can account for the mechanism of cell injury in the presence of a relatively high blood flow rate, glutamate may relate to selective neuronal damage as the distribution of neuronal death in our experiments was similar to that seen with neuropathological changes after transient global cerebral ischemia.

Thus, selective neuronal damage was also observed following permanent BCAL in Wistar rats. This model can serve as one of chronic ischemia and may be useful for evaluating behavioral changes after ischemia or the effects of the new drugs to treat patients with cerebral ischemia.

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