European Journal of Pharmacology, 111 (1985) 211–220
Elsevier

MATERNAL AGGRESSION IN MICE: EFFECTS OF TREATMENTS WITH PCPA, 5-HTP AND 5-HT RECEPTOR ANTAGONISTS

JOHN R. IENI * and JOHN B. THURMOND

Neuropsychopharmacology Program, Department of Psychology, University of Louisville, Louisville, Kentucky 40292, U.S.A.

Received 7 November 1984, revised MS received 8 January 1985, accepted 12 February 1985

J.R. IENI and J.B. THURMOND, *Maternal aggression in mice: effects of treatments with PCPA, 5-HTP and 5-HT receptor antagonists*, European J. Pharmacol. 111 (1985) 211–220.

Drug treatments which influence brain serotonergic systems were administered to lactating female mice during the early postpartum period, and their effects on aggressive behavior, locomotor activity and brain monoamines were examined. P-chlorophenylalanine (200 and 400 mg/kg) and 5-hydroxytryptophan (100 mg/kg) inhibited fighting behavior of postpartum mice toward unfamiliar male intruder mice. These drug-treated postpartum females showed increased latencies to attack male intruders and also reduced frequencies of attack. In addition, postpartum mice treated with the serotonin receptor antagonists, mianserin (2 and 4 mg/kg), methysergide (4 mg/kg) and methiothepin (0.25 and 0.5 mg/kg), displayed significantly less aggressive behavior than control mice, as measured by reduced number of attacks. Whole brain monoamine and monoamine metabolite levels were measured after drug treatments. The behavioral results are discussed in terms of drug-induced changes in brain chemistry and indicate a possible role for serotonin in the mediation of maternal aggressive behavior of mice.

Maternal aggression Locomotor activity 5-HT receptor antagonists Serotonin Monoamines PCPA

1. Introduction

Postpartum females of various mammalian species including mice, hamsters and rats display intense aggressive behavior towards unfamiliar animals (see Svare, 1977, 1981, for review). Generally the aggressive behavior is displayed most intensely during the early lactation period, then declines over the latter days of the lactation period (Svare and Gandelman, 1973; Erskine et al., 1978).

Offspring are necessary stimuli for maintenance of this behavior. Removal of pups from the nest for 4-5 h attenuated the aggressive behavior of postpartum females (Svare and Gandelman, 1973; Erskine et al., 1978). Replacing these offspring in the nest for only 5 min restored the maternal fighting response (Svare and Gandelman, 1973). Thus, short-term maintenance of maternal fighting behavior may require the presence of offspring, but direct physical contact may not be crucial (Svare, 1981).

Suckling by offspring appears to activate the aggressive behavior of postpartum animals (Svare, 1977, 1981). Female mice thelectomized (nipple removal) during pregnancy failed to display aggression during the postpartum period although these female mice maintained litters. Thelectomy performed 24 h after birth, but not 48 h after birth significantly reduced maternal aggression (Svare and Gandelman, 1976). In addition, when suckling stimulation was prevented by removal of pups immediately after birth, postpartum aggression was blocked (Gandelman and Simon, 1980). These studies suggest a critical period of suckling exposure (24-48 h postpartum) which is necessary for initiation of maternal aggressive behavior (Svare and Gandelman, 1976; Svare, 1977).

* To whom all correspondence should be addressed at present address: Department of Psychiatry, Rm. G49E Bldg. F, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, N.Y. 10461, U.S.A.

Suckling may activate transmitter systems in the central nervous system (CNS) which innervate structures implicated in aggressive behavior. However, little is known of transmitter systems which control maternal aggressive behavior. Suckling activity has been reported to increase hypothalamic dopamine (DA) synthesis (Voogt and Carr, 1974) and increase serotonin (5-HT) release (Mena et al., 1976). Both DA and 5-HT have been implicated in the regulation of aggressive behavior of male rodents (Barr et al., 1979; Kostowski and Valzelli, 1974; Kantak et al., 1981). Svare (1983) found that treatment of postpartum mice with para-chlorophenylalanine (PCPA), suppressed maternal fighting. Since it has been demonstrated that suckling-induced release of prolactin from the pituitary involves activation of brain 5-HT neurons (Kordon et al., 1973; Mena et al., 1976), perhaps suckling activates aggressive behavior of postpartum mice through a serotonergic mechanism.

The purpose of this research was focused on further characterizing the potential role for 5-HT in maternal aggression. In experiment 1, aggressive behavior and locomotor activity, as well as whole brain monoamines, norepinephrine (NE), DA and 5-HT, and their metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were measured in postpartum mice treated with PCPA and/or 5-hydroxytryptophan (5-HTP). In experiment 2, the acute effects of treatment with three purported 5-HT receptor antagonists, mianserin (MIAN), methysergide (METHY) and methiothepin (METHIO) on aggressive behavior, locomotor activity and brain monoamines as well as their metabolites were examined.

2. Materials and methods

2.1. Animals

Nulliparous CD-1 female mice (70-150 days of age) that were either bred in this laboratory or purchased from Charles River Breeding Labs (Wilmington, MA) were used for all experiments. No brother-sister matings were permitted. CD-1 male mice (Charles River, Wilmington, MA) were used either as studs or intruders. Males used as studs were never used as intruders.

2.2. Mating process

All nulliparous females were mated in community cages (3-4 females and 1 male). Females were checked daily for vaginal plugs. When plugs were found, the females were removed from the group cages, and individually housed in transparent plastic mouse cages (Lab Products, Inc., Rochelle Park, NJ). Pregnant females were housed in a separate air-conditioned vivarium (25°C) with a 12 h day/12 h night cycle; night commenced at 0800 h. Pregnant females were given shredded paper for nest-building several days prior to pup delivery. The date of delivery was recorded and designated as postpartum day zero (PP-0). Litters were culled to eight pups at birth.

2.3. Behavioral tests

Prior to testing, the cage containing the dam and pups was removed to an adjoining testing room. The aggression test began when the experimenter placed an intruder male into the dam's home cage. Latency for the postpartum female to attack the intruder and total number of attacks during the 5 min test period were recorded. Attacks consisted of either lunging at the intruder with mouth open or biting the intruder mouse. Intruders were used for a maximum of four aggression tests and never used twice with the same experimental postpartum female. Locomotor activity was assessed in an automated motimeter apparatus (see Kramarcy et al., 1984, for description). Activity levels consisted of total counts during a 10 min period. All testing was done during the first half of the dim(active) portion of the light/dark cycle.

2.4. Drug treatments and procedure

PCPA methyl ester HCl (Sigma), 200 and 400 mg/kg and 5-HTP ethyl ester HCl (Sigma), 50 and 100 mg/kg, were dissolved in 0.9% saline (SAL). Mianserin maleate (Organon), 1, 2 and 4 mg/kg, and METHY (Sandoz), 1, 2 and 4 mg/kg,

were dissolved in distilled water (H_2O). Methiothepin maleate (Roche), 0.125, 0.25 and 0.5 mg/kg, was dissolved in 0.4% methyl cellulose (VEH) (Sigma). All drugs were administered intraperitoneally. Drug doses are expressed as the free base except for METHY and METHIO which are expressed in salt form.

In experiment 1, postpartum females were given daily doses of PCPA or SAL from PP-1 through PP-8. On postpartum days when behavioral measures were taken, PCPA or SAL were injected after completion of behavioral tests. On PP-5, the SAL and the two PCPA groups were subdivided into three groups which would receive either low or high doses of 5-HTP or SAL. Animals were tested for maternal aggression on PP-5, PP-7, and PP-9, 1 h after their respective SAL or 5-HTP challenge. Locomotor activity measures were recorded on PP-6 and PP-8, 1 h after 5-HTP or SAL challenge. Immediately following the aggression test on PP-9, 5 animals from each of the 9 groups were sacrificed for biochemical determinations (see below).

In experiment 2 (treatment with 5-HT receptor antagonists), postpartum females were injected with SAL on PP-5, pretested for aggression 30 min later, and tested for locomotor activity 90 min post-injection. Only those dams that displayed aggressive behavior were assigned to a drug treatment condition. Animals were assigned to treatment groups in order to attain similar mean pretest aggression scores for all groups. On PP-7, animals were injected with their respective drug or vehicle (H_2O or VEH), tested for aggression 30 min later, and tested for activity 90 min post-injection. Immediately after the activity test, 5 animals from each of the 12 treatment groups were sacrificed for biochemical determinations.

2.5. Biochemical analysis

Animals were sacrificed by cervical dislocation and their brains quickly removed and rinsed in ice-cold saline (0.9%). Brains were dissected into two hemispheres, weighed and frozen (−20°C) until assayed (less than 72 h later). Half-brains were assayed for NE and DA using the high pressure liquid chromatography with electrochemical detection (LC-EC) procedure of Wagner et al. (1979). The analytical column used was a BIO-RAD ODS-5S reverse phase column (250 × 4 mm). The detector potential was set at 0.9 mV versus Ag/AgCl reference electrode. Mobile phase was composed of 7 mM phosphate/15 mM citrate buffer with 5% methanol and 25-50 mg of sodium octyl sulfate as the ion pair agent (pH 3.85). The remaining half-brains were assayed for DOPAC, HVA, 5-HIAA and 5-HT according to the LC-EC procedure of Perry and Fuller (1979). The column used was a C6 HiCHROM reversible column (250 mm, Regis Chemical Co.). The detector potential for the carbon paste electrode was set at 0.9 mV vs. Ag/AgCl reference electrode. The mobile phase was composed of 0.1 M phosphate/0.05 M citrate buffer with 10% methanol (pH 4.80).

2.6. Statistical analysis

Behavioral data obtained from the PCPA-5-HTP experiment were analyzed using a three factor, PCPA × 5-HTP × TRIALS, analysis of variance (ANOVA) with repeated measures on the TRIALS factor. Significant main effects and interactions were probed using the post-hoc Newman-Keuls test. Behavioral data from animals treated with the 5-HT receptor antagonists were analyzed using two factor, DRUG × DOSE, analysis of covariance (ANCOVA) with pretest scores on the behavioral measures as the covariates. Only comparisons (a priori) of a specific drug treatment with its respective control were examined based on the procedure for planned comparisons described by Winer (1971). Biochemical data were analyzed using ANOVA. A priori planned comparisons of drug treatment effects on brain monoamine and metabolite levels with the levels from the specific control groups were analyzed (Winer, 1971).

3. Results

3.1. Effects of PCPA and/or 5-HTP on aggressive behavior

Fig. 1 illustrates that PCPA increased attack latency in a dose-dependent manner. The 100

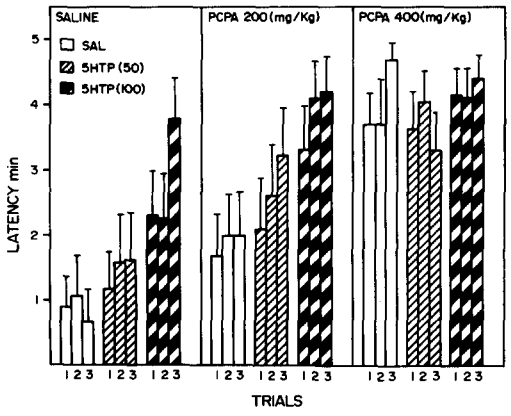

Fig. 1. Mean latency to attack for postpartum mice treated with PCPA and/or 5-HTP. Each bar represents the mean and S.E.M. for that trial. Each group represents data from 10 animals. Significant effects of PCPA $F(2,81) = 12.8$, $P < 0.001$), 5-HTP ($F(2,81) = 6.56$, $P < 0.0024$) and TRIALS $F(2,162) = 3.59$, $P < 0.03$) were observed. All interactions were non significant. The effects of PCPA on attack latency were dose-dependent (P's < 0.01, Neuman-Keuls test) while only the 100 mg/kg dose of 5-HTP increased attack latency ($P < 0.01$, Newman-Keuls test). Latency scores for the drug treatment groups were significantly longer on trial 3 than trial 1 ($P < 0.05$, Newman-Keuls test).

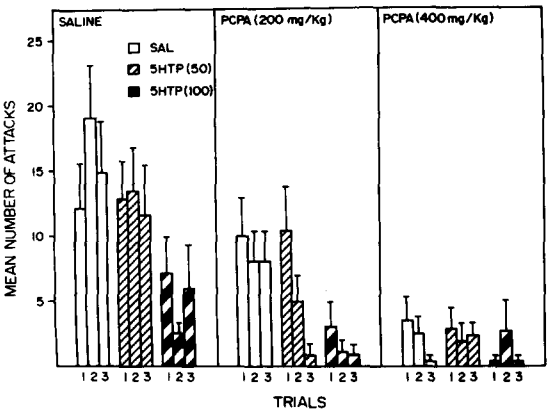

Fig. 2. Mean number of attacks for postpartum mice treated with either PCPA and/or 5-HTP. Each bar represents the mean and S.E.M. for that trial. Each group represents data from 10 animals. Only significant PCPA ($F(2,81) = 13.19$, $P < 0.001$) and 5-HTP ($F(2,81) = 5.83$, $P < 0.005$) effects were observed. PCPA (200 and 400 mg/kg) and 5-HTP (100 mg/kg) significantly reduced attack behavior of postpartum mice (P's < 0.01, Newman-Keuls test).

mg/kg dose of 5-HTP was also effective in increasing the latency to attack. These drugs seemed to have cumulative suppressive effects on aggressive behavior since the latency scores of the treatment groups were significantly longer on trial 3 (PP-9) than on trial 1 (PP-5). The effects of PCPA and 5-HTP on number of attacks are presented in fig. 2. The 100 mg/kg dose of 5-HTP as well as both doses of PCPA were effective in suppressing the number of attacks emitted by postpartum mice.

3.2. Effects of PCPA and/or 5-HTP on locomotor activity

As may be seen in fig. 3, both doses of PCPA had activity reducing effects only on trial 2 (PP-8). Only the 100 mg/kg dose of 5-HTP reduced activ-

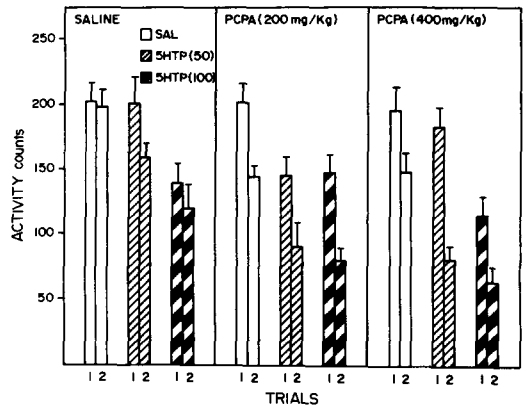

Fig. 3. Mean activity scores for postpartum mice treated with PCPA and/or 5-HTP. Each bar represents the mean and S.E.M. for that trial. Each group represents data from 10 animals. Analysis of activity data yielded significant effects of PCPA ($F(2,81) = 8.01$, $P < 0.001$), 5-HTP ($F(2,81) = 22.63$, $P < 0.001$) and TRIALS ($F(1,81) = 73.73$, $P < 0.001$) as well as a TRIALS × PCPA interaction ($F(2,81) = 5.26$, $P < 0.007$). The interaction was probed using procedures outlined by Winer (1971, p. 563). Post hoc analysis (Newman-Keuls test) revealed that on trial 1, 5-HTP (100 mg/kg) lowered activity when compared to 5-HTP (50 mg/kg) ($P < 0.01$) and SAL ($P < 0.01$). However, 5-HTP (50 mg/kg) did not significantly reduce activity ($P > 0.05$). PCPA treatment did not affect activity levels on trial 1. For trial 2, both PCPA, 200 and 400 mg/kg, decreased activity compared to SAL ($P < 0.01$), but PCPA (200 mg/kg) and PCPA (400 mg/kg) did not differ ($P > 0.05$). Both 5-HTP, 50 and 100 mg/kg, lowered activity on trial 2 compared to SAL ($P < 0.01$) but 5-HTP (50 mg/kg) and 5-HTP (100 mg/kg) did not differ ($P > 0.05$).

ity on trial 1 (PP-6), while both doses of 5-HTP reduced activity on trial 2 (PP-8).

3.3. Effects of 5-HT receptor antagonists on aggressive behavior

Fig. 4 reveals that only the 0.5 mg/kg dose of METHIO was effective in increasing latency to attack scores when compared to VEH. However, the 5-HT receptor antagonists generally had anti-aggressive effects as measured by number of attacks. MIAN (2 and 4 mg/kg) significantly depressed attack scores. The middle and high doses of METHIO also reduced number of attacks, while only the high dose of METHY lowered attack scores (fig. 5).

3.4. Effects of 5-HT receptor antagonists on locomotor activity

The data presented in fig. 6 illustrate the suppressive effects of 5-HT receptor antagonists on locomotor activity. The highest dose of MIAN and the middle dose of METHY reduced locomotor activity. Both the 0.25 and 0.50 mg/kg doses of METHIO suppressed activity. Several animals in

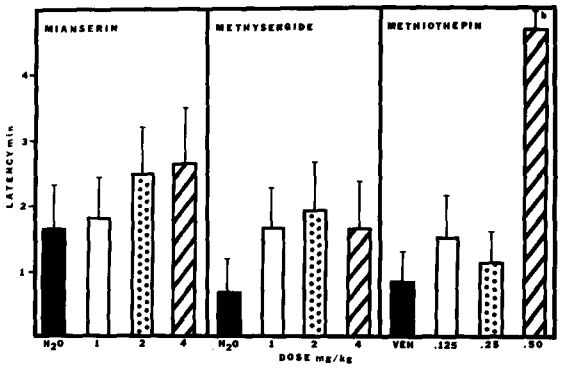

Fig. 4. Post-test latency to attack scores for postpartum mice treated with 5-HT receptor antagonists. Each bar represents the mean and S.E.M. for n = 10 animals. Two factor, DRUG × DOSE, ANCOVA yielded only a significant DOSE effect ($F(3, 107) = 5.52$, $P < 0.001$). Planned comparisons, described by Winer (1971), revealed that only methiothepin (0.50 mg/kg) significantly elevated latency to attack compared to VEH ($F(1,107) = 18.57$, $P < 0.01$). All other drug treatment conditions were without effect on attack latency (comparisons made with their respective control groups). b = $P < 0.01$.

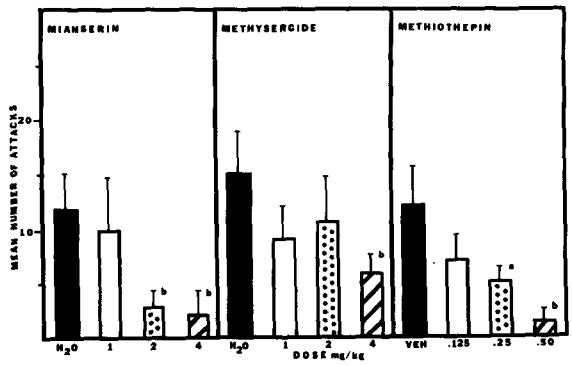

Fig. 5. Post-test number of attack scores for postpartum mice treated with 5-HT receptor antagonists. Each bar represents the mean and S.E.M. for n = 10 animals. Overall ANCOVA yielded significant effects due to DRUG ($F(2,107) = 4.62$, $P < 0.012$) and DOSE ($F(3,07) = 10.15$, $P < 0.001$). Planned comparisons revealed the following effects: mianserin (2 and 4 mg/kg) significantly reduced number of attacks ($F(1,107) = 9.20$ and $F(1,107) = 10.09$, $P < 0.01$ compared to H_2O control, respectively). Only methysergide (4 mg/kg) significantly lowered attack scores ($F(1,107) = 9.61$, $P < 0.01$, compared to its H_2O control). Both methiothepin (0.25 and 0.50 mg/kg) significantly reduced attack scores ($F(1,107) = 4.62$, $P < 0.05$, and $F(1,107) = 8.62$, $P < 0.01$, compared to VEH, respectively). a = $P < 0.05$; b = $P < 0.01$ compared to respective control group.

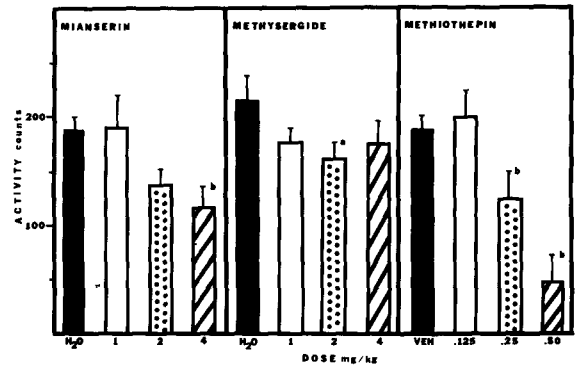

Fig. 6. Post-test locomotor activity scores for postpartum mice treated with 5-HT receptor antagonists. Each bar represents the mean and S.E.M. for n = 10 animals. The overall ANCOVA revealed effects due to DRUG ($F(2,107) = 4.21$, $P < 0.02$), DOSE ($F(3,107) = 16.52$, $P < 0.001$) as well as a significant DRUG × DOSE interaction ($F(6,107) = 2.64$, $P < 0.02$). Planned comparisons revealed that MIAN (4 mg/kg) ($F(1,107) = 8.23$, $P < 0.01$) and methysergide (2 mg/kg) ($F(1,107) = 5.27$, $P < 0.05$) reduced locomotor activity compared to their respective H_2O controls. The 0.25 and 0.50 mg/kg doses of methiothepin also significantly depressed activity ($F(1,107) = 9.71$, $P < 0.05$, and $F(1,107) = 37.30$, $P < 0.01$ compared to VEH). a = $P < 0.05$; b = $P < 0.01$ compared to respective control group.

the 0.50 mg/kg METHIO group displayed cataleptic responses. The mean activity scores for this group were reduced to 23% of pre-test activity levels.

3.5. Effects of PCPA and/or 5-HTP on brain monoamines and metabolites

The most consistent and profound effects of PCPA and 5-HTP were observed on brain 5-HT and 5-HIAA levels (table 1). PCPA effectively depleted whole brain 5-HT and 5-HIAA and 5-HTP effectively elevated whole brain 5-HT and 5-HIAA levels. 5-HTP was effective in reversing the serotonergic depletion effects of PCPA. In fact, the 100 mg/kg dose of 5-HTP restored 5-HT to normal levels and elevated 5-HIAA levels beyond control levels in animals previously treated with PCPA. In addition, several PCPA and 5-HTP treatment groups had altered levels of catecholamines and/or their metabolites. Most notably, several PCPA groups had depressed DOPAC levels, and the 50 mg/kg 5-HTP groups had elevated DA levels, regardless of PCPA treatment. No drug treatment altered brain HVA, whereas NE levels were depressed only in the 400 mg/kg PCPA group.

3.6. Effects of 5-HT receptor antagonists on brain monoamines and metabolites

Few biochemical effects were observed in response to treatment with 5-HT receptor antagonists (table 2). The 2 mg/kg dose of METHY elevated 5-HT levels and the low dose of METHIO significantly decreased brain 5-HT levels. No changes in whole brain 5-HIAA were observed as a result of treatment with any 5-HT receptor antagonist. The highest doses of both MIAN and METHY depressed NE, while only the highest dose of METHY decreased DA. The most profound biochemical effects observed were those of METHIO

TABLE 1

Whole mouse brain levels of monoamines and their metabolites from postpartum mice treated with PCPA and/or 5-HTP. The overall PCPA×5-HTP ANOVAs for each transmitter and metabolite are presented as follows: NE, PCPA effect ($F(2,36) = 3.85$, $P < 0.03$); DA, 5-HTP effect ($F(2,36) = 11.32$, $P < 0.001$) and PCPA×5-HTP interaction ($F(4,36) = 5.27$, $P < 0.002$); DOPAC, PCPA effect ($F(2,36) = 5.27$, $P < 0.01$); HVA, PCPA effect ($F(2,36) = 3.38$, $P < 0.05$); 5-HT, PCPA effect ($F(2,36) = 68.63$, $P < 0.001$), 5-HTP effect ($F(2,36) = 81.73$, $P < 0.001$) and PCPA×5-HTP interaction ($F(4,36) = 9.21$, $P < 0.001$); and 5-HIAA, PCPA effect ($F(2,36) = 13.39$, $P < 0.001$), 5-HTP effect ($F(2,36) = 201.26$, $P < 0.001$) and PCPA×5-HTP interaction ($F(4,36) = 3.77$, $P < 0.012$). Planned comparisons between each treatment and SAL control were performed for each transmitter and metabolite.

Group (n = 5)	NE	DA	DOPAC	HVA	5-HT	5-HIAA
SAL	413.0 [a]	1166.4	244.7	285.5	717.4	709.2
	24.7	65.7	26.2	25.5	41.9	44.5
5-HTP (50)	412.7	1520.6 [c]	232.9	279.9	969.6 [c]	1657.3 [c]
	24.7	97.3	11.3	32.0	41.5	109.2
5-HTP (100)	438.4	1316.3	178.8 [b]	326.9	943.8 [c]	2104.8 [c]
	19.6	30.2	7.8	15.7	47.5	198.5
PCPA (200)	441.0	1292.5	245.3	222.8	403.5 [c]	198.5 [c]
	19.9	41.2	29.5	19.4	15.8	26.7
PCPA (200)	402.7	1209.9	162.1 [c]	271.7	521.7 [c]	981.8
5-HTP (50)	9.4	6.7	19.7	21.4	26.0	58.6
PCPA (200)	422.5	1234.5	212.3	253.0	784.0	2078.9 [c]
5-HTP (100)	18.0	14.3	16.6	14.0	47.0	156.8
PCPA (400)	351.9 [b]	1146.0	171.3 [b]	285.7	176.3 [c]	93.2 [c]
	12.3	30.8	18.8	30.7	28.4	6.8
PCPA (400)	409.0	1535.8 [c]	170.0 [b]	279.8	578.0 [b]	1059.8 [b]
5-HTP (50)	14.9	95.9	2.6	23.9	46.3	120.0
PCPA (400)	395.1	1299.9	168.1 [c]	235.1	815.7	2149.4 [c]
5-HTP (100)	17.3	53.5	23.7	16.7	51.9	98.4

[a] Mean ± S.E.M. (ng/g tissue). [b] $P < 0.05$ compared to SAL. [c] $P < 0.01$ compared to SAL.

TABLE 2

Whole mouse brain levels of monoamines and their metabolites from postpartum mice treated with 5-HT receptor antagonists. The overall DRUG × DOSE ANOVAs for each transmitter and metabolites are as follows: NE, DOSE effect ($F(2,48) = 3.27$, $P < 0.03$); DA, DRUG × DOSE interaction ($F(6,48) = 2.86$, $P < 0.02$); DOPAC, DRUG ($F(2,48) = 24.49$, $P < 0.001$), DOSE ($F(3,48) = 4.85$, $P < 0.005$) and DRUG × DOSE interaction ($F(6,38) = 5.76$, $P < 0.001$); HVA, DRUG effect ($F(2,48) = 18.72$, $P < 0.001$ and DOSE effect ($F(3,48) = 3.70$, $P < 0.02$); 5-HT, DRUG effect ($F(2,48) = 5.83$, $P < 0.005$) and DRUG × DOSE interaction ($F(6,48) = 2.63$, $P < 0.03$); and 5-HIAA, no significant DRUG and DOSE effects or interaction. Planned comparisons between drug treatment and specific control group were performed.

Group (N = 5)	NE	DA	DOPAC	HVA	5-HT	5-HIAA
H_2O	411.0 [a]	1242.1	168.9	225.4	624.7	472.0
	14.1	13.4	10.4	8.9	23.8	30.8
MIAN (1)	410.5	1208.4	188.3	238.9	555.1	516.1
	19.3	51.7	13.0	22.7	30.9	19.0
MIAN (2)	436.7	1390.4	193.8	271.2	544.2	516.2
	8.0	53.1	6.9	42.3	31.3	43.9
MIAN (4)	362.2 [b]	1374.4	180.6	264.1	537.6	465.5
	8.6	77.6	14.9	19.6	37.0	32.2
H_2O	438.6	1318.4	200.4	240.8	585.7	514.7
	12.5	21.3	13.7	18.7	28.5	30.3
METHY (1)	403.3	1228.6	180.7	265.5	667.4	433.7
	14.3	42.5	8.3	21.1	41.6	17.3
METHY (2)	434.1	1306.9	187.3	271.7	678.3 [b]	515.8
	26.5	68.0	14.5	11.4	29.1	26.8
METHY (4)	389.8 [b]	1123.7 [b]	182.5	274.3	621.5	476.8
	18.8	48.7	13.2	8.5	28.9	32.1
VEH	433.8	1254.5	199.9	292.5	655.0	505.0
	21.3	47.1	11.7	12.6	33.4	20.1
METHIO (0.125)	423.0	1316.3	284.4 [b]	352.1	532.7 [c]	515.2
	10.0	34.3	19.5	25.9	13.9	21.8
METHIO (0.25)	393.5	1198.5	244.4	414.4 [b]	576.2	517.1
	14.6	66.7	33.3	65.1	41.5	21.3
METHIO (0.50)	411.0	1236.4	420.7 [c]	482.5 [c]	587.1	484.8
	22.9	84.7	63.3	73.9	18.3	39.7

[a] Mean ± S.E.M. (ng/g tissue). [b] $P < 0.05$ compared to control. [c] $P < 0.01$ compared to control.

treatments on the dopamine metabolites, DOPAC and HVA. Treatment with METHIO generally resulted in elevations of whole brain levels of both DOPAC and HVA.

4. Discussion

Since both enhancement and inhibition of serotonergic activity have been shown to attenuate fighting, the specific role, if any, of 5-HT in the mediation of maternal aggressive behavior remains to be elucidated. The results of the present study, however, are consistent with previous reports demonstrating inhibition of isolation-induced aggression in males by treatment with 5-HTP (Hodge and Butcher, 1974), PCPA or METHY (Malick and Barnett, 1976), and with Svare's (1983) finding that PCPA suppressed fighting in postpartum mice.

Behaviorally, chronic PCPA treatment disrupted maternal aggressive behavior in CD-1 mice, both increasing latency scores and decreasing total number of attack scores. Presumably, the drug-induced behavioral effects of PCPA were a consequence of depletion of whole brain 5-HT and 5-HIAA concentrations (confirmed by assay). Since suckling by young offspring seems prerequisite for proper expression of postpartum aggression (Svare, 1977, 1981) and that suckling

activates CNS 5-HT neurons (Kordon et al., 1973; Mena et al., 1976), perhaps suckling-induced activation of maternal aggressive behavior was impaired by the loss of brain 5-HT through inhibition of 5-HT synthesis with PCPA. Brain areas, including the hypothalamus, septum, hippocampus and amygdala, which have been implicated in the regulation of maternal aggressive behavior (Valzelli, 1978) receive prominent serotonergic input from 5-HT-containing cell bodies of the raphe nuclei (Dahlström and Fuxe, 1964).

The anti-aggressive effects of MIAN, METHY and METHIO may be related to their pharmacologic actions on 5-HT receptors. Within the CNS, at least two different 5-HT receptors have been identified: 5-HT_1 and 5-HT_2 (Peroutka et al., 1981). Serotonin agonists and antagonists differ in their ability to bind to these receptors. METHIO, MIAN and METHY all preferentially antagonize 5-HT_2 receptors (Peroutka et al., 1981). It has been suggested that 5-HT_2 receptors may be involved with excitatory 5-HT synapses, whereas 5-HT_1 receptors may be involved with inhibitory 5-HT synapses (Peroutka et al., 1981). Both METHY and METHIO antagonized excitatory but not inhibitory 5-HT synapses (Boakes et al., 1970; Haigler and Aghaganian, 1974). The observed behavioral effects of MIAN, METHY and METHIO may have been a consequence of antagonism of only the 5-HT_2 subpopulation of 5-HT receptors, resulting in blockade of the excitatory effects of 5-HT on maternal aggressive behavior. This supposition must await further analysis of the behavioral effects of pharmacological manipulations aimed at both 5-HT_1 and 5-HT_2 receptors.

However, one cannot rule out that secondary actions of the pharmacological agents used may have contributed to the observed behavioral effects. For example, it has been reported that PCPA, which has a long half-life, can be metabolized by the L-aromatic amino acid decarboxylase enzyme to p-chlorophenylethylamine (PCPEA) which has 5-HT agonist activity (Sloviter et al., 1978). Therefore, behavioral effects of PCPA could have been due, in part, to the 5-HT agonist activity of PCPEA. The effects of 5-HTP in suppressing maternal aggressive behavior reported here support such a notion. Secondary effects of 5-HTP treatment include the ability of this drug to be taken up by catecholamine neurons, synthesized into 5-HT, which in turn displaces the catecholamines present in the nerve terminals (Fuxe et al., 1971). However, biochemical profiles of postpartum females treated with 5-HTP in the present study do not support this displacement hypothesis (see table 1, normal NE levels and elevated DA levels). In addition, others have failed to demonstrate any displacement of catecholamines with doses of 80-100 mg/kg of 5-HTP (Modigh, 1973; Everett, 1974).

Behavioral effects which resulted from treatments with the 5-HT receptor antagonists could have been due to non-serotonergic activity. MIAN has significant central presynaptic α-adrenergic activity (Baumann and Maitre, 1977). α-Adrenergic blockade with MIAN resulted in increased NE release (Baumann and Maitre, 1977) and subsequent increased 3-methoxy-4-hydroxyphenylglycol levels (Tang et al., 1979). The 4 mg/kg dose of MIAN decreased whole brain NE levels, consistent with this reported α-adrenergic effect. METHIO has been reported to have significant DA receptor antagonist activity (Monachon et al., 1972). Postpartum mice treated with METHIO had elevated whole brain levels of DOPAC and HVA; profiles consistent with DA receptor antagonism. Chlorpromazine and haloperidol, two DA receptor antagonists, have been shown to increase brain DOPAC and HVA (Andén et al., 1964). Behaviorally, acute haloperidol treatment also suppressed postpartum fighting in mice (Ieni and Thurmond, 1983). Therefore, the behavioral effects observed in the present study from treatment with MIAN and METHIO could have been due to α-adrenergic antagonism or DA receptor antagonism, respectively.

Modulation of brain serotonergic systems by some drug treatments in the present study significantly suppressed locomotor activity. Thus, the anti-aggressive effects of these treatments could have been the consequence of non-specific reduction in arousal. However, reduction in activity did not always accompany drug-induced changes in aggressive behavior. For example, PCPA suppressed aggression without any significant effects on activity. The anti-aggressive dose of METHY

did not significantly suppress locomotor activity. In addition, only the 4 mg/kg dose of MIAN suppressed activity but both the 2 and 4 mg/kg doses of MIAN suppressed aggressive behavior. On the other hand, 5-HTP and METHIO suppressed activity and aggression at the same doses. The activity-reducing effects of 5-HTP were consistent with the report of Modigh (1972) who demonstrated the activity reducing effects of 5-HTP. METHIO produced profound activity changes; the 0.5 mg/kg dose induced cataleptic responses in several animals. Since locmotor activity has been reported to be related to catecholaminergic systems (Laverty and Taylor, 1970), possibly the activity-reducing and cataleptic effects of METHIO were related to DA receptor antagonism, as well as 5-HT antagonist activity.

Pharmacological manipulations in the present study which produced effects on maternal aggression parallel effects reported for variants of male aggression. Depletion of brain 5-HT with 5,6-dihydroxytryptamine (Krsiak et al., 1981) or PCPA (Malick and Barnett, 1976) have reportedly antagonized aggression induced by prolonged isolation. Depletion of brain 5-HT and 5-HIAA by raphe lesions completely abolished aggression in isolated mice (Kostowski and Valzelli, 1974). Territorial aggression in male mice is reduced by 5-HTP treatment (Kramarcy et al., 1984). Though the stimuli which serve to elicit aggression in male and female members of a species may be different, the underlying transmitter systems and CNS areas which modulate aggression may be similar.

The specific functions of 5-HT in maternal behaviors, including aggressive behavior, are still in question. Though the pharmacologic manipulations aimed at disruption of brain 5-HT systems suggest a serotonergic role in regulating aggressive behavior of postpartum mice, the specific nature is unclear. Analysis with more specific drugs may help clarify the relationship of 5-HT to aggressive behavior. Further research identifying the transmitter systems involved in maternal aggression and understanding of the regulatory function of hormones on these transmitter systems may provide important information concerning emotionality in human populations. It has been suggested that the study of maternal aggressive behavior may increase our understanding of mood changes (e.g. postpartum depression) in women (Svare, 1977).

Acknowledgements

This study was performed as partial fulfillment of requirements for Ph. D. in Psychology in the Neuropsychopharmacology Program at the University of Louisville. This research was supported by a grant from the University of Louisville Academic Excellence Commission (J.R.I.) and the Department of Psychology. We would like to thank Drs. John Brown, Laurence Carr, Richard Smith and James Smothers for guidance in this research. We would also like to acknowledge Dr. Gordon Barr for his helpful comments in preparing this manuscript. In addition, we would like to thank Organon, Hoffman-LaRoche and Sandoz for their generous supplies of drugs.

References

Andén, N.E., B.E. Roos and B. Werdinius, 1964, Effects of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum, Life Sci. 3, 149.

Barr, G.A., N.S. Sharpless and J.L. Gibbons, 1979, Differences in the level of dopamine in the hypothalamus of aggressive and non-aggressive rats, Brain Res. 166, 211.

Baumann, P.A. and L. Maitre, 1977, Blockade of presynaptic alpha-receptors and of amine uptake in the rat brain by the antidepressant mianserine, Naunyn-Schmiedeb. Arch. Pharmacol. 300, 31.

Boakes, R.J., P.B. Bradley, I. Briggs and A. Dray, 1970, Antagonism of 5-hydroxytryptamine by LSD 25 in the central nervous system: a possible neuronal basis for the actions of LSD 25, Br. J. Pharmacol. 40, 202.

Dahlström, A. and K. Fuxe, 1964, Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons, Acta Physiol. Scand. 62, Suppl. 232, 1.

Erskine, M.S., R.J. Barfield and B.D. Goldman, 1978, Intraspecific fighting during late pregnancy and lactation in rats and effects of litter removal, Behav. Biol. 23, 206.

Everett, G.M., 1974, Effects of 5-hydroxytryptophan on brain levels of dopamine, norepinephrine and serotonin in mice, Adv. Biochem. Pharmacol. 10, 261.

Fuxe, K., L. Butcher and J. Engel, 1971, DL-5-hydroxytryptophan-induced changes in central monoamine neurons after peripheral decarboxylase inhibition, J. Pharm. Pharmacol. 23, 420.

Gandelman, R. and N.G. Simon, 1980, Postpartum fighting in the rat: nipple development and the presence of young, Behav. Neural Biol. 28, 350.

Haigler, H.J. and G.K. Aghajanian, 1974, Peripheral serotonin antagonists: failure to antagonize serotonin in brain areas receiving a prominent serotonergic input, J. Neural. Trans. 35, 257.

Hodge, G.K. and L.L. Butcher, 1974, 5-Hydroxytryptamine correlates of isolation-induced aggression in mice, European J. Pharmacol. 28, 326.

Ieni, J.R. and J.B. Thurmond, 1983, Pharmacological manipulations of dopaminergic and serotonergic transmitter systems alter maternal aggressive behavior of CD-1 mice, Soc. Neurosci. Abst. 9, 557.

Kantak, K.M., L.R. Hegstrand and B. Eichelman, 1981, Facilitation of shock-induced fighting following intraventricular 5,7-dihydroxytryptamine and 6-hydroxydopa, Psychopharmacology 74, 157.

Kordon, C., C.A. Blake, J. Terkel and C.H. Sawyer, 1973, Participation of serotonin-containing neurons in the suckling-induced rise in plasma prolactin levels in lactating rats, Neuroendocrinology 13, 213.

Kostowski, W. and L. Valzelli, 1974, Biochemical and behavioral effects of lesions of raphe nuclei in aggressive mice, Pharmacol. Biochem. Behav. 2, 277.

Kramarcy, N.R., J.W. Brown and J.B. Thurmond, 1984, Effects of drug-induced changes in brain monoamines on aggression and motor behavior in mice, European J. Pharmacol. 99, 141.

Krsiak, M., A. Sulcova, Z. Tomasikova, N. Dlohozkova, E. Kosar and K. Masek, 1981, Drug effects on attack, defense and escape in mice, Pharmacol. Biochem. Behav. 14, (Suppl. 1), 47.

Lavery, R. and K.M. Taylor, 1970, Effects of intraventricular 2,4,5-trihydroxyphenylethylamine (6-hydroxydopamine) on rat behaviour and brain catecholamine metabolism, Br. J. Pharmacol. 40, 836.

Malick, J.B. and A. Barnett, 1976, The role of serotonergic pathways in isolation-induced aggression in mice, Pharmacol. Biochem. Behav. 5, 55.

Mena, F., A. Enjalbert, L. Carbonell, M. Priam and C. Kordon, 1976, Effects of suckling on plasma prolactin and hypothalamic monoamine levels in the rat, Endocrinology 99, 445.

Modigh, K., 1972, Central and peripheral effects of 5-hydroxytryptophan on motor activity in mice, Psychopharmacologia 23, 48.

Modigh, K., 1973, Effects of chlorimipramine and protryptiline on the hyperactivity induced by 5-hydroxytryptophan after peripheral decarboxylase inhibition in mice, J. Neural Trans. 34, 101.

Monachon, M.A., W.P. Burkard, M. Jalfre and W. Haefely, 1972, Blockade of central 5-hydroxytryptamine receptors by methiothepin, Naunyn-Schmiedeb. Arch. Pharmacol. 274, 192.

Peroutka, S.J., R.M. Lebovitz and S.H. Snyder, 1981, Two distinct central serotonin receptors with different physiological functions, Science 12, 827.

Perry, K.W. and R.W. Fuller, 1979, Assay procedure for 5-HIAA, HVA and DOPAC, Soc. Neurosci. Abst. 5, 1158.

Sloviter, R.S., E.G. Drust and J.D. Connor, 1978, Serotonin agonist actions of p-chlorophenylalanine, Neuropharmacology 17, 1029.

Svare, B.B., 1977, Maternal aggression in mice: influence of the young, Biobehav. Rev. 1, 151.

Svare, B.B., 1981, Maternal aggression in mammals, in: Parental Care in Mammals, eds. D.J. Gubernick and P.H. Klopfer (Plenum Press, New York).

Svare, B., 1983, Psychobiological determinants of maternal aggressive behavior. in: Aggressive Behavior: Genetic and Neural Approaches, eds. E.C. Simmel, M.E. Hahn and J.K. Walters (Lawrence Erlbaum Assoc., Hillsdale, NJ) p. 129.

Svare, B. and R. Gandelman, 1973, Postpartum aggression in mice: experiential and environmental factors, Hormones Behavior 4, 323.

Svare, B. and R. Gandelman, 1976, Postpartum aggression in mice: the influence of suckling stimulation, Hormones Behavior 7, 407.

Tang, S.W., D.M. Helmeste and H.C. Stancer, 1979, Interaction of antidepressants with clonidine on rat brain total 3-methoxy-4-hydroxyphenylglycol, Can. J. Physiol. Pharmacol. 57, 435.

Valzelli, L., 1978, Human and animal studies on the neurophysiology of aggression, Prog. Neuro-Psychopharmacol. 2, 591.

Voogt, J.L. and L.A. Carr, 1974, Plasma prolactin levels and hypothalamic catecholamine synthesis during suckling, Neuroendocrinology 16, 108.

Wagner, J., M. Palfreyman and M. Zraika, 1979, Determination of DOPA, dopamine, DOPAC, epinephrine, norepinephrine, alpha-monofluorodopa and alpha-d-fluoromethyldopa in various tissues of mice and rats using reverse phase ion pair liquid chromatography with electrochemical detection, J. Chromatog. 164, 41.

Winer, B.J., 1971, Statistical Principles in Experimental Design, 2nd ed. (McGraw-Hill Book Company, New York).