Original Contribution

Effects of induced hyperthermia on canine brain

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Abstract: The cerebral blood flow (CBF), intracranial pressure (ICP), brain metabolism, and auditory evoked potentials (AEP) of 30 mongrel dogs were measured to evaluate the effects of induced hyperthermia (43°C for 1h) on brain function. The intracranial pressure increased significantly during hyperthermia, but cortical or subcortical blood flow, cerebral oxygen uptake, glucose uptake or lactic acid uptake showed no significant changes. Although the amplitude of components in the auditory brain stem response (ABR) was reduced, the configuration of each component was maintained. In the auditory evoked middle latency response (MLR), the late components disappeared at 42°C and higher. The rate of production of super oxide dismutase (SOD) increased significantly during hyperthermia. Considering the CBF and brain metabolism, the brain appeared to be better protected during systemic hyperthermia than were the abdominal organs. However, considering central nervous system activity, although the brain stem function was relatively well preserved at 42°C and higher, brain function at higher levels was greatly inhibited and the super oxide seemed to be involved in this inhibition. With respect to brain function, thus, the critical thermal maximum (CTM) in the dog is about 42–43°C.

Key words: Induced hyperthermia, Cerebral blood flow, Intracranial pressure, Brain metabolism, Auditory evoked potentials

Introduction

Hyperthermia occurs in patients suffering from conditions such as heatstroke, malignant hyperthermia, infectious disease, and certain types of poisoning. Systemic hyperthermia has been used as a means of treating malignant tumors. Some studies of the effects of hyperthermia on the brain reported that cerebral blood flow
(CBF) is increased\(^2-6\), while others report that it is not\(^7,8\); no definitive evidence has yet been obtained. Brain metabolism has been evaluated according to brain tissue pH, and the oxygen pressure in brain surface tissue\(^6\), but no study has been based on cerebral oxygen consumption. There is a need for further research on brain electrophysiological activity. Therefore, the effect of systemic hyperthermia on the brain was assessed by evaluating CBF, intracranial pressure (ICP), cerebral oxygen consumption, and the concentrations of glucose, lactic acid, and super oxide dismutase (SOD) in the cerebral venous sinus blood of dogs during hyperthermia. Auditory brain stem response (ABR) and auditory evoked middle latency response (MLR) were also assessed as indices of cerebral electrophysiological activity.

I. Materials and Methods

This experiment was performed in accordance with the guidelines for animal experiments of Oita Medical University and the study protocol was approved by the animal care committee of this university.

1. Materials

Thirty mongrel dogs, 10 to 15kg, was assigned to three groups of 10 animals each, viz., a CBF group, an ICP and brain metabolism group, and an ABR and MLR group.

2. Anesthesia

Animals in both the CBF and the ICP/brain metabolism group were given pentobarbital sodium, 25mg/kg i.v., and pancuronium bromide, 0.1 – 0.2mg/kg i.v., to facilitate tracheal intubation. Continuous positive pressure ventilation was provided with an animal respirator (Aika model R-60, Aika, Tokyo, Japan). Tidal volume was maintained at 12 – 15ml/kg, and oxygen flow and respiratory rate were adjusted so that PaO\(_2\) was at least 100mmHg and PaCO\(_2\) was about 35mmHg. Anesthesia was maintained by continuous intravenous infusion of pentobarbital sodium, 1mg/kg/h, and supplemental pancuronium bromide.

Since both ABR\(^9,10\) and MLR\(^11-13\) are reportedly greatly influenced by intravenous and inhalation anesthetics, in the ABR-MLR group, anesthesia was induced with thiamylal sodium, ultrashort acting barbiturate, 5mg/kg i.v., and pancuronium bromide, 0.1mg/kg i.v.; artificial ventilation was provided in the same manner as in the other two groups. Since opiates reportedly have little effect on ABR and MLR\(^14\), morphine HCl, 1mg/kg i.v., was administered to maintain anesthesia.

3. Body temperature

Brain tissue temperature and blood temperature are reportedly well correlated\(^10\). Honda\(^15\) reported that pulmonary artery blood temperature was the most stable indicator of body temperature. In the present study, brain temperature was evaluated via blood temperature. A catheter-type thermometer probe (Nihon Kohden, Tokyo, Japan) was inserted into the left femoral vein as far as the inferior vena cava for measurement of blood temperature.
4. Cerebral blood flow

In the CBF group, electromagnetic flow probes were attached to the left internal carotid artery and the left vertebral artery for measuring internal carotid artery blood flow (CABF) and vertebral artery blood flow (VABF), respectively. At a site approximately 1.5cm lateral to the midline on a line connecting the external acoustic meatus of each side, a 2-mm-long platinum electrode was inserted into the cerebral cortex and a laser Doppler blood flowmeter probe was attached to the surface of the cerebrum. They were connected to a hydrogen clearance blood flowmeter (Model UPS 400, Unique Medical, Tokyo, Japan) and a laser Doppler blood flowmeter (ALF21, Advance, Tokyo, Japan), respectively, for measurement of cortical blood flow. An 8-mm-long platinum electrode was inserted into the white matter at the same place for measurement of subcortical blood flow.

5. Intracranial pressure

In the ICP/brain metabolism group, at the same site as in the CBF group, an intracranial pressure measurement catheter (Model TM-200T, Nihon Kohden, Tokyo, Japan) was inserted into the extradural space and ICP was monitored continuously.

6. Cerebral metabolic activity

Oxygen content (CaO$_2$) was measured in femoral artery blood. Retroglenoidal venous blood (from a branch of the external maxillary vein) was drawn from the cerebral venous sinuses for measurement of cerebral venous blood oxygen content (CcvO$_2$). The rate of cerebral oxygen uptake was calculated from CaO$_2$ and CcvO$_2$ values. A hemoximeter (OSM3, Radiometer, Copenhagen, Denmark) was used to measure the oxygen content of arterial and venous blood. Blood glucose concentration (arterial blood glucose: Gla; cerebral venous sinus blood glucose: Glv), lactic acid concentration (arterial blood lactic acid: La; cerebral venous sinus blood lactic acid: Lv) and SOD (arterial blood SOD: Sa; cerebral venous sinus blood SOD: Sv) in arterial blood and cerebral venous sinus blood were also measured, and SOD in cerebrospinal fluid (Sf) was also measured. The rates of cerebral glucose uptake (Glu-R), cerebral lactic acid uptake (Lac-R), and cerebral SOD production (SOD-R) were calculated from the following formulas:

\[ \text{Glu-R} = (\text{Gla} - \text{Glv}) \times 100/\text{Gla} \]
\[ \text{Lac-R} = (\text{La} - \text{Lv}) \times 100/\text{La} \]
\[ \text{SOD-R} = (\text{Sv} + \text{Sf} - \text{Sa}) \times 100/\text{Sv} + \text{Sf} \]

The electrode method was used for measuring blood glucose concentration, the enzymatic method was used for measuring lactic acid concentration, and the NBT (nitro blue tetrazolium) reduction method which was reported by Oberley et al. was used for measuring SOD.

7. ABR and MLR

ABR was used as an index of brain stem electrical activity and MLR was used as an index...
of electrical activity from brain stem to the cerebral cortex. The responses were studied with an induced-response recorder (MEB 5100, Neuropack-2, Nihon Kohden, Tokyo, Japan). Active electrodes for both ABR and MLR were attached to the parietal area, reference electrodes were attached to the mastoid process area, and ground electrodes were attached to the anterior forehead. After the positioning of headphones over the ears, the left ear was stimulated with 90-dB, 10-Hz clicks for the ABR, and with 90-dB, 5-Hz clicks for the MLR. The right ear was simultaneously exposed to white noise as a masking stimulus. A 100 to 1000-Hz filter was used for the ABR, and 2048 arithmetical averaging was done. A 20 to 500-Hz filter was used for the MLR, and 1024 arithmetical averaging was done.

Amplitudes, latencies, and rates of appearance of each component of both ABR and MLR were assessed. The amplitude of ABR components was defined as the negative peak to positive peak. The amplitudes of individual wave components of MLR, when positive, were defined as for the ABR; when negative, as the length of the line extending perpendicularly from the bottom to the peak of the immediately preceding positive wave, and were expressed as absolute values. The number of wave components that appeared at each temperature was calculated as the rate of appearance of individual wave components; their rates of appearance at 41°C and below and at 42°C and above were compared.

8. Method of elevating body temperature

After stabilizing the dog's hemodynamics, each animal was wrapped in a vinyl sheet and immersed it in a 120 × 45 × 30cm hot-water tub, and then body temperature was increased at a rate of 1°C/45min by adjusting the Thermal Unit (Model 302, Taiyo Co., Tokyo, Japan) so that the difference between the temperature of water in the tub and that of the animal's blood was less than 10°C.

During the temperature increase, end tidal partial pressure of carbon dioxide (ETCO₂) was measured with a Normocap (NR82279, Datex, Finland), and ventilation conditions were adjusted so that ETCO₂ was the same as that of controls.

During operating period, saline solution was given for the most part at 10ml/kg/h, and at 20ml/kg/h during heating, and supplemental saline solution was given to maintain CVP constant. Glucose, 0.06g/kg/h, was also infused i. v. to maintain an arterial blood glucose level of at least 80 mg/dl. Measurements of all parameters were made at seven points in time, at 38°C (the control), 39°C, 40°C, 41°C, 42°C, 43°C and when 43°C had been maintained for 1h.

9. Statistical analysis

Data are reported as mean ± standard deviation (S. D.) ANOVA and the Dunnett's multiple-comparison test were used to analyze changes in CBF, cerebral oxygen uptake, uptake of blood glucose, lactate, and SOD, and in the amplitude and latency of ABR and MLR and to compare body weight between individual groups. Appearance rates of ABR and MLR waves were compared by use of the chi-square test. Differences were considered statistically signi-
significant when p values were less than 0.05.

II. Results

1. Mean body weight

Mean body weight was 14.6 ± 3.8kg in the CBF group, 13.7 ± 4.5kg in the ICP/brain metabolism group, and 13.5 ± 5.5kg in the ABR/MLR group. The differences between groups were not significant.

2. Cerebral circulation (Fig. 1, 2)

Internal carotid artery blood flow (CABF) increased from 96.6 ± 16.6 at 38°C to 195.1 ± 37.5ml/min at 42°C and to 246 ± 46.8ml/min at 43°C, declining significantly thereafter to 91.0 ± 23.3ml/min when body temperature was maintained for 1hr at 43°C (Fig. 1).

Vertebral artery blood flow (VABF) did not change significantly from the control value (58.3 ± 10.1ml/min) until 43°C, but when 43°C had been maintained for 1h, VABF decreased significantly to 28.0 ± 7.2ml/min (Fig. 1).

As measured by the hydrogen clearance method, neither cerebral cortical blood flow nor subcortical blood flow changed significantly from control (75.9 ± 15.6 and 30.5 ± 6.0ml/min/100g, respectively) (Fig. 2A), nor were there any significant changes in cerebral

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Fig. 1 Blood flow values of internal carotid artery and vertebral artery at various temperatures. ICA: internal carotid artery, VA: vertebral artery, points represent mean ± S.D., *: p < 0.01 vs. control, **: p < 0.01 vs. 43°C.

Fig. 2 Blood flow values in brain cortex and medulla at various temperatures. Cx: brain cortex, Mb: brain medulla, points represent mean ± S.D., there was no significant change. A: Blood flow in brain cortex and medulla was measured by inhalation hydrogen method. B: Blood flow in brain cortex was measured by lazer dopplar method.
cortical blood flow from control (71.8 ± 12.6 ml/min/100g) as measured by the laser Doppler method (Fig. 2B).

3. Intracranial pressure (ICP) (Fig. 3)

ICP rose gradually from the 38°C control value (15.1 ± 2.7 mmHg), and increased significantly to 26.4 ± 3.9 mmHg at 42°C and 31.8 ± 5.0 mmHg at 43°C, but after 1h at 43°C it decreased significantly to 17±4.8 mmHg (Fig. 3).

4. Brain metabolism (Table 1)

There was no significant difference between the rate of cerebral oxygen extraction at the control temperature of 38°C (14.3 ± 2.4%) and at 43°C (15.7 ± 2.5%) (Table 1). There was no significant increase in cerebral glucose extraction (Glu-R) during hyperthermia (Table 1). Cerebral lactic acid extraction (Lac-R) at 38°C was 24.5 ± 6.2%, and a slight tendency to increase was noted at 43°C (29.8 ± 6.3%) and when 43°C had been maintained for 1h (32.5 ± 5.7%) but the changes were not statistically significant (Table 1).

<table>
<thead>
<tr>
<th>temp (°C)</th>
<th>O₂ExR (%)</th>
<th>Glu-R (%)</th>
<th>Lac-R (%)</th>
<th>SOD-R (%)</th>
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<tr>
<td>38</td>
<td>14.30±2.7</td>
<td>4.5±1.3</td>
<td>24.5±6.2</td>
<td>11.46±1.8</td>
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<td>39</td>
<td>14.29±2.2</td>
<td>4.2±1.1</td>
<td>25.2±4.3</td>
<td>11.71±1.8</td>
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<td>30</td>
<td>14.21±2.1</td>
<td>3.8±0.9</td>
<td>22.0±3.9</td>
<td>11.52±1.8</td>
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<td>41</td>
<td>14.32±2.3</td>
<td>4.8±1.2</td>
<td>26.9±5.5</td>
<td>11.73±2.1</td>
</tr>
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<td>42</td>
<td>15.00±2.4</td>
<td>4.0±1.1</td>
<td>21.2±4.1</td>
<td>12.84±1.7</td>
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<td>43</td>
<td>15.68±2.5</td>
<td>5.1±1.4</td>
<td>29.8±6.3</td>
<td>13.50±1.8*</td>
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<tr>
<td>43-1h</td>
<td>16.62±4.0</td>
<td>5.2±1.5</td>
<td>32.5±5.7</td>
<td>14.58±2.4**</td>
</tr>
</tbody>
</table>

O₂ExR: oxygen extraction ratio, Glu-R: glucose extraction, Lac-R: lactate extraction ratio, SOD-R: SOD production ratio. The values represent means ± S. D., *: p < 0.05, **: p<0.01.

5. SOD (Table 1)

No significant change in the rate of cerebral production of SOD (SOD-R) was observed up to 42°C (12.84 ± 1.7%), but the production rate was significantly higher at 43°C (13.50 ± 1.8%) and after 1h at 43°C (14.58±2.4%) (Table 1).

6. ABR and MLR (Fig. 4, Fig. 5)

In ABR (Figs. 4 A and 4 B), although the amplitudes of waves I, III, and V tended to
decrease, the differences were not significant. After 1h at 43°C, the amplitude of waves II and IV (0.26 ± 0.22 and 0.08 ± 0.08 μV, respectively) was significantly lower than in the control (1.29 ± 0.31 and 0.54 ± 0.23 μV, respectively; Fig. 4A). As shown in Fig. 4B, although the peak latency of waves iii, iv, and v tended to decrease, no significant differences were observed (Fig. 4B).

In MLR (Figs. 5A and 5B), although the amplitude of P0 and Na tended to decrease, none of the difference was significant. The amplitudes of No and Pa were significantly lower at 43°C (0.33 ± 0.14 and 0.18 ± 0.04 μV, respectively) and after 1h at 43°C (0.23 ± 0.06 and 0.20 ± 0.07 μV, respectively) than in the control (0.74 ± 0.20 and 0.71 ± 0.31 μV, respectively). Nb disappeared at 43°C or above and Pb disappeared at 42°C or above (Fig. 5A). No significant changes in the peak latency of No, P0, or Na, were observed until 43°C had been maintained for 1h. In the peak latency of Nb and Pb, no significant differences were observed at any body temperature at which those waves appeared (Fig. 5B).

The rate of appearance of all the ABR waves, from wave i to v, was 100% at every
temperature; there were no differences between rates of appearance at any individual temperature. In MLR, on the other hand, although the rate of appearance of No, Po, and Na was 100% at every temperature, the rate of appearance of the Pa wave was 40/40 (100%) at 41°C and below cf. 16/30 (53.3%) at 42°C and above; by chi-square test, the difference was significant (Table 2). The rate of appearance of the Nb wave was 40/40 (100%) at 41°C and below, as opposed to 14/30 (46.7%) at 42°C and above, and this difference was also significant (Table 2). The rate of appearance of the Pb wave was 40/40 (100%) at 41°C and below cf. 4/30 (13.3%) at 42°C and above; again, a significant difference was detected (Table 2).

### III. Discussion

A critical thermal maximum (CTM) has been reported in hyperthermic humans\(^{17,18}\). According to Honda\(^{19}\), it seems to be about 42°C; Bynun et al.\(^{20}\) reported a similar level in dogs. Although many reports on the effects of hyperthermia on the body are available—(including studies of our department; cardiac function\(^{21}\), intestinal blood flow and endotoxins\(^{22}\), renal circulation\(^{23}\), liver circulation and metabolism\(^{24}\)—, its effects on the brain are particularly important. Thus, changes in CBF during hyperthermia were investigated in this study. Yamada\(^3\) reported an increased blood flow in the brain of the rabbit during local hyperthermia, as did Lyons et al.\(^4\) in the dog, and Moriyama\(^5\) in the monkey. During systemic hyperthermia, Meyer et al.\(^2\) and Katsumura et al.\(^6\) reported CBF increased, but Oda et al.\(^8\) and Nemoto et al.\(^7\) reported no significant change in CBF, the established theory has not provided. The present study showed no increase in cerebral cortical or subcortical blood flow during hyperthermia despite increases in internal carotid artery blood flow. Whereas Katsumura et al.\(^9\) elevated the body temperature 3.5°C (from 38°C to 41.5°C) in 45 min, in our study body temperature was increased at the rate of 1°C per 45 min. Ogura et al.\(^{25}\) studied intracerebral arterioles of rats in vitro and observed biphasic changes; i.e., vasoconstriction during the initial period of hyperthermia, followed by vasodilatation. It seems that when body temperature is increased slowly, as it was in our study, similar biphasic changes occur, and blood flow does not increase. Scheier et al.\(^{26}\) and Song et al.\(^{27}\) reported increase in muscle blood flow as body temperature rose. In dogs, some branches of the internal carotid artery are distributed to the brain; i.e., to the anterior, middle, and posterior cerebral arteries, but other branches are ex-

### Table 2. Comparison of appearance ratio of Pa wave, Nb wave and Pb wave at low temperatures (<42°C) and high temperatures (42°C <).

<table>
<thead>
<tr>
<th></th>
<th>&lt;42°C (n=40)</th>
<th>42°C &lt; (n=30)</th>
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<tbody>
<tr>
<td>Pa wave (+)</td>
<td>40/40</td>
<td>16/30*</td>
</tr>
<tr>
<td>(-)</td>
<td>0/40</td>
<td>14/30*</td>
</tr>
<tr>
<td>Nb wave (+)</td>
<td>40/40</td>
<td>14/30*</td>
</tr>
<tr>
<td>(-)</td>
<td>0/40</td>
<td>16/30*</td>
</tr>
<tr>
<td>Pb wave (+)</td>
<td>40/40</td>
<td>4/30*</td>
</tr>
<tr>
<td>(-)</td>
<td>0/40</td>
<td>26/30*</td>
</tr>
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</table>

*: p<0.01
tracerebral; i.e., the posterior intercarotid artery, the internal ophthalmic artery, and the internal ethmoidal artery. The results indicate that blood flow to muscle and blood flow within these extracerebral arteries increased, but that blood flow within the brain tissue did not.

During hyperthermia, ICP significantly increased at 42°C and 43°C, but declined significantly at 1 hr of 43°C. Hosotani et al. reported that ICP rose during systemic hyperthermia in cats. There are several reports on the autoregulation of CBF. Katsumura et al. reported that ICP in dogs increased during hyperthermia, CBF and ICP changed in parallel with fluctuations in blood pressure, and autoregulation of CBF was diminished. In our study, however, CBF showed no significant change, despite an increase in ICP; when the ICP increases gradually, the autoregulation of CBF seems to be maintained.

Uptake of cerebral oxygen, of glucose, and of lactic acid are all used as means of assessing cerebral metabolism. Busija et al. and Nowak reported that hyperthermia had a marked effect on cerebral energy metabolism, but Chopp et al. claimed that cerebral tissue pH did not change during hyperthermia in non-ischemic brain, and that hyperthermia had little effect on brain metabolism. No significant change in cerebral oxygen uptake was observed in the present study. This appears to be attributable to the fact in order to make the conditions the same as when the various individual organs were investigated in our laboratory in earlier experiments, pentobarbital anesthesia was used. Thus, cerebral metabolism seemed to have inhibited by the brain-protective of pentobarbital. Our results showed no changes in the rates of uptake of glucose or lactic acid; if adequate supplemental infusions and adequate glucose supplementation are administered, as they were in this experiment, the brain, unlike other organs, seemed to be highly protected.

In evaluating the electroencephalograms (EEG) of rabbits during hyperthermia, Yamada reported that slow waves appeared at 44°C or higher and the EEG became flat. Oda et al. reported that in dogs and monkeys, the slow waves appeared at 41°C and that the EEG became flat at 42°C and above. It is conceivable, therefore, that hyperthermia has a marked effect on EEG. In this study, we recorded ABR and MLR during hyperthermia to assess the electrical activity of the brain stem and above. It was found the the amplitude of II and IV components in ABR was decreased significantly, but there were no significant change in the amplitude of the other components, and no significant changes were detected in the peak latency of components. All of wave components were observed at every temperature, yielding a 100% rate of appearance. In MLR, however, the amplitude of N0 and Pa decreased significantly starting at 43°C, and the rate of appearance of Pa, Nb, and Pb decreased significantly with 42°C as the boundary. Nb almost disappeared at 43°C and above, and Pb almost disappeared at 42°C and above. As for the effect of body temperature on the ABR, several reports claimed that the peak latency of all the wave components was shortened and that their amplitude was also diminished. In this study, in a species different from those used in those studies, peak latency of ABR did not become shorter during hyperthermia, but the amplitudes of
II and IV components decreased. No major changes, such as the disappearance of components during hyperthermia observed in the MLR, occurred, however, and the appearance of components was relatively well maintained. In MLR, on the other hand, 42°C was the boundary for the decrease in the appearance of waveforms. Mustafa et al.\textsuperscript{39} reported that the ABR was preserved during hyperthermia in conscious sheep, but that the MLR disappeared at 42.9±0.6°C. The sources of components in the ABR have been identified\textsuperscript{40-41}, viz.: wave I, the acoustic nerve; wave II, the cochlear nucleus; wave III, the superior olivary complex; wave IV, the lateral lemniscus; and wave V, the inferior colliculus. There have been various reports\textsuperscript{41-47} on the many parts of the source of MLR wave components, on the other hand, it remains unclear. These waves definitely seem to reflect electrical activity from above the brain stem to the cortical auditory area. Based on the results of this study, during hyperthermia above 42°C, electrical activity in part of brain stem may be affected while its function is preserved. In contrast, electrical activity of the upper brain above the brain stem seems to be greatly affected by thermal stimulation, and central nervous system activity from the brain stem to the cortical auditory area appears to be inhibited.

To determine whether the superoxide in the brain are involved in the inhibition of nervous activity, SOD was measured and SOD-R was found to have increased significantly. Issels et al.\textsuperscript{48} reported that when the temperature of Chinese hamster ovary cells in vitro was increased, \( \text{O}_2 \text{ }^\cdot \) production and SOD levels increased dramatically. Omar et al.\textsuperscript{49}, using mouse embryo cells in vitro concluded that increased \( \text{O}_2 \text{ }^\cdot \) production during hyperthermia probably has a cell-damaging effect. Several papers\textsuperscript{50-53} claim that free radicals have considerable effect on the production of heat shock proteins. The finding of increased SOD-R in this experiment suggests that the super oxide are involved in inhibition of central nervous system activity during hyperthermia.

The effect of hyperthermia on brain was evaluated during the body temperature increasing, in this study, but it was not evaluated during the body temperature falling again, because maintenance of systemic circulation was very difficult while 43°C had been maintained for 1h. Noguchi\textsuperscript{21} reported that cardiac function was suppressed greatly at 43°C.

In conclusion, concerning CBF and cerebral metabolism, these results show that the brain appears to be better protected than the abdominal organs during systemic hyperthermia. Concerning central nervous system activity, however, although brain stem activity that is correlated with vegetative function was relatively well maintained at 42°C and above, brain function was considerably inhibited at higher levels. The super oxide appear to be related to this inhibition. Thus, concerning brain function, the CTM of dogs appeared to be about 42−43°C.

**Acknowledgments**

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