Pharmacology Biochemistry & Behavior Vol 38, pp 253–257 © Pergamon Press plc, 1991 Printed in the U S A 0091-3057/91 $3 00 + 00

Circadian Rhythms of Body Temperature and Drinking and Responses to Thermal Challenge in Rats After PCPA

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Received 3 August 1990

SATINOFF, E , S KENT, H LI, D MEGIRIAN AND J M TOMKOWIAK. *Circadian rhythms of body temperature and drinking and responses to thermal challenge in rats after PCPA* PHARMACOL BIOCHEM BEHAV **38**(2) 253–257, 1991 —Body temperature (Tb) and drinking were measured for five days in male and female rats On day 6 (S1) the rats were injected with saline On day 7 (P1) they were injected with PCPA (300 mg/kg IP) Measurements continued for 12 days Immediately after PCPA Tb dropped After that, the amplitude of the daily Tb rhythm was significantly decreased from days P2–P5 Females were more affected than males Nocturnality of drinking was decreased on days P2–P4 Because the peak of the Tb rhythm advanced after PCPA, while the peak of the drinking rhythm was delayed, we conclude that the attenuation of the Tb rhythm was a direct result of PCPA treatment rather than a masking effect due to the attenuation of other rhythms Other rats were thermally challenged during the first week post-PCPA. There were no differences in ability to regulate Tb in the cold, and the small variations in the heat were overshadowed by gender differences

Body temperature Circadian rhythms Drinking Cold challenge Heat challenge Serotonin depletion Rats Gender differences PCPA

SEROTONIN (5-HT) has been implicated in the control of thermoregulation since 1964, when Feldberg and Myers (7) suggested that body temperature (Tb) was regulated by the hypothalamic balance between 5-HT and norepinephrine. This theory soon proved to be overly simple The effects of 5-HT, indeed of most neurotransmitters and neuromodulators, depend on species, dose and route of administration (3) One method that has been commonly used to examine the role of 5-HT in thermoregulation is injection of parachlorophenylalanine (PCPA). PCPA inhibits tryptophan hydroxylase, the rate-limiting enzyme in the formation of 5-HT from tryptophan (15). An intraperitoneal dose of 300 mg/kg induces a total inhibition of tryptophan hydroxylase after 3 to 4 hours (25). The decrease of endogenous cerebral 5-HT reaches its minimum level by 36 hours (24) and it can take a week or more for brain 5-HT levels to return to normal (9). Catecholamines are also depleted, albeit to a lesser extent (18).

All of the above implies that there should be changes in Tb for several days after PCPA injections, yet several investigators have failed to find them. For example, at ambient temperatures (Ta's) of 19–23°C, there were no changes in Tb 24 (4) or 48 hours (13) after PCPA injection (300–315 mg/kg IP). Even where animals are thermally challenged, results have been minor. For instance, Giacchino et al (10) reported that PCPA-pretreated rats exposed to a Ta of 32°C for one hour, 48–96 hours after injection, had a greater increase in mean core Tb than did control rats, but the difference was only about 0.25°C. Others, challenging the rats with higher Ta's or higher doses of PCPA, sometimes reported hyperthermia above control levels (5,21) and sometimes did not (26). In the cold, the Tb of PCPA-pretreated rats dropped less than that of controls (10). All of these results imply that serotonergic pathways, insofar as 5-HT is depleted by PCPA, have little to do with thermoregulation. At most, it can be said that PCPA-pretreated rats do not lose heat to the environment as readily as do controls, and that 5-HT acts as a neuromodulator in thermoregulatory pathways rather than as a neurotransmitter transferring specific thermal information to effectors (27).

Another aspect of thermoregulation involves the rhythm of Tb After PCPA, daily rhythms of feeding, drinking, and motor activity are attenuated for several days (2). Rhythms of locomotor activity, ACTH and plasma corticosterone are also diminished (14,23). In the present report we measured Tb continuously for twelve days postinjection at Ta 23°C, and found clear abnormalities in the diurnal rhythm of Tb, which was larger in females than in males To see if this change in Tb rhythm had any functional significance for homeostasis, we tested rats at various intervals within the first week post-PCPA in both hot and cold environments. We found no differences in their ability to regulate their Tb in the cold, and any variations in the heat were overshad-

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owed by gender differences.

METHOD

General Design

Starting at least one week after surgery, Tb and drinking were measured for five days On day 6 (S1) the rats were injected with saline at the beginning of lights-on. On day 7 (P1) they were injected with PCPA methyl ester dissolved in sterile saline (300 mg/kg in 1 ml, IP) and monitoring continued for twelve more days A separate group of rats was housed at 23°C, injected with PCPA at the beginning of lights-on, and on alternate days beginning 24 h postinjection (P2), were placed in either cold (0°C) or hot (35°C) environments for three hours, while Tb was monitored. Control rats injected with an equal volume of saline were tested simultaneously

Subjects and Surgery

Subjects were male and female rats of the Sprague-Dawley strain that were 3 months old at the time of surgery. The rats were maintained under a 12 12 light/dark cycle at a Ta of 23 ± 1°C. Food and water were available ad lib. The rats were anesthetized with ketamine (80 mg/kg) and xylazine (Rompum; 15 mg/kg), a small (1–2 cm) incision was made in the peritoneal cavity and a temperature transmitter (Model M, Mini-Mitter Co., Sunriver, OR) was placed inside. The rats that were used for the rhythm studies weighed 238–320 g (males) and 225–250 g (females) at the time of surgery However, by the time the injection was made 1–2 weeks later, the male rats had gained so much more weight than the females that the body weight distributions no longer overlapped the weight range for the males was 332–405 g and for the females it was 267–310 g. The rats that were used in the cold and heat stress tests were four months old and weighed 370–514 g (males) and 260–377 g (females) at the time of the tests

Housing and Measurements

After surgery, the rats were housed individually in plastic cages (20 × 18 × 20 cm) containing a metal platform (12 × 10 × 2 cm) below the water bottle. The cages were placed in larger light-proof ventilated boxes Ta was 23 ± 0.5°C and lights were on from 08.00 to 20 00 hours.

Tb measurements for each animal (9 males and 9 females) began immediately after surgery and continued at 10-min intervals for the duration of the experiments Mini-mitters produce beats detectable by an AM radio. Beat frequency is proportional to surrounding temperature. There was a radio on the side of each cage which amplified and conditioned the signal before sending it to an Apple II+ computer. Data were stored on floppy disks for later statistical analyses. Drinking was measured by a standard drinkometer circuit Wires were connected to the metal platform on the cage floor and to the water bottle spout. The platform was situated such that to reach the water spout the rat had to step on the platform When it did, and licked at the spout, the circuit was closed. The number of licks was also summed and stored every 20 min.

Different rats were used to determine any inefficiency in the ability to maintain normal Tb in the face of heat (35°C) or cold (0°C) challenge Rats were implanted with telemetry devices as described above. At least one week after surgery one group was injected with PCPA (300 mg/kg IP; males, n = 6, females, n = 6) or an equivalent volume of saline (males, n = 5, females, n = 5) at the beginning of lights-on. [In ongoing work we have found that this dose of PCPA depletes hypothalamic 5-HT content by 66–69% (Li and Satinoff, unpublished).] Then, 24, 72 and 120 hours postinjection, they were placed at Ta 35 ± 1°C for three hours. Another group (PCPA, males, n = 4, females, n = 5, saline, males, n = 4, females, n = 5) was treated similarly, except that they were placed at Ta 0 ± 0 5°C for three hours Tb was monitored every ten min from the time of surgery for at least one week after injection

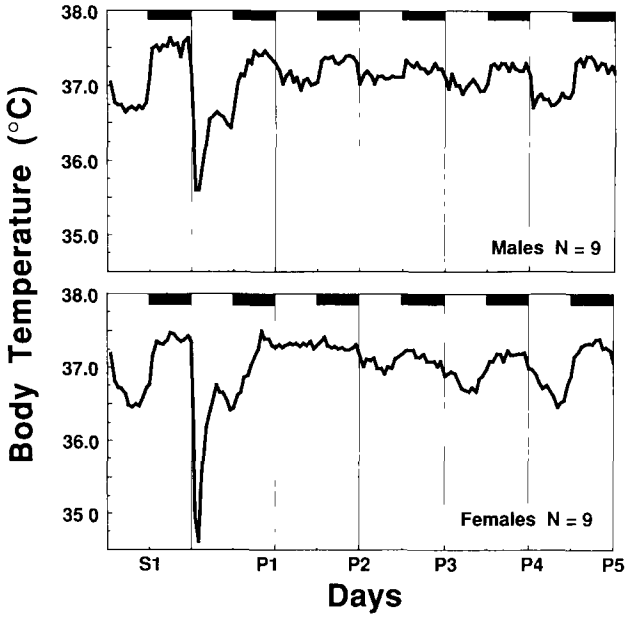

FIG 1 Mean hourly Tb for the day of saline injection (S1), PCPA injection (P1), and the next four days (P2–P5) Line at top indicates 12 h of lights-off Top males Bottom females For male SEM, minimum = 0 04, maximum = 0 22, mean = 0 11°C For female SEM, minimum = 0 07, maximum = 0 25, mean = 0 12°C

Data Analysis

For daily Tb rhythm analysis, means discussed in the text are the means of the Tb for each animal recorded every 10 min Means on the figures are hourly averages. For drinking, means discussed are the cumulated number of licks in 24 h A cosine wave was fitted to the Tb and drinking data to determine the acrophase (peak) of the daily rhythm. The percentage of drinking that occurred during the 12 h of lights-off was also computed. All data were analyzed with a 2 (sexes) × 8 (days) ANOVA with repeated measures on days Planned comparisons were conducted between each day post-PCPA and S1. For cold and heat challenge tests, data were analyzed in hourly means with a 2 (Ta) × 2 (sexes) × 2 (drug) × 3 (test) × 3 (hour) ANOVA with repeated measures on test and hour

RESULTS

Body Temperature Rhythms

Saline injections did not significantly affect Tb. Immediately post-PCPA injection, the mean minimum Tb (± sem) of the males dropped 1.2 ± 0.2°C to 34.9 ± 0.2°C (compared to the mean daily minimum of 36.2 ± 0.1°C for the week before the saline injection, $p < 0.0001$; Fig. 1, top) The Tb of the females dropped a mean of 2.1 ± 0.2°C to 34.0 ± 0 2°C, (compared to the previous week of 36 1 ± 0.1°C, $p < 0.0001$, Fig 1, bottom) The Tb of females dropped significantly more than did that of the males

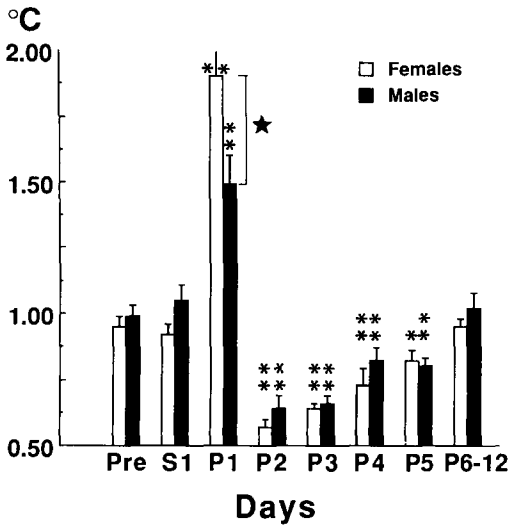

FIG 2 Tb amplitude (hourly means) for males and females $^{**}p<0.001$, $^{*}p<0.01$ compared to S1 $\bigstar p<0.001$, males vs females

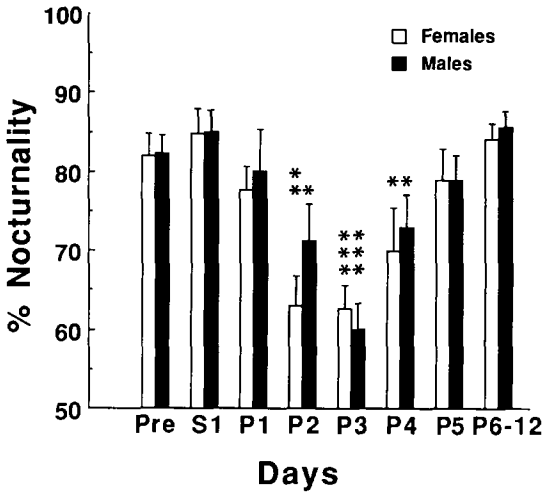

FIG 3 Percent nocturnality of drinking $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to S1

($p<0.005$). Tb returned to normal levels by 5–6 hours post-injection.

Because this initial drop and recovery, Tb amplitude was increased on P1, the day of the PCPA injection. After this day Tb's in the light were higher, and in the dark, lower than normal The net result was that the amplitude of the daily Tb ((Tb max − Tb min)/2) was significantly decreased in both sexes from days P2–P5. This flattening was caused both by an elevated Tb in the light, and a lowered Tb in the dark, which gradually returned to baseline. The recovery to normal levels is shown in Fig 2. Daily mean Tb did not change

Tb acrophase (the peak of the 24-h Tb curve as determined by cosinor analysis) on the day of saline injection was $267 \pm 3°$ (5 h, 48 min after lights-off; $15° = 1$ h, beginning of lights-on $= 0°$) for males and $277 \pm 3°$ (6 h, 28 min after lights-off) for females ($p<0.05$) The day after the PCPA injection (P2) the mean acrophase of males was advanced $27 \pm 8°$ compared to S1 (to 240°, $p<0.02$), $55 \pm 25°$ on P3 (not significant because the variance was so large, although all the rats showed a phase advance), and was nonsignificantly advanced by $19 \pm 15°$ on P4. It was meaningless to determine an acrophase for the females the day after the injection, because the Tb rhythm was so flat However, on P3, when a Tb rhythm was again discernible, the acrophase was advanced $80 \pm 17°$ compared to S1 (to 197°, $p<0.01$) It was also back to normal on P4.

Drinking Rhythms

Because the absolute amount of drinking varied between animals, we standardized the number of licks, with the presaline week equal to 100%. Drinking was slightly though not significantly reduced on P1. After this, both males and females increased the number of licks. For males the greatest increase was on P3 ($129 \pm 9\%$, $p<0.01$) and for females it was on P2 ($192 \pm 37\%$, $p<0.04$). In the week preinjection, and on S1, $82–86 \pm 3\%$ of drinking occurred in the dark (Fig. 3). Post-PCPA, percents of nocturnal drinking on P2 ($63–71 \pm 5\%$), P3 ($59–62 \pm 3\%$) and P4 ($68–73 \pm 5\%$) were decreased. There were no gender differences either in percent nocturnality or in lick acrophase. The only significant change in acrophase was on P3 males were delayed $47 \pm 11°$ ($p<0.01$) These changes were opposite those seen in Tb acrophase. For the females, the difference between lick and Tb acrophases on day S1 was $1 \pm 7°$, on P3 it was $102 \pm 29°$ ($p<0.003$). This means that on the day of the saline injection the peaks of Tb and drinking curves were within 4 min of each other; two days post-PCPA they were 6 h 45 min apart. For the males, the difference on S1 was $7 \pm 8°$, on P3 it was $90 \pm 34°$ ($p<0.03$).

Effects of PCPA on Tb During Cold or Heat Challenge

The effects of pretreatment with PCPA depended on the type of thermal challenge. In the cold, PCPA-pretreated rats were no different from controls (Fig. 4) However, 24 and 72 h postinjection, females maintained higher Tb's than did males ($p<0.001$) In the heat, on the other hand, PCPA-pretreated rats maintained a lower Tb than did the controls, regardless of gender ($p<0.03$,

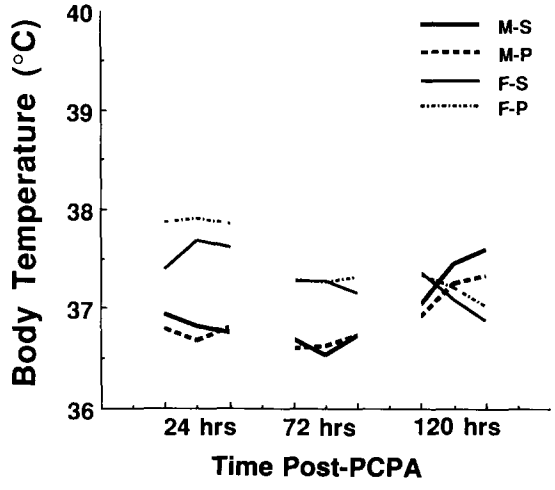

FIG 4 Mean hourly Tb of male and female rats at 0°C M = male, F = female, S = saline, P = PCPA Females maintained significantly higher Tb's than did males for the first two tests There were no significant differences between PCPA and saline groups

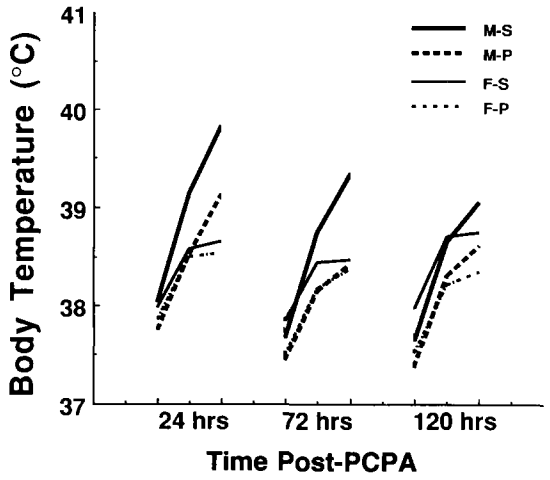

FIG 5 Mean hourly Tb of male and female rats at 35°C Symbols same as Fig. 4 PCPA-pretreated rats maintained lower Tb's than saline controls on all three tests ($p<0$ 03) Two male rats in the PCPA group and one in the saline group died after the first heat test Therefore, n = 6 for the males 24 h post-PCPA, and n = 4 thereafter

Fig 5). In addition, males showed a continuous increase in Tb, whereas the Tb's of female rats levelled off from the second to the third hour. For males 24 h posttreatment, mean Tb for h 1, (37.7 ± 0.1°C) vs. h 2, (38 6 ± 0.1°C), and h 2 vs. h 3 (39.1 ± 0 2°C) were significant at $p<0$ 0001 and $p<0$ 006, respectively. For female rats, h 1 mean Tb (37 8 ± 0.2°C) vs h 2 (38 4 ± 0.1°C), $p<0.0001$, h 2 vs. h 3 (38.5 ± 0.1°C), n.s. Consequently, males had higher mean Tb in the last hour of each test than did the females ($p<0.03$). Two of six PCPA-pretreated males and 1/5 controls died in the heat with Tb's above 41°C.

Within each of the four thermal challenge conditions, saline-cold, PCPA-cold, saline-heat, and PCPA heat, we pooled male and female rats and correlated their weights with their mean Tb's over 3 h for each test using Pearson correlation analysis. The only significant correlation was 24 h post-PCPA in the saline-heat condition heavier rats tended to have higher Tb's ($r = .74$, $p<0.02$).

DISCUSSION

The first result of this study was that PCPA caused hypothermia immediately postinjection, and females were more affected than males their Tb dropped a mean of 0 9°C lower This effect may be due to some unknown peripheral effect of the injection, alone or in combination with brain 5-HT depletion, since brain 5-HT is significantly lower than control levels even five h postinjection (19). In any case it is unlikely to be caused by a lowered thermal setpoint. We have found that rats given PCPA and immediately allowed to press a bar to change the ambient temperature, worked to counteract the hypothermia in a cold environment (Li and Satinoff, unpublished results) The second result is that in both sexes PCPA decreased the amplitude of the Tb rhythm on the second, third, and fourth day postinjection. Again, females were more affected than males on P2 their Tb rhythm was obliterated.

There were other gender differences With respect to Tb rhythms, females were worse off on P2 and had started to recover by P3. With respect to drinking, females mainly increased their fluid intake on P2, although the percent nocturnality was equally low on P2–P4 Males, on the other hand, showed the lowest amplitude Tb rhythms on both P2 and P3, but the strongest decline in drinking nocturnality on P3.

One possible reason for these differences is that PCPA might have depleted 5-HT more in females than in males. In other work on males of the same strain we have found that 300 mg/kg PCPA decreases brain 5-HT levels by 67% on P2 at Ta 20°C (Li and Satinoff, unpublished). It also decreased catecholamine levels, although to a lesser extent [Li and Satinoff, unpublished, (20)] We did not measure depletion in females and we know of no other papers that have done so Another reason might be that the PCPA was acting on preexistent 5-HT differences in the brains of males and females, of which there are many There are gender-specific changes in binding by 5-HT_1 receptors in separate areas of the brain (8) There is sexual differentiation of serotonergic control of anterior pituitary secretion (1) Hippocampal 5-HT synthesis is greater in female rats than in males and more decreased by the 5-HT_{1A} agonist 8-OH-DPAT (13) The suprachiasmatic nuclei, the area of the brain most responsible for circadian rhythm regulation, has very high concentrations of 5-HT (22), is sexually dimorphic (11), and shows differences in 5-HT uptake in slices taken from female and male brains (17).

It is impossible to tell from the present experiments whether the decreased Tb amplitude was a primary effect of 5-HT (and other amines) depletion or was due to masking. As mentioned earlier, rhythms of locomotor activity, eating, drinking and several hormones are attenuated for several days after PCPA treatment To the extent that the Tb rhythm is influenced by these behavioral and hormonal changes, it might be attenuated secondarily. However, the surprising finding that the peaks of the Tb and drinking rhythms were 6 h 45 min apart from each other in females and 6 h apart in males on P3, argues that PCPA was directly lowering Tb amplitude.

There were no effects of the PCPA treatment on ability of the rats to regulate Tb at 0°C. The only significant differences were between the genders 24 and 72 hours postinjection female rats maintained significantly higher Tb's than did males This agrees with previous work by McDonald et al. (16), who reported that female Fischer 344 rats withstood cold stress better than males did. Because oxygen consumption was the same in both sexes, the authors concluded that the females must have better heat conservation mechanisms. In another study (6), no significant differences were found between male and female Wistar rats during acute exposure to 0°C. However, these authors did find that female rats showed significantly less of an increase in oxygen consumption. Strain differences may account for these discrepancies, but the conclusion from both studies is the same Female rats respond to cold challenge more competently than do males, probably by having more efficient heat conservation mechanisms

In the heat all the females maintained their Tb between the second and third hour of the test The Tb of the males, on the other hand, continued to rise Doi and Kuroshima (6) also noted that females had less of an increase in heat production after exposure to 35°C If the same held true for the rats in the present study, it would explain why males' Tb increased more in the heat The Tb of all PCPA-pretreated rats was significantly lower than saline controls throughout the tests.

It might be supposed that the dissimilarities in body weight had something to do with the gender variations in Tb, but in fact the only significant correlation between body weight and Tb was in the heat the day after the saline injection: the Tb of the heavier rats rose higher In any case, it would be difficult to understand on the basis of body weight why females, who weigh less than males, should maintain a higher Tb in the cold, or why the PCPA-pretreated males should be able to maintain a lower Tb in the heat than saline-injected males

In summary, it appears that the diurnal rhythm of Tb can be interfered with without having much of an effect on homeostatic

regulation against heat or cold. These results, together with others showing attenuation of other rhythms, and little effect on thermal homeostasis, imply that incomplete depletion of brain 5-HT can modify the expression of diurnal rhythmicity of body temperature, but is not critical for thermal homeostasis.

ACKNOWLEDGEMENTS

Support to E S from NIMH Grant 1 RO1 MH 41138 is gratefully acknowledged S K was partially supported by NIMH Training Grant 1 T32 MH18412

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