

Protective Effects of Combinations of Hypothermia and Barbiturates in Cerebral Hypoxia in the Rat

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The protective effects of phenobarbital and hypothermia in cerebral hypoxia were studied in Wistar rats with unilateral carotid ligation. The animals were exposed to hypoxia (P_{aO_2} , 25–15 torr) for 25 minutes. Cerebral protection was evaluated by means of effects on cerebral tissue ATP, phosphocreatine (PCr), lactate, and nicotinamide-adenine dinucleotide (NADH) values. At P_{aO_2} , 25 torr, cerebral blood flow (CBF) and cerebral oxygen consumption (CMR_{O_2}) were decreased by 25 per cent either with phenobarbital, 50 mg/kg, or by hypothermia, 32 C, or decreased by 40–50 per cent with hypothermia, 27 C, phenobarbital, 150 mg/kg, or the combination of 32 C and phenobarbital, 50 mg/kg. The group given phenobarbital, 50 mg/kg, as well as the normothermic hypoxic control group, had marked metabolic changes, with a 30–50 per cent decrease in PCr and a four- to sixfold increase in lactate levels on the ligated side compared with the hypothermic rats. The hypothermic rats showed almost no metabolic sign of hypoxia. All rats in the group that received phenobarbital, 150 mg/kg, developed cardiac arrhythmias and decreases in blood pressure. No animal in this group survived.

At P_{aO_2} , 15 torr, hypothermia to 32 C alone or combined with phenobarbital, 50 mg/kg, decreased PCr 40–50 per cent and increased lactate values four- to fivefold on the ligated side compared with hypothermia to 27 C alone. With the exception of an elevated lactate level on the ligated side, there was no metabolic sign of hypoxia with hypothermia to 27 C.

As judged by metabolic criteria, hypothermia offers better cerebral protection from hypoxia than does a dose of phenobarbital that gives the same decreases in CBF and CMR_{O_2} . Hypothermic rats had less severe metabolic acidosis and higher arterial oxygen contents, which may partly explain the greater protective effect. (Key words: Brain: anoxia; blood flow; lactate; oxygen consumption. Anesthetics, intraperitoneal: phenobarbital. Hypothermia. Hypoxia.)

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BOTH BARBITURATES AND HYPOTHERMIA have been shown to protect brain tissue in a variety of hypoxic-ischemic models.¹⁻² Large doses of barbiturates, however, have been necessary to obtain protection, causing cardiovascular side effects such as a decrease in arterial blood pressure and cardiac arrhythmias.³ Also, at body temperatures below 30 C, severe cardiac arrhythmias are common.⁴ In order to minimize these side effects, a combination of barbiturates and hypothermia has been tried as a treatment in patients with severe head injuries.⁵ In previous studies in the rat we showed that either phenobarbital, 50 mg/kg, or a decrease of body temperature to 32 C decreased cerebral blood flow (CBF) and cerebral metabolism (CMR_{O_2}) by about 25 per cent.^{6,7} A combination of phenobarbital, 50 mg/kg, and 32 C body temperature decreased CBF and CMR_{O_2} by 44 per cent. Our conclusion was that the effects of phenobarbital and hypothermia on CBF and CMR_{O_2} were additive.^{7,8} As the protective mechanisms for both hypothermia and barbiturates in cerebral hypoxia and ischemia are unclear,^{1,2} the present study was designed to determine whether the protective effects of hypothermia and phenobarbital are additive in cerebral hypoxia. Protection was evaluated by means of influence on cerebral cortical concentrations of adenosine triphosphate (ATP), phosphocreatine (PCr), lactate, and nicotinamide-adenine dinucleotide (NADH) values during hypoxia. ATP and PCr levels decrease in hypoxia and lactate and NADH levels increase.^{9,10} The absence of, or the decrease in, such changes has been regarded as protective.²

Methods

Two to five days following unilateral carotid ligation during halothane anesthesia, unstarved male Wistar rats of 250–350-g body weight were anesthetized in a closed jar with halothane, 2.5–3 per cent. When the rat was unresponsive to tilting, tracheotomy was performed and anesthesia during surgical preparation continued with halothane, 0.7–1.2 per cent, in nitrous oxide, 70 per cent, via a Harvard apparatus small-animal ventilator. One femoral artery and vein were cannulated, a midline scalp incision was made for subsequent insertion of a freezing funnel, a rectal probe was placed for continuous monitoring of body

TABLE 1. Series A, Physiologic Variables at the End of the Study

| | Temperature (C) | MABP (torr) | Pa _{o₂} (torr) | Pa _{co₂} (torr) | pH | Ca _{o₂} (ml/100 ml) | Hemoglobin (g/100 ml) |
|--|--------------------|----------------|---------------------------------------|--|-----------------|--|--------------------------|
| Group I 37 C | 37.1 ± 0.1 | 122 ± 5 | 25.7 ± 0.6 | 26.6 ± 1.4 | 7.22 ± 0.02 | 3.97 ± 0.16 | 14.8 ± 0.3 |
| Group II 37 C, 50 mg/kg phenobarbital | 36.6 ± 0.2 | 123 ± 1 | 25.7 ± 0.2 | 24.6 ± 1.1 | 7.23 ± 0.02 | 4.73 ± 0.38 | 15.7 ± 0.7 |
| Group III 32 C | 31.3 ± 0.1 | 134* ± 5 | 26.7 ± 0.6 | 23.6 ± 1.1 | 7.42* ± 0.02 | 8.39* ± 0.26 | 13.8 ± 0.9 |
| Group IV 32 C, 50 mg/kg phenobarbital | 31.3 ± 0.1 | 126 ± 6 | 25.3 ± 1.0 | 25.9 ± 1.7 | 7.40* ± 0.03 | 7.50* ± 0.88 | 14.0 ± 0.5 |
| Group V 27 C | 26.5 ± 0.2 | 151* ± 7 | 26.5 ± 0.9 | 32.8* ± 2.6 | 7.41* ± 0.01 | 12.52* ± 0.53 | 15.6 ± 0.8 |

Values are means ± SEM. Six animals in each group.

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

temperature, and silver-silver chloride fronto-occipital electroencephalographic (EEG) electrodes were affixed. At this point, halothane was discontinued and *d*-tubocurarine, 1.0 mg/kg, was given intravenously. Ventilation was adjusted to maintain arterial carbon dioxide partial pressure (Pa_{CO₂}) at 35–40 torr. Two series of experiments were done.

SERIES A

In Series A, there were six groups with six animals in each. Groups I and II were kept at 37 C. Rats in Group II, in addition, received injections of phenobarbital, 50 mg/kg, intraperitoneally. Groups III, IV, and V had their body temperatures lowered through surface cooling, Groups III and IV to 32 C and Group V to 27 C. Rats in Group IV also received injections of phenobarbital, 50 mg/kg. Rats in Group VI re-

ceived injections of phenobarbital, 150 mg/kg. Carbon dioxide, 0.5 per cent, was added to the inspired gas mixture during hypothermia in the 27 C group to maintain arterial carbon dioxide comparable to values in the other groups. At least 15 min after the injection of phenobarbital, and at comparable times in the other groups, the inspired oxygen concentration was lowered by replacing oxygen with nitrogen to give an arterial partial pressure for oxygen (Pa_{O₂}) of 25 torr. After 25 min of hypoxia, a plastic funnel was accommodated over the skull, liquid nitrogen was poured into the funnel, and the brain was frozen *in situ*.¹¹

Artificial ventilation was continued during freezing, and arterial blood pressure was maintained for 1–2 min. The brains were chiseled out and stored at the temperature of liquid nitrogen. Later, samples of cortical tissue were taken separately from the cerebral

TABLE 2. Series A: ATP, Phosphocreatine, Lactate and NADH Values on Unligated (UL) and Ligated (L) Sides

| | mmol·kg ⁻¹ | | | | | | | |
|--|-----------------------|-----------------|-----------------|-----------------|---------------|---------------|-------------------|-------------------|
| | ATP | | PCr | | Lactate | | NADH | |
| | UL | L | UL | L | UL | L | UL | L |
| Group I 37 C | 2.82 ± 0.04 | 2.42 ± 0.23 | 4.22 ± 0.15 | 2.51 ± 0.55 | 6.9 ± 1.0 | 16.9 ± 3.6 | 0.020 ± 0.001 | 0.024 ± 0.002 |
| Group II 37 C, 50 mg/kg phenobarbital | 2.92 ± 0.17 | 3.10* ± 0.08 | 4.06 ± 0.20 | 3.66 ± 0.36 | 4.7 ± 0.6 | 11.8 ± 1.6 | 0.014* ± 0.001 | 0.017* ± 0.001 |
| Group III 32 C | 2.88 ± 0.05 | 2.95* ± 0.05 | 5.09* ± 0.23 | 4.88* ± 0.34 | 2.8* ± 0.3 | 3.0* ± 0.3 | 0.014* ± 0.001 | 0.015* ± 0.001 |
| Group IV 32 C, 50 mg/kg phenobarbital | 2.78 ± 0.06 | 2.78 ± 0.06 | 4.85* ± 0.22 | 4.98* ± 0.18 | 2.2* ± 0.3 | 2.2* ± 0.4 | 0.016* ± 0.001 | 0.016* ± 0.002 |
| Group V 27 C | 2.82 ± 0.04 | 2.86* ± 0.04 | 5.05* ± 0.21 | 5.34* ± 0.19 | 1.0* ± 0.1 | 1.4* ± 0.6 | 0.011* ± 0.001 | 0.010* ± 0.001 |

Values are means ± SEM. Six animals in each group.

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

TABLE 3. Series B, Physiologic Variables at the End of the Study

| | Temperature (C) | MABP (torr) | Pa _o ₂ (torr) | Paco ₂ (torr) | pH | Ca _o ₂ (m/100 ml) | Hemoglobin (g/100 ml) |
|--|-----------------|-------------|-------------------------------------|--------------------------|-----------------|---|-----------------------|
| Group I 32 C | 31.7 ± 0.2 | 128 ± 5 | 15.3 ± 0.3 | 28.7 ± 2.0 | 7.18 ± 0.03 | 2.98 ± 0.11 | 15.6 ± 0.6 |
| Group II 32 C, 50 mg/kg phenobarbital | 31.7 ± 0.2 | 117 ± 3 | 15.8 ± 0.2 | 26.9 ± 2.0 | 7.18 ± 0.02 | 2.75 ± 0.20 | 15.2 ± 0.4 |
| Group III 27 C | 27.0 ± 0.1 | 122 ± 2 | 15.2 ± 0.3 | 31.5 ± 1.0 | 7.34 ± 0.03* | 4.67 ± 0.71* | 15.2 ± 0.6 |

Values are means ± SEM. Six animals in each group.
* Significantly different from Group I, *P* < 0.05.

TABLE 4. Series B: ATP, Phosphocreatine, Lactate and NADH Values on Unligated (UL) and Ligated (L) Sides

| | mmol · kg ⁻¹ | | | | | | | |
|--|-------------------------|----------------|-----------------|-----------------|---------------|---------------|-------------------|-------------------|
| | ATP | | PCr | | Lactate | | NADH | |
| | UL | L | UL | L | UL | L | UL | L |
| Group I 32 C | 2.81 ± 0.05 | 2.78 ± 0.05 | 3.48 ± 0.10 | 2.29 ± 0.53 | 8.6 ± 1.6 | 17.7 ± 2.8 | 0.027 ± 0.003 | 0.036 ± 0.004 |
| Group II 32 C, 50 mg/kg phenobarbital | 2.75 ± 0.04 | 2.79 ± 0.07 | 4.34* ± 0.14 | 2.66 ± 0.22 | 5.7 ± 1.9 | 15.1 ± 2.7 | 0.021* ± 0.001 | 0.031 ± 0.002 |
| Group III 27 C | 2.87 ± 0.04 | 2.86 ± 0.03 | 4.68* ± 0.12 | 4.65* ± 0.12 | 1.6* ± 0.2 | 3.4* ± 0.8 | 0.015* ± 0.001 | 0.014* ± 0.001 |

Values are means ± SEM. Six animals in each group.
* Significantly different from Group I, *P* < 0.05.

hemispheres for microfluorometric analyses of ATP, PCr, lactate and NADH.¹² Pa_o₂, Paco₂ and pH values were measured before hypoxia and twice during hypoxia. The values were corrected for temperature differences.¹³ Total arterial oxygen content (Ca_o₂) was measured at the same times according to the method of Fabel and Lübbers.^{14,15}

SERIES B

This series consisted of three groups with six rats in each. All three groups had body temperatures lowered, Groups I and II to 32 C and Group III to 27 C. Rats in Group II in addition received injections of phenobarbital, 50 mg/kg. Apart from a decrease of the inspired oxygen content to give a Pa_o₂ of 15 torr, all the other procedures and measurements were the same as in Series A.

Statistical differences were tested with Student's *t* test. *P* 0.05 was regarded as significant.

Results

In Series A, there was no significant difference in physiologic variables between Groups I and II (table 1). All hypothermic groups had higher pH and arterial oxygen content values compared with the two normothermic groups. Mean arterial pressure was higher

in Group V than in the other groups. Paco₂, initially close to 35 torr, decreased in all groups during hypoxia, but less in Group V than in the other groups. Data for Group VI are omitted since severe hypotension and arrhythmias developed in all animals and they died within 5 minutes of the onset of hypoxia. Pa_o₂ measured shortly before death was more than 25 torr in the animals of Group VI. Compared with Group I, all other groups had higher ATP values on the ligated side and significantly lower levels of NADH on both ligated and unligated sides (table 2). In Group I there were significant side-to-side differences, with a lower PCr value and a higher lactate value on the ligated side. Group II had a side-to-side difference in only lactate values, which were higher on the ligated side. In the hypothermic groups there was no side-to-side difference. There was no significant difference between Group III and Group IV. When Groups III and IV were compared with Group V, the latter had slightly lower lactate and NADH values bilaterally.

In Series B, the only significant intragroup physiologic differences were in Group III, which had higher pH and total oxygen content values than the other groups (table 3). Both Group I and Group II had side-to-side differences in biochemical values, with lower PCr and higher lactate values on the ligated side (table 4). Group II had a higher PCr value on the unligated

side than Group I. In Group III there was a higher lactate level on the ligated side. PCr was higher and lactate and NADH values were lower in Group 3 than in the other two groups bilaterally.

Discussion

We chose the Levine model¹⁶ with one-sided carotid ligation as a model for studying cerebral protection during hypoxia because the extent of hypoxia needed to damage the brain is otherwise so large that the heart is affected, causing arrhythmias and a decrease in arterial blood pressure. Previous studies with the same model, also in rats, have shown that acute clamping of one carotid artery decreases cerebral blood flow by less than 10 per cent on the clamped side. This reduction does not cause any difference in PCr, ATP, or lactate levels between the clamped and the unclamped sides. Furthermore, during hypoxia cerebral blood flow increases two times on the clamped side and five times on the unclamped side.¹⁷ Thus, provided the blood pressure is maintained, one-sided carotid clamping does not cause ischemia, but it reduces the compensatory increase of CBF during hypoxia. Another study by the same investigators, using the same model, showed that in hypoxia, the metabolic changes on the ligated side were similar to, but more pronounced than, those on the unligated side.¹⁸ Other studies of hypoxia have shown that PCr values decrease before ATP values, and that a decrease in PCr and an increase in lactate values are the first signs of metabolic alterations in hypoxia.^{9,10}

Previous studies in the Wistar rat showed that phenobarbital, 50 mg/kg, decreased CMR_{O_2} by 25 per cent and 150 mg/kg, by about 40 per cent.^{7,19} Hypothermia depresses CMR_{O_2} by 5 per cent per degree decrease in temperature.⁶ The combination of 32 C and phenobarbital, 50 mg/kg, decreased CMR_{O_2} by about 45 per cent.⁷ Changes in CBF are of the same magnitude. We consider these measurements valid also for the present study, as one-sided carotid clamping has only minimal effects on CBF and no detectable influence on energy metabolism in normoxia. In the present experiments we therefore had decreases of approximately 25 per cent in CBF and CMR_{O_2} in Series A, Groups II and III, and Series B, Group I, and 40–45 per cent decreases in CBF and CMR_{O_2} in Series A, Groups IV, V, and VI, and Series B, Groups II and III.

The results in Series A showed that phenobarbital, 50 mg/kg, offered less protection to the brain during hypoxia than hypothermia to 32 C. The biochemical changes in the barbiturate-treated group were very similar to those of the control group. Hypothermia to 32 C, however, seems to have been almost fully protective, with normal levels of energy metabolites

in the brain tissue. Mean arterial blood pressure was slightly higher in the hypothermic animals. They also had normal pH and higher total oxygen content values in arterial blood, although Pa_{O_2} and hemoglobin concentration values did not differ from those in the other groups. This finding is in agreement with the findings of Carlsson *et al.*² who studied Wistar rats exposed to hypoxia during hypothermia, and found that hypothermic animals had less acidosis and a higher oxygen content in arterial blood than normothermic, hypoxic animals. They concluded that hypothermia protects not only by decreasing blood flow and metabolic rate, but also through a temperature effect on arterial oxygen content.

The low lactate levels of Group III in Series A made it difficult to evaluate whether the addition of barbiturate to hypothermia to 32 C (Group IV) resulted in metabolic protection. Thus, in Series B the inspired oxygen concentration was decreased further. There were only minor biochemical differences between Groups I and II, indicating that the addition of phenobarbital, 50 mg/kg, offered only slight protection during hypoxia. At 27 C, pH and arterial oxygen content values were higher than in the other groups and, with the exception of an elevated lactate value on the ligated side, there was no metabolic sign of hypoxia.

Decreases in CBF and CMR_{O_2} by barbiturates seem to play a relatively small protective role in hypoxia, as the difference between Groups I and II in Series B was very slight despite the fact that the latter group had CBF and CMR_{O_2} values that were further decreased by 20–25 per cent by the addition of phenobarbital, 50 mg/kg. As judged by metabolic criteria, hypothermia offers better cerebral protection from hypoxia than does a dose of phenobarbital that gives the same decreases in CBF and CMR_{O_2} . The hypothermic rats had less acidosis and higher arterial oxygen contents. Our data do not allow us to conclude whether these are the only important differences between hypothermia and barbiturates for protection in cerebral hypoxia.

All the rats in the group receiving phenobarbital, 150 mg/kg, died when the inspired oxygen concentration was decreased. Other investigators have found that large doses of barbiturates cause severe circulatory side effects in hypoxia and ischemia,³ which may decrease the clinical usefulness of barbiturates for cerebral protection in ischemia and hypoxia.

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