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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.

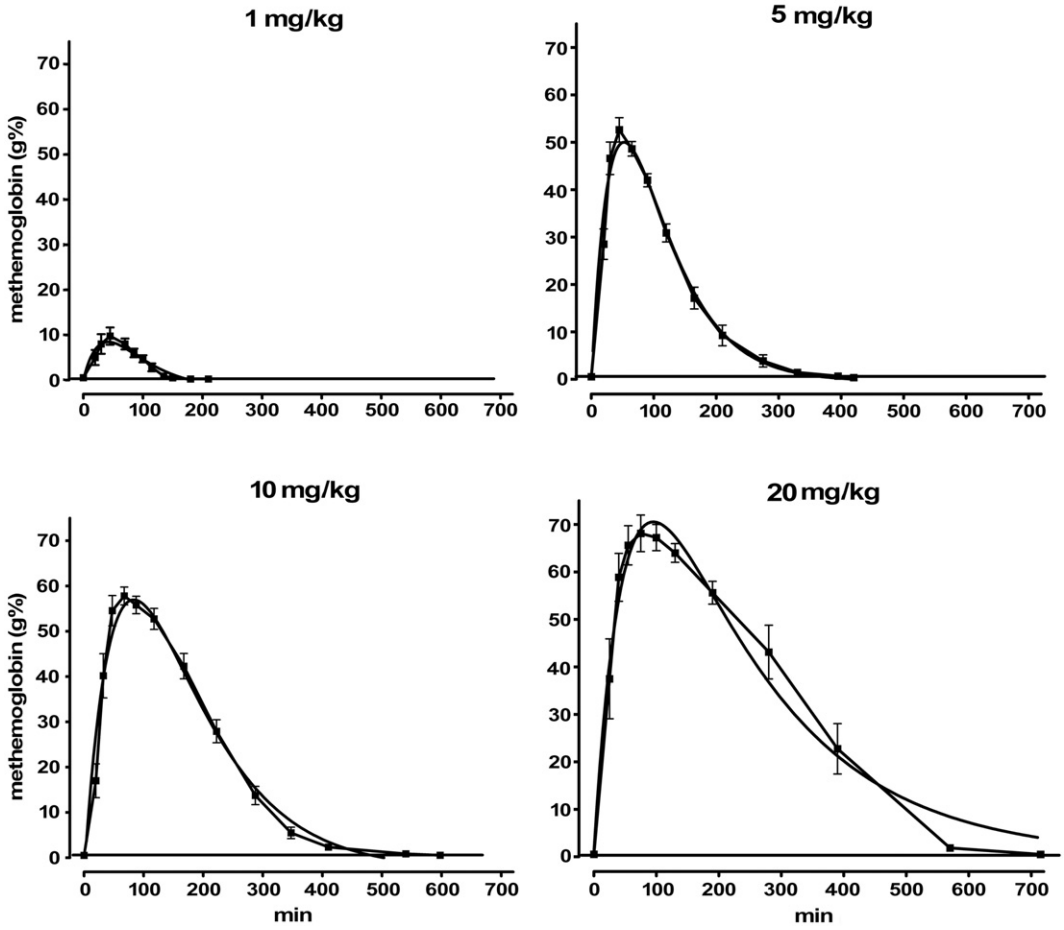

Fig. 1. Methemoglobin (±SEM) is shown at different times after the delivery of 1, 5 10, or 20 mg of PAPP. Each dose was delivered to a separate group. The smooth black functions are best-fitting curves to each configuration of metHb concentrations. (See text for further explanation).

In a second analysis, Rstrip™ an exponential stripping and curve fitting program was used to identify the best-fitting curve for the mean metHb values at each dose of PAPP. Statistics provided by Rstrip™ were used to describe the kinetics of metHb formation.

3. Results

Fig. 1 shows the mean obtained metHb values (±SEM) at different times after the delivery of each dose of PAPP. The horizontal line above the *x*-axis represents mean metHb [0.53 g/dl (g%)] for the PEG200 group. Mean metHb was significantly different among groups ($F=87.9$, $p<0.0001$) and was significantly different among groups at different times after the delivery of PAPP ($F=65.2$, $p<0.0001$). Comparisons among means before the delivery of PAPP did not reveal any significant difference among group mean metHb concentrations. However, at 20 min after the delivery of PAPP, mean metHb increased for all groups and differed significantly ($p<0.05$) among groups. Mean metHb for the 5, 10, and 20 mg/kg groups was significantly larger than mean metHb for the Sham group, mean metHb for the 5, 10, and 20 mg/kg was significantly larger than for the 1 mg/kg; and mean metHb for the 20 mg/kg group was significantly larger than for the 10 mg/kg and 1 mg/kg. At 30 and 45 min after PAPP, mean metHb continued increasing and differed significantly ($p<0.05$) among groups. At these times, mean metHb for the 1, 10, and 20 mg/kg groups was significantly greater than for the Sham group, mean metHb for the 5, 10, and 20 mg/kg doses was significantly greater than mean metHb for the 1 mg dose, and mean metHb for the 20 mg/kg group was greater than mean metHb for the 1 and 10 mg/kg groups. At 75 min after the delivery of PAPP, mean metHb differed significantly among all groups. Mean metHb appeared to peak for the 10 and 20 mg/kg groups and was near peak, but decreasing, for the 1 and 5 mg/kg groups.

Table 1
Parameters of least square fits*

Dose	$metHb_{max}$	AUC
1 mg/kg	11 g%	850
5 mg/kg	51 g%	7002
10 mg/kg	58 g%	12,653
20 mg/kg	70 g%	22,660

*Maximum metHb ($metHb_{max}$,) and area under the curve (AUC) resulting from nonlinear least square fits of Equation1 to the metHb concentrations shown in Fig. 1.

The smooth curves in Fig. 1 resulted from using Rstrip™ to identify the best fitting curve for the mean metHb concentrations at each dose of PAPP. For every dose of PAPP, Rstrip™ identified a biexponential function (Eq.(1)) that accounted for between 0.96 and 0.98% of the total variance. Table 1 shows the maximum predicted metHb concentration, $metHb_{max}$, and the area under the curve (AUC), a measure of the bioavailability of metHb, of each fitted function. As the dose of PAPP increased, $metHb_{max}$ and AUC increased.

$$C(t) = A(1)\cdot e^{-K(1)\cdot t} + A(2)\cdot e^{-K(2)\cdot t} \quad (1)$$

[$C(t)$ is the concentration of metHb at time t, $A(1)$ and $A(2)$ are coefficients that define metHb concentration at $t=0$, and $K(1)$ and $K(2)$ are the slopes that describe the rates of oxidation and reduction of Hb].

In what follows, the best-fitting curves were used to evaluate the relation between the kinetics of metHb formation and the changes in response output at the different doses of PAPP.

4. Experiment II: kinetics of methemoglobin formation and response output

4.1. Methods and materials

4.1.1. Animals

Sixty male Harlan Sprague–Dawley rats were used. At the beginning of this experiment, the weights of all rats were between 300 and 350 gm.

4.1.2. Housing and equipment

Rats were confined 24 h/day to individual rodent test cages (23.5 cm wide × 30 cm long × 29 cm high). Individual cages were hung over one side of a stainless steel wall that supported a running wheel (11 cm wide × 36 cm in diameter) that could be accessed through a rectangular opening along the left side wall of each test cage; a red cuelight protruded into each running wheel. A small lightweight lever protruded through the left front wall of each test cage and a recessed food trough was adjacent to the lever. Each trough was connected to a pellet dispenser that was located at the back of the front wall. A tube protruded through the ceiling of each test cage and permitted each rat free access to water 24 h/day.

An environmental chamber was used to house 12 test cages. Outside of this chamber, a computer interface was tethered to the running wheel, lever, and pellet dispenser of each test cage and also was linked to a PDP® 11/73 (Digital Equipment Corporation, MA) microcomputer that used SKED11® software to control the 12 hour light/dark cycle of the overhead lights within the chamber, pellet deliveries, and record all leverpresses and wheelturns. The overhead lights within the chamber were on between 0900 and 2100 h and off the remaining 12 h.

4.1.3. Training

The doses of PAPP used in Experiment 2 were identical to those that were used in Experiment 1 (1, 5, 10, or 20 mg/kg or PEG 200). For each dose of PAPP or PEG200, 12 rats were injected with the same dose of PAPP or vehicle however, 6 of these rats were required to leverpress for food and the remaining 6 were required to run in an activity wheel for food. The 4-stage procedure that was used to train each group of 12 rats is described below.

During Stage 1 of training, all 12 rats were first trained to eat from the recessed food trough and press the lever for the delivery of single 45 mg Noyes Formula A® pellets that consisted of a balanced mixture of the major macronutrients, vitamins, and minerals. Initially, access to the wheels was blocked and a single leverpress resulted in the delivery of a single food pellet at any time during the light or dark period. During Stage 1, each the lever was baited daily with peanut butter and both the lever and trough were baited with pellets between 0900 and 1000 h. Most rats learned to press within 2 to 3 days however, if a rat did not learn, it was trained to leverpress by the method of successive approximations. Within 4 days after the levers were first baited, all rats learned to leverpress repeatedly for their entire daily intake of food pellets. The nominal cost of a food pellet remained fixed at a ratio of 1 press per pellet (FR1) for 1 week, which was the duration of Stage 1.

During Stage 2, 6 of the 12 rats were assigned to the Lever group and the remaining 6 were assigned to the Wheel group. The entrance to the wheel was unblocked for the rats in the Wheel group and wheelrunning was allowed to stabilize for 10 additional days. Previously published data have shown that between one and two weeks of adaptation are necessary for food intake and wheelrunning to reach an equilibrium (Bauman, 1992). During Stage 2, the ratio of presses/pellet remained at FR1.

Stage 3 began immediately after Stage 2. During Stage 3, the training program was changed for rats in the Wheel group so that leverpresses no longer activated the pellet dispenser. Instead, a single leverpress by a rat in the Wheel group turned on the cuelight within the wheel and a single rotation of the running wheel produced a pellet and turned off the cuelight. After a rat had collected and eaten a pellet, it could choose to eat more by executing an additional leverpress and a wheel-turn, it could simply choose to run in the activity wheel, or neither eat nor run. In the absence of the cuelight however, wheelturns would not result in the delivery of food pellets and these wheel turns were recorded separately from those that occurred while the cuelight was on. This leverpress–cuelight contingency was maintained for five days. In a previous experiment (Bauman et al., 1997), five days were sufficient for the rats in the Wheel group to learn that a food pellet was delivered only after a complete wheelturn.

During Stage 4, the ratio of turns per pellet for the rats in the Wheel group and the Lever group were increased from 1:1 to 5:1. This ratio discouraged hoarding and presumably increased the disparity between the energetic cost of leverpressing and wheel-running for food. Food intake and wheel-running were allowed to stabilize for an additional two weeks, at the end of which time daily food intake was not consistently increasing or decreasing.

4.1.4. Experimental design and the delivery of PAPP

Four groups of rats were used to evaluate the effect of 4 different doses (1, 5, 10, and 20 mg/kg) of PAPP on leverpressing

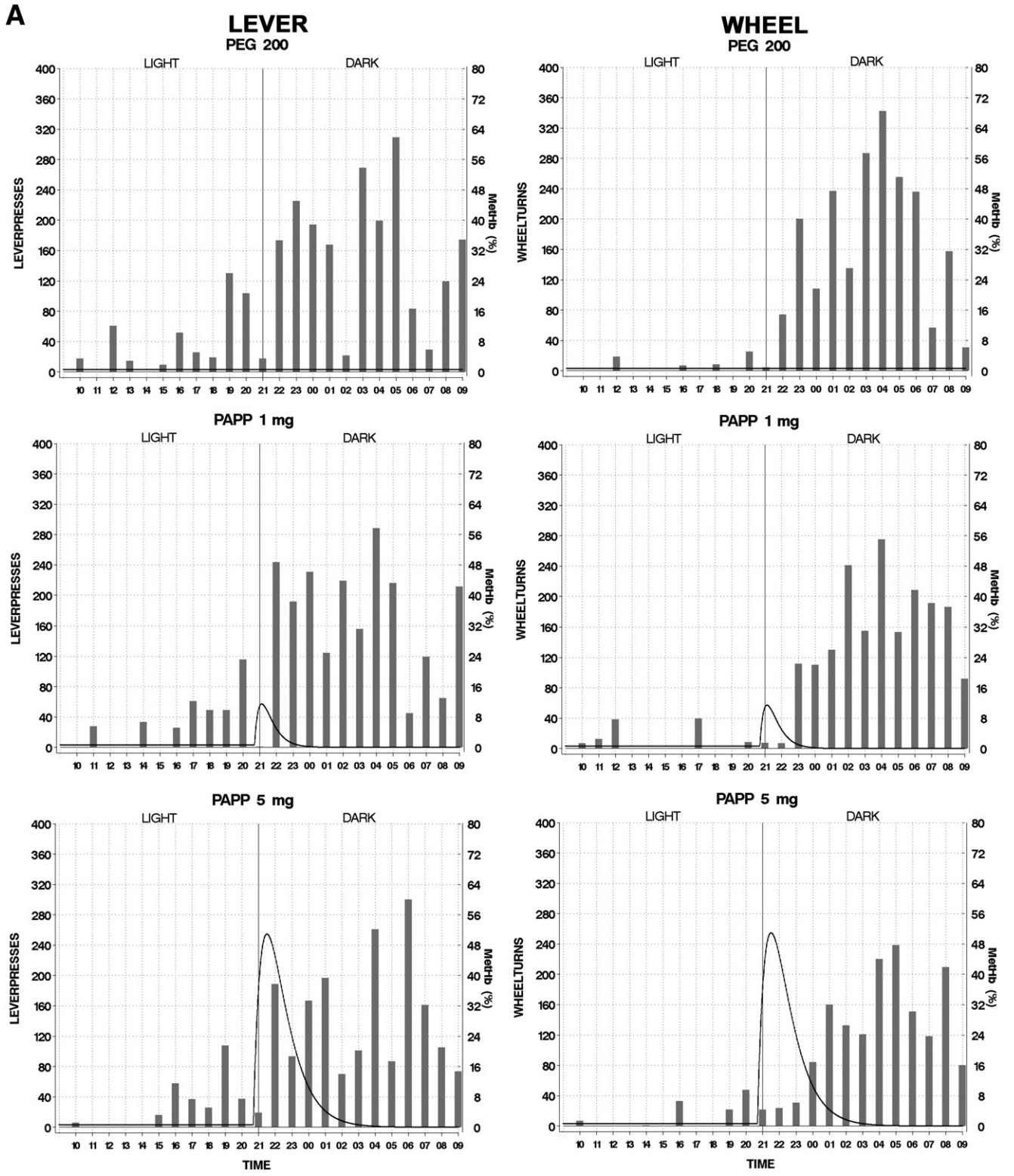

Fig. 2. The left *y*-axis is the scale for the bras that represent mean hourly output of leverpresses and wheelturns during the light and dark periods after 1, 5, 10, or 20 mg of PAPP or after PEG 200. The *x*-axis is scaled in 24 h time beginning at 0900 h, the time of light onset, and ending at 2100 h, the time of light offset. The right *y*-axis is the scaled as percent metHb and the solid black lines in each plot are the fitted metHb curves for each dose of PAPP or PEG200. Each curve begins between 2030 and 2100 h, the time period within which PAPP was injected.

and wheelrunning. One of four doses of PAPP (1, 5, 10, and 20 mg/kg) or PEG200 was delivered to a separate group of rats via a gastric gavage tube between 2030 and 2100 h, the onset of darkness; the PAPP was prepared immediately before each dosing. For any dose of PAPP, the 6 rats in the wheel group and the 6 rats in the lever group were gavaged with the same dose. The

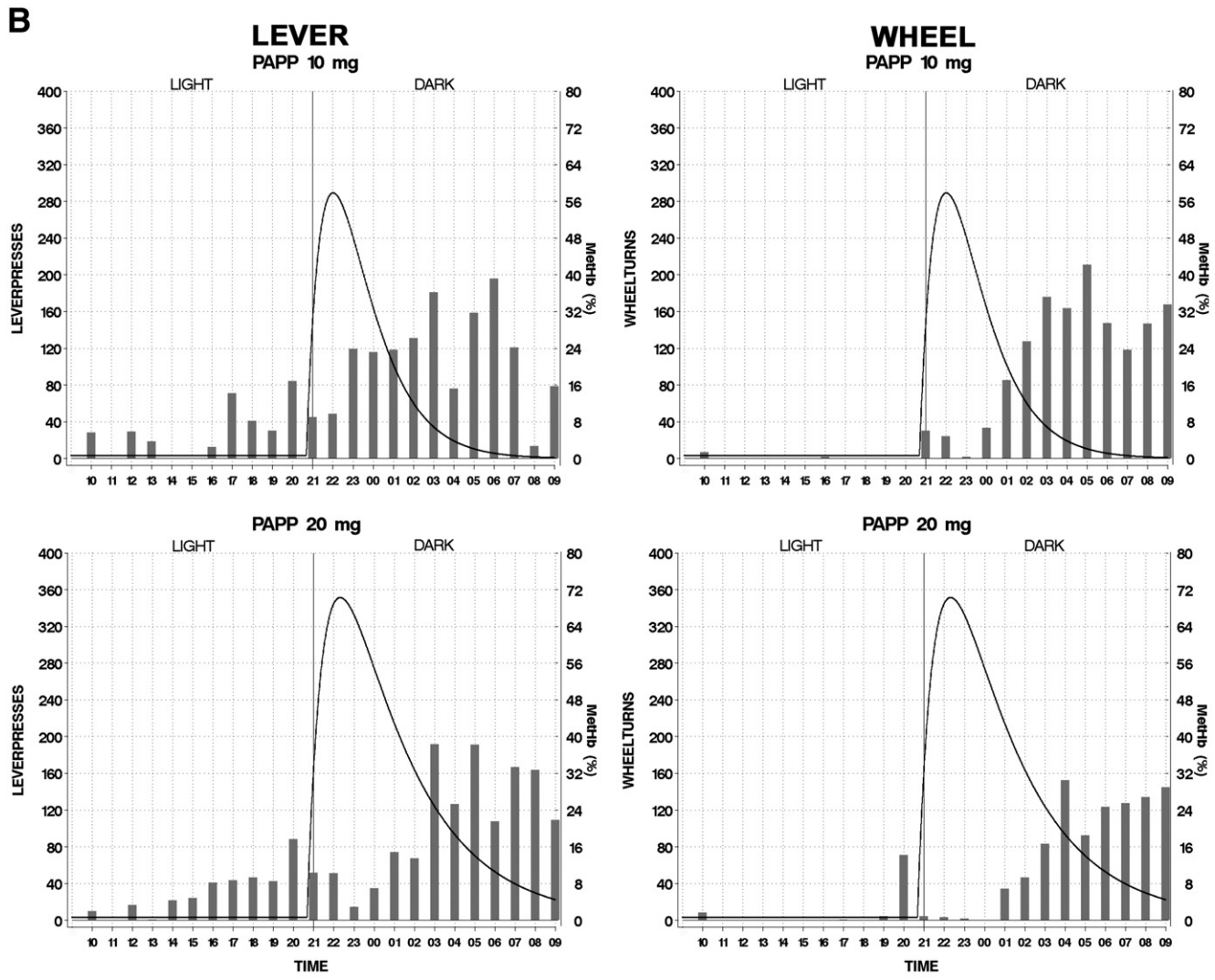

Fig. 2 (*continued*).

chosen doses of PAPP and PEG200 were delivered in an irregular order (5, 1, PEG200, 20, and 10 mg/kg).

Leverpressing, wheelrunning and food intake were recorded in 60-minute blocks of time for the 12 h after the delivery of either PAPP or PEG 200. This 12-hour period was chosen because existing data suggested that, for all doses of PAPP but the largest (20 mg/kg), PAPP and its active metabolite PHAPP (*p*-hydroxyaminopropiophenone) would be cleared totally 12 h after it was delivered (Bright, 1987). Since 12 cages were available and the four doses of PAPP and PEG200 were delivered to separate groups of rats in lever and wheel treatment conditions, it was necessary to replicate the same experiment 5 times to yield an *n* of 6 rats per group for the lever and wheel conditions at each dose of PAPP and PEG200.

4.1.5. Data analysis

A SAS® general linear models analysis of variance with a repeated measures option was used to evaluate the effect of dose of PAPP on response output during the dark period (Time × PAPP dose interaction) and the interaction of PAPP dose and output of wheelturns and leverpresses in the dark (PAPP dose × Time × Response interaction). An F-score was considered to be statistically significant if its *p* value was 0.05 or less.

In addition, the best-fitting curve at each dose from Experiment I was used to characterize the relation between the metHb kinetics of PAPP and the food intake and instrumental output of the Lever and Wheel groups.

5. Results

In Fig. 2, the mean number of leverpresses and wheelturns for each hour of the light and dark periods are plotted for each dose of PAPP. Equivalent drug treatments for the lever and wheel groups are displayed in adjacent panels within a row while the response treatments (lever versus wheel) are displayed within columns. (It should be noted that the food intake graphs are not shown because food intake for any given hour is simply 1/5 of the total leverpresses for that hour.) Within each graph, the left *y*-axis is for leverpresses while the scale along the right y-axis is for metHb (g%). The flat line above the *x*-axis within

the PEG 200 plots represents the mean metHb (0.53 g%) in the absence of PAPP. The smooth black curves in each plot are the best fitting curves to the mean metHb concentrations shown in Fig. 1. Each of these curves begins between 2030 and 2100 h, which is the time period within which the doses of PAPP were delivered.

In general, Fig. 2 show that the hourly response output for rats in the Wheel groups (right column) was more restricted to the dark period than the response output for the rats in the Lever groups (Time × Response, $F=3.31$ $p<0.0001$). For example, in the top two panels of Fig. 2 (PEG200 treatment), the rats in the Lever group press significantly more in the light period than the rats in the Wheel group run. Rats in the Wheel group restrict their wheelrunning for food almost entirely to the dark period. This same effect is also evident for each of the doses of PAPP.

The plots for the Lever and Wheel groups in Fig. 2 also clearly show that the delivery of PAPP results in a dose dependent reduction of response output in the dark period ((PAPP dose × Time, $F=2.40$, $p<0.0001$), which would be expected since PAPP was delivered shortly before the onset of darkness. The reduction of output for the Lever and Wheel groups is most evident during the first 6 or so hours of darkness. During this time, wheelrunning and leverpressing are increasingly reduced as the dose of PAPP increases.

Table 1 showed that the maximum metHb concentration ($metHb_{max}$) and the area under the metHb curve (AUC), a measure of the bioavailability of metHb, increased as the dose of PAPP increased. Consequently, when the fitted metHb curves from Fig. 1 are superimposed on the *x*-axis in Fig. 2 such that the beginning of each curve is fixed at the time when rats were gavaged with PAPP (2030–2100 h), there appears to be an inverse relation between $metHb_{max}$ or AUC and hourly response output. As metHb increases, response output for leverpresses and wheelturns decrease and as metHb decreases, response output recovers. However, a comparison of the left and right columns in Fig. 2 shows that the PAPP-induced reduction of response output was greater for the Wheel group than for the Lever group (Time × PAPP dose × Response, $F=1.31$, $p=0.0296$). For all doses of PAPP, the output of leverpresses during the first two hours of darkness was reduced relatively little compared to the reduction of wheelturns during the same period. This difference in output reduction is larger for the 10 mg/kg and 20 mg/kg doses, both of which catastrophically suppressed the output of wheelturns for the first 5–7 hours of darkness. In particular, rats executed many more leverpresses than wheelturns during the first five hours of darkness at the 10 mg/kg dose and the first 7 hours at the 20 mg/kg dose. This differential effect of PAPP on response output implies that although the same doses of PAPP were delivered to the Lever and Wheel groups, increasingly larger AUC (bioavailability of metHb) is correlated with significantly greater response output reductions in the Wheel group than in the Lever group.

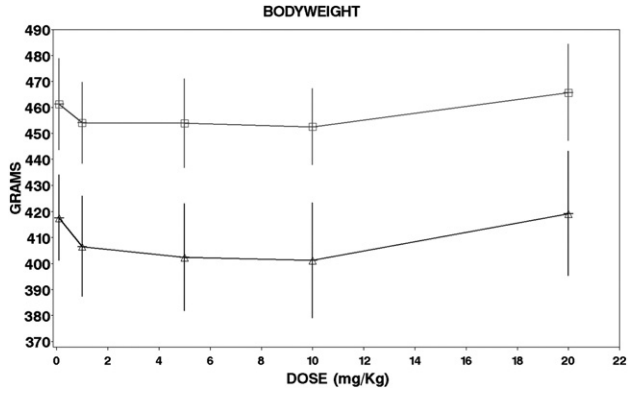

Fig. 3. Mean bodyweight (±SEM) at each dose of PAPP or PEG200.

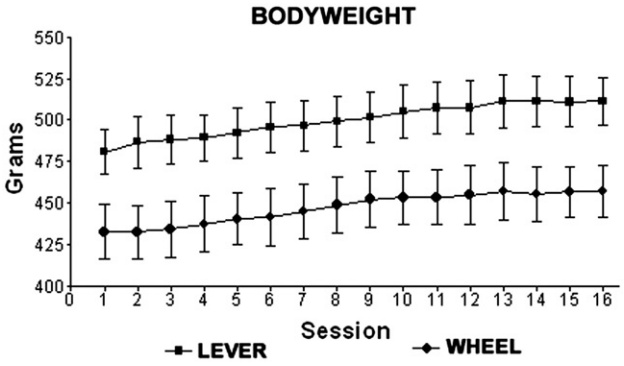

Fig. 4. Mean bodyweight during a 16 day adaptation period for rats that were required to execute 5 leverpresses for each pellet of food (squares) and for rats that were required to execute five wheelturns for each pellet of food (circles).

Fig. 3 shows mean bodyweights (±SEM) for the Lever and Wheel groups at each dose of PAPP. The mean bodyweight of the Lever groups was significantly less than the mean bodyweight for the Wheel groups ($F=16.3$, $p=.0002$) however, there was no statistically significant effect of dose of PAPP ($F=.28$, $p=0.89$) nor was there an interaction of group and dose ($F=0.02$, $p=0.99$).

Fig. 4 shows the mean bodyweights (±SEM) of Lever and Wheel groups over time. The rats in these groups were not used in Experiment 2 however, they were trained identically to the rats in Experiment 2. The mean bodyweight of the Wheel group was significantly less than the mean bodyweight of the Lever group ($F=5.65$ $p=0.0388$) and mean bodyweight increased significantly across days ($F=23.00$, $p<0.0001$). Mean bodyweight did not increase more or less rapidly for one group or the other ($F=0.27$, $p=0.9981$).

6. Discussion

6.1. Methemoglobinemia and response output

When considered together, the results of Experiments I and II are consistent with the hypothesis that sufficiently high levels of PAPP-induced methemoglobinemia engendered a cytotoxic hypoxemia that differentially affected wheelrunning more than leverpressing because wheelrunning is a more energetically demanding instrumental response than leverpressing. One might legitimately ask whether PAPP resulted in hypoxemia. Although blood gases were not collected for rats in either the

Leverpress or the Wheel groups, anecdotal evidence suggested that PAPP did in fact induce hypoxemia. At the 10 and 20 mg/kg doses, the normally bright red eyes and the vascular beds in the ears, pads of the feet, and testicles of rats were a pale pink, not the normal deep red. Although this induced hypoxemia may not have been sufficient to be cytotoxic, it is conceivable that wheelrunning was reduced more than leverpressing because the relatively high density of mitochondria in skeletal muscle might predispose them to even small restrictions on the availability of O_2 (Tepperman et al., 1946).

The correlation between the time course of metHb formation and the reductions of leverpresses and wheelturns does not imply that metHb cause these reductions of operant output.

It is conceivable that the reductions in leverpressing and wheelrunning might have resulted from malaise or reduced gastrointestinal motility; increasingly larger doses of PAPP might have resulted in greater malaise or reduced motility and greater reductions in response output. However, even if true, it would not be clear why wheelrunning would be reduced more than leverpressing, although one might invoke post-hoc that more effortful activities cause greater malaise. In the absence of additional data, the most parsimonious explanation is that PAPP-induced methemoglobinemia resulted in hypoxemia that reduced response output, but does so more for wheelrunning because the metabolic demand of wheelrunning is significantly greater than leverpressing when thousands of wheelturns are required for a rat's daily food intake. No direct evidence (e.g. oxygen consumption or heat production) is available from the present study to support the assumption that wheelrunning is more energetically demanding than leverpressing. However, the bodyweights in Figs. 3 and 4 are consistent with this assumption. The mean bodyweight of rats required to run in a wheel for food was significantly less than the mean bodyweight of rats that were required to press a lever for food and the approximately parallel nature of these curves in Fig. 4 would appear to suggest a proportional reduction of bodyweight by the wheelrunning requirement.

The relatively energetic nature of wheelrunning was also used by Bauman et, al. (1997) to evaluate the reduction of operant output by air blast overpressure. In this study, rats were required either to run in individual activity wheels or press levers for their entire daily food intake before being exposed to a whole-body blast at the end of an air-driven shock tube. Results from this study clearly showed that rats exposed to blast overpressure suffered injury to the pulmonary epithelium, which presumably limited diffusion of O_2 from the air to hemoglobin in the alveoli. Consistent with the hypothesis of increased metabolic demand imposed by wheelrunning, wheelrunning was reduced more than leverpressing by the blast wave.

Swimming is also a relatively energetic operant that D'Mello (1986, 1987) used to evaluate the protective efficacy of PAPP against exposure to cyanide. In these studies, guinea pigs were trained to swim along a narrow straight alley to the safety of a platform. Subsequently, 25 mg/kg of PAPP was delivered intraperitoneally before injections of sodium cyanide ($NaCN^-$). Demello reported that 25 mg/kg of PAPP resulted in a peak methemoglobinemia of between 7–15%, which did not affect swimming performance, but protected against impairments resulting from several LD_{50}s of $NaCN^-$. In contrast to Demello's experiments, the 1 mg/kg dose of PAPP in the present experiment resulted in a peak metHb of about 10 g%, but at a 5 mg dose of PAPP, the peak metHb level had increased to 55 g% and at 20 mg/Kg, the peak metHb level rose to near 70 g%. It is not clear what procedural differences (e.g. rats vs. guinea pigs, IP versus gastric infusion) might have contributed to the very different peak metHb levels at identical doses of PAPP. The species difference is probably not a significant contributor because, although the activity of metHb reductase is species specific (Rockwood et al., 2003; Smith and Beutler, 1966; Robin and Harley, 1966), the rate of metHb reduction *in vitro* is not significantly different for rats and guinea pigs (Robin and Harley, 1966).

7. Conclusion

Although D'Mello (1987), Bright (1987), and Marrs (1987) acknowledged the potential risk associated with using metHb as a scavenger for the cyanide anion, no tests have been performed to evaluate the behavioral toxicity of elevated metHb levels. The principal purpose of the present series of experiments was to characterize the behavioral toxicity of metHb levels higher than the 12–15 g% that is considered to be protective against exposure to several LD_{50}s of cyanide (Ballantyne, 1987) and determine whether the energetic demands of an operant response modulated the behavioral toxicity of metHb. The significantly greater reduction of wheelrunning, an energetic operant, compared to leverpressing, a sedentary operant, is a finding that may be important to military medicine. In particular, this finding suggests that if a metHb former is used as a prophylaxis for acute cyanide poisoning in soldiers, it might be necessary to consider the energetic demands of the activities or tasks that these soldiers will be required to perform because even low (10–12%) levels of drug-induced methemoglobinemia could significantly interfere with the aerobic demands of battlefield activities. In this regard, Fig. 2A shows that even the 1 mg dose of PAPP resulted in a near zero output of wheelrunning during the first hour of darkness.

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