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EFFECT OF STRESS AND *PARA*-CHLOROPHENYLALANINE UPON BRAIN SEROTONIN, 5-HYDROXYINDOLEACETIC ACID AND CATECHOLAMINES IN GROUPED AND ISOLATED MICE*

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Abstract—Para-chlorophenylalanine (pCpA), administered to mice only a few hours before they were sacrificed, lowered whole brain serotonin (5-HT) only slightly but caused a marked (27%) reduction in 5-hydroxyindoleacetic acid (5-HIAA). Restraint stress for 2·75 hr markedly elevated 5-HIAA in saline controls and largely prevented its reduction by pCpA. The elevation of brain 5-HT otherwise induced by fighting and by restraint stress was prevented by pCpA, although stress did not significantly accelerate the pCpA-induced depletion of 5-HT. Levels of 5-HT and 5-HIAA, and the 5-HIAA/5-HT ratio, were lower in mice that had been isolated for 3 months than in their grouped littermates. Contingent upon future elucidation of possible effects of stress and of pCpA upon active transport of 5-HIAA from the brain, these observations suggest: (1) that very pronounced changes in the normal metabolism of 5-HT may be produced by treatments that modify total endogenous stores only slightly; (2) that the elevation of 5-HT by stress may be associated with an increase, not with a decrease, in the metabolism of serotonin; (3) that an appreciable proportion of the 5-HIAA formed under normal non-stressed conditions may be derived from newly synthesized 5-HT; but (4) that during stress a considerable amount of 5-HIAA may be formed from 5-HT that is released from stores; and (5) that the basal rate of formation of 5-HIAA from 5-HT is faster in grouped than in isolated mice.

In unstressed mice, norepinephrine (NE) and dopamine (DA) were lowered by pCpA to about the same small degree as 5-HT. They were lowered more in pregrouped than in preisolated mice. Stress elevated DA and, by so doing, counteracted its tendency to be reduced by pCpA. This resulted in a reduction of the NE/DA ratio in stressed mice, particularly in mice pretreated with pCpA. The normal tendency for preisolated mice to fight when placed together was immediately abolished by pCpA concomitant with an immediate small reduction of NE in the pons and medulla oblongata; aggressive tendencies gradually returned over a period of 1·5–5 hr.

VARIOUS stressors elevate brain levels of serotonin, dopamine, and under some circumstances norepinephrine (cf. refs 1–6).† In the studies herein reported, we have used *p*-chlorophenylalanine (pCpA), a drug recently introduced as an inhibitor of serotonin biosynthesis,[7] to help elucidate the mechanism of brain amine changes in two stress situations.

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† The following abbreviations will be employed: 5-HT = serotonin; NE = norepinephrine; DA = dopamine; 5-HIAA = 5-hydroxyindoleacetic acid; pCpA = *para*-chlorophenylalanine.

METHODS

p-Chlorophenylalanine (360 mg/kg) was administered i.p. to mice as a neutral suspension in 0·2 ml of 0·9% NaCl. Control animals received vehicle only. Whole brains were removed, weighed, and frozen on dry ice immediately after decapitation of the animals. While still frozen, whole brains were individually homogenized in 3 ml ice-cold 0·01 N HCl containing 10 mg EDTA, and extracted into 25 ml of salt-saturated butanol by the method of Shore and Olin;[8] 23 ml of the butanol was transferred to 40 ml of washed heptane, and the amines were collected in 1·5 ml of 0·01 N HCl. Appropriate aliquots were taken for the analysis of serotonin,[9] dopamine,[10] and norepinephrine,[11] with the serotonin samples subsequently serving as individual brain blanks for the dopamine analyses. Three concentrations of each of the three amines (50, 100, 150 ng norepinephrine; 100, 200, 300 ng each of dopamine and serotonin) were added in duplicate to brain tissue homogenates and carried throughout the entire procedure. Recovery of the internal standards ranged from 76–80 per cent for norepinephrine, from 70–79 per cent for dopamine and from 74–80 per cent for serotonin; the S.E. of the mean of multiple standards in the same analytical run was $\pm$0·3 to 0·6 per cent.

In the multifactorial experiment reported in Table 5, 5-hydroxyindoleacetic acid (5-HIAA) was also determined by employing an adaptation of the ether extraction method of Udenfriend *et al.*[12] Recoveries of 5-HIAA ranged from 83–86 per cent. Our method for the simultaneous determination of 5-HIAA and the three amines in individual mouse brains will be described in detail in a separate publication devoted to that subject (in preparation).

In their introductory work, Koe and Weissman[7] used three consecutive daily doses of pCpA at 316 mg/kg to achieve a 75 per cent lowering of brain serotonin in mice. However, in our preliminary experiments we noted that two or three consecutive daily doses of 300 mg/kg sometimes (but not always) resulted in gaseous distention of the stomach and small intestine, apparently due to impairment of normal gastrointestinal motility. In order to minimize such problems, we sacrificed our mice 3–6 hr after a single dose of pCpA at 360 mg/kg.

All manipulations with the animals and later with the chemical analyses were patterned to rotate systematically among the treatment conditions every four animals in order to balance any rhythmic or otherwise uncontrollable variations. In each experiment except the last, all samples from different treatments compared were analyzed in the same analytical run. In the last experiment a multifactorial design of five analytical runs of 32–40 brains each was employed.[13] Within each run, each treatment was represented by an identical number of brains. Run-to-run differences were removed by analysis of variance.[14]

In the fighting experiments, male white Swiss mice which had been made aggressive by 8 weeks of social isolation were paired to fight either 10 min or 5 hr after injection with pCpA or placebo. By using uniform handling procedures, two drug-treated or two saline-treated mice were gently placed into a strange cage, and the latency before the first attack was measured in seconds. In the succeeding 5-min period, each definitive biting contact was scored with a mechanical counter, and these were recorded as "fights". One drug-treated and one saline-treated pair were observed simultaneously. The nonparametric Wilcoxon two-sample test was employed for statistical comparisons.[15] The latency of attack and the number of fights were expressed in two ways: (1) as

the mean based upon the pairs in which fighting actually occurred; (2) as the mean based upon all pairs tested, irrespective of whether the mice fought or not. In the latter case, pairs which did not fight were assigned a latency of 300 sec, which is the length of the period of observation.

RESULTS

Reduction of brain amines after pCpA. Table 1 shows that small but statistically significant reductions in brain serotonin can be achieved within 3–6 hr with either 115 mg/kg or 345 mg/kg of pCpA in female Jackson SWR/J mice.

Table 2 shows that brain norepinephrine, but not brain dopamine or serotonin, was lowered significantly within 10–15 min after administration of 360 mg/kg of pCpA. The greatest lowering occurred in the pons and medulla.

Fighting. In the first stress experiment, male white Swiss mice which had been made aggressive by 8 weeks of individual housing were administered either 360 mg/kg of

TABLE 1. SHORT-TERM EFFECT OF *p*-CHLOROPHENYLALANINE UPON WHOLE BRAIN SEROTONIN IN MICE*

	Serotonin (ng/g)	P <
Controls	914 ± 27 (12)†	
pCpA (115 mg/kg)		
3 hr	819 ± 20 (12)	0·025
6 hr	800 ± 20 (8)	0·01
pCpA (345 mg/kg)		
3 hr	760 ± 14 (12)	0·001
6 hr	704 ± 13 (12)	0·001

* Female Jackson Laboratory SWR/J mice were given 115 mg/kg or 345 mg/kg pCpA and sacrificed 3 or 6 hr later.

† Values for all tables are mean ± S.E.M.; the number of individual whole-brain determinations is indicated in parentheses.

TABLE 2. LOWERING OF BRAIN NOREPINEPHRINE BUT NOT DOPAMINE OR SEROTONIN WITHIN 10–15 MIN AFTER ADMINISTRATION OF *p*-CHLOROPHENYLALANINE*

	N	Norepinephrine (ng/g)	Dopamine (ng/g)	Serotonin (ng/g)
Jackson BALB/c				
Control	6	393 ± 19	994 ± 81	653 ± 15
360 mg/kg, 10 min	7	379 ± 14†	902 ± 70	648 ± 15
Jackson C_3H				
Control	15	472 ± 9	834 ± 16	823 ± 32
360 mg/kg, 10 min	15	448 ± 9†	817 ± 12	905 ± 29
Cumberland C_3H				
Telencephalon				
Control	15‡	271 ± 5	1119 ± 17	491 ± 7
360 mg/kg, 15 min	15	272 ± 4	1163 ± 21	505 ± 6
Dien. + mesencephalon				
Control	15	705 ± 19	306 ± 11	1076 ± 16
360 mg/kg, 15 min	15	702 ± 18	330 ± 12	1092 ± 17
Pons + medulla				
Control	15	744 ± 16		
360 mg/kg, 15 min	15	688 ± 15§		

* Whole brains of individual mice were analyzed except where brain parts are indicated. All values are given as the mean ± S.E.M.

† $P < 0.05$ for Jackson BALB/c and C_3H combined; Students' *t*-test (two-tailed hypothesis).

‡ There were 30 mice for each treatment. Brain parts of 2 mice were pooled for each analysis.

§ $P < 0.025$.

TABLE 3. INHIBITION OF THE STRESS-INDUCED ELEVATION OF BRAIN SEROTONIN AND DOPAMINE BY *p*-CHLOROPHENYLALANINE*

	Saline	pCpA	P <
A. Fighting†			
Serotonin (ng/g)			
Non-fighting content	675 ± 17 (16)	602 ± 34 (16)	0·05
Fighting-induced change	+40 ± 5 (16)	−9 ± 11 (16)	0·01
P <	0·06	n.s.	
Norepinephrine (ng/g)			
Non-fighting content	503 ± 11 (16)	467 ± 9 (16)	0·02
Fighting-induced change	−4 ± 15 (16)	−5 ± 13 (16)	n.s.
P <	n.s.	n.s.	
B. Restraint‡			
Serotonin (ng/g)			
Unstressed content	811 ± 22 (10)	743 ± 12 (10)	0·025
Stress-induced change	+43 ± 9 (10)	+9 ± 10 (10)	0·03
P <	0·06	n.s.	
Dopamine (ng/g)			
Unstressed content	845 ± 31 (10)	892 ± 57 (10)	n.s.
Stress-induced change	+225 ± 88 (10)	−78 ± 20 (10)	0·001
P <	0·05	0·07	
Norepinephrine (ng/g)			
Unstressed content	445 ± 18 (10)	418 ± 14 (10)	n.s.
Stress-induced change	+1 ± 3 (10)	−11 ± 3 (10)	0·05
P <	n.s.	n.s.	

* Paired vehicle-treated and drug-treated stressed and unstressed mice were compared by the Wilcoxon two-sample test.[15] All values are given as mean ± S.E.M. N for each treatment is in parentheses.

† Male white Swiss mice were made aggressive by isolating them for 8 weeks. They were given 360 mg/kg pCpA or vehicle and were sacrificed 6 hr later. Experimental mice were placed in pairs and allowed to fight during the last hour prior to sacrifice.

‡ Male C3H mice were given 360 mg/kg pCpA or vehicle and were sacrificed 4·5 hr later. Experimental mice were subjected to restraint stress by taping them to the edge of a table for the last 2 hr prior to sacrifice.

pCpA or vehicle alone, and they were sacrificed exactly 6 hr later; half of the mice from each treatment were placed in pairs and allowed to fight for exactly the last 60 min of this time. Mice injected with vehicle or drug and returned to their cages for 6 hr before sacrifice served as baseline controls; they were sacrificed in the animal room in which they had been housed, and care was taken to cause them minimal disturbance. Table 3 shows that fighting caused an increase in brain serotonin in vehicle-injected mice, but that serotonin was lowered to virtually the same level irrespective of whether the mice fought or not if they had been pretreated with pCpA; the difference between the effect of stress upon brain serotonin in the drug-treated and in the non-drug animals was significant ($P < 0{\cdot}01$).

Brain norepinephrine was significantly lowered by the pCpA ($P < 0{\cdot}02$), but it was not measurably altered by fighting. The effect of pCpA upon the incidence of fighting in these mice is shown in Table 4. Mice paired for testing 5 hr after injection with pCpA fought less than saline-injected controls, but this difference was not significant statistically. Mice paired only 10 min (Table 2) after pCpA injection did not fight at all (Table 4); 85 min later, when the animals were observed again, the saline pairs were still fighting, although at a reduced rate, and the drug-treated animals had begun sporadic light nipping at one another. When placed in groups of four 10 min later (95 min after the first pairing), the pCpA-treated mice fought slightly less than their saline-treated controls, but the difference was no longer statistically significant.

TABLE 4. EFFECT OF *p*-CHLOROPHENYLALANINE ON ISOLATION-INDUCED AGGRESSIVENESS*

	Mice paired 10 min after injection						Mice paired 5 hr after injection†	
	Observed 10 min after injection		Observed after 85 min in original pairs		Groups of 4 mice formed from preceding after 95 min in original pairs		Observed 5 hr after injection	
	Control	pCpA	Control	pCpA	Control	pCpA	Control	pCpA
No. pairs fighting	8	0‡	6	3	4	4	8	6
No. pairs formed	8	8	8	8	4	4	8	8
Latency of attack (sec)								
Basis: groups actually fighting	81 ± 17	no fights‡			34 ± 17	38 ± 12	93 ± 29	89 ± 15
Basis: all groups (300 sec assigned non-fighters)	81 ± 17	300 ± 0‡			34 ± 17	38 ± 12	93 ± 29	141 ± 34
No. fights in 5 min								
Basis: groups actually fighting	52 ± 7	0 ± 0‡	23 ± 5	6 ± 2§	76 ± 12	54 ± 15	97 ± 15	92 ± 12
Basis: all groups (avg.)	52 ± 7	0 ± 0‡	17 ± 5	2 ± 1§	76 ± 12	54 ± 15	97 ± 15	69 ± 16

* pCpA, 360 mg/kg, administered i.p. as a neutral suspension in 0·2 ml of 0·9% saline. All values given as the mean ± S.E.M.
† Brain amines of these mice are given in Table 3.
‡ Differ from controls $P < 0.001$.
§ Differ from controls $P < 0.05$.

Restraint stress. In a second stress experiment, (Table 3), male C_3H mice were given vehicle or 360 mg/kg pCpA, and $2\frac{1}{2}$ hr later they were subjected to restraint by taping them lightly to the edge of a table. Exactly 2 hr later they were sacrificed. Vehicle- or drug-injected unstressed mice were the baselines for judging the stress effects. As in the previous experiment, serotonin was elevated by stress in the saline controls, but it was lowered to virtually the same level irrespective of whether the mice were stressed or not if they had been pretreated with pCpA; again, the difference between the stress responses of the drug-treated and non-drug animals was significant ($P < 0{\cdot}03$). Dopamine was significantly elevated by stress in the saline controls, but both it and norepinephrine were slightly lowered by stress in mice pretreated with pCpA; as was the case for serotonin, the difference between the stress responses of the drug-treated and non-drug animals was significant for both catecholamines, viz. $P < 0{\cdot}001$ and $P < 0{\cdot}05$ respectively.

If the serotonin data for the two first experiments are considered together statistically as representing two units of a single study of stress, then both the stress-induced elevation in the controls and the difference between the stress responses of the drug-treated and non-drug mice are highly significant ($P < 0{\cdot}001$).

Multifactorial restraint stress experiment. Male white Swiss mice which had been housed in isolation or in groups of 10 for 3 months were administered vehicle or 360 mg/kg pCpA exactly 7 hr before sacrifice. Some mice were subjected to restraint stress, as described above, for the last $2\frac{3}{4}$ hr of this time. A total of 184 mice were distributed equally among the 8 treatments. The results are presented in Tables 5 and 6.

5-HT. In no case was 5-HT lowered more than 10–13 per cent by pCpA. Stress tended to elevate 5-HT in saline controls, although the effect was not as marked as in the preceding experiments; it had a very slight tendency to accelerate the lowering of 5-HT caused by pCpA. *P*-Chlorophenylalanine tended to lower 5-HT slightly more in mice that were pregrouped or stressed than in mice that were preisolated or were not stressed, but these differences were not quite significant statistically ($P < 0{\cdot}15$). Serotonin levels were significantly higher in pregrouped than in preisolated saline-treated mice ($P < 0{\cdot}01$); after pCpA, they were slightly, although insignificantly, lower.

5-HIAA. p-Chlorophenylalanine caused a marked drop in 5-HIAA ($P < 0{\cdot}001$), whereas stress caused a marked increase in 5-HIAA both in the saline-treated controls ($P < 0{\cdot}001$) and in the mice pretreated with pCpA ($P < 0{\cdot}001$). The increase induced by stress, however, was significantly less in the mice pretreated with pCpA ($P < 0{\cdot}025$) than in those treated with vehicle alone, although the elevation was not adequate to return 5-HIAA to the level of the unstressed saline-treated controls. The stress-induced return of 5-HIAA levels toward the levels of unstressed saline controls was more nearly complete in preisolated than in pregrouped mice ($P < 0{\cdot}15$). Overall, the pregrouped mice had significantly higher levels of brain 5-HIAA than the preisolated mice, whether they had been stressed or not and whether they had been pretreated with pCpA or not, although this difference tended to be abolished during stress due to the slightly greater relative elevation of 5-HIAA in the isolates.

Ratio of 5-HIAA/5-HT. In general, the ratio reflected the relative lability of 5-HIAA as opposed to the relative stability of 5-HT. It dropped significantly after pCpA. It was significantly elevated by stress, and significantly more in the saline-treated controls than in the mice pretreated with pCpA ($P < 0{\cdot}001$). Preisolated mice pretreated with

TABLE 6. PROBABILITIES OF SIGNIFICANT COMPARISONS NOT INDICATED IN TABLE 5*

					Ratios			
	5-HIAA	5-HT	NE	DA	5-HIAA/5-HT	NE/DA	NE/5-HT	DA/5-HT
Stressed *vs.* Unstressed								
Overall	<0·001	—	—	<0·001	<0·001	<0·001	—	<0·001
Pregroup	<0·001	—	—	<0·001	<0·005	<0·005	—	<0·001
Preisolate	<0·001	—	—	<0·001	<0·001	<0·001	—	<0·025
Saline	<0·001	—	—	<0·001	<0·001	<0·05	—	<0·025
pCpA	<0·001	—	—	<0·001	<0·001	<0·001	—	<0·001
Saline *vs.* pCpA								
Overall	<0·001	<0·001	<0·001	<0·05	<0·001	—	<0·025	<0·08
Pregroup	<0·001	<0·001	<0·001	<0·05	<0·001	—	<0·07	~0·15
Preisolate	<0·001	<0·005	<0·005	—	<0·001	—	<0·18	<0·05
Stressed	<0·001	<0·001	<0·025	—	<0·001	—	<0·05	<0·025
Unstressed	<0·001	<0·005	<0·01	<0·01	<0·001	—	—	—
Pregroup *vs.* preisolate								
Overall	<0·001	~0·15	—	—	<0·005	—	—	—
Stressed	<0·025	—	—	—	<0·15	—	—	—
Unstressed	<0·001	—	—	—	<0·01	<0·05	—	—
Saline	<0·001	<0·01	—	—	<0·025	—	—	—
pCpA	<0·025	—	—	—	<0·05	—	—	—
Stress + saline	<0·02	~0·15	—	—	~0·18	—	—	—
Unstressed + saline	<0·01	~0·15	—	—	<0·06	~0·09	—	—
Stressed + pCpA	—	—	—	—	—	—	—	—
Unstressed + pCpA	<0·02	—	—	<0·05	<0·05	—	—	—

* Comparisons represented by the coincidence of blocks which contain only dashes do not approach significance.

pCpA had their 5-HIAA/5-HT ratios elevated closer to unstressed, saline-treated controls by stress than was the case for pregrouped mice ($P < 0{\cdot}001$). Pregrouped mice had significantly higher 5-HIAA/5-HT ratios than preisolated mice, in spite of their significantly higher levels of 5-HT in this experiment.

Norepinephrine. NE was significantly lower in all pCpA-pretreated mice than in their saline controls, whether they were stressed or not and whether they were preisolated or pregrouped. The lowering of NE by pCpA was greater in pregrouped than in preisolated mice ($P < 0{\cdot}01$). It was not significantly affected by stress in this experiment.

Dopamine. p-Chlorophenylalanine caused a significant lowering of DA in unstressed mice ($P < 0{\cdot}01$), However, stress caused a marked elevation of DA ($P < 0{\cdot}001$), and this elevation was somewhat greater in the pCpA-pretreated mice than in their saline-treated controls ($P < 0{\cdot}05$); stress tended, therefore, to obscure the lowering of DA which was otherwise produced by pCpA. The lowering of DA induced by pCpA in unstressed mice tended to be less for preisolated than for pregrouped mice ($P < 0{\cdot}07$).

Ratio of NE/DA. Stress significantly lowered the NE/DA ratio, reflecting the relative elevation of DA induced by stress. In pre-isolated (but not in pregrouped) mice, the stress-induced lowering of the NE/DA ratio was greater if the mice had been pretreated with pCpA ($P < 0{\cdot}06$). The tendency for pCpA to lower the NE/DA ratio below unstressed, saline-treated control values was significantly greater in stressed than in unstressed mice ($P < 0{\cdot}001$). The ratio was significantly higher in saline-treated, unstressed, pregrouped mice than in saline-treated, unstressed preisolates ($P < 0{\cdot}05$).

Ratio of NE/5-HT. Overall, the NE/5-HT ratio was significantly elevated by pCpA ($P < 0{\cdot}025$), reflecting the relatively greater lowering of 5-HT. This effect was significant in stressed ($P < 0{\cdot}05$) but not in unstressed mice, and it approached significance in pregrouped ($P < 0{\cdot}07$) but not in pre-isolated mice ($P < 0{\cdot}18$).

Ratio of DA/5-HT. The DA/5-HT ratio was significantly elevated by pCpA in stressed but not in unstressed mice. Stress significantly elevated the DA/5-HT ratio ($P < 0{\cdot}001$); this effect tended to be greatest in the pCpA-treated mice ($P < 0{\cdot}12$), reflecting the simultaneous enhancement by stress of the lowering of 5-HT and the elevation of DA. The elevation of this ratio above unstressed, saline-treated control values induced by pCpA was greater for stressed than for unstressed mice ($P < 0{\cdot}005$).

DISCUSSION

Our results show that *p*-chlorophenylalanine may interfere with normal dopamine and noradrenaline metabolism as well as with that of serotonin. Thus, they support other evidence that the general physiological and behavioral effects of this drug cannot be attributed solely to its effects upon the synthesis of serotonin.[16]

Nevertheless, pCpA is a sufficiently effective inhibitor of serotonin biosynthesis to be useful as an aid to the study of normal control mechanisms in serotonin metabolism, provided that interpretations are appropriately restrained by regard for its other effects.

Since tryptophan hydroxylase can be inhibited both *in vitro* and *in vivo* by pCpA,[7, 17] it is probable that the small elevation of serotonin which is normally induced by stress was prevented in our experiments by the inhibition of normal on-going sero-

TABLE 5. EFFECTS OF STRESS, *p*-CHLOROPHENYLALANINE AND PRIOR HOUSING UPON WHOLE BRAIN AMINES AND 5-HYDROXYINDOLEACETIC ACID IN MICE*

	Preisolated			Pregrouped		
	Unstressed	Restraint stress	Induced change	Unstressed	Restraint stress	Induced change
5-HIAA (ng/g ± S.E.M.)						
Saline	392 ± 13·8	489 ± 19·5	+97 ± 23·9 (<0·001)†	462 ± 21·7	561 ± 23·6	+99 ± 32·0 (<0·005)†
pCpA	287 ± 7·9	361 ± 13·8	+74 ± 15·9 (<0·001)	333 ± 16 4	379 ± 15·6	+46 ± 22·6 (<0·05)
Induced change from:						
1) Treatment saline	−105 ± 15·9 (<0·001)	−128 ± 23·9 (<0·001)		−129 ± 27·2 (<0·001)	−182 ± 28·3 (<0·001)	
2) Unstressed saline	−105 ± 15·9 (<0·001)	−31 ± 19·5 (~0·15)‡		−129 ± 27·2 (<0·001)	−83 ± 26·7 (<0·002)§	
5-HT (ng/g ± S.E.M.)						
Saline	691 ± 14·9	711 ± 16·0	+20 ± 21·9 (n.s.)‖	724 ± 15·4	749 ± 26·0	+25 ± 30·2 (n.s.)‖
pCpA	649 ± 21·1	644 ± 16·6	−5 ± 26·8 (n.s.)	648 ± 23·7	637 ± 16·7	−11 ± 29·0 (n.s.)
Induced change from:						
1) Treatment saline	−42 ± 25·8 (~0·11)	−67 ± 23·1 (<0·005)		−76 ± 28·3 (<0·01)	−112 ± 30·9 (<0·001)¶	
2) Unstressed saline	−42 ± 25·8 (~ 0·11)	−47 ± 22·3 (<0·05)		−76 ± 28·3 (<0·01)	−87 ± 22·7 (<0·001)	
NE (ng/g ± S.E.M.)						
Saline	339 ± 7·1	339 ± 6·0	0 ± 9·3 (n.s.)	355 ± 7·6	347 ± 7·7	−8 ± 10·8 (n.s.)
pCpA	328 ± 7·7	324 ± 8·6	−4 ± 11·5 (n.s.)	323 ± 8·8	326 ± 8·4	+3 ± 12·2 (n.s.)
Induced change from:						
1) Treatment saline	−11 ± 10·5 (n.s.)	−15 ± 10·5 (~0·15)		−32 ± 11·6 (<0·01)	−21 ± 11·4 (0·07)Ø	
2) Unstressed saline	−11 ± 10·5 (n.s.)	−15 ± 11·2 (~0·16)		−32 ± 11·6 (<0·01)	−29 ± 11·3 (0·02)	
DA (ng/g ± S.E.M.)						
Saline	581 ± 12·1	634 ± 15·8	+53 ± 19·9 (<0·01)**	586 ± 16·1	670 ± 27·9	+84 ± 32·2 (0·01)**
pCpA	563 ± 12·9	643 ± 26·2	+80 ± 29·2 (<0·01)	516 ± 17·9	640 ± 18·9	+124 ± 26·0 (<0·001)
Induced change from:						
1) Treatment saline	−18 ± 17·7 (n.s.)	+9 ± 30·6 (n.s.)		−70 ± 24·1 (<0·005)††	−30 ± 33·7 (n.s.)	
2) Unstressed saline	−18 ± 17·7 (ns.)	+62 ± 28·9 (<0·05)‡‡		−70 ± 24·1 (<0·005)	+54 ± 24·8 (<0·005)‡‡	
5-HIAA/5-HT (Ratio × 100 ± S.E.M.)						
Saline	56·9 ± 2·00	69·8 ± 2·82	+12·9 ± 3·46 (<0·001)§§	64·2 ± 3·16	75·9 ± 3·60	+11·7 ± 4·79 (<0·02)§§
pCpA	45·4 ± 1·73	56·2 ± 2·64	+10·8 ± 3·16 (<0.001)	51·0 ± 2·00	59·0 ± 1·73	+8·0 ± 2·64 (<0·005)
Induced change from:						
1) Treatment saline	−11·5 ± 2·64 (<0·001)	−13·6 ± 3·86 (<0·001)		−13·2 ± 3·74 (<0·001)	−16·9 ± 3·99 (<0·001)	
2) Unstressed saline	−11·5 ± 2·64 (<0·001)	−0·7 ± 3·31 (n.s.)‖‖		−13·2 ± 3·74 (<0·001)	−5·2 ± 3·60 (~0·15)‖‖	
NE/DA (Ratio × 100 ⊥ S.E.M.)						
Saline	61·9 ± 1·41	58·2 ± 1·41	−3·7 ± 1·99 (<0·06)¶¶	65·7 ± 1·73	54·2 ± 6·55	−11·5 ± 6·77 (~0·1)
pCpA	62·1 ± 1·73	52·9 ± 1·41	−9·2 ± 2·23 (<0·001)	65·7 ± 2·00	54·5 ± 1·41	−11·2 ± 2·45 (<0·001)
Induced change from:						
1) Treatment saline	+0·2 ± 2·23 (n.s.)	−5·3 ± 1·99 (<0·01)		0·0 ± 2·64	+0·3 ± 6·7 (n.s.)	
2) Unstressed saline	+0·2 ± 2·23 (n.s.)	−9·0 ± 1·99 (<0·001)ØØ		0·0 ± 2·64	−11·2 ± 2·23 (<0·001)ØØ	
NE/5-HT (Ratio × 100 ± S.E.M.)						
Saline	49·1 ± 1·00	48·1 ± 1·00	−1·0 ± 1·41 (n.s.)	49·4 ± 1·41	47·1 ± 1·41	−2·3 ± 1·99 (n.s.)
pCpA	51·5 ± 1·73	50·0 ± 1·00	−1·5 ± 2·00 (n.s.)	51·5 ± 2·23	51·8 ± 1·41	+0·3 ± 2·64 (n.s.)
Induced change from:						
1) Treatment saline	+2·4 ± 2·00 (n.s.)	+1·9 ± 1·41 (~0·16)		+2·1 ± 2·64 (n.s.)	+4·7 ± 1·99 (<0·02)	
2) Unstressed saline	+2·4 ± 2,00 (n.s.)	+0·9 ± 1·41 (n.s.)		+2·1 ± 2·64 (n.s.)	+2·4 ± 1·99 (~0·02)	
DA/5-HT (Ratio × 100 ± S.E.M.)						
Saline	83·7 ± 2·82	88·7 ± 3·16	+5·0 ± 4·24 (n.s.)	80·4 ± 2·44	90·9 ± 4·47	+10·5 ± 5·09 (<0·05)***
pCpA	86·9 ± 2·82	100·5 ± 4·69	+13·6 ± 5·47 (<0·02)	81·8 ± 4·00	99·6 ± 3·16	+17·8 ± 5·10 (<0·001)
Induced change from:						
1) Treatment saline	+3·2 ± 3·99 (n.s.)	+11·8 ± 5·66 (<0·05)		+1·4 ± 4·69 (n.s.)	+8·7 ± 5·47 (~0·11)	
2) Unstressed saline	+3·2 ± 3·99 (n.s.)	+16·8 ± 5·47 (<0·005)†††		+1·4 ± 4·69 (n.s.)	+19·2 ± 3·99 (<0·001)†††	

* Male white Swiss mice which had been housed in isolation or in groups of 10 for 3 months were given vehicle or 360 mg/kg pCpA i.p. in 0·3 ml pH 7·0 saline. They were sacrificed exactly 7 hr later. Some mice were stressed by taping them lightly to the edge of a table for the last 2 ½ hr of this time. There were 184 mice equally distributed among the 8 treatments.

† Stress elevated 5-HIAA less in pCpA-pretreated than in saline-pretreated mice ($P < 0·025$).

‡ pCpA reduced 5-HIAA further below unstressed, saline-treated control values in unstressed than in stressed mice ($P < 0·005$).

§ Stress raised 5-HIAA closer to unstressed, saline-treated control values in preisolated than in pregrouped mice pretreated with pCpA ($P \sim 0·15$).

‖ The difference between the tendencies for stress to slightly elevate 5-HT in saline-treated mice and to slightly lower it in pCpA-treated mice approached significance ($P \sim 0·15$).

¶ pCpA tended to lower 5-HT more in pregrouped than in preisolated mice ($P \sim 0·17$).

Ø pCpA lowered NE more in pregrouped than in pre-isolated mice ($P < 0·01$).

** Stress tended to elevate DA more in mice pretreated with pCpA than with saline ($P < 0·05$) and to elevate it more in pregrouped than in preisolated mice ($P \sim 0·17$).

†† pCpA tended to lower DA more in unstressed pregrouped mice than in unstressed preisolated mice ($P < 0·07$).

‡‡ The difference between the tendency for DA to be lowered below the levels of unstressed, saline-treated controls in unstressed pCpA-treated mice and the tendency for it to be raised above them in stressed pCpA-treated mice approached significance ($P < 0·08$).

§§ Stress elevated the 5-HIAA/5-HT ratio more in saline-treated than in pCpA-treated mice ($P < 0·001$).

‖‖ pCpA lowered the 5-HIAA/5-HT ratio below unstressed, saline-treated controls more in unstressed than in stressed mice ($P < 0·001$).

¶¶ Stress lowered the NE/DA ratio less in preisolated mice pretreated with saline than in those pretreated with pCpA ($P < 0·06$).

ØØ pCpA lowered the NE/DA ratio below unstressed, saline-treated controls less in unstressed than in stressed mice ($P < 0·001$).

*** Stress tended to elevate the DA/5-HT ratio less in saline-treated than in pCpA-treated mice ($P \sim 0·12$).

††† pCpA elevated the DA/5-HT ratio above unstressed, saline-treated control values less in unstressed than in stressed mice ($P < 0·005$).

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tonin biosynthesis. However, pCpA is also a potent inhibitor *in vivo* of phenylalanine hydroxylase and it causes elevated serum levels of phenylalanine,[18] an amino acid which is capable of decreasing transport of both 5-hydroxytryptophan[19] and tyrosine[20] into brain. Decreased transport of these amino acids could be partially responsible for the observed effects of pCpA upon both serotonin and the catecholamines.

Although the lowering of norepinephrine and dopamine by *p*-chlorophenylalanine was slightly greater in pregrouped than in pre-isolated mice, stress did not produce different drug responses in mice which had lived under these two different housing conditions. Norepinephrine was not detectably modified by stress in this experiment but dopamine was markedly increased. In the smaller of the two restraint stress experiments, pCpA seemed to retard importantly the stress-induced elevation of dopamine, but the opposite effect was observed in the larger multifactorial experiment. The effect of pCpA upon brain catecholamines appears to be complex. A partial inhibition of catecholamine biosynthesis could contribute to our result inasmuch as tyrosine hydroxylase can be inhibited to a degree by either phenylalanine[21] or pCpA.[7] However, the possibility that pCpA or, more likely, a decaroboxylated derivative[22] may have a mild releasing action is suggested by the finding that brain norepinephrine may be measurably lowered in mice (particularly in the pons and medulla) within 10–15 min after pCpA administration, at a time when serotonin is not significantly altered (Table 2). A third, and equally likely possibility is that the effect of pCpA upon brain catecholamines is not primarily biochemical, but instead reflects nonspecific modifications of activity of catecholamine-containing neurons which follow secondarily as a consequence of modified activity of serotonergic neurons with which they interact. The findings that levels of 5-HIAA are significantly higher and that pCpA tends to lower serotonin and 5-HIAA more in pregrouped than in preisolated mice are consistent with other evidence that the turnover of serotonin may be faster in mice that live in groups.[23, 24] Further, the observation that stress tended to return 5-HIAA levels more nearly to unstressed, saline-treated control levels in preisolated pCpA-treated mice than in pregrouped pCpA-treated mice fits well with the greater behavioral reactivity of the preisolates.

The marked increase in 5-HIAA which is induced by stress is probably the result of an increased rate of serotonin metabolism; if so, this is evidence that the increase in brain serotonin which occurs during stress is associated with an increase, and not with a decrease, in its rate of metabolism. Unfortunately, however, because the removal of organic acids from the brain is an active energy-requiring process,[25, 26] inferences concerning the rate of serotonin metabolism which are based upon changing 5-HIAA levels must be tempered by recognition of the fact that a change in transport could contribute to the changes in 5-HIAA levels observed.

5-HIAA is significantly elevated by stress even in mice pretreated with *p*-chlorophenylalanine, although the elevation induced is significantly less than that produced by stress in saline-treated controls. This increased production of 5-HIAA in stressed pCpA-treated mice may simply reflect a stimulus-facilitated release of serotonin from stores, which indeed is implied by the tendency for stress to facilitate the pCpA-induced reduction of 5-HT. Other evidence has been offered that stimulus may accelerate the release of serotonin from central nervous system tissues.[27, 28]

The relatively slight effect of pCpA upon serotonin levels in mice as compared with that in rats[7] may be due in part to incomplete inhibition of tryptophan hydroxylase

by the drug. Nevertheless, it is significant that the elevations of brain serotonin normally induced by fighting and by restraint stress were blocked by pCpA at a time when endogenous stores of serotonin were lowered only slightly. Further, very small pCpA-induced reductions of 5-HT in unstressed mice were accompanied by very large reductions in 5-HIAA. To us, this emphasizes the general irrelevance of amine levels, as such, and suggests that more importance must be attached to evaluating the integrity of the functional pool when studying the effects of treatments thought to have physiologically important effects upon the metabolism of brain biogenic amines.[29, 30]

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REFERENCES

1. J. S. BRACHAS and D. X. FREEDMAN, *Biochem. Pharmac.* **12**, 1225 (1963).
2. C. BREITNER, A. PICCHIONI and L. CHIN, *J. Neurochem.* **5**, 153 (1963).
3. S. GARATTINI and L. VALZELLI, in *Psychotrophic Drugs* (Eds. S. GARATTINI and V. GHETTI), p. 428. Elsevier, Amsterdam (1957).
4. C. C. TOH, *J. Physiol., Lond.* **151**, 410 (1960).
5. B. L. WELCH, *Science* **155**, 878 (1967).
6. L. KATO, B. GOZSY, P. B. ROY and V. GROH, *Int. J. Neuropsychiat.* **3**, 46 (1967).
7. B. K. KOE and A. WEISSMAN, *J. Pharmac. exp. Ther.* **154**, 499 (1966).
8. P. S. SHORE and J. S. OLIN, *J. Pharmac. exp. Ther.* **122**, 295 (1958).
9. D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE and S. UDENFRIEND, *J. Pharmac. exp. Ther.* **117**, 82 (1956).
10. G. F. MURPHEY and T. L. SOURKES, *Revue can. Biol.* **18**, 379 (1959).
11. R. J. CROUT, *Stand. Meth. clin. Chem.* **3**, 62 (1961).
12. S. UDENFRIEND, E. TITUS and H. WEISSBACH, *J. biol. Chem.* **216**, 499 (1955).
13. D. R. COX, *Planning of Experiments*. John Wiley, New York (1958).
14. E. F. LINDQUIST, *Design and Analysis of Experiments in Psychology and Education*. Houghton Mifflin, Boston (1953).
15. C. L. RUMKE and H. DE JONGE, in *Evaluation of Drug Activities: Pharmacometrics* (Eds. D. R. LAURENCE and A. L. BACARACH), p. 47. Academic Press, New York (1964).
16. A. S. WELCH and B. L. WELCH, *J. Pharm. Pharmac.* **19**, 632 (1967).
17. E. JÉQUIER, W. LOVENBERG and A. SJOERDSMA, *Molec. Pharmac.* **3**, 274 (1967).
18. M. A. LIPTON, R. GORDON, G. GUROFF and S. UDENFRIEND, *Science* **156**, 250 (1967).
19. A. YUWILER, E. GELLER and G. G. SLATER, *J. biol. Chem.* **240**, 1170 (1965).
20. G. GUROFF and S. UDENFRIEND, *J. biol. Chem.* **237**, 803 (1962).
21. M. IKEDA, M. LEVITT and S. UDENFRIEND, *Biochem. biophys. Res. Commun.* **18**, 482 (1965).
22. A. PLETSCHER, M. DA PRADA, G. BARTHOLINI, W. P. BURKHARD and H. BRUDERER, *Life Sci.* 4, 2301 (1965).
23. S. GARATTINI, E. GIACALONE and L. VALZELLI, *J. Pharm. Pharmac.* **19**, 338 (1967).
24. B. L. WELCH and A. S. WELCH, *Fedn Proc.* **25**, 623 (1966).
25. L. D. PROCKOP, L. S. SCHANKER and B. B. BRODIE, *J. Pharmac. exp. Ther.* **3**, 266 (1962).
26. B. WERDINIUS, *J. Pharm. Pharmac.* **18**, 546 (1966).
27. N. E. ANDÉN, A. CARLSSON, N.-Å. HILLARP and T. MAGNUSSON, *Life Sci.* **3**, 473 (1964).
28. T. N. CHASE, G. R. BREESE and I. J. KOPIN, *Science* **157**, 1461 (1967).
29. A. CARLSSON, in *Handbook of Experimental Pharmacology* (Eds. O. EICHLER and A. FARAH), new series, vol. 19, p. 34. Springer-Verlag, Berlin (1965).
30. B. B. BRODIE and C. COSTA, *Psychopharmac. Serv. Cent. Bull.* **5**, 1 (1962).