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Effect of High vs. *Low Arterial Blood Oxygen Content on Cerebral Energy Metabolite Levels during Hypoxia with Normothermia and Hypothermia in the Rat*

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The effects of different levels of arterial blood oxygen content (Ca_{O_2}) on brain tissue adenosine triphosphate (ATP), phosphocreatine (PCr), lactate, and reduced nicotinamide adenine dinucleotide (NADH) were studied during cerebral hypoxia in normothermic and hypothermic male Wistar rats with unilateral carotid ligation. Animals were exposed to hypoxia (Pa_{O_2} 19–26 torr) for 25 min, and brain tissue metabolite values measured microfluorometrically were compared with those of normothermic normoxic controls. Ca_{O_2} was 4.0 ± 0.2 ml/dl (mean ± SEM) at Pa_{O_2} 26 torr in normothermic animals. Ca_{O_2} was increased to 8.2 ± 0.3 ml/dl at Pa_{O_2} 26 torr by means of bicarbonate infusion producing a leftward shift of the oxyhemoglobin-dissociation curve in one normothermic hypoxic group. In all normothermic hypoxic groups ATP and PCr decreased and lactate and NADH increased significantly compared with control values. There was no significant difference in brain tissue metabolite values among these groups despite an increase in Ca_{O_2} by twofold in one group. Hypothermia (32 C) resulted in Ca_{O_2} 8.4 ± 0.2 ml/dl at Pa_{O_2} 26 torr. This was decreased to 4.0 ± 0.2 ml/dl by decreasing Pa_{O_2} to 19 torr in another group at the same temperature. ATP and PCr were well preserved in both groups despite the difference in Ca_{O_2}s. Although the lactate and NADH levels were increased in the hypothermic group with Ca_{O_2} 4.0 ± 0.2 ml/dl, they were significantly lower than those values in normothermic hypoxic groups. These results indicate that the increase in Ca_{O_2} produced by hypothermia is not a major determinant in hypothermic protection during cerebral hypoxia. (Key words: Brain: anoxia; lactate; metabolism; oxygenation; protection. Hypothermia: brain. Oxygen: content.)

HYPOXIC STRESS induces characteristic changes in brain tissue levels of lactate, phosphocreatine (PCr), adenosine triphosphate (ATP), and reduced nicotinamide adenine dinucleotide (NADH).[1–2] Hypothermia can prevent these hypoxic changes, and this has been interpreted to represent a "protective" effect. Hypothermia produces a leftward shift in the oxyhemoglobin-dissociation curve, which effect is exaggerated during hypoxia by absence of metabolic acidosis in hypothermic animals.[1,2] Thus, at 32 C, a Pa_{O_2} of 25 torr maintained for 25 min in the Wistar rat would produce an arterial blood oxygen content (Ca_{O_2}) of 8.0 vol per cent, while at 37 C, a Pa_{O_2} of 25 torr maintained for 25 min would produce a Ca_{O_2} of 4.0 vol per cent.[1,2] Is this increase in arterial oxygen content critical for the protective effect? Or does preservation of metabolite levels depend upon a decrease of brain energy consumption by hypothermia?[3,4]

This study was designed to examine whether an increase in Ca_{O_2} produced by a leftward shift in the oxyhemoglobin-dissociation curve produced by infusion of bicarbonate is protective in cerebral hypoxia at normal body temperature, and also to evaluate whether equal Ca_{O_2}s mean equal resistances to cerebral hypoxia in normothermic and hypothermic animals. Cerebral metabolic state was evaluated by measuring the concentrations of cerebral cortical tissue ATP, PCr, lactate, and NADH.

Methods

Male Wistar rats weighing 250–300 g were anesthetized with halothane, 2–3 per cent, in oxygen. Through a neck incision, the right carotid artery was separated from the sympathetic trunk and divided between silk ligatures. After wound closure animals were allowed to recover from anesthesia and returned to their cages. Free access to food and water was allowed until the time of the study.

Forty-eight hours after carotid ligation, these animals were again anesthetized with halothane, 2–3 per cent, in oxygen. Following tracheostomy, anesthesia was continued with halothane, 0.5–0.75 per cent, and nitrous oxide, 70 per cent, in oxygen, delivered via a Harvard® small-animal ventilator. The femoral arteries were cannulated for continuous arterial pressure recording via a Statham P23DB transducer and for sampling for blood-gas analysis. One femoral vein was

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TABLE 1. Rectal Temperature, Mean Arterial Pressure (MAP), Pa_{O_2}, Pa_{CO_2}, pH_a, Total Arterial Oxygen Content (Ca_{O_2}), and Hemoglobin Concentration in Group I (Control) and Hypoxic Groups

	Rectal Temperature (C)	MAP (torr)	Pa_{O_2} (torr)	Pa_{CO_2} (torr)	pH_a	Ca_{O_2} (ml/dl)	Hemoglobin (g/dl)
Group I	37.0 ± 0.1	174 ± 5.0	162 ± 8	34.7 ± 0.5	7.41 ± 0.02	19.8 ± 0.7	14.4 ± 0.3
Group II	36.9 ± 0.1	145* ± 6	25.8* ± 0.5	42.4* ± 1.4	7.55* ± 0.01	8.3* ± 0.3	14.9 ± 0.3
Group III	36.9 ± 0.03	156 ± 4	26.4* ± 0.5	36.6* ± 1.7	7.14* ± 0.02	4.0* ± 0.2	14.8 ± 0.5
Group IV	37.1 ± 0.1	135* ± 2	25.7* ± 0.6	26.6* ± 1.4	7.27* ± 0.01	4.0* ± 0.2	14.9 ± 0.3
Group V	31.8* ± 0.1	134* ± 5	26.7* ± 0.6	23.6* ± 1.1	7.42 ± 0.08	8.4* ± 0.2	13.8 ± 0.9
Group VI	32.0* ± 0.1	133* ± 5	19.3* ± 0.4	31.4 ± 2.4	7.24* ± 0.01	4.0* ± 0.2	13.9 ± 0.3

Values are mean ± SE.

* Significantly different from Group I, $P < 0.05$, Student t test.

cannulated for drug and fluid administration. A rectal temperature probe was inserted, and body temperature was controlled at the desired level by means of a heat-lamp servomechanism. The head was immobilized in a stereotaxic head holder and the skull was exposed through a midline incision in the scalp for placement of a plastic freezing funnel.

Upon completion of the surgical procedure, halothane administration was discontinued, *d*-tubocurarine, 1 mg/kg was given intravenously, and ventilation was adjusted to maintain arterial blood carbon dioxide tension (Pa_{CO_2}) at 35–40 torr *prior to exposure to hypoxia*. All animals' lungs were ventilated with nitrous oxide, 70 per cent, in oxygen, at least 30 min before exposure to hypoxia.

Six groups were studied, with eight animals in Group II and six in each of the other groups. Group I, body temperature 37 C, was the control group. These animals' lungs were ventilated with nitrous oxide, 70 per cent, in oxygen, for 25 min. Groups II, III, and IV also had body temperatures of 37 C, but arterial blood oxygen tension (Pa_{O_2}) was decreased to 25–26 torr (normothermic hypoxic groups). Animals in Group II received an intravenous infusion of sodium bicarbonate, 7 mEq/kg (8.4 per cent) via a Harvard pump to maintain an alkaline pH (7.5–7.6) during hypoxia. This caused a leftward shift in the oxyhemoglobin-dissociation curve, thereby increasing total Ca_{O_2}. This also significantly increased serum sodium and osmolality from normal values and caused an increase in Pa_{CO_2}. Animals in Group III received an intravenous infusion of sodium chloride, 5 per cent solution, to maintain serum sodium and osmolality close to levels of those in Group II (table 2). Carbon dioxide was also added to the inspired gases in this group to attain a Pa_{CO_2} comparable to the corresponding value in Group II (table 1). Normal values of serum sodium and serum osmolality were determined in a separate group of five normoxic, normothermic Wistar rats, referred to as control in table 2. No other measurements were made in this group. Groups IV, V, and VI did not receive any infusion, and CO_2 was not added to the inspired gases during hypoxia. Animals in Group IV had exposure to hypoxia at 37 C identical to that of Groups II and III, but without infusion of bicarbonate or sodium chloride. Groups V and VI had body temperatures of 32 C with Pa_{O_2} decreased to 25 torr in Group V and to 19 torr in Group VI (hypothermic hypoxic groups). The decrease in Pa_{O_2} was achieved by replacing oxygen with nitrogen in the inspired gases.

Arterial pressure was maintained above 120 torr throughout hypoxia by administration of phenylnephrine (0.05 mg) when necessary. Arterial blood-gas and pH values were measured before and three times during the experiment, and the values were

TABLE 2. Serum Sodium Concentration and Osmolality in a Control Group and after Infusion of Sodium Bicarbonate in Group II and Sodium Chloride, 5 Per Cent, in Group III

	Serum Sodium (mEq/l)	Serum Osmolality (mosm/l)
Control (n = 5)	144 ± 1	295 ± 4
Group II (n = 5)	162* ± 1	339* ± 2
Group III (n = 6)	166* ± 1	338* ± 3

Values are means ± SE.

* Significantly different from control, $P < 0.05$, Student t test.

TABLE 3. Cerebral Cortical Tissue Concentrations of Adenosine Triphosphate, Phosphocreatine, Lactate, and NADH from Right and Left Cortices (Right Cortex Homolateral to Carotid Ligation)

	Adenosine Triphosphate (μmol/g)		Phosphocreatine (μmol/g)		Lactate (μmol/g)		NADH (μmol/g Wet Weight)	
	Right Cortex	Left Cortex	Right Cortex	Left Cortex	Right Cortex	Left Cortex	Right Cortex	Left Cortex
Group I	2.79 ± 0.03	2.76 ± 0.02	4.65 ± 0.11	4.37 ± 0.10	1.9 ± 0.43	2.1 ± 0.5	0.016 ± 0.02	0.015 ± 0.002
Group II	2.36* ± 0.09	2.65 ± 0.10	3.15* ± 0.3	4.12 ± 0.1	11.4* ± 1.5	5.1* ± 0.9	0.024* ± 0.006	0.015 ± 0.002
Group III	2.44* ± 0.05	2.68 ± 0.06	2.58* ± 0.3	3.5* ± 0.1	13.9* ± 4.0	6.5* ± 1.6	0.023* ± 0.001	0.020* ± 0.002
Group IV	2.42* ± 0.03	2.82 ± 0.04	2.51* ± 0.5	4.22 ± 0.1	16.6* ± 3.6	6.9* ± 1.0	0.024* ± 0.002	0.020* ± 0.001
Group V	2.95* ± 0.05	2.88 ± 0.05	4.88 ± 0.34	5.09 ± 0.23	3.0 ± 0.3	2.8 ± 0.3	0.015 ± 0.001	0.014 ± 0.001
Group VI	2.80 ± 0.03	2.77 ± 0.03	4.24 ± 0.28	4.31 ± 0.12	5.7* ± 0.4	4.4* ± 0.5	0.020* ± 0.002	0.018 ± 0.001

Values are mean ± SE.

* Significantly different from Group I (control), $P < 0.05$ Student t test.

corrected for temperature differences.[5] Total arterial oxygen content was measured at the same points by the method of Fabel and Lübbers.[6,7]

After 25 min of hypoxia and the corresponding period in the control group, liquid nitrogen was poured into the affixed freezing funnel and the brain was frozen *in situ*.[8] Ventilation was continued during freezing and the arterial pressure was maintained for at least 1–2 min. The frozen brain was removed *en bloc* and stored in liquid nitrogen until cortical tissue samples were prepared separately from cerebral hemispheres for microfluorometric analysis of adenosine triphosphate (ATP), phosphocreatine (PCr), lactate, and NADH.[9]

Statistical analysis of the results employed the Student t test, and $P < 0.05$ was considered significant.

Results

Mean arterial pressure (MAP) was uniformly decreased in the hypoxic groups as compared with the control group, except in Group III (table 1). Pa_{CO_2} was significantly higher in Groups II and III, and lower in Groups IV and V than in the control group, while pH_a was significantly higher in Group II and lower in Groups III, IV, and VI compared with the control group (table 1). The increases in Pa_{CO_2} and pH_a values in Group II were the result of sodium bicarbonate infusion. The increased Pa_{CO_2} in Group III was the result of carbon dioxide added to the inspired gases in this group. Infusion of sodium bicarbonate in Group II and sodium chloride in Group III increased serum sodium and osmolality in these groups significantly when compared with normal values (table 2). Ca_{O_2} decreased significantly in all hypoxic groups (table 1).

In normothermic hypoxic animals (Groups II, III, and IV), ATP and PCr values decreased and lactate and NADH values increased significantly in cortical hemisphere homolateral to the ligated carotid artery (right side) compared with the control group (table 3). In Group III, phosphocreatine was significantly decreased in the hemispheres contralateral to the ligated carotid (left side). Otherwise, ATP and PCr were not decreased in cortex contralateral to carotid ligation in normothermic hypoxic animals (Groups II, III, and IV). Lactate and NADH concentrations were significantly increased in all normothermic hypoxic groups (II–IV) on the right side (carotid ligated). Except for NADH in Group II, lactate and NADH were consistently increased on the left side (carotid intact) in Groups II, III, and IV. Concentrations of NADH were lower on the left side in Group II than in Groups III and IV. Increases in serum sodium concentration and osmolality did not have any significant effect on the cerebral cortical tissue metabolites in group III compared with corresponding values in Group IV.

There was no side-to-side difference in the hypothermic hypoxic animals (Groups V and VI). In Group V the ATP concentration was significantly higher than control on the right side. In Group VI there was a significant increase in lactate on both sides and NADH on the right side compared with the control group. When Group V (Ca_{O_2} 8.4 ml/dl) was compared with Group VI (Ca_{O_2} 4.0 ml/dl), the ATP concentration was

significantly higher on the right side and the lactate concentration was lower on both sides in Group V than in Group VI.

When Group II (normothermic, Ca_{O_2} 8.3 ml/dl) was compared with Groups V and VI (hypothermic, Ca_{O_2}s 8.4 and 4.0 ml/dl, respectively), the concentrations of ATP and PCr were significantly higher and those of lactate and NADH were significantly lower on the right side in Groups V and VI than in Group II. This was also true when Groups III and IV (normothermic, Ca_{O_2} 4 ml/dl) were compared with Groups V and VI. Thus, an increase in Ca_{O_2} by twofold did not change the effect of hypoxia upon the brain tissue metabolites in normothermic animals, and a decrease in Ca_{O_2} by 50 per cent did not cause any change in high-energy phosphate levels in the brains of hypothermic rats.

Discussion

Since the severity of hypoxia necessary to produce profound alterations in cerebral metabolism causes cardiovascular instability and arterial hypotension, we used rats with unilateral carotic ligation.[10] Occlusion of one carotid artery does not cause cerebral ischemia or alteration in brain tissue ATP, PCr, and lactate levels in normoxic Wistar rats.[11] This is in agreement with our results in Group I, which showed no difference between metabolite values in the two hemispheres (table 3). Other studies have shown that changes in brain tissue metabolites due to hypoxia are similar in the two hemispheres but more profound on the side of occlusion.[2,12] In the present study differences between brain tissue metabolite levels in normothermic hypoxic groups and the control group were far greater on the side of carotid ligation.

Our results indicate that increasing Pa_{O_2} in normothermic hypoxic rats by a leftward shift in the oxyhemoglobin-dissociation curve did not prevent the harmful effects of hypoxia on brain tissue metabolites. Thus, while animals in Group II had Ca_{O_2}s twice as high as those in Groups III and IV, extents of deterioration in brain tissue metabolites were of the same magnitude in all of these groups (table 3). Brain tissue high-energy phosphate levels were well preserved in hypothermic hypoxic groups. Decreasing Ca_{O_2} in animals in Group VI to half of those in Group V by decreasing Pa_{O_2} to 19 torr caused no change in brain tissue ATP and PCr, but increased the lactate and NADH levels in group VI animals. However, these lactate and NADH levels were far lower than those of normothermic hypoxic groups.

This observation contradicts the hypothesis that the higher arterial blood oxygen content in hypothermia plays a major role in cerebral protection during hypoxia.[2] The mechanism by which hypothermia protects the brain is not totally clear. Our results indicate that increases in arterial blood oxygen content cannot be the primary mechanism for hypothermic protection of brain tissue during hypoxia. It remains to be determined whether the protective effects of hypothermia are mediated solely by a decrease in metabolic rate, or whether other mechanisms are involved.

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