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Operant leverpressing and wheelrunning were differentially reduced by PAPP (*p*-aminopropiophenone)-induced methemoglobinemia☆

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Abstract

Cyanide is a potent toxin that binds to cytochrome oxidase blocking electron transfer and the synthesis of adenosine triphosphate (ATP). Many antidotes to cyanide poisoning oxidize hemoglobin to methemoglobin (metHb), which serves as a scavenger of the cyanide anion. However, sufficiently high levels of metHb can be toxic because metHb cannot bind O_2 until it is reduced. The purpose of the proposed study was twofold: (1) Characterize the time course of metHb formation for different doses of *p*-aminopropiophenone (PAPP), a drug that oxidizes hemoglobin and can be used as an antidote to cyanide intoxication; and (2) Determine whether the effort of an operant response affects the behavioral toxicity of metHb, since more effortful responses presumably are more energetically demanding. In Experiment I, the oral metHb kinetics of *p*-aminopropiophenone (PAPP) were studied; four doses of PAPP (1, 5, 10, and 20 mg/kg) or the vehicle, polyethylene glycol 200 (PEG200), were delivered via a gavage tube to separate groups of rats. In Experiment II, rats were trained to press a lever or run in an activity wheel at any time during a 12-hour light/dark cycle for their entire daily food intake; five presses or turns were required for the delivery of each food pellet. The same doses of PAPP were delivered p.o. shortly before the onset of darkness, 2100 h. Results from Exp I showed that PAPP induced a dose-dependent rapid increase and relatively slower exponential-like decline in metHb concentration. In Exp. II, the same doses of PAPP induced a dose-dependent reduction in hourly outputs of leverpresses and wheelturns however; wheelturns were reduced significantly more than leverpresses. When the best-fitting metHb curves from Experiment I were superimposed on the time scale for outputs of wheelturns and leverpresses, reduction of output was inversely related to the kinetics of metHb formation. These findings are consistent with the conclusion that PAPP-induced metHb formation reduced the output of wheelrunning more than leverpressing because the more energetically demanding response of wheelrunning was more affected by metHb induced hypoxemia. Furthermore, these data suggest that although certain longacting metHb formers might be useful prophylactics for warfighters, it will be critical to determine the energetic loads of required battlefield activities because even low (10%) therapeutic metHb levels might impair the performance of those activities.

Keywords: Cyanide; *p*-aminopropiophenone; Methemoglobin; Energy metabolism; Operant behaviour

1. Introduction

Cyanide is used in the metal trades, mining, electroplating, jewelry manufacture, and X-ray film recovery, and has been used as a weapon by individuals and nations (Baskin and Brewer, 1989). The principal molecular mechanism of cyanide toxicity is well documented (Ballantyne, 1987). When ingested, inhaled, or absorbed through the skin, the cyanide anion (CN^-) penetrates the outer mitochondrial membrane and binds to cytochrome oxidase, the terminal enzyme complex in the electron transport chain. In so doing, CN^- blocks the transfer of electrons to O_2 and, as a consequence, the movement of hydrogen ions (H^-) across the inner membrane, resulting in a greatly reduced synthesis of adenosine triphosphate (ATP) and compensatory anaerobic glycolysis that results in lactic acidosis. The CN^--induced reduction or elimination of the H^- gradient also promotes cell death by opening the mitochondrial transition pore sufficiently to permit the release

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of intermembrane and matrix proteins [cytochrome c (CytC), apoptosis inducing factor (AIF) and endonuclease G (endoG)] to the cytosol, where these proteins contribute to caspase dependent and independent cell death (Stavroskaya and Kristal, 2005; van Gurp et al., 2003).

Almost all therapies for cyanide poisoning require the delivery of an antidote that oxidizes hemoglobin to methemoglobin (metHb), which binds the CN^- with high affinity. One such potential antidote is *p*-amino-propiophenone, the parent compound in a class of compounds that induces a relatively rapid, long-lasting methemoglobinemia. The methemoglobin kinetics of PAPP are well characterized (Bright and Marrs, 1986a,b; Marrs and Bright, 1986; Marino et al., 1997). When PAPP was delivered intravenously to beagle bitches (Bright and Marrs, 1986a), percent (g/deciliter) metHb increased rapidly to a maximum, beyond which metHb decreased very slowly over a period of hours to the preinjection level. Similarly, the oral delivery of PAPP results in a relatively rapid increase of percent metHb that decays slowly.

The principal purpose of the present experiments was to determine whether the behavioral toxicity of PAPP-induced methemoglobinemia is affected by energetic demands of an operant response. The principal objectives of the proposed experiments were to: (1) evaluate the methemoglobin kinetics of PAPP and (2) evaluate whether the temporal course of metHb formation and peak metHb generated by different doses of PAPP differentially affect the behavioral output of rats that are either required to execute repeatedly an energetically costly or minimally costly instrumental response for their entire daily food intake. In an effort to satisfy the second objective, rats were required to repeatedly execute 5 leverpresses or 5 wheelturns for the delivery of each food pellet. Since no food is available other than what as delivered for executing leverpresses or wheelturns, an adult rat who eats an average of 500 to 600 pellets/day, the 5:1 ratio required a 24 hr output of approximately 2500 to 3000 leverpresses or wheelturns. If these responses are energetically different; i.e. five wheelturns/pellet costs more in terms of O_2 consumption than 5 leverpresses, then a sufficiently high methemoglobinemia will reduce food intake more for the groups that must execute wheelturns than for the groups that must execute leverpresses for food, especially since the density of mitochondria and corresponding O_2 demands of skeletal muscle (as well as heart, liver and brain tissue) are relatively high.

2. Experiment I: metHb kinetics of PAPP

2.1. Methods and materials

2.1.1. Animals and housing

Thirty-six adult male Harlan Sprague–Dawley rats were used. The weights of all rats at the beginning of this study were between 300 and 350 g. All rats had free access to food and water and were housed in individual Lucite cages that were maintained in a temperature controlled room and were used in accordance with an experimental protocol that was approved by the Walter Reed Army Institute of Research Institute Animal Care and Use Committee (WRAIR IACUC). All experiments were conducted in laboratories that were approved by the American Association for Accreditation of Laboratory Animal Care.

2.1.2. Dosing and the measurement of methemoglobin

Four doses of PAPP (1, 5, 10, and 20 mg/kg) were used. Each dose was dissolved in polyethylene glycol 200 (PEG200), the vehicle, and new drug solutions were prepared daily. Between 10 and 10:15 A.M., PAPP or PEG200 was delivered at an injection volume of 1 ml/kg via a gavage tube inserted into the rat's stomach. Separate groups of rats were gavaged with 1, 5, or 10 mg/kg of PAPP or PEG200 (8 rats/dose) and 4 rats were gavaged with the 20 mg/kg dose. The doses of PAPP were used in the following irregular order: 20, 5, 1, 10 mg/kg. The vehicle was used last.

At various times after the delivery of each dose of PAPP, a modified 26 ga butterfly needle and attached catheter were used to remove 30–35 μl blood samples from the lateral tail veins of individual rats. This was accomplished in the following way. For each blood sample, the catheter for a butterfly needle was cut near the base of the needle and a hematocrit tube was inserted into the remaining piece of catheter. Each tail was immersed in warm water for 30 to 60 s to dilate the veins and, beginning at the distal end of the tail, the needle was inserted into either the left or right tail vein and a sample was removed. Once the needle penetrated the vein and blood flowed into the hematocrit tube, the tube was immediately detached, the sample was aspirated into an OSM3® Hemoximeter (Radiometer, Copenhagen), analyzed for metHb and pressure was applied with a 2×2 gauze pad to the location where the needle penetrated the tail vein.

The goal of this experiment was to collect blood samples that would be sufficient in number to completely characterize the metHb kinetics, i.e. the increase and decrease of metHb, at each dose. The number of blood samples at each dose was determined by the time between samples, which in turn was determined by the rates of oxidation and reduction of hemoglobin at each dose. Since PAPP oxidized hemoglobin relatively rapidly, the first few samples could be taken at approximately the same time after the delivery of each dose. However, since the decrease of metHb was more prolonged at larger doses and the number of samples that could be effectively and safely removed from a rat's tail was limited, we needed to spread out the sampling time for this segment of the metHb curve. At a dose of 1 mg/kg, 7 samples were sufficient to completely characterize the metHb kinetics of PAPP however, 12 to 13 samples were necessary at all larger doses.

2.1.3. Data analysis

A SAS® combined between and within group repeated measures analysis of variance (ANOVA) was used to compare mean metHb concentrations of the different groups at 0, 20, 30, 45, 70 min after the delivery of PAPP. If the p value of the F score for the interaction of Group X Sample Time was less than or equal to 0.05, Tukey's test was used to determine the specific pair(s) of mean metHb values that differed significantly.