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Mechanisms of Action of p-Chlorophenylalanine in Stimulating Sexual Receptivity in the Female Rat

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WILSON, C. A., R. C. BONNEY, D. M. EVERARD, R. F. PARROTT AND J. WISE. *Mechanisms of action of p-chlorophenylalanine in stimulating sexual receptivity in the female rat.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 777–784, 1982.—PCPA stimulates lordotic activity in oestrogen-primed ovariectomised rats 4 to 6 hours, after administration. It does not act via depletion of brain indoleamines since the time course of this effect and its behaviour effect are different. In addition, the effect on sexual behaviour cannot be reversed by 5HTP, which restores the depleted brain indoleamines back to normal. PCPA may stimulate receptivity by causing stimulation of progesterone secretion, or a transient depletion of noradrenaline. Evidence is given that the effect of PCPA is due to its active metabolite PCPEA, which may be responsible for the depletion of noradrenaline. Alternatively, PCPEA may stimulate female sexual behaviour by inducing a transient release of 5HT.

p-Chlorophenylalanine Sexual receptivity Lordosis 5HTP Serotonin Noradrenaline

THE hypothesis that central seronergic activity is inhibitory to sexual behaviour is based mainly on pharmacological evidence, in particular on the work carried out using the pharmacological agent p-chlorophenylalanine (PCPA) in males [1, 6, 23, 24, 25, 33, 34, 39, 41, 43, 45, 52] and in females [16, 17, 19, 20, 36, 37, 56]. PCPA inhibits the synthesis of 5HT [29] and the subsequent depletion of brain 5HT has been associated with the increased sexual behaviour seen in both males and females after PCPA treatment.

The experiments carried out on male behaviour, all support this hypothesis, in that any treatment that lowers 5HT activity within the CNS increases male sexual activity in both male and female rats [1, 6, 25, 34, 39, 41, 47, 49, 50, 52, 53]; the time of maximum reduction of 5HT, but not of the catecholamines, correlates with peak sexual activity; and raising 5HT activity in both PCPA treated and untreated animals inhibits male behaviour [3, 24, 33, 34, 39, 50, 52].

However, in the female there are conflicting reports. Administration of a single injection of PCPA stimulates receptivity 4–8 hours later in ovariectomised females primed with either a single [2, 37, 56] or multiple injections of oestrogen [13, 16, 17, 19]. In some reports, the PCPA was still active 24 and 48 hours after administration [13,17] although this was not seen by others [2,37]; the reason for this is not clear. The extended effect may be due to the higher doses of oestradiol benzoate used to prime the rats (i.e., 2 μg/kg daily, compared with a single dose of 10 μg/rat or 1 μg/kg daily), or perhaps due to a longer lasting depletion of noradrenaline in some rats. For instance PCPA treatment causes a depletion in hypothalamic noradrenaline (NA) lasting 3 days

in male Sprague-Dawley rats [38] while in female Wistar rats NA levels had returned to normal by 26 hours [2].

When PCPA is given chronically either daily or every 48 hours, to oestrogen primed ovariectomised animals, or even as a single dose 24 hours before priming, it is ineffective in stimulating receptivity [42,50] and in fact reduces the stimulatory action on lordosis normally produced by progesterone [15, 27, 42, 47]. Similarly, conflicting reports have been published on the effects of other 5HT depletors such as p-chloroamphetamine and 5,7 dihydroxytryptamine, two reports showing a stimulatory effect correlated with depletion [18,57] and another showing no effect at all [51].

The stimulatory effects of PCPA seen at 4–8 hours after administration do not occur at times of maximum 5HT depletion and Ahlenius *et al.* [2] has suggested there is a better correlation between the effects of PCPA on lordosis and its transitory depletion of the catecholamines. There is also confusion as to whether PCPA may be acting via the adrenals or not, presumably stimulating secretion of endogenous progesterone; thus according to two reports PCPA is inactive in oestrogen primed plus adrenalectomised rats [15,27] while others have shown that removal of the adrenals has little or no effect on PCPA activity [19,56]. These differences do not seem to be due to the different times of testing after administration of PCPA.

This paper confirms the lack of correlation between the stimulatory effects of PCPA on female sexual behaviour and depletion of brain 5HT and attempts to investigate the other possible mechanisms of action of PCPA on stimulating lordosis in the female rat.

METHOD

Female Sprague-Dawley rats (Tuck & Son, Essex) were kept in a reversed lighting system of 10 hr dark; 14 hours light (lights off 10.00–20.00 hr). At least 3 weeks before the experiments, the rats were all ovariectomised and for one experiment adrenalectomised as well, under ether anaesthesia. Permanent cannulae were placed into the third ventricle stereotaxically under pentobarbitone sodium (35 mg/kg IP) anaesthesia.

Female rats were tested for lordosis behaviour under red light, 4 hours into the dark period, by first priming with 2 μg/rat oestradiol benzoate (OB) and then 52 hours later placing them with vigorous males and noting the number of lordotic responses to 20 mounts. The results are expressed as lordosis quotients (LQ=Number of lordoses/Number of mounts×100) and also the number of receptive rats per group. A rat was considered receptive when its LQ was greater than 50%. At the end of the testing period some rats were decapitated, the hypothalami dissected out and stored at −20°C until assayed for amines. In one experiment blood was collected from the cervical cut, at autopsy, centrifuged at 400 g for 15 minutes and stored at −20°C until assayed for progesterone.

Drugs

All drugs, unless indicated otherwise, were obtained from Sigma (Poole; Dorset), dissolved in saline and given intraperitoneally (IP).

In the experiments shown in Table 1, p-chlorophenylalanine methyl ester was given in doses of 100, 150, 200 and 300 mg/kg expressed as the base (i.e., 120, 180, 240 and 360 mg/kg of the ester) 24 or 48 hours after the OB injection, that is 28 or 4 hours before testing for lordosis. In one experiment 150 mg/kg PCPA was given 0, 24 and 48 hours after OB priming.

In the experiments shown in Table 2, PCPA was always given at 150 mg/kg, 48 hours after OB priming (4 hours before testing) and the following compounds were given at the same time: 5-hydroxytryptophan, ethyl ester (5HTP; 20 mg/kg); dihydroxyphenylalanine ethyl ester (DOPA; 200 mg/kg), dihydroxyphenylserine as the base (DOPS; 250 mg/kg) and clonidine (Catapres; Boehringer-Ingelheim; 1 mg/kg). In another experiment also shown on Table 2 noradrenaline hydrochloride (NA) was given in 0.01 ml/rat saline intra-ventricularly to rats bearing permanent implants placed in the 3rd ventricle. Doses of 50, 100 and 200 μg/rat were used (that is 0.15, 0.3 and 0.6 μg/kg) and tests were made at 15 and 30 minutes. For the final experiment 100 μg/rat was used and the test made at 15 minutes.

Phenylalanine was given at 150 mg/kg, 48 hours after OB priming. p-Chlorophenylethylamine (PCPEA) was given to groups of OB primed rats at 10, 20 and 40 mg/kg 48 hours after OB priming and tested 15, 30 and 60 minutes later. For the final experiment 20 mg/kg was given since it was not toxic and testing for receptivity carried out 60 minutes later when peak activity was seen. Benserazide (R4–4602; Hoffman-La Roche) was used at 250 mg/kg and given 15 minutes before the substance being tested for its stimulatory effect.

Hypothalamic Amine Assays

5-Hydroxytryptamine (5HT) and 5-hydroxyindole acetic acid (5HIAA) was assayed according to the method of Curzon and Green [9] and noradrenaline (NA) and dopamine (DA) by the method of Shellenbergher and Gordon [44] after an extraction procedure based on that of Welch and Welch [54].

The recoveries were noted by measuring internal standards in the presence of tissue and the yields were 105% (5HT), 76% (5HIAA), 78% (NA) and 74% (DA). Results were not corrected for recovery. The intra and inter-assay coefficients of variance were 6.0% and 10.2% (5HT), 4.3% and 17.7% (5HIAA), 3.6% and 17% (NA), 10% and 23% (DA).

Plasma Progesterone Measurement

The progesterone was measured by radioimmunoassay according to the method reported in detail elsewhere [7].

Statistics

The concentration of hypothalamic amines in different groups were compared for significant difference by Dunnet's procedure for comparing all means with a control. Plasma progesterone concentration changes and some of the brain amine results were compared by the Student *t*-test. Differences between lordosis quotients in the groups were compared by the Mann-Whitney U test, except in the experiments where noradrenaline was given to rats pretreated with PCPA or progesterone; in this case the Wilcoxon Matched-Pairs signed rank test was used.

RESULTS

Effect of PCPA on Lordosis and Hypothalamic Amines

Table 1 shows the effect of graded doses of PCPA given

TABLE 1

EFFECT OF PCPA GIVEN 4 HOURS BEFORE, ON LORDOSIS AND HYPOTHALAMIC AMINE CONCENTRATION

Treatment mg/kg IP	Number receptive rats	Mean LQ% (with range)	Hypothalamic Concentration 5HT	5HIAA	NA (ng/100 mg ± SE)[a]	DA
Saline	1/23	7.2 (0–65)	163 ± 19	122 ± 29	233 ± 12	320 ± 38
100	9/24*††	39.6**††† (0–100)	180 ± 29	107 ± 14	300 ± 31*	568 ± 43
150	19/22***	71.6*** (0–100)	157 ± 14	42 ± 9**	197 ± 10[b]	323 ± 35
200	12/15***	42.8***† (0–95)	—	—	—	
300	13/20***	54.7***† (0–90)	175 ± 10	37 ± 5**	188 ± 8	365 ± 83

Significance between saline group and treated groups: *$p<0.05$; **$p<0.01$; ***$p<0.001$.
Significance between group given 150 mg/kg PCPA and other PCPA treated groups: †$p<0.05$; ††$p<0.01$; †††$p<0.002$.
[a]Amine estimations carried out on 7 rats/group.
[b]Significantly different from saline group at $p<0.05$ according to Student t-test.

TABLE 2

EFFECT OF PCPA, GIVEN 24 HOURS BEFORE, ON LORDOSIS AND HYPOTHALAMIC AMINE CONCENTRATIONS

Treatment mg/kg IP	Number receptive rats	Mean LQ% with range	Hypothalamic Concentration 5HT	5HIAA	NA (ng/100 mg ± SE)[a]
Saline	0/7	0	195 ± 10	115 ± 10	209 ± 13
100	1/8	12.9 (0–90)	108 ± 8**	74 ± 30***	189 ± 14
150	0/6	0	51 ± 6***	14 ± 3***	193 ± 12
300	1/6 (2 sick)	9.0 (0–55)	59 ± 8***	not detectable	234 ± 34
150×3[b]	0/13	5.2 (0–40)	54 ± 4***	not detectable	191 ± 10

Significance between saline group and treated groups: **$p<0.01$; ***$p<0.001$.
[a]Amine estimations carried out on 6 rats/group.
[b]150×3; PCPA given at 150 mg/kg 4, 24 and 48 hours before testing.

48 hours after oestrogen priming i.e., 4 hours before testing for lordosis and approximately 5 hours before autopsy. Although the lower doses of PCPA had no effect on hypothalamic 5HT concentration in this particular experiment (depletion of 5HT was seen in one of the later experiments; see Table 3), there was a graded effect on depleting hypothalamic 5HIAA levels with increasing doses of PCPA indicating an increasing inhibition of 5HT synthesis. There was a tendency towards lower noradrenaline (NA) and dopamine (DA) concentrations with the higher doses of PCPA (150 and 300 mg/kg). Reports in the literature show that PCPA can induce significant depletion of NA [2, 30, 38] and indeed when the group treated with 150 mg/kg PCPA was compared with the control group by an unpaired student t-test, NA levels were significantly lower ($p<0.05$) in the PCPA group.

All doses of PCPA stimulated lordosis behaviour, but the doses of 100, 200 and 300 mg/kg were significantly less effective than 150 mg/kg, so that there was not a straight dose response curve of behaviour with increasing doses of PCPA.

Table 2 shows the effect of PCPA 24 hours after the OB injection, that is approximately 28 hours before testing for lordosis and autopsy. None of the doses of PCPA stimulated lordosis behaviour, while the depletion of 5HIAA levels was very marked. 5HT levels were also lowered in a dose-graded manner, but NA was not affected. Dopamine (DA) was not measured in this experiment.

When 150 mg/kg PCPA was given daily for 3 days there was almost complete depletion of the indoleamines. No effect on the catecholamines and no stimulatory effect on lordosis behaviour was noted (see Table 2).

Attempts to Reverse the Effects of PCPA

Groups of oestrogen primed rats were treated with 150 mg/kg PCPA 48 hours after OB and tested for lordosis 4 hours later. At the same time as the PCPA, groups received either 5HTP, DOPA or DOPS. The 5HTP was used to reverse the PCPA depletion of 5HT and 5HIAA and the DOPA and DOPS the depletion of noradrenaline.

Table 3 shows that PCPA alone depleted 5HT and

TABLE 3

REVERSAL OF THE EFFECTS OF PCPA, GIVEN 4 HOURS BEFORE, ON LORDOSIS AND AMINE DEPLETION

Treatment	Dose mg/kg IP	LQ% (range)	Hypothalamic Concentration 5HT	5HIAA	NA	DA
			ng/100 mg ± SE[a]			
Saline (18)	—	23 (0–65)	156 ± 15	190 ± 11	269 ± 21	219 ± 14
PCPA + (30)	150	76.5*** (0–100)	99 ± 8*	82 ± 14***	187 ± 18	181 ± 7
Saline	—					
PCPA + (20)	150	80.7*** (5–100)	215 ± 23*†	139 ± 23	194 ± 14	197 ± 34
5HTP	20					
PCPA + (11)	150	73.0*** (20–100)	80 ± 9**	121 ± 20*	171 ± 25	255 ± 20
DOPA	200					
PCPA + (4)[d]	150	74.1*** (70–100)	98 ± 5*	87 ± 17***	149 ± 11	217 ± 39
DOPS	250					
PCPA + (14)	150	2.0††† (0–65)				
Clonidine	1.0					
PCPA + (8)	150	54.0**† (24–100)				
Noradrenaline	0.3[b]					
Progesterone (7)	2.0[c]	84.4*** (65–100)				
Progesterone +	2.0	55.4**† (0–100)				
Noradrenaline (7)	0.3[b]					

All drugs were given intraperitoneally concomitantly with the PCPA except: [b]0.3 mg/kg noradrenaline given intraventricularly 15 minutes before testing; [c]progesterone given SC. ( )Number of rats per group for behavioural test.

[a]Amine estimations carried out on 8 rats/group, except [d] the group treated with PCPA plus DOPS (n=4) which was not included in the tests for significance.

Significance between saline group and other treated groups: *$p<0.05$; **$p<0.01$; ***$p\pm0.001$.

Significance between group given PCPA alone and other treated groups: †$p<0.05$; †††$p<0.001$.

Significance between progesterone alone and progesterone plus noradrenaline: †$p<0.05$.

5HIAA, 4 hours after administration; and that concomitant 5HTP treatment was able to maintain normal 5HIAA levels and significantly higher than normal 5HT levels, in the presence of PCPA and thus overcome its depletory effect. However 5HTP treatment did not prevent the stimulatory effect of PCPA on sexual behaviour. DOPA and DOPS also had no effect on the PCPA action on lordosis, but neither drug maintained normal NA levels (which according to the Student *t*-test was lowered by PCPA ($p<0.02$). For this reason clonidine was given (an NA agonist administered in a dose that stimulates post-synaptic receptors; 1 mg/kg [26]). This dose alone is not toxic, however when clonidine was given to PCPA treated rats it proved to be very toxic causing flaccid paralysis in most animals. Noradrenaline (NA) itself, was then given intraventricularly at 50, 100 and 200 μg/rat. Two hundred μg did not appear to affect motor activity and was therefore used in this experiment. When PCPA treated rats were tested for receptivity and then given 100 μg/rat NA and tested again 15 minutes later, the NA treatment significantly reduced their receptivity ($p<0.05$). This treatment also reduced the lordosis quotient in rats treated with 2 μg OB followed 48 hours later by 2 mg/kg progesterone.

Action of PCPA on the Adrenals

Experiments were carried out to see whether PCPA may be acting via stimulation of the adrenals. Oestrogen primed ovariectomised plus adrenalectomised animals given progesterone (1 mg/rat SC 48 hours after the OB) showed full sexual behaviour (LQ 86%; n=8); however, 2 weeks later when the same rats were given 150 mg/kg PCPA, the LQ was 0%, four hours later; 4 rats were paralysed and 2 of these died 24 hours later. A different approach was taken, therefore, and 150 mg/kg PCPA was given to a group of oestrogen-primed ovariectomised rats (with their adrenals intact) and plasma was collected four hours later. Progesterone plasma levels in the PCPA treated rats were significantly higher than the saline controls (PCPA 3.1±0.25 ng/ml; n=5; saline 2.2±0.18 ng/ml, n=6; $p<0.01$) indicating that PCPA may have an effect on steroidogenesis in the adrenals.

Effect of Phenylalanine and PCPEA (a PCPA Metabolite) on Sexual Behaviour

The basic amino acid structure of PCPA, phenylalanine, has no effect on sexual receptivity (Table 4) when given at the same dose and time before testing as the most effective PCPA treatment.

PCPEA is the chief metabolite of PCPA and its effect on lordosis was investigated at several doses and times before final tests were made. Doses higher than 20 mg/kg were toxic in our rats (5 died after 40 mg/kg, although this dose was reported as non-toxic by Sloviter *et al.* [48]). At 5, 10 and 20 minutes after administration of 5, 10 and 20 mg/kg, the rats demonstrated the shaking and head waving syndrome associated with increased 5HT activity [48]. Lordosis activity was seen at 30 and 60 minutes after injection and so tests were finally carried out at 60 minutes using 20 mg/kg. This treatment induced a singificant increase in LQ compared with the saline controls. The results were "all or none" in that the animals either became highly receptive, or showed

TABLE 4

EFFECT OF A PCPA ANALOGUE AND METABOLITE ON LORDOSIS AND HYPOTHALAMIC AMINE CONCENTRATION

Treatment	Dose (mg/kg)	Time of Administration (hours before testing)	Mean LQ% (range)	Hypothalamic Concentration (ng/100 mg ± SE)[a] 5HT	5HIAA	NA	DA
Saline (11)	—	4	3.6 (0–20)	144 ± 5	100 ± 9	197 ± 6	246 ± 27
Phenylalanine (6)	150	4	0	—	—	—	—
PCPEA (12)	20	1	55.0** (0–90)	116 ± 11*	119 ± 16	143 ± 6**	188 ± 12

( )Number of rats per group.
[a]Seven rats/group for amine assays.
Significance between saline group and treated groups: *$p<0.05$; **$p<0.02$.

TABLE 5

EFFECT OF BENSERAZIDE (AN AMINO-ACID DECARBOXYLASE INHIBITOR) ON THE STIMULATORY ACTION OF CERTAIN COMPOUNDS ON SEXUAL BEHAVIOUR

Treatment	Dose (mg/kg)	Time of Administration (hours before testing)	Mean LQ% (range)	Hypothalamic Concentration ng/100 mg/SE[a] 5HT	5HIAA	NA	DA
Saline (11)	—	4	3.6 (0–20)	144 ± 5	100 ± 9	197 ± 6	246 ± 27
PCPA (11)	150	4	73.0*** (20–100)	134 ± 6	56 ± 5*	165 ± 6[b]	315 ± 16
PCPA +	150	4	16.0† (0–100)	173 ± 24	71 ± 12*	202 ± 18	279 ± 26
Benserazide (7)	250	4.25					
PCPEA (8)	20	1	36.0* (0–100)				
PCPEA +	20	1					
Benserazide (5) (3/8 died)	250	1.25	33.0* (0–100)				
Progesterone (5)	2	4	83.7*** (60–100)				
Progesterone +	2	4					
Benserazide (5)	250	4.25	92.0*** (80–100)				

( )Number of rats per group.
[a]Seven rats/group for amine assays.
[b]Significantly different from saline group at $p<0.02$ according to Student t-test.
Significance between saline group and treated groups: *$p<0.05$; **$p<0.02$; ***$p<0.001$.
Significance between PCPA group and PCPA plus Benserazide: †$p<0.02$.

no lordosis at all. Unlike PCPA, PCPEA did not affect 5HIAA hypothalamic levels, although 5HT and NA concentrations were both significantly reduced confirming the results of Sloviter *et al.* [48].

PCPA is converted to PCPEA by aromatic acid decarboxylase and so the former was given together with an inhibitor of this enzyme, in order to prevent the conversion. Benserazide is reported to inhibit aromatic acid decarboxylase within the CNS at 200 mg/kg IP [48]. In our rats, this dose was ineffective, however when 250 mg/kg IP was given 15 minutes before PCPA treatment, it significantly inhibited the sexual receptivity induced by PCPA alone (Table 5). This was in spite of the fact that the 5HIAA levels were reduced as in the group given PCPA alone. Interestingly, Benserazide prevented the effect of PCPA in reducing NA levels.

Benserazide did not antagonise the stimulatory action of progesterone or PCPEA on receptivity indicating that it was acting specifically on PCPA and not as a general depressant on sexual behaviour. The combination of benserazide and PCPEA was toxic as $^3/_8$ rats died.

Effect of Progesterone on Sexual Receptivity and Hypothalamic Indoleamine Concentrations

Two groups of 10 ovariectomised-oestrogen primed rats were given either 0.1 ml corn oil as controls or 0.5 mg/rat SC progesterone (P), 48 hours after the OB and tested for receptivity 4 hours later and then autopsied. The progesterone induced sexual receptivity (control LQ 0%; P LQ 66%), a significant reduction in hypothalamic 5HT concentration (control 220±9; P 196±6; $p<0.05$) and maintenance of normal 5HIAA levels (control 114±5; P 126±4; NS). All concentrations expressed as ng/100 mg ±standard error of the mean. The reduction in 5HT levels without a reduction in 5HIAA levels indicates that progesterone has stimulated the release of 5HT.

DISCUSSION

The results reported here show that a single injection of PCPA can stimulate lordosis behaviour in oestrogen primed rats when tested four hours after administration. This is in full agreement with many other reports (e.g. [19, 36, 56]). However, when the PCPA is given 24 hours before testing for lordosis or as three daily injections, it is ineffective. Segan and Whalen [42] also found that multiple doses of PCPA had no stimulatory effect, indeed PCPA treatment, whether given as a multiple injection or 24 hours before oestrogen priming inhibited lordosis normally induced by progesterone [27,42]. We have found that a single injection of PCPA can inhibit the usual effects of progesterone in oestrogen primed animals seven days later (unpublished results).

When the stimulatory effect of PCPA on lordosis was correlated with its effect on depletion of hypothalamic amines, it was clear that there was no relationship between stimulation of lordosis and inhibition of 5HT synthesis. PCPA given 24 hours before the test or given in multiple doses was ineffective on behaviour and yet caused significant or even complete depletion of 5HT and 5HIAA. PCPA given four hours before testing showed a graded dose response on inhibition of 5HT synthesis as shown by the lowered 5HIAA levels [29] but the dose response curve for stimulation of lordosis was not parallel and the higher dose of 300 mg/kg was less effective than 150 mg/kg. There was no sign of any toxic effects after a single injection of 300 mg/kg. One hundred fifty mg/kg PCPA also significantly lowered NA levels at 4 hours but not 24 hours after administration. This is in agreement with the findings of Ahlenius *et al.* [2] who suggested that the behavioural effect of PCPA may be due to its transitory depletion of catecholamines. After PCPA treatment, attempts were made to selectively restore the depleted amine levels by simultaneous administration of the precursors 5HTP, DOPA and DOPS. 5HTP brought the 5HT and the 5HIAA levels back to normal, but did not reverse the PCPA effect of sexual behaviour. DOPA and DOPS were also unsuccessful in reversing the behavioural effects, but in fact did not restore NA levels back to normal either. DOPS is supposed to be converted directly to NA without the intermediary dopamine step, so this result is unexpected.

In order to stimulate NA activity, the selective agonist, clonidine was given, but in a dose which had no effect by itself it was very toxic when given to rats pre-treated with PCPA. NA was then given intraventricularly and, in a dose that did not depress motor activity, it reduced sexual activity in PCPA treated animals. This effect may however be non-specific, since NA also reduced the action of progesterone on receptivity. However the possibility that alpha-adrenergic activity in the CNS is inhibitory to sexual behaviour in the female rat has been suggested [12,22] and our results perhaps support this. The reversal of PCPA by NA plus the fact that the time course of NA depletion correlates with sexual activity after PCPA could indicate that this depletion may be one of the mechanisms by which PCPA stimulates receptivity.

We have found that PCPA is very toxic in adrenalectomised animals, as did Hamberger-Bar *et al.* [28]; but others have carried out similar experiments successfully. Two reports indicate that PCPA is inactive in ovariectomised-adrenalectomised animals [15,27], while two others have shown that removal of the adrenals or adrenal suppression by dexamethasone did not significantly reduce stimulation of lordosis by PCPA [19,56] although certain aspects of sexual behaviour were inhibited [19].

We have found that PCPA stimulated progesterone secretion in the rat. Although the rise in progesterone levels did not seem very great, it is possible that one component of the mechanisms by which PCPA acts on sexual behaviour may be via stimulation of steroidogenesis.

Coscina *et al.* [8] have shown that the amino-acid structure of PCPA, phenylalanine, has similar effects to PCPA in stimulating feeding behaviour, at the same dose and same time course of action, and suggested this was due to a similarity in physico-chemical properties. However Dalhousie [10] found no effect of phenylalanine on male mounting and we found that 150 mg/kg phenylalanine did not stimulate lordosis 4 hours after administration.

PCPA is metabolised by amino acid decarboxylase to PCPEA and recently Sloviter *et al.* [48] has suggested that any short term effect of PCPA may be due to conversion to PCPEA. We have found that PCPEA can stimulate lordosis behaviour 1 hour after administration and, in addition, when the conversion of PCPA to PCPEA is prevented, the effect of PCPA on lordosis is significantly reduced. So it is possible that the action of PCPA on female sexual behaviour is due to conversion to an active metabolite—PCPEA. The action of benserazide is a specific one on PCPA, since it does not antagonise sexual receptivity induced by progesterone or PCPEA.

The mechanism of action of PCPEA can be speculated upon; PCPEA does not inhibit 5HT synthesis since normal 5HIAA levels are maintained in its presence. However, like PCPA it causes depletion of hypothalamic NA and in fact when PCPA conversion to PCPEA is prevented NA depletion does not occur. It is possible, therefore, that increased sexual receptivity is due to NA depletion and both these effects seen after PCPA treatment are due to its active metabolite. Another possibility is that the small rise in progesterone seen after PCPA may actually be due to the action of PCPEA on the adrenal.

An alternative hypothesis for the mechanism of action of PCPEA can be based on the fact that PCPEA induces a transient release of 5HT [35,48] and this is supported by the results in Table 4, showing that PCPEA caused a fall in hypothalamic 5HT concentration. It is possible, therefore, that while in the male 5HT is inhibitory to sexual activity, in the female the presence of 5HT is essential for inducing receptivity. We suggest that a transient increase in hypothalamic 5HT activity stimulates receptivity, but a long-lasting increase is inhibitory, perhaps due to a desensitization effect by maintenance of high levels of 5HT. This may be the explanation for the biphasic effects of 5HT agonists on lordosis; they are stimulatory and inhibitory in low and high doses respectively [21].

There are several pieces of evidence to support this hypothesis:

(a) Inhibitors of 5HT uptake (which therefore raise 5HT activity) can stimulate female receptivity [28,55].

(b) At a time when progesterone stimulates receptivity it appears to stimulate release of 5HT in the hypothalamus (see Results). Chronic administration of progesterone over 2 or 4 days (which would inhibit receptivity [40]), also stimulates 5HT turnover [31] and this longer lasting increase could cause desensitization of the neural substrate. A single injection of progesterone enhances the inhibitory effect of LSD (a 5HT agonist) [46] perhaps because their additive effect on increasing 5HT activity leads to greater desensitization.

(c) Long-term depletion of brain 5HT, by PCPA, inhibits female receptivity, normally induced by oestrogen-progesterone priming; presumably the progesterone cannot act on the depleted serotonergic nervones [42]. PCPA given 24 hours before the expected time of receptivity in intact cyclic females also exerts an inhibitory effect and this can be reversed by 5HTP [5]. Other depletors of brain 5HT have not been tested in receptive females for an inhibitory effect [18, 51, 57].

(d) Meyerson and Lewander [37] showed that the tryptophan hydroxylase inhibitor, α-propyldopacetamide, which does not induce a transient release of 5HT, does not stimulate female sexual activity. They showed indirectly that PCPA does release 5HT by giving it with a monoamine oxidase inhibitor. This would convert a transient release into a longer lasting and greater rise of 5HT, which would exert an inhibitory effect on lordosis as was, in fact, noted. In addition, other depletors of brain 5HT have not been shown to stimulate receptivity satisfactorily. PCA only stimulated lordosis in oestrogen-progesterone primed rats, but in rats given oestrogen alone it was ineffective [51,57], 57 DHT was not stimulatory in oestrogen primed rats in one report [51] and in another was effective between days 1–7 and 20–160; but not days 7–14 even though at this time significant destruction of hypothalamic nerve terminals had occurred [18].

In summary PCPA stimulates lordosis in the female rat by one or more of three mechanisms; (1) stimulation of adrenal production of progesterone, (2) transient depletion of NA and (3) conversion to an active metabolite which may act via depletion of NA or possibly by stimulating a transient release of 5HT.

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