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Circadian Rhythm-Dependent Development of Melatonin Effects and Tolerance to PHNO in Rats

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MUNRO, J. D. AND M. T. MARTIN-IVERSON. *Circadian rhythm-dependent development of melatonin effects and tolerance to PHNO in rats*. PHARMACOL BIOCHEM BEHAV **65**(3) 495–501, 2000.—Male Sprague–Dawley rats were given 12 days of continuous infusions of (+)-4-propyl-9-hyroxynapthoxazine (PHNO, 5μg/h), a highly selective dopamine D_2 receptor agonist, via subcutaneous ALZET® osmotic pumps. Motor stimulant effects (locomotion and rearing) were monitored throughout the treatment period, including after the animals were injected with 2-iodo-melatonin (0.5 mg/kg) on days 8–10 and 13 after initiation of PHNO infusions. The rats (maintained on 12 L:12 D cycle) developed tolerance to the motor stimulant effects of PHNO during the day, and behavioral sensitization to PHNO during the night. Arousing rats with a vehicle injection transiently blocked the daytime tolerance. A more sustained environmental noise without handling of animals, which had a stronger effect on increasing motor activity of control rats, reversed tolerance to sensitization. Therefore, graded levels of arousal produce graded increases in motor activity in rats otherwise tolerant to the effects of PHNO. Daytime tolerance to PHNO was reversed to sensitization by 2-iodo-melatonin. This effect was more than an additive effect of drug + injection procedure stress. The differential development of nocturnal sensitization and diurnal tolerance to PHNO effects on motor activity may depend upon circadian rhythms in melatonin release, as well as on state of arousal. © 2000 Elsevier Science Inc.

Arousal Locomotion Rearing Dopamine agonist PHNO D_2 receptor Sensitization Tolerance Circadian rhythms Stress

(+)-4-PROPYL-9-HYDROXYNAPHTHOXAZINE (PHNO) is an extremely potent dopamine (DA) agonist with a marked affinity and high selectivity for the DA D_2 receptor subtype (17). Seeman et al. (17) argued that PHNO is selective for the D_2 receptor subtype, excluding significant binding to D_4 receptors because clozapine does not displace tritiated PHNO from its brain binding sites at concentrations that occupy D_4 receptors (K_i = 508 nM). Binding of PHNO to D_3 receptors was excluded based on PHNO's binding being sensitive to guanilylimidodiphosphate, a characteristic of D_2 but not D_3 receptors. Rats given continuous infusions of PHNO (5 μg/h, SC) with Alzet osmotic pumps exhibit both tolerance and sensitization to PHNO's motor stimulant effects as a function of the day–night cycle (12). Rats show a loss of motor stimulant effect (tolerance) during days and progressively greater locomotor stimulant actions (sensitization) during successive nights. Most of the diurnal tolerance occurs by the second day of treatment, with complete tolerance developing by the fifth to seventh day. Nocturnal activity gradually increases each night, with a maximal level occurring after about 8 nights of continuous treatment. Associative (classical conditioning) models of drug tolerance and sensitization do not easily explain this pattern, as no environmental cues are uniquely associated with the drug administration.

Reversing the light–dark schedule, from lights on between 0900–2100 h to lights on from 2100–0900 h, results in tolerance shifting (within 3–4 days) to follow "daylight" hours. Concurrently, sensitization switches to the opposite "nighttime" schedule, lights on from 0900–2100 h rather than 2100–0900 h (13). Sensitization and tolerance, however, are not exclusively dependent on light cues. Rats maintained in constant darkness show a free running ~25-h rhythm with peaks and troughs of activity. Sensitization is observed during the active periods and tolerance during the resting phases (14).

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Rats exhibit fluctuations in tolerance and sensitization that match their free-running motor activity rhythms even in the absence of external circadian cues. This observation is further evidence against a strictly associative explanation of tolerance and sensitization to stimulants.

Dopamine D_2 receptor density changes are an unlikely explanation for the observed phenomenon, because the reversal from tolerance to sensitization and from sensitization to tolerance occurs in the first hour after the lights go off or on. DA receptor densities do not appear to alter so rapidly: the half-life of the turnover rate for D_2 receptors is 94.2 h (5). Moreover, studies with D_2 agonists have not reported an increase in D_2 receptors after prolonged treatment (9).

If rats are kept under constant light conditions for a period of 3 weeks, they do not exhibit circadian rhythms in activity, and PHNO has no motor stimulant effects, even on the first day of treatment (14). The complete lack of motor stimulant response persists for the duration of the PHNO treatment. However, a motor stimulant effect appears if animals are cotreated with a dopamine D_1 receptor agonist (SKF 38393) at a dose that has little effect on its own (14). This observation, along with other evidence for D_1-receptor regulation of sensitization and tolerance to PHNO (11), suggests that tolerance to PHNO arises from a decrease in endogenous dopamine release, and a consequent, reduction in D_1 receptor activation, necessary for the expression of motor stimulation effects of PHNO. However, it is not clear what mechanism regulates dopamine release according to circadian rhythms. That PHNO has no measurable effect on locomotor activity in the absence of circadian rhythms in locomotion suggests that constant light may be inhibiting some factor or system regulating PHNO's effect on behavior, probably via actions on dopamine release.

The pineal gland is an obvious candidate for a mechanism that may be mediating responsiveness to PHNO, because it releases melatonin in a 24-h cycle, and melatonin release is suppressed under constant light. The rhythmic release of melatonin at night in mammals is thought to serve as a resetting signal for the endogenous biological clock (18), although its role phase-shifting circadian rhythms in the rat is controversial, depending on the rat strain and other factors (15). Recently, melatonin has also been shown to interact with DA D_2 receptors to influence striatal neuronal activity (4).

It might be argued that the observed diurnal tolerance and nocturnal sensitization is merely a function of basal activity per se. The influence of a mechanism that establishes a circadian rhythm may simply depend on its effects on regulating basal activity. By this argument, sensitization would occur when activity is high, and tolerance when activity is low. This is certainly consistent with the arousal effects at reversing tolerance. However, this possibility is refuted by the observation that rats housed under constant light show tolerance to continuous infusions of PHNO during times of high activity (14). Vehicle and PHNO-reated rats maintained under constant light show nonperiodic variations in locomotor activity, with peaks in activity identical in magnitude to the periodic peaks observed in rats kept under constant darkness that exhibit clear circadian rhythms. Despite this similarity in activity level, rats in constant light do not exhibit sensitization. This observation discounts the possibility that sensitization and tolerance are simply activity dependent.

Inhibition of melatonin release from the pineal gland may be responsible for diurnal tolerance, and increasing melatonin release responsible for nocturnal sensitization. Two predictions follow this argument: 1) injecting rats that show daytime tolerance to PHNO with a potent melatonin agonist such as 2-iodo-melatonin (18) should reverse diurnal tolerance into sensitization; and 2) pinealectomy should prevent the nocturnal development of sensitization to PHNO. The experiment in this report tested the first of these predictions.

METHOD

Animals

Forty-eight experimentally naive, male Sprague–Dawley rats, weighing 300–370 g at the beginning of the experiment, were randomly assigned to one of four equal groups ($n = 12$). The animals were purchased from the Animal Resources Centre of Western Australia and individually housed in cages on a timer controlled 12 L:12 D cycle (0700–1900 H light), with ad lib access to food and water.

Apparatus

Each of 48 cages [21 (W) × 18 (H) × 33 (L) cm] had a stainless steel mesh floor (1 cm^2) and a stainless steel grill roof that doubled as a food dispenser and supported a water bottle. Underneath each cage was a waste tray containing pine chaff. The individual cages were positioned on two adjacent cage racks and arranged in four rows of six cages (i.e., each cage rack held 24 cages). Eight holes, 1 cm in diameter, were drilled into the clear Plexiglas walls to accommodate the infrared photocell beams. Each cage was positioned between an eight photocell apparatus, which allow continuous monitoring of motor activity. Four photocells were positioned 2 cm from the floor of the cage, and four photocells placed 6 cm from the roof, all spaced equally 8 cm apart. Each photocell produces a beam of light from an emitter, which forms a circuit to a receiver. Sensitivity of the photocells was set such that rapid repeated interruptions (i.e., movements of the head, tail, paws) were not counted to ensure that only gross locomotor activity was recorded. A PC computer recorded interruptions in the photocell circuit and accumulated the counts in 1-h blocks. The computer recorded two different types of photocell interruptions: locomotion (defined as interruption of one of the lower four beams that was NOT preceded by interruption of the same beam), and rearing (interruption of one of the higher four beams that was NOT preceded by interruption of the same beam). It has been previously shown that nocturnal sensitization and diurnal tolerance to locomotion and rearing occurs to PHNO whether the behavior is measured as beam interruptions or as visually scored behaviors (10). However, the reader should bear in mind that the terms locomotion and rearing used in this report refer to interruptions of photobeams only.

Drugs

(+)-4-Propyl-9 hydroxynaphthoxazine hydrochloride (PHNO) was provided courtesy of Merck Sharp & Dohme Ltd. PHNO HCl (10.9 mg/ml) was dissolved in distilled water and Alzet Osmotic Pumps (model #2002) were filled with the solution to provide a release rate of approximately 5.0 μg/h, which gave an average dose of 16.8 $\mu g\ kg^{-1}\ h^{-1}$. The dose of PHNO was chosen based on previous work (11,13). 2-Iodo-melatonin (0.5 mg/ml) was purchased from Research Biochemicals Incorporated and dissolved into a solution of 1% ethanol and distilled water. The dosage was chosen based on the literature (18). All drug dosages are expressed in weights of their salts.

Surgery

Rats were anesthetized with halothane in nitrous oxygen:oxide (3:1). Hair was shaved off each animal in the midscapular region of the back, and the skin was cleaned and disinfected with an alcohol solution. An incision was made between the shoulder blades and hemostats were used to create a small pocket in the connective tissue beneath the skin into which the osmotic pump was inserted. The incision was closed with wound clips, and a local anaesthetic (xylocaine) was applied to the wound.

Procedure

The rats were habituated to the cages for 3 weeks before the beginning of drug treatment. In addition, the animals were habituated to a regular housekeeping routine, in which food, water, and litter were changed between the hours of 0900 and 1100 h on Mondays, Wednesdays, and Fridays. This procedure was accompanied with a fair amount of noise and disruption, lasting for about 50 min, and constitutes the "housekeeping disturbance" variable. Average hourly activity counts were calculated over blocks of 12 h for nocturnal activity, 10 h for diurnal activity, and 2 h (beginning with the initiation of the care of the animals or the equivalent periods on days without care) for housekeeping effects.

Day 0 of the experiment proper (day 22 in the cage) marked the beginning of psychomotor drug treatment, with the implantation of the pumps. The order of drug treatments in the 48 rats was randomized within a counterbalanced format, so that each row of 6 boxes contained a similar number of rats from each group. Half the animals ($n = 24$) received pumps containing PHNO, and the rest received pumps containing vehicle. Recording of data began at 1900 h. The continuous recording of motor activity continued up to and including day 7, after which the second part of the experiment began.

Day 8 was the beginning of the diurnal 2-iodo-melatonin/vehicle injections. Three injections were given on days 8, 10, and 12. The injection procedure began at 1230 h on each day, and typically lasted 30 min for all animals. Each animal was given an injection (IP) of either 2-iodo-melatonin or vehicle. Half of the rats in each of the two groups (vehicle pump or PHNO pump, $n = 12$ for each) were given a vehicle injection, and the rest received 2-iodo-melatonin. All rats were given an injection of vehicle on day 9. Recording of motor activity began immediately after each animal was injected and returned to its cage.

Statistics

The four separate ANOVAs in this study all used a mixed design with repeated-measures ANOVA. Planned comparisons were achieved using the multiple *F*-test (8). The ANOVA used to analyze the effects of continuous administration of PHNO on diurnal activity utilized one between factor, PHNO, with two levels (0 or 5 μg/h), and one within factor, days, with seven levels (days 1–7).

The second analysis tested the effects of continuous administration of PHNO on nocturnal activity. This analysis had one between factor, PHNO, with two levels (0 or 5 μg/h) and one within factor, nights, with eight levels (nights 1–8).

The third analysis examined the effects of the housekeeping disturbance on diurnal tolerance. The housekeeping analysis involves one between factor, PHNO, with two levels (0 or 5 μg/h) and one within factor, housekeeping, with two levels (day 7 with housekeeping, or the same time from day 6 without housekeeping).

The last ANOVA was on the motor activity 1 h before and 1 h after 2-iodo-melatonin injections, averaged across the 3 treatment days. The ANOVA had two between factors with two levels, PHNO (0 or 5 μg/h) and 2-iodo-melatonin (0 or 0.5 mg/kg), and one within factor with two levels, treatment time (pre- or postinjection).

In all figures, the error terms refer to the critical difference for planned comparisons at $\alpha = 0.05$. The critical difference is determined by: square root($2 \times F$) $\times$ square root(MS_{ERROR}/n), where "F" is the value of F with 1 degree of freedom for the numerator, and the degrees of freedom associated with the MS_{ERROR} in the denominator (the MS_{ERROR} is derived from the within-cells term of the ANOVA for the interaction analysis), and "n" = the size of the groups.

RESULTS

Effects of PHNO on Diurnal Activity

The pattern of diurnal locomotor activity after administration of the PHNO/vehicle minipump is displayed in Fig. 1. For mean hourly locomotor activity over days, analysis of variance revealed a significant drug $\times$ days interaction, $F(6, 276) = 21.54$, $p < 0.001$. Figure 1 indicates that the interaction was due to a decreasing difference in activity between PHNO and vehicle-treated groups on successive days. The largest difference occurred between days 1 and 2. Planned comparisons using the multiple *F*-test show that locomotor activity in the PHNO group was significantly higher than the vehicle controls on all days ($p < 0.05$), except for day 7. There was no difference in the level of activity between groups on the last day before surgery (DBS).

Similar to locomotion, mean hourly diurnal rearing showed a significant drug $\times$ days interaction, $F(6, 276) = 16.78$, $p < 0.001$. The interaction was also due to a decreasing difference between the PHNO and vehicle-treated groups on successive days, as can be seen in Fig. 2. Again, the largest dif-

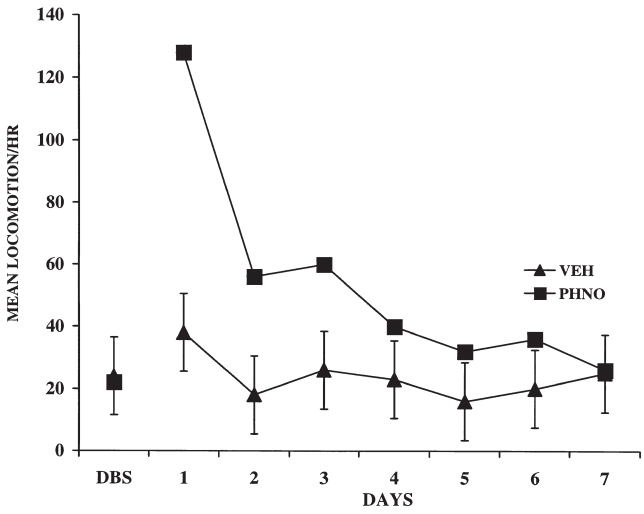

FIG. 1. Mean daily locomotion averaged over the 10-h light period (0700–0900 and 1100–1900 h) for rats treated with continuous infusions of PHNO (5 μg/h) or continuous infusions of vehicle (VEH). $n = 24$ for each group. DBS represents the last day before surgery. The error bars represent the critical difference between means derived from the multiple *F*-test for planned comparisons ($p < 0.05$).

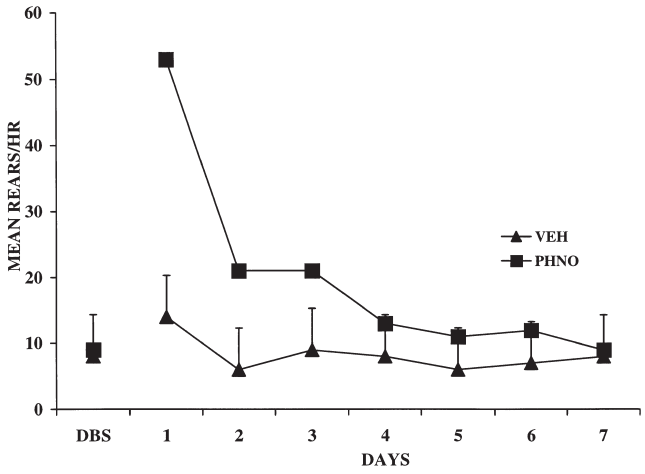

FIG. 2. Mean daily rears averaged over the 10-h light period (0700–0900 and 1100–1900 h) for rats treated with continuous infusions of PHNO (5 μg/h) or continuous infusions of vehicle (VEH). $n = 24$ for each group. DBS represents the last day before surgery. The error bars represent the critical difference between means derived from the multiple *F*-test for planned comparisons ($p < 0.05$).

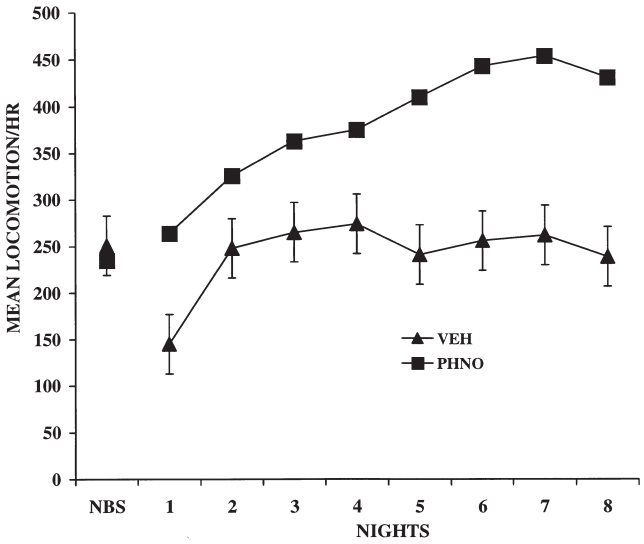

FIG. 3. Mean nocturnal locomotion averaged over the 12-h dark period (1900–0700) for rats treated with continuous infusions of PHNO (5 μg/h) or continuous infusions of vehicle (VEH). $n = 24$ for each group. NBS represents the last night before surgery. The error bars represent the critical difference between means derived from the multiple *F*-test for planned comparisons ($p < 0.05$).

ference in average rearing occurred between days 1 and 2. Comparisons using the multiple *F-test* show that rearing in the PHNO group was significantly higher than the vehicle controls on days 1, 2, and 3 ($p < 0.05$), but rearing on days 4–7 did not significantly differ. On the last day before surgery (DBS) the rearing in the vehicle and PHNO groups was not significantly different.

Effects of PHNO on Nocturnal Activity

Figure 3 shows the effects of PHNO/vehicle minipump treatment on nocturnal locomotor activity over the 12-h dark periods. Analysis of variance indicated a significant drug by nights interaction, $F(7, 322) = 8.5$, $p < 0.001$. As can be seen in Fig. 3, the difference in locomotion between the vehicle and the PHNO group increases over successive nights, beginning from night 1. Pairwise comparisons within the PHNO group show that nights 2, 3, 5, and 6 are significantly greater than the night before, and that nights 2–8 are all significantly higher than night 1. There was no difference between groups in the level of activity on the last night before surgery (NBS). Activity in the vehicle group decreased on night 1, compared to the night before surgery.

Average nocturnal rearing showed a significant drug by nights interaction, $F(7, 322) = 12.94$, $p < 0.001$. The interaction was due to an increasing difference between the PHNO and the vehicle group over successive nights, depicted in Fig. 4. Planned comparisons show that average nocturnal rearing in the PHNO group was significantly higher than in the vehicle group across all 8 nights ($p < 0.05$). Individual pairwise comparisons within the PHNO group show significant increases in rearing on nights 3, 4, and 5, and the difference between night 1 and night 8 was significant. The last night before surgery (NBS) shows that the vehicle and PHNO groups did not differ in average rearing before the implanting of the pumps. On night 1, the vehicle groups activity was diminished, but returned to presurgery levels by night 2.

Effect of Housekeeping Disturbance on Diurnal Locomotion and Rears

Figure 5 compares the effects of the housekeeping disturbance on the average locomotor activity of both the PHNO and the vehicle groups across 1 h of day 6 (no housekeeping) and the same hour on day 7 (with housekeeping). Analysis of variance revealed a significant PHNO by housekeeping inter-

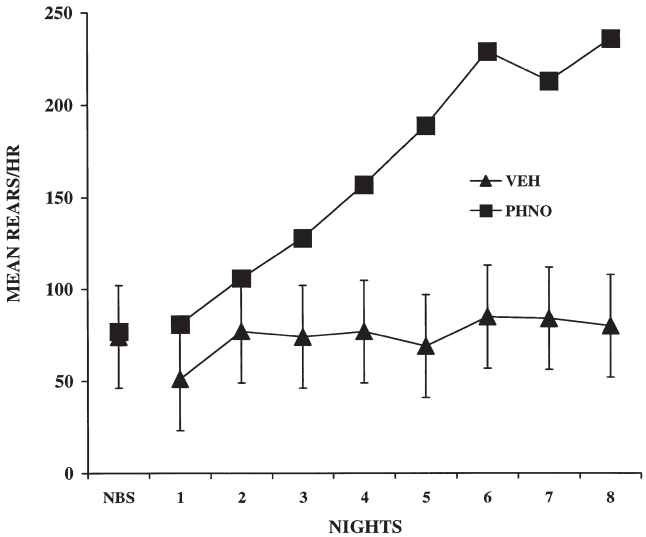

FIG. 4. Mean nocturnal rears averaged over the 12-h dark period (1900–0700 h) for rats treated with continuous infusions of PHNO (5 μg/h) or continuous infusions of vehicle (VEH). $n = 24$ for each group. NBS represents the last night before surgery. The error bars represent the critical difference between means derived from the multiple *F*-test for planned comparisons ($p < 0.05$).

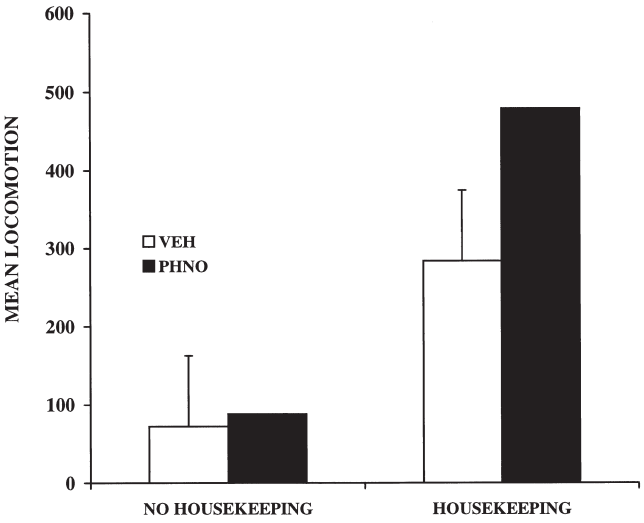

FIG. 5. Mean locomotion during a 1-h period of mild environmental disturbance (housekeeping) or at the equivalent time on the corresponding day without disturbane (no housekeeping) for rats given continuous infusions of vehicle (VEH) or PHNO (5 μg/h). $n = 24$ for each group. Data from days 6 and 7 are represented here, after tolerance to PHNO had been established. The error bars represent the critical difference between means derived from the multiple F-test for planned comparisons ($p < 0.05$).

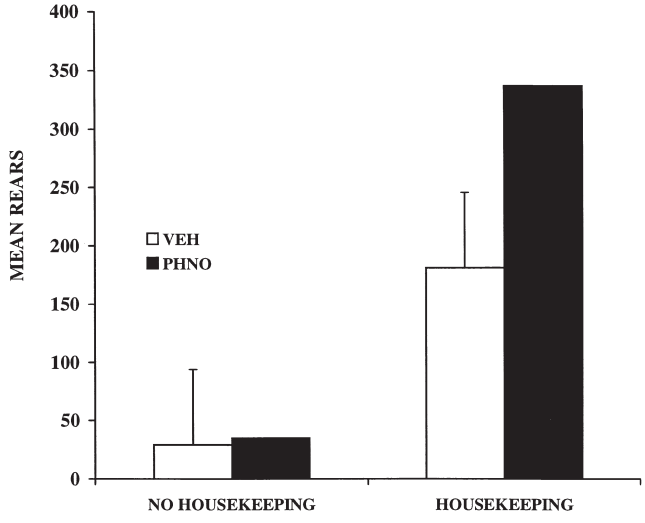

FIG. 6. Mean rears during a 1-h period of environmental disturbance (housekeeping) or at the equivalent time on the corresponding day without disturbance (no housekeeping) for rats given continuous infusions of vehicle (VEH) or PHNO (5 μg/h). $n = 24$ for each group. Data from days 6 and 7 are represented here, after tolerance to PHNO had been established. The error bars represent the critical difference between means derived from the multiple F-test for planned comparisons ($p < 0.05$).

action, $F(1, 46) = 6.36$, $p < 0.02$. Figure 5 shows that on the day with no housekeeping the PHNO group did not differ from the vehicle group in mean hourly locomotion. However, under the influence of housekeeping the PHNO group's mean hourly locomotion was significantly increased in comparison to the vehicle group. There were no significant differences between groups in hourly locomotor activity on the day with no housekeeping (day 6). On the day with housekeeping (day 7), individual pairwise comparisons revealed a significant difference in locomotor activity between the PHNO and the control groups. Housekeeping increased locomotion significantly in both groups (main effect of housekeeping: $F(1, 46) = 72.4$, $p < 0.001$), but the effect was greater in the PHNO group than in the vehicle group.

Similar effects were observed on rears, as depicted in Fig. 6. There was a significant housekeeping by PHNO interaction, $F(1, 46) = 10.8$, $p < 0.005$. Housekeeping increased rears in both groups [main effect: $F(1, 46) = 99$, $p < 0.001$), but more so in the PHNO-infused group than in the vehicle group. Again, there was no difference in rears between the PHNO and vehicle group on the hour without housekeeping, but a significant difference between the two groups on the day with housekeeping.

Effects of 2-Iodo-melatonin Injections on Diurnal Locomotion and Rears

Locomotion (averaged across 3 injection days) 1 h before and another hour after 2-iodo-melatonin injections is represented in Fig. 7. Analysis of variance revealed a significant PHNO by treatment time (pre- vs. postinjection) interaction, $F(1, 44) = 10.68$, $p < 0.001$. That is, there was no apparent effect of PHNO prior to injection, but an effect of PHNO was apparent after injection, whether 2-iodo-melatonin or vehicle were injected. There was a significant melatonin by treatment time interaction, $F(1, 44) = 5.07$, $p < 0.05$. Animals receiving 2-iodo-melatonin were more active than those receiving vehicle were. Locomotion in all groups increased after receiving an injection, whether it was vehicle or 2-iodo-melatonin, $F(1, 44) = 123$, $p < 0.001$. However, individual comparisons indi-

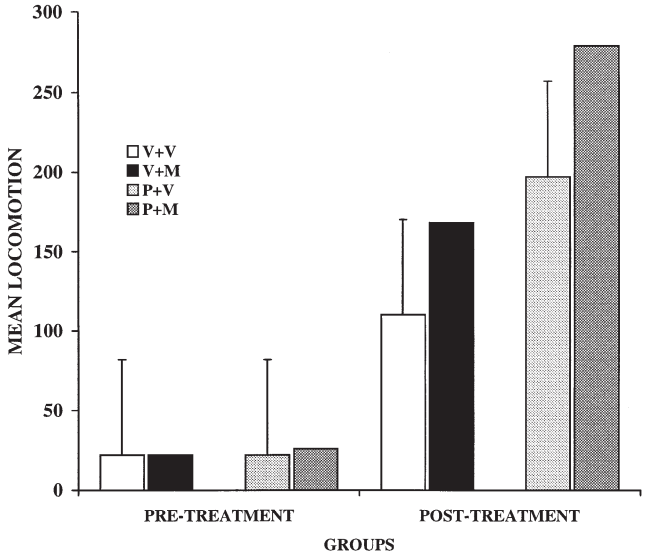

FIG. 7. Mean locomotion 1 h before (pretreat) and 1 h after (posttreat) 2-iodo-melatonin (0.5 mg/kg) or vehicle injections, for rats administered continuous PHNO (5 μg/h) or vehicle. The groups are V+V: vehicle minipump + vehicle injection; V+M: vehicle minipump + 2-iodo-melatonin injection; P+V: PHNO minipump + vehicle injection; and P+M: PHNO minipump + 2-iodo-melatonin injection. The error bars represent the critical difference between means derived from the Multiple F-test for planned comparisons ($p < 0.05$).

cated that only activity in the group receiving both 2-iodo-melatonin and PHNO was significantly greater than its respective vehicle control. The vehicle infused group given 2-iodo-melatonin did not significantly differ from its vehicle-injected control group. The PHNO by melatonin and the PHNO by treatment time by melatonin interactions were not significant.

The effects of 2-iodo-melatonin on rears were similar, but less robust, to those on locomotion. The injection procedure itself increased rears in all groups, $F(1, 44) = 122$, $p < 0.001$. There was also a significant PHNO by treatment time interaction, $F(1, 44) = 10.32$, $p < 0.005$, and the 2-iodo-melatonin by treatment time interaction was near significance level, $F(1, 44) = 3.44$, $p < 0.07$. Planned comparisons indicated that rats receiving PHNO + 2-iodo-melatonin exhibited significantly more rears than those receiving PHNO + vehicle or vehicle + 2-iodo-melatonin (Fig. 8). The vehicle + 2-iodo-melatonin group did not exhibit significantly more rears than the vehicle + vehicle group.

DISCUSSION

This study investigated a possible link between circadian melatonin release and the development of tolerance and sensitization to the continuous infusion of PHNO (5 μg/h), a selective DA D_2 agonist. Circadian melatonin release by the pineal gland could be a mediating factor in the development of diurnal tolerance and nocturnal sensitization to continuous infusion of PHNO. It was hypothesized that injecting animals that are tolerant to PHNO with a potent melatonin agonist such as 2-iodo-melatonin (18) would reverse this tolerance.

The results replicate previous findings (11–13). Continuous administration of PHNO in rats results in rapid tolerance developing to its locomotor stimulant effects during the day, with a more gradual sensitization developing at night. The findings are extended to an automated measure of rearing behavior, where a similar pattern is observed. However, rearing differs from locomotion in degree: tolerance developed more rapidly and sensitization was greater for rearing than for locomotion.

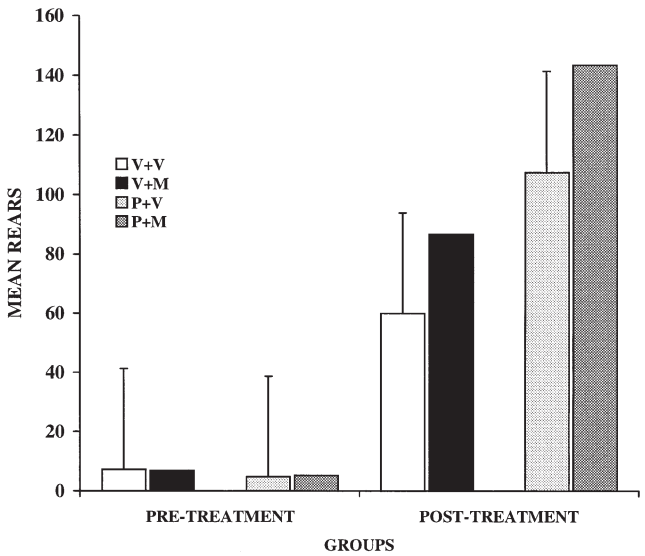

FIG. 8. Mean rears 1 h before (pretreat) and 1 h after (Posttreat) 2-iodo-melatonin (0.5 mg/kg) or vehicle injections, for rats administered continuous PHNO (5 μg/h) or vehicle. The groups are V+V: vehicle minipump + vehicle injection; V+M: vehicle minipump + 2-iodo-melatonin injection; P+V: PHNO minipump + vehicle injection, and P+M: PHNO minipump + 2-iodo-melatonin injection. The error bars represent the critical difference between means derived from the multiple *F*-test for planned comparisons ($p < 0.05$).

Tolerance and sensitization to PHNO did not follow the same time course. The greatest drop in responsiveness to PHNO occurred between days 1 and 2, whereas nocturnal sensitization develops in a more linear fashion. This suggests that tolerance and sensitization may not result simply from converse actions of the same mechanism.

This study also replicated the earlier finding that the arousing effects associated with changing food, water, and cage litter can reverse diurnal tolerance to PHNO (11–13). This latter finding was further extended to the procedure of vehicle injection. Rats receiving vehicle infusions through an osmotic pump exhibit a significant increase in both locomotion and rearing for 1 h after a vehicle injection. Rats receiving continuous PHNO and showing virtually complete tolerance on days 8, 9, 10, and 12 of treatment also showed an increase in locomotion and rearing after vehicle injections. The magnitude of the effect in PHNO-infused rats was significantly larger than in vehicle-infused rats. This could be interpreted as an arousal-induced reversal of tolerance to PHNO, or as a PHNO potentiation of arousal effects, when PHNO's direct effects on motor activity would otherwise be absent.

Expression of PHNO's motor stimulant effects is dependent on the concomitant activation of the D_1 receptor subtype (11,14). This observation is supported by the finding that PHNO's diurnal and nocturnal motor stimulant effects can be blocked with coadministration of the D_1 receptor antagonist SCH 23390. Stress reversal of tolerance to PHNO is blocked by SCH 23390, whereas stress-induced activity in vehicle-treated rats is not blocked by SCH 23390. Moreover, tolerance to continuous infusions of PHNO can be reversed with administration of a D_1 agonist. Additionally, extensive DA depletion blocks the motor stimulation of bromocriptine, a D_2 selective agonist (7). Agents that increase DA release or increase D_1 activation, reinstate the actions of bromocriptine (6). It has been suggested that the circadian rhythm in tolerance and sensitization to PHNO can be explained by a circadian rhythm in endogenous dopamine release acting at D_1 receptors. Additionally, arousal reversal of tolerance may be related to arousal-related release of dopamine that then acts at D_1 receptors.

This explanation leaves unexplained how dopamine release might be regulated following circadian rhythms. Melatonin release is high at night when sensitization to PHNO's motor stimulant effects is observed and low in the day when tolerance develops. Constant light results in complete or nearly complete loss of melatonin release and loss of circadian rhythms in rats; the latter can be reinstated with daily melatonin injections (1,3). Significantly, rats kept under constant light show no behavioral effects to PHNO even on the first day of treatment (14). Therefore, the present experiment tested the possibility that injections of a selective melatonin agonist, 2-iodo-melatonin, could reinstate the motor stimulant effects of PHNO at a time of treatment when complete tolerance to PHNO is apparent.

The effects of 2-iodo-melatonin are not straightforward. There is an effect of the injection procedure itself, as can be observed in both the vehicle- and PHNO-infused rats given vehicle challenges. This appears similar, although less in degree, to the effect of "housekeeping" (providing the rats with fresh food and water and cleaning the litter at the bottom of

the cage). The injection effect is, therefore, likely to be an arousal or stress effect. Furthermore, there was a slight, but statistically insignificant, trend for 2-iodo-melatonin to increase motor activity in vehicle-infused rats. Importantly, the increase in motor activity in the PHNO + 2-iodo-melatonin group was significantly greater than the effect observed in the PHNO + vehicle group or the vehicle + 2-iodo-melatonin group. The actual level of activity achieved in the PHNO + 2-iodo-melatonin group was about double that observed on the first day of PHNO treatment. This is consistent with the view that the melatonin agonist reversed tolerance into sensitization.

The possibility that the effect of 2-iodo-melatonin is independent of the development of tolerance to PHNO cannot be presently discounted. It may represent an interaction with arousal or stress. If the melatonin hypothesis is accurate, pinealectomised rats will not develop nocturnal sensitization to continuous PHNO infusion. Indeed, rats exposed to continuous light, which results in a virtual pinealectomy, do not exhibit sensitization to PHNO or even acute stimulant effects of PHNO (14).

The development of both tolerance and sensitization to a continuously administered D_2 agonist may have important implications for schizophrenia. If schizophrenics have a continuously high activation of D_2 receptors, then tolerance may have developed. The reversal of tolerance into sensitization by even moderate levels of arousal or stress could explain the episodic nature of psychosis in schizophrenia. The possibility that tolerance to D_2 receptor activation in humans may be reversed by melatonin deserves consideration.

Patients with Parkinson's disease treated with dopaminergic drugs often exhibit fluctuations in the efficacy of these drugs. When treated with sustained release formulations of PHNO they rapidly develop tolerance to its therapeutic effects (2). Moreover, patients given 12-h infusions with lisuride, a D_2 receptor agonist, do not appear to develop tolerance. Rats given 12-h infusions of PHNO also do not develop tolerance. Parkinson's patients on 12-h lisuride infusions are less likely to suffer from psychiatric side effects than those given continuous infusions (16).

In conclusion, this study is consistent with the view that diurnal tolerance and nocturnal sensitization to the continuous administration of PHNO are mediated to a certain degree by circadian melatonin release. Low melatonin release in the day may produce a loss of behavioral effect of PHNO, and increasing melatonin release at night may result in an augmented effect of PHNO. However, it remains possible that melatonin potentiates the effects of arousal, without its presence necessarily regulating nocturnal sensitization or its absence regulating tolerance. Further experiments in pinealectomised rats would resolve this issue.

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