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RAPID COMMUNICATION

Diurnal Variation of Phenylpropanolamine Anorexia in Rats[1]

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DAVIES, B. T. AND P. J. WELLMAN. *Diurnal variation of phenylpropanolamine anorexia in rats.* PHARMACOL BIOCHEM BEHAV **37**(1) 201–203, 1990.—An examination of the effects of the diurnal cycle on phenylpropanolamine (PPA) anorexia was conducted using two groups of rats, differentiated on the basis of time (Night or Day) of drug injection (0, 10, 20, or 30 mg/kg PPA). The results demonstrate that PPA, over a range of doses, has greater anorectic potency during the dark phase of the diurnal cycle. Moreover, the effect of PPA on water intake was not influenced by the diurnal cycle, suggesting that the diurnal effect was limited to the inhibitory action of PPA on feeding. Other studies are cited which, combined with the present results, suggest that the diurnal cycle may modulate feeding control processes, and that the impact of an anorectic agent such as PPA on feeding behavior may be, in part, a function of the diurnal cycle.

Phenylpropanolamine Feeding Anorexia Diurnal cycle Paraventricular nucleus

PHENYLPROPANOLAMINE (PPA) has been noted to suppress feeding behavior and induce weight loss in a variety of species (8). The threshold for the induction of anorexia in rats given systemic injections of PPA is about 5 mg/kg, whereas near-maximal suppression of feeding may be obtained with 35–50 mg/kg PPA (2). Upon surveying the PPA literature, one may note considerable variability in the potency of PPA on feeding behavior across different experimental protocols. One salient factor which may contribute to this variability is the time of testing within the diurnal cycle (compare 1 with 3). Thus, the purpose of the present study was to evaluate the effect that time of testing within the diurnal cycle may have on the anorectic potency of PPA.

METHOD

Seventeen male Sprague-Dawley albino rats (Harlan Industries, Houston, TX), weighing between 250–275 g at the beginning of the study, were maintained ad lib on tap water and rodent pellets (Teklad), except as required by the experimental protocol. The rats were housed individually in standard plastic rodent cages in a colony room maintained at 23°C under a 12-hr/12-hr illumination schedule (lights on at 13:00 hr).

Solutions of phenylpropanolamine (10, 20, and 30 mg/kg) were prepared by dissolving phenylpropanolamine hydrochloride (d,l-norephedrine, Sigma Chemical Co., Lot No. 21-F-0215) into sterile distilled water. The drug solutions were calculated as the weight of chemical (base and salt) per volume.

One week after arrival of the animals in the lab, the illumination schedule for the colony room was altered such that the lights were on from 13:00 to 01:00 hr. All procedures carried out during the dark cycle were conducted under the illumination of a single 25-watt red light bulb. The animals were rank-ordered according to weight and alternately assigned to one of two Treatment Groups: Night Tests (n = 9) and Day Tests (n = 8), reflecting that period of the illumination schedule during which testing occurred. The animals were allowed 7 days to adapt to the lighting schedule, during which a 15-hr food and water deprivation schedule was initiated. During the last 3 days of the adaptation period, vehicle injections were administered and baseline food and water intakes were measured and recorded.

On each day of vehicle administration, at 01:00 hr (Night Tests), and 13:00 hr (Day Tests), fresh cardboard pads were placed beneath the grid floors of the cages. At 01:30 or 13:30 hr (respectively), the injections were administered, and one-half hour later (02:00 or 14:00 hr) the 30-min testing period began. Food intake was measured to the nearest 0.1 gram, corrected for spillage collected on the cardboard pad from each cage. Water intake was measured to the nearest 0.1 gram using weighed water bottles. Following recordings of the 30-min intakes, an additional portion of food pellets was placed in the cage and the weighed water bottles were returned. At 06:00 or 18:00 hr, the intakes were again measured and recorded as above. The animals then received food and water ad lib until the 15-hr deprivation began (11:00 or 23:00 hr).

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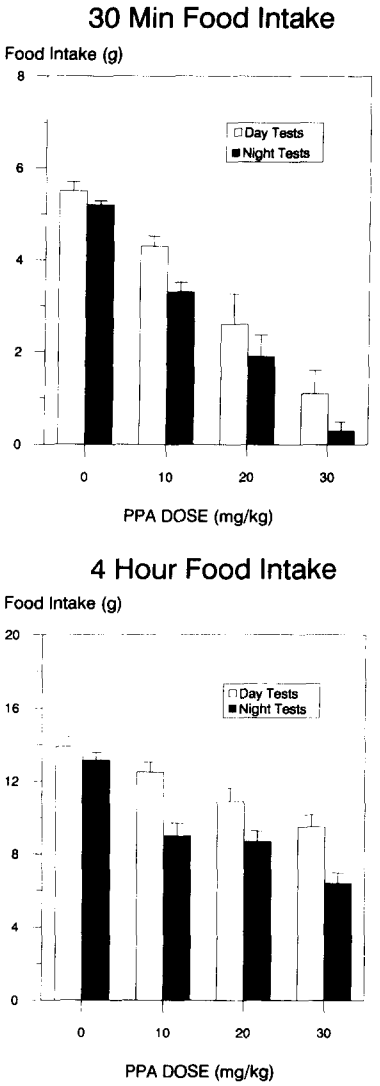

FIG. 1. Mean food intake during 30-min tests (a) and 4-hr tests (b) for the two Treatment Groups (Day Tests, Night Tests) across four doses of PPA (0, 10, 20 and 30 mg/kg).

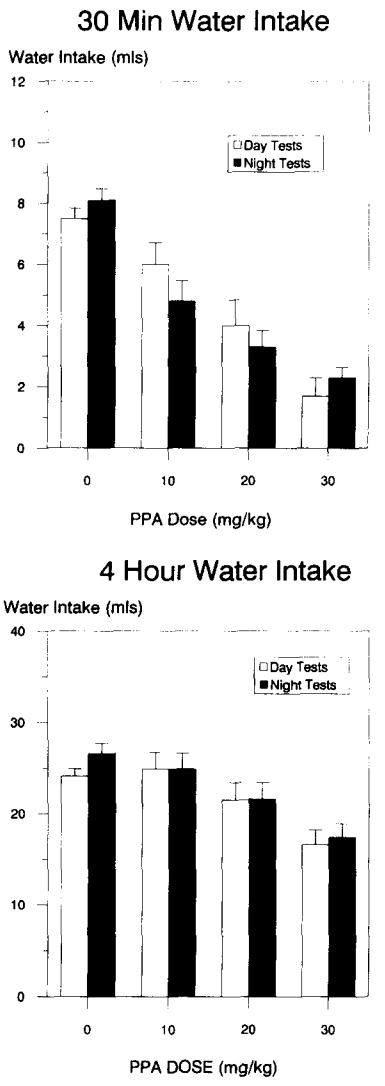

FIG. 2. Mean water intake during 30-min tests (a) and 4-hr tests (b) for the two Treatment Groups (Day Tests, Night Tests) across four doses of PPA (0, 10, 20 and 30 mg/kg).

The procedures used during the drug tests were identical to those outlined above except that, within each Treatment group, each rat was randomly assigned a specific drug test sequence consisting of single injections of 10, 20, and 30 mg/kg PPA interspersed with two vehicle injections.

Food intake and water intake data were analyzed using an overall Analysis of Variance for each testing period (30 min, 4 hr). The design of this analysis was a mixed factorial with a between-group factor of Treatment Group (Night Tests, Day Tests) and a within-group factor of Drug Dose (0, 10, 20 and 30 mg/kg PPA). Subsequent Newman-Keuls analyses were used for multiple comparisons between drug doses. The criterion level for all statistical tests was $p<0.05$.

RESULTS

Food Intake

Figures 1a and b present the mean food intake during the 30-min and 4-hr tests, respectively, for the two Treatment Groups (Night Tests/Day Tests) across the four Drug Doses (VEH, PPA-10, PPA-20, PPA-30). A posteriori contrasts using t-tests failed to reveal a significant difference between the two Treatment Groups at either 30 min or 4 hr after vehicle injection, t's(15)<0.90, p's>0.05. Overall 2 × 4 mixed ANOVA's for each test period revealed significant Drug Dose effects, F's(3,45)>42.3, p's<0.001, and significant Treatment effects, F's(1,15)>7.7, p's<0.05, but no interaction between the factors of Drug Dose and Treatment Group. Subsequent Newman-Keuls analyses comparing the different Drug Doses revealed that, during both the 30-min and 4-hr tests, PPA-10, PPA-20, and PPA-30 all differed significantly from VEH, and PPA-30 differed significantly from both PPA-10 and PPA-20 (p's<0.05). Thus, PPA produced a dose-dependent suppression of feeding, and this effect was greater for night tests than for day tests.

Although the baseline food intakes after vehicle treatment were not significantly different for Day Tests and Night Tests, slight differences between the groups may have contributed to the significant effect of Treatment Group noted for the food intake

data. Accordingly, an additional Analysis of Variance was computed for the 4-hr food intake data using baseline 4-hr food intake after vehicle injection as the covariate. This analysis demonstrated that the significantly greater inhibitory effect of PPA noted during the Night Tests was independent of the variation of baseline food intake values, $F(2,30) = 17.6$, $p<0.001$.

Water Intake

Figures 2a and b present the mean water intake during the 30-min and 4-hr tests, respectively, for the two Treatment Groups across the four Drug Doses. A posteriori contrasts using *t*-tests failed to reveal a significant difference between the two Treatment Groups for the VEH dose during either the 30-min or 4-hr test, t's(15)<1.2, p's>0.05. Overall 2 × 4 mixed ANOVA's revealed only significant Drug Dose effects, F's(3,45)>22.0, p's<0.001. Thus, while there were no significant differences in the effect of PPA on water intake between the Night Tests and the Day Tests, the suppression of water intake noted during the 30-min and 4-hr tests was a function of dose. Subsequent Newman-Keuls analyses comparing the different Drug Doses revealed that, during the 30-min test, PPA-10, PPA-20, and PPA-30 all differed significantly from VEH, and both PPA-30 and PPA-20 differed significantly from PPA-10. During the 4-hr test, PPA-30 differed significantly from VEH, PPA-10 and PPA-20, p's<0.05.

DISCUSSION

The results of the present study demonstrate that PPA is more effective in suppressing food intake during the early dark phase of the diurnal cycle. If one collapses the recorded changes in feeding behavior over the drug doses, PPA suppressed feeding by approximately 69 and 36%, respectively, during the 30- min and 4-hr Night Tests, whereas the comparable values for PPA during the Day Tests were 51 and 22%. Thus, the magnitude of the increase in PPA anorexia noted during the Night Tests was +26% for the 30-min test and +39% for the 4-hr test.

PPA produced a dose-dependent suppression of feeding behavior at both testing intervals. In contrast, although the effect of PPA on drinking was a function of dose during the 30-min trial, only the 30 mg/kg PPA dose suppressed water intake during the 4-hr trial. These results support the growing body of evidence which suggests that, although PPA exerts an inhibitory effect on drinking, this effect is of a smaller magnitude than its effect on feeding (5). Furthermore, the enhanced effect of PPA presently noted during the Night Tests was specific to feeding behavior rather than drinking behavior.

The diurnal difference in PPA potency demonstrated in the present study is intriguing for at least 2 reasons. Firstly, this difference may account for a portion of the variability noted in past studies of the effect of PPA on feeding behavior. Secondly, the diurnal effect may offer some insight into the mechanism by which PPA suppresses feeding behavior. In a recent experiment, Wellman and Davies (10) noted that microinjection of PPA into the paraventricular hypothalamus (PVN) results in a suppression of feeding behavior, whereas PPA does not induce such anorexia when microinjected into the perifornical area of the hypothalamus (9). The neurotransmitters norepinephrine (NE) and serotonin (5-HT) are known to play a role in the modulation of feeding behavior within the PVN. Microinjections of NE elicit feeding, whereas similar injections of 5-HT suppress feeding (4). Interestingly, these neurotransmitters exhibit marked diurnal rhythms, with a peak in activity that occurs during the early portion of the dark phase (6,7). Whether PPA exerts its effects on feeding via an action on either NE or 5-HT within the PVN remains to be determined.

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