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COMPARATIVE EFFECTS OF *p*-CHLOROPHENYLALANINE, *p*-CHLOROAMPHETAMINE AND *p*-CHLORO-*N*-METHYLAMPHETAMINE ON RAT BRAIN NOREPINEPHRINE, SEROTONIN AND 5-HYDROXYINDOLE-3-ACETIC ACID*

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Abstract—The time-course of effects of a single dose of *p*-chlorophenylanine on rat brain amines shows a significant lowering of serotonin (5HT) and 5-hydroxyindole-3-acetic acid (5HIAA) beginning on day 1 and lasting for about 8 days. In most brain areas, levels of norepinephrine (NE) are also lowered significantly on days 1 through 5. In contrast, both *p*-chloroamphetamine and *p*-chloro-*N*-methylamphetamine decrease brain 5HT and 5HIAA, beginning at 2–4 hr after dosage and continuing for > 4 days, with no depleting effect on brain NE.

A SEARCH has been on for a selective depletor of brain serotonin (5HT) since the first reports of the presence of this amine in the mammalian central nervous system.[1–3] Numerous studies with reserpine demonstrated that this compound[4] as well as others such as tetrabenazine[5] and the benzoquinolizine Ro 4-1284[6] lowered brain 5HT levels. However, this effect was not limited to 5HT; brain levels of norepinephrine (NE) were also lowered. More recently, compounds such as *p*-chloroamphetamine (PCA) and *p*-chloro-*N*-methylamphetamine (PCMA) have been reported to be selective depletors of brain 5HT by mechanisms yet to be explained.[7, 8] In addition, the amino acid derivative, *p*-chlorophenylalanine (PCPA) has been shown to lower brain 5HT levels by impairing the biosynthesis of the amine at the rate-limiting tryptophan hydroxylase step.[9, 10]

Despite this wealth of publications, no one has reported a comparative study of these more specific depletors of brain 5HT in animals of a similar strain, sex and age. A recent paper from this laboratory presented a more sensitive method for the determination of 5HT and NE in discrete areas of rat brain.[11] Intrastrain differences in the levels of these amines in brains of Sprague–Dawley rats obtained from different breeder-suppliers have also been reported.[12] The present paper describes the time-course of action of PCPA, PCA and PCMA on brain levels of 5HT, NE and 5-hydroxyindole-3-acetic acid (5HIAA) in adult, male rats obtained from a single supplier.

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MATERIALS AND METHODS

Adult, male, Sprague–Dawley rats (250–350 g) obtained from Hormone Assay Laboratories, Chicago, Ill., were maintained on a diet of Purina laboratory chow and tap water *ad lib.* for at least 7 days prior to experimental use. All chemicals and solvents used were reagent grade, purchased from commercial sources. PCPA was purchased from Pierce Chemical Co., Rockford, Ill., PCA was generously supplied by Eli Lilly & Co., Indianapolis, Ind., and PCMA was generously supplied by Hoffmann-La Roche, Inc., Nutley, N.J.

Drugs were given by i.p. injection in volumes such that 1 ml/kg body weight delivered the desired dosage in mg/kg. PCA and PCMA, as the hydrochloride salts, were dissolved in distilled water; PCPA was administered as a suspension in peanut oil.

Acidified *n*-butanol was prepared by adding 0·85 ml of 12 N HCl to 1 l. of *n*-butanol. *o*-Phthalaldehyde (OPT) reagent (10 mg% in 10 N HCl) was stored in the refrigerator. To prepare 0·1 M EDTA, 37·2 g of disodium ethylenediamine tetraacetate dihydrate was dissolved in 900 ml of 1 M sodium acetate solution. After adjustment of the pH to 6·7–7·0 by addition of solid NaOH, the solution was made up to a final volume of 1 l. with distilled water and stored in the refrigerator to retard bacterial growth. Alkaline sulfite reagent was prepared just before use by addition of 9 ml of 5 N NaOH to 1 ml of an aqueous solution containing 2·5 g of anhydrous Na_2SO_3 in 10 ml of distilled water.

Rats were sacrificed by decapitation, brains removed, washed in cold 0·9% saline, blotted dry and stored at −20° until dissection for assay of amines. To obtain meaningful biochemical results from analysis of specific brain areas, a simple technique was devised for dividing rat brains into five reproducible discrete parts. The cerebellum (CB) was removed first (Fig. 1a); then the brain was transsected at the fovia inferior, and just forward of the superior peduncles (Fig. 1b); the area caudal to the fovia inferior was discarded and the part described by the transsections was retained and called medulla (MED). Next, the cerebral hemispheres were peeled away from the midline, revealing the corpus callosum, which was divided down the midline and peeled back similarly to the cerebral hemispheres (Fig. 1c). Now, with the thalamic area visible, a piece was literally "punched out" using a circular tool 4·5 mm in diameter, made for brains of adult rats in the weight range 250–350 g; this piece was referred to as hypothalamus (HTH) (Fig. 1d). Finally, transsections were made just forward of the anterior colliculi on either side of the brain (Fig. 1e); the piece caudal to these transsections was retained and referred to as midbrain (MB), while the remaining brain tissue was called cerebral hemispheres (CH). These parts are referred to as such in the text. While not anatomically perfect, the reproducibility is excellent for biochemical studies.

The levels of 5HT, 5HIAA and NE were determined in single brain areas by a modification of the method of Maickel *et al.*[11] The various areas of rat brain were homogenized in acidified *n*-butanol: areas weighing >280 mg (CH) were homogenized in 10 vols.; other areas, weighing <280 mg, were made up to 280 mg with distilled water, and were then homogenized in 2·8 ml. All homogenizations were performed in a glass homogenizer tube with a motor-driven Teflon pestle. The homogenate was transferred to a centrifuge tube and centrifuged for 5 min at 2000 rpm (IEC Model UV Centrifuge). An aliquot (2·5 ml) of the butanol was then transferred to a 13-ml glass-stoppered centrifuge tube containing 7 ml of *n*-heptane and 0·2 ml of 0·1 N HCl. After

FIG. 1a.

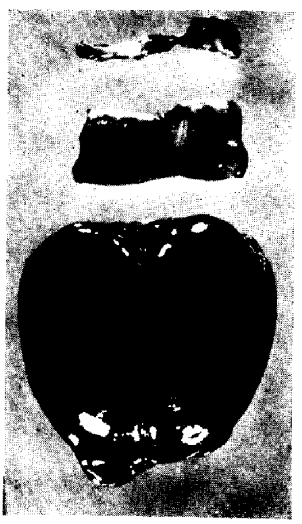

FIG. 1b.

FIG. 1c.

Fig. 1 continued overleaf

FIG. 1d.

FIG. 1e.

FIG. 1. Rat brain parts. See text for details.

mechanical shaking for 5 min, the tubes were centrifuged for 5 min at 2000 rpm. An aliquot (8·5) of the organic phase was transferred to a clean 13-ml glass-stoppered centrifuge tube for assay of 5HIAA. The remainder of the organic phase, including the tissue disc at the organic-aqueous interface was removed by aspiration and discarded. Aliquots of 0·1 ml of the acid phase were then transferred to 10 × 75 mm test tubes for the determination of 5HT and NE as described below.

To tubes containing the organic phase (5HIAA), 0·2 ml of 0·033 M $NaHCO_3$ was added. The tubes were shaken for 1 min, then centrifuged for 5 min at 2000 rpm. The organic phase plus tissue disc were removed by aspiration and discarded. A 0·1-ml aliquot was transferred to a 10 × 75 mm test tube for determination of 5HIAA as described below.

For the assay of 5HT or 5HIAA, 0·5 ml of a 10 mg% solution of OPT in 10 N HCl was added to tubes containing 0·1 ml of 0·1 N HCl or 0·033 M $NaHCO_3$, obtained as described above. After mixing with a vibrating mixer, the tubes were placed in a boiling water bath for 10 min, then removed and cooled in tap water. Fluorescence was measured in an Aminco–Bowman spectrophotofluorometer using 1 cm × 1 cm quartz cuvettes. Activation and emission wavelengths were 360 mμ and 470 mμ respectively. The instrument was equipped with an IP28 phototube, activation and emission slits were set at 3·0 mm, and the phototube slit width was set at 1·0 mm. Under these conditions, linearity was achieved over the range 0·025–3·5 μg of both 5HT and 5HIAA.

For the assay of NE, test tubes containing 0·1 ml of 0·1 N HCl, obtained as described above, were treated with 0·1 ml of 0·4 N HCl and 0·2 ml of EDTA reagent. The contents of the tubes were mixed and 0·1 ml of iodine reagent was added. After mixing, the tubes were allowed to stand exactly 2 min at room temperature, then 0·2 ml of freshly prepared alkaline sulfite solution was added to each tube. The tubes were mixed again; then, after exactly 1·5 min, 0·2 ml of 10 N acetic acid was added and the tubes were shaken and heated in a boiling water bath for 2 min. After cooling in tap water, the fluorescence was measured at activation and emission wavelengths of 385 mμ and 485 mμ, respectively, using the slit settings as described for 5HT and 5HIAA. Fluorescence was linear over the range 0·05–3·0 μg of NE.

Recovery of exogenous 5HT, 5HIAA, and NE added to tubes prior to the first shaking step was 95 to 105 per cent for 5HT, 55–65 per cent for 5HIAA, and 80–90 per cent for NE.

RESULTS

Constancy of brain area weights and levels of 5HT, 5HIAA and NE in normal rats. As a check on the reproducibility of the brain dissection technique and the assay procedure, brains from thirty-two normal rats were assayed in groups of 4–6 over a span of several months. The results are presented in Table 1. The excellent reproducibility of the dissection technique is demonstrated by the area weights. The largest standard deviation is that of cerebral hemispheres (9·5 per cent), while the smallest was that of hypothalamus (7·3 per cent). The consistency of the levels of 5HT, 5HIAA and NE is also quite good. With the exception of 5HT in the medulla, 5HIAA in the cerebellum, and 5HIAA and NE in the hypothalamus, the standard deviations are all <10 per cent of their respective means.

Effects of PCPA on levels of 5HT, 5HIAA and NE in discrete areas of rat brain. The

TABLE 1. BRAIN AREA WEIGHTS AND LEVELS OF 5HT, 5HIAA AND NE*

Brain area	Symbol	Weight (g)	5HT (μg/g)	5HIAA (μg/g)	NE (μg/g)
Cerebral hemispheres	CH	1·074 ± 0·102	0·65 ± 0·05	0·82 ± 0·06	0·42 ± 0·03
Cerebellum	CB	0·247 ± 0·023	0·19 ± 0·01	0·29 ± 0·06	0·21 ± 0·02
Hypothalamus	HTH	0·068 ± 0·005	0·97 ± 0·11	1·55 ± 0·19	1·24 ± 0·22
Midbrain	MB	0·177 ± 0·015	1·06 ± 0·08	1·48 ± 0·09	0·64 ± 0·06
Medulla	MED	0·128 ± 0·011	0·57 ± 0·07	0·94 ± 0·09	0·56 ± 0·05

* Each value is the mean ± S. D. of values obtained from thirty-two rats.

TABLE 2. EFFECTS OF PCPA LEVELS OF 5HT, 5HIAA AND NE IN DISCRETE AREAS OF RAT BRAIN*

Time (days)	Compound	CH (μg/g)	CB (μg/g)	HTH (μg/g)	MB (μg/g)	MED (μg/g)	WB† (μg/g)
0	5HT	0·65±0·05	0·19±0·01	0·87±0·11	1·06±0·08	0·57±0·07	0·63±0·06
	5HIAA	0·82±0·06	0·29±0·06	1·55±0·19	1·48±0·09	0·94±0·09	0·85±0·10
	NE	0·42±0·03	0·21±0·02	1·24±0·22	0·64±0·06	0·56±0·05	0·46±0·05
1	5HT	0·23±0·04‡	0·16±0·01‡	0·44±0·04‡	0 37±0 05‡	0·24±0·03‡	0·24±0·03‡
	5HIAA	0·22±0·02‡	0·14±0·06‡	0·41±0·06‡	0·47±0·09‡	0·28±0·04‡	0·25±0·06‡
	NE	0·29±0·02‡	0·15±0·02‡	0·81±0·12‡	0·51±0·05‡	0·49±0·04	0·32±0·03‡
2	5HT	0·20±0·01‡	0·16±0·01‡	0·36±0·02‡	0·32±0·02‡	0·20±0·02‡	0·21±0·01‡
	5HIAA	0·14±0·02‡	0·12±0·04‡	0·06±0·02‡	0·30±0·01‡	0·21±0·01‡	0·16±0·03‡
	NE	0·33±0·06‡	0·14±0·01‡	0·69±0·08‡	0·42±0·06‡	0·44±0·07‡	0·33±0·04‡
3	5HT	0·18±0·01‡	0·15±0·01‡	0·38±0·09‡	0·35±0·04‡	0·24±0·01‡	0·20±0·02‡
	5HIAA	0·12±0·01‡	0·08±0·04‡	0·10±0·05‡	0·36±0·03‡	0·25±0·01‡	0·15±0·04‡
	NE	0·31±0·04‡	0·17±0·01‡	0·80±0·08‡	0·52±0·05‡	0·55±0·09	0·35±0·04‡
5	5HT	0·20±0·01‡	0·14±0·01‡	0·40±0·06‡	0·46±0·04‡	0·29±0·02‡	0·23±0·02‡
	5HIAA	0·14±0·02‡	0·10±0·02‡	0·32±0·12‡	0·51±0·13‡	0·32±0·04‡	0·16±0·04‡
	NE	0·33±0·02‡	0·17±0·01‡	0·90±0·22	0·49±0·04‡	0·57±0·05	0·36±0·04‡
8	5HT	0·38±0·03‡	0·17±0·02	0·66±0·02‡	0·81±0·10‡	0·46±0·02‡	0·41±0·03‡
	5HIAA	0·37±0·04‡	0·15±0·02‡	0·84±0·26‡	1·20±0·11‡	0·56±0·12‡	0·46±0·08‡
	NE	0·39±0·02	0·20±0·02	0·94±0·08	0·60±0·10	0·60±0·04	0 42±0 04
12	5HT	0·54±0·02‡	0·19±0·01	0·80±0·10	0·86±0·07‡	0·59±0·08	0·54±0·05‡
	5HIAA	0·57±0·05‡	0·25±0·04	1·34±0·22	1·41±0·18	1·71±0·18	0·65±0·10‡
	NE	0 40±0 02	0·18±0·02	0·98±0·25	0·58±0·02	0·61±0·05	0·42±0·04
16	5HT	0·65±0·02	0·19±0·01	0·75±0·12	1·13±0·19	0·68±0·04	0·64±0·06
	5HIAA	0·65±0·09‡	0·27±0·04	1·50±0·35	1·51±0·09	0·87±0·16	0·74±0·12
	NE	0·40±0·01	0·18±0·02	1·14±0·12	0·64±0·04	0·70±0·06	0·44±0·03
20	5HT	0·64±0·02	0·17±0·02	0·80±0·04	1·06±0·09	0·66±0·10	0·62±0·05
	5HIAA	0·76±0·07	0·25±0·04	1·46±0·23	1·36±0·19	0·83±0·03	0·77±0·08
	NE	0·41±0·02	0·17±0·02	1·18±0·15	0·58±0·08	0·65±0·04	0·43±0·04

* Rats were given a single i. p. dose of PCPA (400 mg/kg in oil) at time = 0 and killed at the times indicated. Each value is the mean ± S. D. of values obtained from 4 animals, except control (n = 20).

† Whole brain (WB) values were estimated from the weighted means for discrete areas.

‡ Values significantly different from time = 0 ($P < 0.05$, Student's t-test).

time-course of effects of a single dose of PCPA (400 mg/kg, in oil, i.p.) on levels of the amines are seen in Table 2. In all areas of the brain the levels of 5HT and 5HIAA fall significantly in the first day after drug dosage. The levels of 5HT remain significantly depressed for 5 days in cerebellum, 8 days in hypothalamus and medulla, and 12 days in cerebral hemispheres and midbrain. In general, the levels of 5HIAA in the various areas follow a time-course similar to those of 5HT. Of particular interest are

the values for whole brain; these follow the pattern of the cerebral hemispheres, a not surprising finding since this area comprises 63 per cent of the total brain weight and 72 per cent of the total 5HT content.

In addition to its effects on brain 5HT and 5HIAA, PCPA also has a marked effect on brain NE. A significant lowering of NE content in cerebral hemispheres, cerebellum, hypothalamus and midbrain is seen 24 hr after the drug. This NE depletion lasts for 3 days in hypothalamus and 5 days in the other parts (Table 2). The effect of PCPA on NE levels in the medulla is unique among the brain areas. A decrease

TABLE 3. EFFECTS OF PCA ON LEVELS OF 5HT, 5HIAA AND NE IN DISCRETE AREAS OF RAT BRAIN*

Time (hr)	Compound	CH (μg/g)	CB (μg/g)	HTH (μg/g)	MB (μg/g)	MED (μg/g)	WB† (μg/g)
0	5HT	0·65±0·05	0·19±0·01	0·97±0·11	1·06±0·08	0·57±0·07	0·63±0·06
	5HIAA	0·82±0·06	0 29±0 06	1·55±0·19	1·48±0·09	0·94±0·09	0·85±0·10
	NE	0·42±0·03	0·21±0·02	1·24±0·22	0·64±0·06	0·56±0·05	0·46±0·05
0·5	5HT	0·68±0·07	0·19±0·01	1·25±0·22	1·29±0·20	0·84±0·11‡	0·70±0·08
	5HIAA	0·61±0·04‡	0·21±0·04	1·40±0·09	1·33±0·02‡	0·53±0·26‡	0·62±0·12‡
	NE	0·51±0·04‡	0·26±0·01‡	1·52±0·20	0·69±0·08	0·70±0·09‡	0·57±0·05‡
1	5HT	0·63±0·02	0·19±0·01	1·24±0·19	1·26±0·15	0·93±0·06‡	0·68±0·04
	5HIAA	0·56±0·04‡	0·18±0·04‡	1·20±0·14‡	0·96±0·08‡	0·47±0·19‡	0·56±0·10‡
	NE	0·52±0·04‡	0·27±0·04‡	1·45±0·26	0·75±0·06	0·80±0·08‡	0·58±0·07‡
2	5HT	0·45±0·04‡	0·19±0·01	0·69±0·09‡	1·08±0·09	0·74±0·02‡	0·53±0·03‡
	5HIAA	0·46±0·03‡	0·21±0·01	1·11±0·05‡	0·99±0·15‡	0·63±0·20‡	0·52±0·07‡
	NE	0·51±0·03‡	0·27±0·02‡	1·46±0·13	0·74±0·06	0·74±0·03‡	0·57±0·04‡
4	5HT	0·30±0·01‡	0·15±0·02‡	0·64±0·16‡	0·83±0·14‡	0·55±0·08	0·37±0·04‡
	5HIAA	0·28±0·05‡	0·14±0·01‡	0·67±0·15‡	0·65±0·08‡	0·46±0·08‡	0·32±0·05‡
	NE	0·49±0·05	0·23±0·05	1·38±0·22	0·68±0·06	0·72±0·06‡	0·55±0·05
24	5HT	0·29±0·01‡	0·16±0·01‡	0·58±0·03‡	0·70±0·04‡	0·46±0·03‡	0·36±0·03‡
	5HIAA	0·17±0·02‡	0·12±0·03‡	0·60±0·14‡	0·73±0·02‡	0·56±0·11‡	0·27±0·04‡
	NE	0·48±0·05	0·24±0·02	1·31±0·22	0·68±0·03	0·58±0·05	0·51±0·05
48	5HT	0·27±0·03‡	0·15±0·01‡	0·58±0·03‡	0·54±0·06‡	0·36±0·03‡	0·30±0·02‡
	5HIAA	0·16±0·04‡	0·12±0·04‡	0·42±0 05‡	0·59±0·11‡	0·46±0·11‡	0·23±0·05‡
	NE	0·42±0·01	0·21 ±0·02	1·08±0·16	0·65±0·07	0·57±0·03	0·46±0·04
96	5HT	0·27±0·02‡	0·16±0·02‡	0·56±0·06‡	0·56±0·09‡	0·40±0·04‡	0·30±0·03‡
	5HIAA	0·18±0·06‡	0·14±0·03‡	0·44±0·12‡	0·67±0·04‡	0·53±0·09‡	0·25±0·05‡
	NE	0·44±0·02	0·20±0·03	1·07±0·11	0·64±0·02	0·56±0·09	0·47±0·04

* Rats were given a single i. p. dose of PCA (3·5 mg/kg, i.p.) at time = 0 and killed at the times indicated. Each value is the mean ± S. D. of values obtained from 4 animals, except control (n = 20).
† Whole brain values obtained as described in Table 2.
‡ Values significantly different from time = 0 ($P < 0·05$, Student's *t*-test).

(though not a statistically significant one) is seen at day 1, a greater (and significant) fall is measurable on day 2, and the level returns to normal by the third day after PCPA. As with 5HT levels, the values for whole brain NE do not reflect the differential area changes occurring; whole brain NE is significantly depressed 1 day after PCPA and remains so for 5 days.

Effects of PCA on levels of 5HT, 5HIAA and NE in discrete areas of rat brain. The time-course of effects of a single dose of PCA (3·5 mg/kg, i.p.) on levels of these amines are seen in Table 3. Several points are of particular interest. The primary initial action of the drug is an elevation of NE levels, with a decrease in 5HIAA and small increases in 5HT. This effect, lasting for about 2 hr, presumably reflects the activity of the PCA as an inhibitor of monoamine oxidase (MAO) as reported by Pletscher *et al.*[8] This

effect is replaced by a prolonged lowering of 5HT and 5HIAA levels in all brain areas, while NE levels return to normal by 4 hr after the dosage and remain there. The effects of PCA on brain 5HT levels are extremely prolonged; preliminary results indicate a return to normal values does not occur until at least 8–10 days after the drug is given.

Effects of PCMA on levels of 5HT, 5HIAA and NE in discrete areas of rat brain. The time-course of effects of a single dose of PCMA (3·7 mg/kg, i.p.) on levels of the amines are shown in Table 4. The overall picture is similar to that seen after PCA. It would appear, however, that the MAO-inhibiting potency of PCMA is somewhat less than that of PCA, as the 0–4 hr effects on levels of NE and 5HIAA are less

TABLE 4. EFFECTS OF PCMA ON LEVELS OF 5HT, 5HIAA AND NE IN DISCRETE AREAS OF RAT BRAIN*

Time (hr)	Compound	CH (μg/g)	CB (μg/g)	HTH (μg/g)	MB (μg/g)	MED (μg/g)	WB (μg/g)
0	5HT	0·65±0·05	0·19±0·01	0·97±0·11	1·06±0·08	0·57±0·07	0·63±0·06
	5HIAA	0·82±0·06	0·29±0·06	1·55±0·19	1·48±0·09	0·94±0·09	0·85±0·10
	NE	0·42±0·03	0·21±0·02	1·24±0·22	0·64±0·06	0·56±0·05	0·46±0·05
0·5	5HT	0·59±0·07	0·18±0·01	1·08±0·08	1·07±0·11	0·66±0 05	0 60±0 05
	5HIAA	0·63±0·13‡	0·25±0·03	1·35±0·45	1·28±0·14	0·58±0·20‡	0·67±0·15
	NE	0·46±0·02	0·25±0·01‡	1·61±0·22	0·75±0·08	0·69±0·03‡	0·54±0·04
1	5HT	0·51±0·05‡	0·17±0·02	0·90±0·03	1·04±0·13	0·69±0·06	0·54±0·04
	5HIAA	0·58±0·13‡	0·22±0·02	1·09±0·31	1·21±0·04‡	0·60±0·11‡	0·61±0·10‡
	NE	0·44±0·04	0·25±0·03	1·93±0·08‡	0·79±0·09‡	0·84±0·08‡	0·55±0·06
2	5HT	0·47±0·04‡	0·16±0·02‡	0·96±0·31	1·05±0·17	0·69±0·10	0·51±0·08
	5HIAA	0·50±0·08‡	0·22±0·04	1·30±0·30	1·18±0·18‡	0·69±0·12‡	0·60±0·12‡
	NE	0·45±0·02	0·24±0·02	1·82±0·34‡	0·72±0·07	0·72±0·06‡	0·53±0·06
4	5HT	0·34±0·04‡	0·16±0·01‡	0·86±0·14	0·87±0·12‡	0·67±0·10	0·41±0·04‡
	5HIAA	0·38±0·07‡	0·16±0·03‡	0·79±0·08‡	1·11±0·09‡	0·64±0·13‡	0·46±0·07‡
	NE	0·42±0·02	0·24±0·02	1·96±0·25‡	0·72±0·08	0·72±0·08‡	0·52±0·04
24	5HT	0·34±0·03‡	0·16±0·01‡	0·77±0·15‡	0·89±0·13‡	0·53±0·18	0·40±0·06‡
	5HIAA	0·24±0·03‡	0·16±0·02‡	0·71±0·10‡	1·14±0·21‡	0·68±0·05‡	0·39±0·05‡
	NE	0·39±0·06	0·24±0·03	1·45±0·19	0·63±0·09	0·67±0·05	0·46±0·06
48	5HT	0·29±0·04‡	0·14±0·02‡	0·63±0·09‡	0·65±0·05‡	0·45±0·06‡	0·32±0·04‡
	5HIAA	0·37±0·07‡	0·18±0·03‡	0·76±0·10‡	1·10±0·14‡	0·46±0·16‡	0·44±0·08‡
	NE	0·36±0·04	0·20±0·02	1·36±0·14	0·64±0·03	0·54±0·04	0·43±0·03
96	5HT	0·28±0·03‡	0·16±0·01‡	0·72±0·05‡	0·70±0·08‡	0·44±0·06‡	0·33±0·04‡
	5HIAA	0·34±0·06‡	0·21±0·04	0·68±0·08‡	0·99±0·17‡	0·65±0·06‡	0·43±0·06‡
	NE	0·40±0·04	0·21±0·03	1·43±0·21	0·63±0·09	0·46±0·06‡	0·45±0·06

* Rats were given a single i. p. dose of PCMA (3·7 mg/kg, i. p.) at time = 0 and killed at the times indicated. Each value is the mean ± S. D. of values obtained from 4 animals, except control (n = 20).
† Whole brain values obtained as described in Table 2.
‡ Values significantly different from time = 0 ($P < 0·05$, Student's *t*-test).

pronounced with the *N*-methyl compound. Significant lowering of 5HT and 5HIAA, the latter presumably not due to MAO inhibition, occurs in 4–24 hr and is maintained for >4 days. Again, as with the other drugs, the values for various brain areas are not necessarily reflected by the whole brain values.

DISCUSSION

The search for drugs that would selectively lower brain stores of 5HT has been continuing since the first reports of the neurohormone as an agent found in the brain.

The reserpinoid compounds, as well as the synthetic benzoquinolizines, cause decreased brain levels of both 5HT and NE with virtually no selectivity.[4–6] The first compound reported to selectively lower brain 5HT was PCPA.[9, 10] This compound has been demonstrated to be a potent inhibitor of tryptophan hydroxylase;[9] thus, it blocks the biosynthetic pathway at the rate-limiting step. As 5HT stores are lowered, the level of 5HIAA also falls,[9] reflecting the decreased turnover of the amine system.

In a paper on the brain 5HT lowering by PCPA, Koe and Weissman[9] reported that levels of brain NE were only slightly lowered in rats given a single dose (316 mg/kg) or three daily doses (100 mg/kg each) of PCPA. However, their data in dogs fed PCPA for 4–10 days, showed a marked lowering of NE in candate nucleus, hypothalamus and thalamus. Since this report by Koe and Weissman,[9] many reports have appeared, using PCPA as a "selective" depletor of brain 5HT in rats, and drawing conclusions with regard to the roles of 5HT and NE in brain function and behavior, based on this supposed selectivity.

In a preliminary report, Miller and Maickel[13] suggested that PCPA had a significant and prolonged effect on rat brain NE, an effect that questioned the conclusions drawn by Weissman[10] on the importance of brain 5HT in behavior. The present paper describes in detail the time-course of effect of PCPA on NE levels in discrete areas of rat brain. The data (Table 2) support the conclusion that a single dose of PCPA (400 mg/kg, i.p.) has a highly significant effect on rat brain NE. The depleting effect of PCPA on NE lasts for 2 days in medulla (maximal depletion, −21 per cent), for 3 days in hypothalamus (maximal depletion, −22 per cent), and for 5 days in cerebral hemispheres, cerebellum and midbrain (maximal depletion, −31, −33 and −34 per cent respectively). The effect of PCPA on 5HT is more prolonged, lasting for 5 days in cerebellum (maximal depletion, −26 per cent), 8 days in hypothalamus (−41 per cent) and medulla (−65 per cent) and 12 days in cerebral hemispheres and midbrain, with maximal depletion of −74 per cent and −70 per cent respectively. Thus, if one wishes to have an animal with depleted brain 5HT stores and normal NE levels, somewhere around 8 days after a single dose of PCPA would seem to be most appropriate, although the levels of 5HT at this time are no longer minimal. Significant differences in behavioral performances evoked by drugs such as Ro 4-1284 may be seen at 1, 8 and 15 days after PCPA.[13] These differences correspond to times of lowered NE-lowered 5HT, normal NE-lowered 5HT and normal NE-normal 5HT respectively.

The data we have presented on PCA and PCMA (Tables 3 and 4) indicate that these compounds are more selective depletors of brain 5HT than is PCPA. After a single dose of PCA, for example, NE levels are normal over the period 24–96 hr after drug, while 5HT levels are lowered by 55–58 per cent in cerebral hemispheres, 16–21 per cent in cerebellum, 40–42 per cent in hypothalamus, 34–49 per cent in midbrain, and 19–37 per cent in medulla. Similarly, in the period 48 to 96 hr after a single dose of PCMA, 5HT levels are lowered by 55–57 per cent in cerebral hemispheres, 16–26 per cent in cerebellum, 28–35 per cent in hypothalamus, 34–39 per cent in midbrain, and 21–23 per cent in medulla, again with normal levels of NE in all areas.

The short-term measurements (0·5–4 hr, post-drug) after PCA and PCMA confirm the previously reported MAO inhibiting potencies of these drugs.[8] At these time points the data show normal or slightly elevated levels of NE and 5HT, with decreased levels of 5HIAA. However, the MAO effect is of short duration; by 24 hr post-drug, the NE levels have returned to normal. After this time, the decreased levels of 5HIAA

presumably are due to corresponding decreases in 5HT. The mechanism by which PCA and PCMA cause lowered brain 5HT remains obscure.[6-8]

The data presented in this paper also confirm and extend previous papers[11-12] emphasizing the importance of measuring amine levels in discrete areas rather than in whole brain. Numerous time points may be seen where highly significant changes occur in one or several discrete areas, despite a lack of significance for the whole brain values. A general conclusion may be drawn from the data; drugs that effect cerebral hemisphere amine contents will have the most marked effect on whole brain levels. In this regard, it is of interest to note that the cerebellar amines are much more resistant to change than other brain areas. This has been previously reported from this laboratory;[11, 14] the possible significance remains a point for further study.

Finally, although the importance of cerebral 5HT in maintenance of normal behavioral function remains obscure, the availability of compounds such as PCA and PCMA should facilitate further research. Drug-induced perturbations of normal animal behavior may be modified by selective depletion of brain 5HT; such effects have been reported.[13] The animal with lowered cerebral 5HT need not show abnormal behavior *per se*—only when faced with a situation demanding functional normalcy of serotonergic systems, such as the response to a benzoquinolizine drug.[13]

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REFERENCES

1. B. M. TWAROG and I. H. PAGE, *Am. J. Physiol.* **175**, 157 (1953).
2. A. H. AMIN, T. B. B. CRAWFORD and J. H. GADDUM, *J. Physiol., Lond.* **126**, 596 (1954).
3. D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE and S. UDENFRIEND, *J. Pharmac. exp. Ther.* **117**, 82 (1958).
4. P. A. SHORE, A. PLETSCHER, E. G. TOMICH, A. CARLSSON, R. KUNTZMAN and B. B. BRODIE, *Ann. N. Y. Acad. Sci.* **66**, 609 (1957).
5. G. P. QUINN, P. A. SHORE and B. B. BRODIE, *J. Pharmac. exp. Ther.* **127**, 103 (1959).
6. A. PLETSCHER, H. BESENDORF and K. F. GEY, *Science, N.Y.* **129**, 844 (1959).
7. R. W. FULLER, C. W. HINES and J. MILLS, *Biochem. Pharmac.* **14**, 483 (1965).
8. A. PLETSCHER, M. DA PRADA, W. P. BURKHARD, G. BARTHOLINI, F. A. STEINER, H. BRUDERED and F. BIGLER, *J. Pharmac. exp. Ther.* **154**, 64 (1966).
9. B. K. KOE and A. WEISSMAN, *J. Pharmac. exp. Ther.* **154**, 499 (1966).
10. A. WEISSMAN, *Bioscience* **17**, 792 (1967).
11. R. P. MAICKEL, R. H. COX, JR., J. SAILLANT and F. P. MILLER, *Int. J. Neuropharmac.* **26**, 708 (1967).
12. F. P. MILLER, R. H. COX, JR. and R. P. MAICKEL, *Science, N.Y.* **162**, 463 (1968).
13. F. P. MILLER and R. P. MAICKEL, *Life Sci.* **8**, part I, 487 (1969).
14. R. P. MAICKEL, R. H. COX, JR., J. SAILLANT and F. P. MILLER, *Fedn Proc.* **26**, 708 (1967).