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Title : ATP PROTECTION IN LETHAL HYPOXIA I: PERIPHERAL INJECTION

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**Introduction.** High energy phosphate metabolism of the brain is not profoundly altered until hypoxemia is severe. In fact, only when arterial oxygen tension is reduced below 20 TORR and hypotension occurs, are changes in cerebral tissue concentrations of adenine nucleotides observed.<sup>1</sup> In view of these considerations, we deemed it important to study the effects of exogenously administered adenine nucleotides in mice exposed to lethal hypoxia.

**Methods.** Male Swiss white mice (25-30g) were used. Control mice were injected intraperitoneally (IP) with 0.9% saline and treatment groups received an IP injection (200 mg/kg) of one of the following drugs: 1) adenosine triphosphate (ATP), 2) adenosine diphosphate (ADP), 3) adenosine monophosphate (AMP), 4) adenosine, 5) adenine phosphate, 6) dibutyl cyclic AMP (db CAMP) and 7) phosphocreatine (CRP). Thirty minutes later, the mice were placed in one of two one-liter airtight glass chambers (2 mice per chamber). A continuous flow at 7 liters/minute of a mixture of 5% oxygen-95% nitrogen was maintained and monitored with a Beckman OM-11 oxygen analyzer. Temperature inside the chambers was maintained between 22.0°-24.0° C. The interval between the introduction of the hypoxic mixture to the chambers and the last respiratory effort was measured for every animal and defined as the survival time (ST). The observation period was arbitrarily truncated at thirty minutes.

**Results.** In all experiments, the oxygen concentration fell to  $5.0 \pm 0.1\%$  within 75 seconds. The mean value of survival time  $\pm$  standard error of the mean (SEM) for saline treated mice was  $2.56 \pm 0.17$  minutes. These mice lost the righting reflex (LRR) within two minutes, rapidly followed by tonic-clonic seizure activity, opisthotonus, urination and respiratory arrest. No untreated mouse survived beyond 4.2 minutes. The mean ST  $\pm$  SEM for ATP, ADP and AMP treated mice were  $22.41 \pm 1.75$ ,  $15.13 \pm 2.48$  and  $13.81 \pm 2.20$  minutes, respectively. (Statistically significant difference for treatment groups from saline treatment ST at 0.0001 level; statistically significant difference of ATP from ADP and AMP groups.) ST was increased 775, 491, and 439% respectively. Treated mice did not LRR until the last 2-3 minutes of life, and exhibited reduced motor activity during the hypoxic period. Ten of 21 ATP treated mice survived the 30 minute exposure; similar findings were observed in ADP and AMP treated mice. No neurologic defects were observed in these mice; some of which survived up to 6 hours hypoxic exposure. Prior

to hypoxic exposure, the mice treated with ATP, ADP and AMP appeared quiet and behaviorally less active than saline treated mice. The other agents tested were ineffective.

**Discussion.** We have demonstrated that treatment with the ATP significantly increases ST in mice during an otherwise lethal hypoxic insult. Moreover, ADP and AMP were ineffective. Several possibilities exist which may underline the protective effects of the nucleotides against hypoxia. During hypoxia, the energy state of the brain is upheld by increases in cerebral blood flow, which may increase four fold.<sup>2</sup> It appears unlikely that ATP increases resistance to hypoxia via a peripheral cardio-vascular effect, since ATP is a recognized cardio-vascular depressant. It is notable that ATP administered systemically has effects beyond the blood-brain barrier.<sup>3</sup> Our finding of sedation in mice treated IP with ATP is similar to effects observed when ATP is centrally administered, and is further evidence suggesting that a direct action within the brain is most likely responsible for the protective effect. Increased ST in mice treated with barbiturates and exposed to 5% oxygen has been demonstrated. ST increased between 158%<sup>4</sup> and 303%<sup>5</sup>, while in this study, ATP increased ST 775%. Unlike barbiturate protection, the protective effect of ATP is completely independent of anesthetic condition. Of further interest are the findings that IP ATP increased ST in mice exposed to lethal concentration of carbon monoxide but not cyanide. Further studies are necessary to determine the physiological mechanism by which ATP functions.

#### References.

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