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BRIEF COMMUNICATION

Sex Differences in Passive Avoidance Depend on the Integrity of the Central Serotonergic System

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HEINSBROEK, R. P. W., M. G. P. FEENSTRA, P. BOON, F. VAN HAAREN AND N. E. VAN DE POLL. *Sex differences in passive avoidance depend on the integrity of the central serotonergic system.* PHARMACOL BIOCHEM BEHAV 31(2) 499–503, 1988.—Effects of the neurotoxin para-chloroamphetamine (PCA) on sex differences in passive avoidance were studied. Seven days prior to passive avoidance training and testing, male and female rats were injected with PCA (5 mg/kg) or physiological saline (SAL). Treatment effects on brain monoamines levels were evaluated in brains collected shortly after the passive avoidance test. Compared to SAL-treated control groups PCA severely reduced both serotonin (5-HT) and 5-hydroxyindole-acetic acid (5-HIAA) in the frontal cortex of males and females. Levels of dopamine (DA) and homovanilic acid (HVA) in the frontal cortex were not affected. These data are indicative of a strong and selective depression of the central 5-HT activity. PCA- and SAL-treated male and female rats were trained and tested in a two-compartment step-through passive avoidance apparatus. Sex differences in passive avoidance were clearly observed in the SAL-treated control groups; a higher number of males did not enter either compartment within the maximum test duration. After PCA treatment sex differences in passive avoidance were abolished, mainly resulting from an increase in the number of PCA-males reentering. Irrespective of sex or treatment subjects seldom failed to choose the nonshock compartment when entering during the passive avoidance test, indicating that disturbance of memory or learning cannot explain for the present results. Rather, the data are discussed in terms of a sex-specific role of central 5-HT in punishment-induced behavioral suppression.

Passive avoidance Sex differences Serotonin Rats

MALE rats have consistently been found to show stronger and longer lasting behavioral suppression than female rats in passive avoidance procedures (6, 20–24). Female rats have been observed to be more active in lever-press avoidance (25) and two-way shuttle-box avoidance (2,15), tasks in which behavioral inhibition or freezing is likely to interfere with response acquisition (3). Recently, it was also found that exposure to a passive avoidance procedure resulted in a decrease in open field ambulation in male rats but not in female rats (7). Sex-dependent depression of activity has also been observed after 2 hours of immobilization stress; a stronger decrease of open field ambulation in male rats as compared to female rats (8). In general, behavioral inhibition as a consequence of stressful stimulation appears to be a more likely response in male rats as compared to female rats.

In addition to sex differences in behavioral effects of stressful stimuli, central serotonergic (5-HT) activity was found to be affected by stress in a sex-dependent way (8). Two hours restraint stress produced increased levels of the major 5-HT metabolite 5-hydroxyindole-acetic acid (5-HIAA) in both male and female rats. However, the 5-HIAA response in the frontal cortex was larger in males as compared to females. In addition, 30 minutes of footshock stress produced a strong 5-HIAA response in different parts of the female brain (including the frontal cortex), but not in the male brain (Heinsbroek *et al.*, submitted). Although this sex difference is in the opposite direction compared to immobilization stress both observations indicate that central 5-HT responses to stress may be sex-dependent. Sex differences in 5-HT activity under nonstress conditions have also been described. In general, higher levels of 5-HT and 5-HIAA were found in the brains of female rats compared to male rats (4), whereas, accumulating evidence suggests that the 5-HT synthetic capacity is also relatively higher in the female rat brain (4,5). Sex differences in central 5-HT activity and/or reactivity may be of particular relevance for sex differences in behavioral adaptation to stressful conditions (8).

Central 5-HT is thought to mediate behavioral inhibition occurring as a consequence of stressful stimulation (18). A decrement in passive avoidance behavior has been found

after manipulations which decrease central 5-HT activity (19), the effect could partially be antagonized by subsequently increasing central 5-HT activity (19). Alternatively, it has been proposed that the behavioral effects of manipulating central 5-HT do not reflect a potentiation or release of inhibition, but involve interference of learning and memory processes. This interpretation is based on behavioral studies in both active and passive avoidance procedures (1, 12, 13). Taken together, there is strong evidence in support of a role of central 5-HT in passive avoidance behavior. Sex differences in passive avoidance behavior may therefore be related to quantitative or qualitative sex differences in the role of central 5-HT in this particular behavior. To study this possibility, the passive avoidance behavior of male and female rats was examined under normal conditions and compared with the behavior after severely depressing the central 5-HT activity.

5-HT activity was depressed by intraperitoneal (IP) injection of the neurotoxin para-chloroamphetamine (PCA) 7 days prior to behavioral testing. PCA produces a long lasting and selective decrease in central 5-HT and 5-HIAA levels, a reduced uptake of 5-HT at nerve terminals and a reduced activity of tryptophan hydroxylase (9,14). These effects are probably related to the degeneration of nerve terminals and cell bodies (14). Effects of PCA on the passive avoidance behavior of male and female rats were studied in two-compartment variant of the step-through passive avoidance box (20). The two-compartment box not only provides an index of shock induced response suppression but also of the choice performance which offers an index of memory functioning. Sex differences in the two-compartment box have been studied (20); male rats again showed longer response latencies after shock compared to female rats. However, regardless of sex, subjects that reentered on te postshock trial seldom failed to choose the nonshock compartment, thus indicating that the relatively short response latencies of female rats cannot be interpreted as due to inferior memory capacities.

METHOD

Subjects

Eight-week-old Wistar rats (36 males and 36 females) were obtained from Animal House (TNO, Zeist, The Netherlands). They were housed in single-sex cages, 4 animals per cage, and were maintained under a reversed light-dark cycle (lights on from 3:30 p.m. to 3:30 a.m.). Food and water were available ad lib. Experimentation started 3–4 weeks after arrival in the laboratory, and took place during the final hours of the animals dark period. Preceding the experiment subjects were weighed twice a week in the testroom under the same light and background noise conditions present during experimentation.

Apparatus

Passive avoidance behavior was studied in a two-compartment passive avoidance apparatus, described in detail elsewhere (19). The apparatus consisted of 2 separate dark compartments of equal dimensions (40×40×40 cm). Both compartments were connected with a brightly lit (25 W bulb) platform, the compartments could be entered through guillotine doors (8×8 cm). The platform was 50 cm wide at its base and 10 cm wide at its end, the guillotine doors were spaced 45 cm apart (center to center). In order to optimize discrimination between the two compartments, one compartment (A) was fitted with an inside box consisting of a wiremesh floor and vertically striped (5 cm wide) black and white inside walls. The other compartment (B) had a grid floor and flat gray inside walls. Scrambled footshocks could be delivered through the grids of compartment B by a Grason-Stadler 700 constant current shock generator. Compartments A and B could be interchanged.

Procedure

On day one of the experiment subjects were adapted to the experimental apparatus. For half of the subjects compartment A was on the left side and compartment B was on the right side, for the other half of the subjects compartment A and B were interchanged. First, subjects were retained in compartment A for a period of 2 minutes. They were then replaced in their home-cage for 2 minutes , and were subsequently placed on the end of the platform facing away from entrances to compartment A and B. Only the entrance to compartment A was open and latency to enter this compartment was measured. After an additional 2 minutes this sequence was repeated for compartment B. On day 2 subjects were again put on the platform in the way described for day one. Only one compartment was accessible and the latency to enter this compartment was measured. This procedure was repeated 6 times with an intertrial interval of 2 minutes. The compartment which could be entered alternated on subsequent trials, compartment A being accessible on the first trial and the sequence ending with compartment B. After entering compartment B on the sixth trial, the door was closed and a 1 mA footshock was delivered for a period of 2 seconds. One hour after shock presentation subjects were replaced on the platform, with both entrances open. This postshock trial was ended after the subject had entered one of the compartments or after a maximum of 5 minutes had elapsed. Latency to enter a compartment as well as which compartment was entered were recorded.

Seven days preceding passive avoidance training and testing (day 2) male and female groups were subdivided in two groups of equal size (n=16). One group was injected intraperitoneally (IP) with 5 mg/kg para-chloroamphetamine hydrochloride (PCA, Sigma). The other group was injected (IP) with an equivalent volume (2 mg/kg) of physiological saline (SAL). In order to establish the effectiveness of the PCA injection, subjects were decapitated within 20 minutes after the final trial of passive avoidance testing. A frontal cortex section was removed from the brain by making a transverse section in a caudal to rostral direction through the frontal pole, ending at the most rostral part of the tuberculum olfactorium (mean weights, males: 77.4 mg ±1.9 s.e.; females: 69.0 mg ±2.2 s.e.). This section was quickly frozen on dry ice and was kept at −80 degrees celsius until assayed. Levels of serotonin (5-HT), 5-hydroxyindole-acetic acid (5-HIAA), dopamine (DA) and homovanilic acid (HVA) were determined in the frontal cortex using HPLC with electrochemical detection. Tissue samples were homogenized in 1 ml 0.1 M perchloric acid to which 25 microl 0.4 mM-ascorbic acid was added. After centrifugation (20 minutes, 5000 g, 4 degrees celsius) supernatants were injected directly in a Hewlett Packard Liquid Chromatograph, model 1090, with a 25 cm reverse phase column provided with a 2 cm guard column (Supelco 5C18-DB) and a Metrohm VA641 electrochemical detector. The mobile phase consisted of 0.3 mM-EDTA, 0.75

TABLE 1

CONCENTRATIONS (ng/g) OF SEROTONIN (5-HT), 5-HYDROXYINDOLE-ACETIC ACID (5-HIAA), DOPAMINE (DA) AND HOMOVANILIC ACID (HVA) IN THE FRONTAL CORTEX OF MALE AND FEMALE RATS DECAPITATED 7 DAYS AFTER INJECTION (IP) WITH 5 mg/kg PARA-CHLORAMPHETAMINE (PCA) OR PHYSIOLOGICAL SALINE (SAL)

	(N)	5-HT	5-HIAA	DA	HVA
Males					
SAL	16	421.8 (±9.7)*	218.2 (±4.8)	36.8 (±1.8)	20.8 (±1.4)
PCA	16	175.7 (±14.1)	107.4 (±7.9)	34.1 (±1.1)	19.4 (±1.5)
Females					
SAL	16	456.3 (±14.6)	247.2 (±13.7)	36.9 (±2.3)	21.4 (±1.6)
PCA	16	157.6 (±10.5)	106.1 (±7.3)	35.4 (±1.9)	23.2 (±1.4)

*S.E.M.

mM heptane sulfonic acid, 25 mM citric acid, 30 mM disodium phosphate and 12% methanol, pH was set at 4.15, flow rate was 1.0 ml/minute. The glassy carbon electrode was set at 800 mV versus the reference electrode.

RESULTS

Table 1 presents the concentrations of 5-HT, 5-HIAA, DA and HVA as measured in the frontal cortex shortly after passive avoidance testing 7 days after PCA or SAL injection. Data for each separate variable were subjected to analysis of variance with sex and treatment as main factors. Significant treatment effects were found for both 5-HT, $F(1,60)=481.02$, $p<0.001$, and 5-HIAA, $F(1,60)=194.50$, $p<0.001$; PCA resulted in a strong decrease of both 5-HT and 5-HIAA in the frontal cortex of both males and females (Table 1). In addition, a significant interaction between sex and treatment was observed for 5-HT, $F(1,60)=4.49$, $p<0.05$. Further analysis of the simple main effects revealed that the treatment effect was larger in the group of females [65.5% reduction; $F(1,60)=289.07$, $p<0.001$] as compared to the group of males [58.3% reduction; $F(1,60)=196.28$, $p<0.001$]. In addition, a marginal significant sex difference in 5-HT levels was found in the SAL groups, $F(1,60)=3.86$, $p<0.10$; SAL females having higher 5-HT concentrations than SAL males. The PCA effect on 5-HIAA also appeared to be sex-dependent (57% reduction in the females and 51% reduction in the males) and 5-HIAA levels were somewhat higher in the frontal cortex of SAL females as compared to SAL males (Table 1). However, these differences were not corroborated by a significant interaction between sex and treatment. Significant effects were not found for frontal cortex concentrations of DA or HVA.

The mean latencies to enter one of the compartments during preshock trials on day 1 and day 2 are shown in Fig. 1. Data for day 1 and day 2 were separately analysed using analysis of variance with sex, treatment and trial as main factors. Trial was a repeated measure having 2 levels on day 1 and 6 levels on day 2. Day 1 only revealed a significant interaction of treatment and trial, $F(1,60)=5.67$, $p<0.05$; during the second preshock trial response latencies increased in the PCA-treated groups and decreased in the SAL-treated groups (Fig. 1). Day 2 showed a significant effect of the main factors sex, $F(1,60)=5.83$, $p<0.05$, and trial, $F(5,300)=3.84$, $p<0.01$, and a significant interaction between sex and trial, $F(5,300)=4.14$, $p<0.01$. Response latencies increased in the males on consecutive trials but not in the females, regardless of treatment.

Data for the final preshock trial (learning trial) and for the postshock retention trial are summarized in Table 2. Postshock data were analysed according to the criterion of entering or not entering within the maximal test duration. Subjecting these dichotomous data to an analysis of variance (10), with sex and treatment as main factors, produced a significant effect of sex, $F(1,60)=7.88$, $p<0.01$; more females reentered a compartment than males. In addition, a marginally significant interaction between sex and treatment was found: $F(1,60)=3.21$, $p<0.10$.

Inspection of Table 2 shows that PCA appeared to specifically increase the number of males who reentered one of the compartments, resulting in a dramatic reduction of the difference between the sexes observed in the SAL groups. Further analysis of simple main effects corroborated this interpretation by showing a strongly significant sex difference in the SAL groups, $F(1,60)=10.54$, $p<0.005$, but not in the PCA groups, $F(1,60)=0.54$, n.s. In addition, treatment was marginally significant in males, $F(1,60)=3.25$, $p<0.10$, but not in females, $F(1,60)=0.54$, n.s. Group differences in postshock latencies were also evaluated by applying Mann-Whitney U-test comparisons (16), including cut-off latencies. Sex differences were again observed in the SAL-treated groups ($z=-2.85$, $p<0.005$), females having shorter reentering latencies as compared to males (Table 2). In contrast, a statistically significant difference between PCA males and PCA females was not found ($z=-1.57$, $p>0.10$). Differences in response latencies between SAL-treated and PCA-treated groups were not statistically significant in the males ($z=-1.17$) nor in the females ($z=-0.02$). Finally, data on the choice performance are also presented in Table 2. Only a small proportion of subjects that reentered a compartment chose the one in which they were previously shocked.

DISCUSSION

The present experiment studied the effects of the 5-HT neurotoxin PCA on choice passive avoidance behavior of male and female rats. In the control groups treated with vehicle solution, the normal pattern of sex differences was found; male rats showed more response suppression than female rats. However, sex differences were abolished after PCA treatment. PCA effectively reduced 5-HT and 5-HIAA

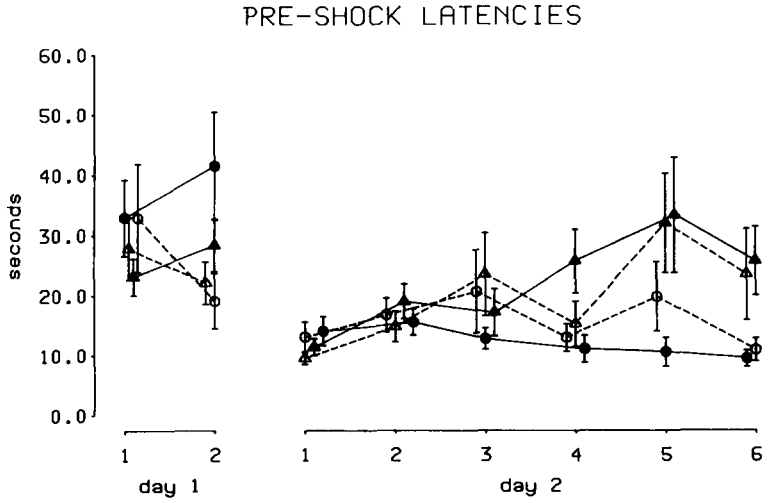

FIG. 1. Mean entering latencies (±S.E.M.) during preshock trials for males (triangles) and females (circles), injected with 5 mg/kg para-chloroamphetamine (dark symbols) or physiological saline (open symbols).

levels in the frontal cortex of both sexes. The PCA effect was somewhat stronger in females as compared to males. Nevertheless, PCA appeared to affect the behavior of male rats preferentially, increasing the number of males reentering the nonshock compartment on the postshock trial.

Short-term behavioral and biochemical effects of PCA can clearly be differentiated from long-term PCA effects (14). Short-term effects, observed within the first few days after PCA treatment, involve changes in both 5-HT and catecholaminergic (CA) variables. While reductions in 5-HT and 5-HIAA levels are maintained, effects on CA variables are only marginal after longer intervals (9, 11, 14). The present data confirm these findings: severe depletions of 5-HT and 5-HIAA were seen 7 days after PCA injection, whereas DA and HVA contents were not affected. The reductions in 5-HT and 5-HIAA as a consequence of PCA were somewhat larger in females as compared to males. Control females were also found to have slightly higher levels of 5-HT and 5-HIAA in the frontal cortex. Female rats have generally been reported to have higher brain levels of 5-HT and 5-HIAA than male rats (4). In addition, the synthetic capacity of 5-HT appears to be higher in the female brain (4,5). Perhaps this latter sex difference is related to the observed sex-dependent effects of PCA.

Long-term effects of PCA treatment resulted in an elimination of sex differences in passive avoidance which was clearly observed in the SAL-treated control groups. The data indicate that this effect was mainly a consequence of a PCA-induced attenuation of the response inhibition in male rats. Although the effects of PCA treatment were only marginally significant in males, they were clearly more salient in males as compared to females. It may be argued that the lack of finding an effect of PCA treatment in females can be attributed to the relatively low level of response inhibition generally found in this sex, thus leaving little room for a reduction in response inhibition. However, comparing preshock and postshock entering latencies of female groups shows a large increase, indicating a broad range to measure behavioral effects of PCA. Nevertheless, in females PCA neither affected the number of subjects reentering nor the response latency during the retention trial. Irrespective of sex or treatment, only a small proportion of subjects that reentered chose the compartment in which they were previously shocked. Reducing central 5-HT activity by lesioning the median raphe nucleus was found to disrupt spatial discrimination (17). Clearly, spatial discrimination was not affected by PCA in the present experiment. Therefore, the behavioral effects found after depletion of central 5-HT did not involve disturbance of learning or memory functions. Alternatively, reducing central 5-HT activity may have attenuated the behavioral suppression normally observed in this task. Central 5-HT

TABLE 2

MEDIAN AND RANGE OF THE ENTERING LATENCIES MEASURED ON THE LEARNING TRIAL AND ON THE RETENTION TRIAL AS WELL AS THE NUMBER OF ANIMALS REENTERING OR NOT REENTERING ON THE RETENTION TRIAL

	(N)	Learning Trial	Retention Trial	S*	NS†	NE‡
Males						
SAL	16 med	14.5	300	1	3	12
	range	2–120	49–300			
PCA	16 med	16.5	186	0	9	7
	range	2–74	53–300			
Females						
SAL	16 med	9	84.5	2	11	3
	range	2–30	2–300			
PCA	16 med	8.5	108.5	1	10	5
	range	2–24	2–300			

*Shock compartment.
†No-shock compartment.
‡Not entering either one compartment.

has been proposed to mediate punishment-induced behavioral inhibition (18). Moreover, the sex difference in passive avoidance behavior appears to involve a relatively stronger behavioral suppression observed in male rats (20–23). The present findings that reducing central 5-HT activity in male and female rats eliminates sex differences in passive avoidance therefore strongly suggest that the role of 5-HT in mediating behavioral inhibition is sex-dependent.

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