

Cerebral Protective Effect of Low-Grade Hypothermia

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Male Wistar rats with unilateral carotid ligation were exposed to arterial hypoxia (P_{aO_2} 20–23 torr for 20 min) while body temperature was controlled at 37° C, 36° C, or 34° C. Brain cortical concentrations of ATP, phosphocreatine (PCr) and lactate were measured microfluorometrically.

Normothermic hypoxic rats had severe metabolic changes with low brain ATP and extremely high brain lactate. When rectal temperature was controlled at 36° C during hypoxia, brain ATP was not different from that observed in normothermic, normoxic rats, and brain lactate was significantly lower than during normothermic hypoxia. At 34° C, brain lactate was even less, but still three times higher than that observed in normothermic normoxic rats. PCr was significantly higher following hypoxia at 34° C than at 37° C. In part, this latter finding may reflect preservation of intracellular pH at 34° C.

A decrease of body temperature of 1–3° C can minimize or prevent brain energy failure during hypoxia as well as decrease the magnitude of brain tissue acidosis. Thus, in experiments examining “cerebral protective effects” of therapies during brain hypoxia-ischemia, stringent control of body temperature is necessary. Furthermore, a possible clinical benefit resulting from modest reduction in body temperature in patients with marginal cerebral oxygenation is suggested. (Key words: Brain: metabolism; protection. Hypothermia.)

INDUCED HYPOTHERMIA has long been known to moderate the response of brain tissue to hypoxic ischemic stress. For example, accidental hypothermia has prevented any obvious neurologic deficit after more than 30 min of submersion in cold water.^{1,2} The brain can tolerate complete circulatory arrest for 31 min at 19° C and 10 min at 30° C.³ Clinical usefulness of this degree of hypothermia is well documented in cardiac and brain surgery.

However, in many clinical circumstances, usefulness of hypothermia is limited by toxic effects which relate to duration of treatment and level of hypothermia.^{4,5} Reduction of body temperature to 34° C can be tolerated for long periods⁶ and, if favorable change in brain tolerance of hypoxic-ischemic stress exists at 34° C, this

level of hypothermia could be an important factor in treatment of head injury, or Reyes Syndrome, for example.

In many studies examining “protective effects” of various therapies upon brain response to hypoxia-ischemia, there has not been precise control of body temperature.⁷ Since minimal decline in body temperature undoubtedly occurs in some of these animal models, hypothermia may be an important contributor to “protective effects.”

To study the possible cerebral protective effect of low-grade hypothermia, we used a hypoxic-oligemic experimental model, that previously has been shown to produce metabolic and histopathological evidence of neuronal damage in the normothermic rat.^{8,9} The severity of the hypoxic stress was determined by measurements of cerebral cortical concentrations of ATP, phosphocreatine (PCr), and lactate.

Methods

Fed male Wistar rats (250–325 g) were anesthetized with halothane 2.5–3.0 per cent in oxygen, tracheotomized, immobilized with *d*-tubocurarine, 1 mg/kg, and ventilated with 0.7 per cent halothane in nitrous oxide (N_2O) 70 per cent and oxygen during surgical preparation. A rectal probe was inserted for continuous measurement of body temperature. One femoral artery was cannulated for monitoring of arterial blood pressure and sampling for arterial blood gases. A scalp incision was made to accommodate a plastic funnel for subsequent freezing of the brain with liquid N_2 . One carotid artery was carefully dissected free via a ventral approach and a ligature was placed loosely around the vessel. The animals were then allowed a 30-min stabilization period on 70 per cent N_2O in oxygen during which temperature was controlled at 37° C, 36° C, or 34° C by a heat lamp servo device. The carotid artery was then ligated and FI_{O_2} simultaneously reduced to about 0.06 (N_2O 70 per cent, balance nitrogen) to give an arterial P_{O_2} of 20–23 torr for 20 min. In addition, one group was maintained normoxic and normothermic, but otherwise identically treated. CO_2 was added to inspired gas of all hypoxic animals to reduce fall in Pa_{CO_2} .

Samples for arterial blood gases (100 μ l) were obtained four times during the hypoxic period to assure that Pa_{O_2} was maintained in the desired range of 20–23 torr. Blood-gas values were corrected for temperature. At the end of the experiment brains were frozen *in situ* with

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TABLE 1. End Hypoxia Physiologic Variables*

| Group | n | Rectal Temperature (°C) | MABP (torr) | Pa _{O₂} (torr) | Pa _{CO₂} (torr) | pH |
|------------|----|-------------------------|-------------|------------------------------------|-------------------------------------|--------------|
| Normoxia I | 6 | 37 ± 0.0 | 160 ± 6 | 131 ± 8† | 41.3 ± 0.9† | 7.29 ± 0.01† |
| Hypoxia II | 10 | 37 ± 0.0 | 124 ± 4 | 21.6 ± 0.3 | 29.7 ± 1.9 | 7.09 ± 0.04 |
| III | 8 | 36 ± 0.0 | 131 ± 4 | 22.1 ± 0.2 | 32.8 ± 6.1 | 7.19 ± 0.02† |
| IV | 12 | 34 ± 0.0 | 155 ± 4† | 21.4 ± 0.2 | 38.6 ± 1.1† | 7.29 ± 0.02† |

* Values are means ± SEM.

† $P < 0.05$ compared to Group II.

liquid nitrogen,¹⁰ the animals decapitated, and the heads stored in liquid nitrogen, until samples (15–20 mg) were taken for microfluorometric analysis of ATP, phosphocreatine (PCr), and lactate.

In the normoxic, 37° C hypoxic and 34° C hypoxic series, arterial oxygen content was measured in five animals of each group using a potassium ferricyanide displacement technique.¹¹

Statistical differences were tested by one way analysis of variance, following which individual means in groups having F test positive at $P < 0.01$ were compared by Student's unpaired *t* test with $P < 0.05$ accepted as significant.

Results

Measured physiological variables are summarized in table 1. Group IV animals (34° C) had a significantly higher mean blood pressure, Pa_{CO₂}, and pH than did the normothermic hypoxic group (Group II). These values in Group IV did not differ from those obtained in normoxic animals (Group I). Animals in Group III (36° C) had less metabolic acidosis but otherwise did not differ from Group II animals. Animals in Group II and Group III had depressed Pa_{CO₂} in spite of addition of CO₂ to inspired gases, reflecting systemic metabolic acidosis during hypoxia at 37° C and 36° C.

Arterial oxygen content during hypoxia (table 2) was 20 per cent lower at 37° C than at 34° C. This difference

TABLE 2. Arterial Oxygen Content*

| Group | n | Ca _{O₂} (ml/dl) |
|--------------------|---|-------------------------------------|
| Normoxia I | 5 | 19.80 ± 0.87† |
| Hypoxia II (37° C) | 5 | 3.34 ± 0.13 |
| IV (34° C) | 5 | 4.09 ± 0.38 |

* Values are means ± SEM.

† $P < 0.05$ compared to Group II.

of means nearly achieved significance at the 0.05 level ($t = 1.83$, $n = 8$).

Cerebral cortical concentrations of ATP, PCr, and lactate from the hemisphere ipsilateral to the ligated carotid artery are summarized in table 3. Groups III and IV (36° C and 34° C) had mean ATP concentrations nearly identical to those of the normoxic groups, while Group II had a significantly lower mean value. The hypothermic groups (Groups III and IV) had less lactic acidosis than Group II, in that there was a stepwise decrease in tissue lactate as the temperature decreased. However, lactate in Groups III and IV remained increased when compared to that observed in normoxic animals. The latter values were somewhat higher than those previously reported from this laboratory,¹² but since the brains of the present study were all analyzed in the same batch this discrepancy does not affect the conclusions. In a reciprocal fashion, PCr values were decreased in all hypoxic animals, but progressively less so in the hypothermic animals.

Discussion

The experimental rat model used in this study with ligation of one carotid artery and severe arterial hypoxia has been well-defined in terms of effects on energy metabolism, cerebral blood flow and histopathology.^{8,9,13-15} Carotid ligation curtails the hypoxia-induced increase in blood flow that otherwise would occur to the ipsilateral hemisphere. The resultant restriction in cerebral oxygen delivery causes a unilaterally disturbed cerebral energy balance and histopathological evidence of neuronal death without any severe systemic complications when Pa_{O₂} is maintained at 20–21 torr for 20 min.^{8,12,15}

In an experimental rat model similar to the one used in the present study changes in brain ATP, PCr, and lactate produced by Pa_{O₂} 25 torr for 25 min at 37° C were eliminated (except for slight lactate elevation) when body temperature was reduced 5° C.¹² In another study in rats without carotid ligation, when body temperature

TABLE 3. Cortical Metabolites*

| Group | n | ATP ($\mu\text{mol/g}$) | PCr ($\mu\text{mol/g}$) | La ($\mu\text{mol/g}$) |
|----------------------|----|---------------------------|---------------------------|--------------------------|
| Normoxia I | 6 | 2.41 \pm 0.05† | 3.56 \pm 0.07† | 3.5 \pm 0.6† |
| Hypoxia II (37°C) | 10 | 1.21 \pm 0.32 | 1.27 \pm 0.53 | 25.7 \pm 3.4 |
| III (36°C) | 8 | 2.44 \pm 0.05† | 2.08 \pm 0.28 | 16.1 \pm 1.0† |
| IV (34°C) | 12 | 2.42 \pm 0.03† | 2.81 \pm 0.13† | 10.7 \pm 1.4† |

* Values are means \pm SEM.† $P < 0.05$ compared to Group II.

was reduced by 10° C, metabolic changes were still small when Pa_{O_2} was further reduced to 11–12 torr for 20 min.¹⁶ Thus, there is clear experimental evidence that induced hypothermia can prevent or curtail metabolic changes secondary to hypoxia.

The effect of hypothermia upon brain tissue response to hypoxia has been assumed to relate to associated reduction of oxygen demand. Previous studies in the Wistar rat have shown that hypothermia reduces brain cortical oxygen consumption by 5 per cent per degree centigrade between 37° C and 22° C.¹⁷

During hypoxia, hypothermia also greatly increases arterial oxygen content.^{12,16,18} This effect is due to the shift of the hemoglobin dissociation curve to the left caused by hypothermia, and to the absence of systemic acidosis during hypoxia in hypothermic animals.¹⁸ While this increase in arterial oxygen content (Ca_{O_2}) may play some role in hypothermic hypoxia, it is not a major factor, since increasing Ca_{O_2} in normothermic hypoxic animals by correcting metabolic acidosis affords no protection from perturbation of cortical metabolites, while hypothermia remains effective in this respect even when Ca_{O_2} is brought to normothermic hypoxic levels by lowering Pa_{O_2} .^{16,18}

The results in the normothermic hypoxic animals (Group II) agree with those reported by Salford *et al.*⁸ using a similar experimental protocol, and can thus be assumed to be associated with the same degree of neuronal damage.^{8,9} Reduction of body temperature to 36° C maintained ATP concentration and may thus have afforded some degree of protection. However, considerable tissue lactic acidosis persisted at 36° C and this tissue acidosis has been proposed to be an important factor in hypoxic neuronal damage.¹⁹ At 34° C, the level of brain cortical lactate was markedly reduced. Low-grade hypothermia both normalized brain cortical ATP level and reduced tissue acidosis and should protect the brain from neuronal damage in the experimental model used.

In the 34° C hypoxia group (Group IV), phosphocreatine was significantly higher than in the 37° C hy-

poxia group (Group II). In part, this result depends upon the preservation of intracellular $p\text{H}$ ($p\text{H}_i$) at 34° C resulting from reduced tissue lactate level. However, Pa_{CO_2} , a major determinant of $p\text{H}_i$ was significantly higher during hypoxia at 34° C than at 37° C. The present data do not permit calculation of $p\text{H}_i$, and therefore the effect it may have had upon the creatine phosphokinase equilibrium cannot be determined. However, it is unlikely that $p\text{H}_i$ difference can explain the observed result.²⁰

Two important observations may be taken from the present study. First, in animal experiments designed to determine the effect of various interventions upon brain response to hypoxia ischemia, it is clearly essential that body temperature be rigidly controlled. Changes of body temperature by 1–3° C are shown here to modify the effect of hypoxia upon brain ATP and lactate, both of which may be determinants of tissue injury.

The second observation is that moderate levels of hypothermia which can be tolerated for long periods of time may afford protection in other forms of stress, such as ischemia or metabolic coma. Thus, studies defining the effects of low-grade hypothermia in ischemia are needed.

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