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The Effect of *p*-Chlorophenylalanine on Cerebral Metabolism and Biogenic Amine Content of Traumatized Brain

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Summary: It was shown previously that focal cortical freezing lesions in rats cause widespread decrease in local cerebral glucose utilization (LCGU) in cortical areas of the lesioned hemisphere. This was interpreted as reflecting a depression of cortical activity. It was then demonstrated that cortical serotonin (5-HT) metabolism was increased throughout the lesioned hemisphere of a focally injured brain. To find out if the changes in the serotonergic system are of functional importance and mediate the observed changes in LCGU, the effects of the inhibition of 5-HT synthesis with *p*-chlorophenylalanine (PCPA) on cerebral metabolism and biogenic amine content in injured brain were studied. PCPA in doses up to 300 mg/kg had little, if any, effect on LCGU in intact brain and in doses up to 100 mg/kg did not modify the depressed LCGU in injured brain. In doses of 200 and 300 mg/kg, PCPA selectively increased cortical glucose utilization in the lesioned hemisphere where it was depressed following injury. PCPA decreased 5-HT levels in the cortical and raphe areas of both intact and injured brain in a dose-dependent manner. However, at doses of PCPA ineffective on LCGU (50 and 100 mg/kg), traumatization still resulted in increased 5-HT metabolism. Doses of PCPA that ameliorated the depression of LCGU in injured brain completely prevented increases in both 5-HT and its metabolite 5-hydroxyindoleacetic acid seen following traumatization in untreated animals. These results provide evidence that decreased LCGU in lesioned brain is due to an activation of the serotonergic system by traumatization. The data are in agreement with the postulated inhibitory role of serotonin in the cortex and its involvement in functional alterations associated with injury. They suggest that blockage of this neurotransmitter system may have a potential in the development of novel therapeutic approaches to brain injury. **Key Words:** Brain injury—*p*-Chlorophenylalanine—Local cerebral glucose utilization—Norepinephrine—Serotonin.

Using focal cortical freezing in the rat as a model of cerebral injury and the deoxyglucose (DG) method as developed and described by Sokoloff et al. (1977) to assess the functional state of traumatized brain, earlier studies (Pappius, 1981) showed that a widespread, but not uniformly distributed, depression of local cerebral glucose utilization (LCGU) developed with time after lesioning. The effects were not related to the location of the lesion, which was regularly placed in the parietal cortex, but could be made more frontally or caudally with the same results (Colle et al., 1986). The most severe metabolic depression occurred in all the cortical regions of the lesioned hemisphere were 3 days after the lesion LCGU was ~50% of normal from frontal to visual cortex (Pappius, 1981). Thus, the effect was not restricted to areas surrounding the lesion or overlying the edematous white matter. The metabolic changes were not associated with parallel alterations in blood flow, which was slightly elevated throughout the whole brain at the time of the greatest decrease in LCGU. In keeping with the hypothesis that the functional state of cerebral tissue is closely coupled to its me-

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Abbreviations used: DG, deoxyglucose; DHBA, dihydroxybenzylamine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; LCGU, local cerebral glucose utilization; NE, norepinephrine; PCPA, *p*-chlorophenylalanine.

tabolism (Sokoloff, 1981), the demonstrated depression of cortical glucose utilization was interpreted as a manifestation of widespread cerebral dysfunction resulting from a focal lesion. In other words, the energy needs of cortical areas in injured brain were thought to be diminished because of a functional depression. Such an interpretation is supported by the demonstration in this model of injury of somatosensory deficits that were significantly correlated with the extent of the depression of glucose utilization in the cortical areas of the lesioned hemisphere (Colle et al., 1986).

In search of mechanisms involved in the functional depression resulting from injury, it was postulated that the changes observed in traumatized brain were mediated through a neurotransmitter system or systems (Pappius and Wolfe, 1983*a*, 1984, 1986). Both the serotonergic and noradrenergic systems appeared to be good potential candidates for such a role. Both innervations are widely distributed to rat cerebral cortex (Beaudet and Descarries, 1976; Descarries et al., 1977; Levitt and Moore, 1978; Moore and Bloom, 1979; Lidov et al., 1980; Morrison et al., 1981; Parent et al., 1981; Foote et al., 1983; Morrison and Magistretti, 1983), the area most affected in traumatized brain, and both systems have been shown to be altered acutely in association with brain injury (e.g., Bareggi et al., 1975; Vecht et al., 1975; Fenske et al., 1976; Finklestein et al., 1983). In cerebral cortex serotonin (5-HT) is thought to be an inhibitory transmitter (Bloom et al., 1972; Sastry and Phillis, 1977; Taylor and Stone, 1981) and is envisaged as exerting profound and global influences on cortical function in general (Lidov et al., 1980), possibly not only as a neurotransmitter, but also as a modulator of neuronal activity in a nonsynaptic fashion (Beaudet and Descarries, 1978; Taylor and Stone, 1981). Major noradrenergic fibers terminate in all layers throughout the cortex and thus similarly can exert their effects in many regions in a manner not related directly to local activity (Foote et al., 1983; Morrison and Magistretti, 1983).

Subsequent studies showed that 5-HT metabolism is increased throughout the cortex of the lesioned hemisphere of a focally injured brain, the decrease in 5-HT level being observed only at 24 h but the increase in the metabolite 5-hydroxyindoleacetic acid (5-HIAA) content persisting for 6 days post lesion (Pappius and Dadoun, 1987). 5-HT was not, however, the only transmitter affected by focal brain injury since cortical norepinephrine (NE) content was also decreased, although in contrast to 5-HT, bilaterally. On the other hand, no changes were found in cortical level of dopamine and its metabolites (Pappius and Dadoun, 1986).

The present studies were designed to elucidate the functional significance of the changes in 5-HT metabolism in the freezing lesion model of injury by determining the effects of inhibition of 5-HT synthesis with *p*-chlorophenylalanine (PCPA) on the depressed cortical glucose utilization in traumatized brain.

MATERIALS AND METHODS

General procedure

Small freezing lesions standardized to produce superficial focal cortical injury in the rat were made in the left parietal region of halothane-anesthetized male Sprague-Dawley rats (300–325 g) by applying a freezing probe (diameter 4 mm) cooled to $-50°$ for 5 s to the exposed dura. After the lesion was made, the wound was sutured and the animals allowed to awaken. At specified intervals after the lesion, either a [^{14}C]DG study was carried out as described below or the animal was decapitated and the brain processed for analysis of 5-HT, 5-HIAA, and NE according to procedures indicated below. The animals were maintained in a temperature- and light-controlled environment throughout and were always killed at the same time of day (for biogenic amine analysis 11:00 a.m. ± 30 min; for LCGU determination between 1:00 and 3:00 p.m.).

PCPA, a known irreversible inhibitor of 5-HT synthesis (Koe and Weissman, 1966; Jéquier et al., 1967), was given as a single intraperitoneal injection in doses of 50, 100, 200, and 300 mg/kg either 24 h before the lesion was made or at an equivalent time in unlesioned animals. Untreated, unlesioned animals served as normals while unlesioned but appropriately treated animals were the PCPA controls.

The physiological state of the conscious fasted animals destined for LCGU determination was assessed by monitoring arterial blood pressure and rectal temperature and by measurement of the hematocrit, serum glucose, and blood gases prior to the DG study. It was assumed that animals decapitated for biogenic amine analysis were in a similar physiological condition.

The values obtained for the measured variables in 18 normal animals before the start of the DG study were (average ± SE) as follow: mean arterial blood pressure 126 ± 2 mm Hg, rectal temperature 36.2 ± 0.2°C, glucose 144 ± 5 mg/dl, P_aO_2 93 ± 2 mm Hg, P_aCO_2 41.0 ± 0.5 mm Hg; pH 7.41 ± 0.01, hematocrit 50 ± 1%. As previously shown (Pappius, 1981), a cortical freezing lesion did not significantly affect any of the above. In the PCPA-treated animals (pooled lesioned and unlesioned), the only significant difference from normal was a fall in P_aCO_2 to 38.3 ± 0.7 mm Hg ($n = 18$, $p < 0.01$) in the 200-mg group and to 35.3 ± 1.1 mm Hg ($n = 14$, $p < 0.01$) in the 300-mg group.

Plasma glucose concentration was measured by means of a YSI glucose analyzer (model 23A; Yellow Springs Instruments, Yellow Springs, OH, U.S.A.) and plasma [^{14}C]DG content was determined in a liquid scintillation counter (model 1219, LKB, Rackbeta liquid scintillation

counter; Allied Scientific Ltd., Montreal, Quebec, Canada) with calibrated [^{14}C]toluene (New England Nuclear Corp., Boston, MA, U.S.A.) used for internal standardization. Blood gases were determined with a micro blood gas analyzer (model ILS 1302, Instrumentation Laboratories, Milan, Italy).

Determination of LCGU

In the present experiments, LCGU was measured 3 days after the lesion was made either in untreated animals or in those given PCPA 24 h before lesioning. This time period was chosen because at that point the depression of cortical glucose utilization is most pronounced in untreated lesioned animals. To have appropriate controls, in unlesioned animals PCPA was given 4 days before the LCGU measurement.

LCGU was determined using the 2-deoxy-D-[^{14}C]glucose quantitative radioautographic method of Sokoloff et al. (1977). Briefly, under general anesthesia with 2% halothane in oxygen and spontaneous respiration, polyethylene catheters were placed in the femoral artery and femoral vein. The animals were restrained from the waist down in a loosely applied plaster cast and allowed to recover from anesthesia for at least 3 h.

After ensuring that physiologic parameters were within normal limits, the LCGU experiment was begun by injecting a bolus of 30 μCi of [^{14}C]DG into the venous catheter over 30 s (2-deoxy-D-[1-^{14}C]glucose specific activity 50–56 μCi/mmol; New England Nuclear). Timed arterial sampling for determination of plasma [^{14}C]DG and glucose concentrations commenced at the start of injection and was continued for 45 min. The animals were then killed by decapitation and the brain was quickly removed and frozen in Freon XII, cooled at −50 to −60°C in liquid nitrogen vapor. Brains were sectioned in 20-μm-thick slices in a cryostat (American Optical Co., Buffalo, NY, U.S.A.) maintained at −22°C. The dried sections were used to prepare autoradiographs. Calibrated [^{14}C]methyl methacrylate standards (New England Nuclear) were included with each autoradiograph. Densitometry was performed on the developed autoradiographs with a Photovolt densitometer (model 52; Photovolt Corp., New York, NY, U.S.A.), using a 0.1-mm aperture and averaging the results of at least five readings from each structure studied. Calculation of LCGU was accomplished using a computer program written for a PDP-12 computer (Digital Equipment Corp., Maynard, MA, U.S.A.).

As in previous studies LCGU was measured in 27 structures and areas. However, only representative results from noncortical structures are given in Tables 1 and 2 since the effects of PCPA were most pronounced in cortical areas.

Determination of 5-HT, 5-HIAA, and NE content in cerebral cortex and raphe nucleus

5-HT, 5-HIAA, and NE were determined in cerebral cortex tissue and the raphe area in both untreated and PCPA-treated animals on the day of the lesion (day 0) and 1 and 3 days following the freezing lesion, as well as in PCPA-treated controls at corresponding time intervals after PCPA injection.

Cortical tissue samples were hand-dissected at room temperature from the brain quickly removed from the skull following decapitation, as previously described (Pappius and Dadoun, 1987). The frontoparietal cortex samples (150–200 mg), from which the lesion area was always discarded and the underlying white matter removed, were rapidly frozen in liquid nitrogen, weighed with care being taken that no thawing occurred, and rapidly homogenized (sonic dismembrator; Artex System Corp., Farmingdale, NY, U.S.A.) in 0.5 ml of ice-cold 0.2 *M* perchloric acid containing 150 pmol of dihydroxybenzylamine (DHBA) as internal standard. The homogenates were centrifuged at 15,000 rpm at 4°C for 15 min. Supernatants were stored at −80°C and thawed just before analysis.

To obtain samples from the raphe area, the cerebellum was removed first and a transverse section made at an angle passing rostrally to the midbrain raphe nuclei, followed by a second cut through the caudal medulla at the level of the obex. Then the raphe area was dissected from the brainstem by two parasagittal cuts 1 mm lateral from the midline and divided along the midline. Thus, each sample contained serotonergic cell bodies with both ascending and descending projections. The weight of each unilateral sample was always between 20 and 30 mg. The samples were weighed after freezing in liquid nitrogen and extracted in 200 μl of 0.2 *M* perchloric acid containing 150 pmol DHBA as internal standard (Mefford, 1981).

The chromatography analyses were performed with a high performance liquid chromatograph equipped with a pump (model 590; Waters Associates, Milford, MA, U.S.A.), an automatic injector (WISP 710B; Waters), an integrator (data module 730; Waters), and a model LC-4B (Bioanalytical Systems) glassy carbon (TL3) amperometric detector.

In a procedure adapted from Warnhoff (1984), a 5-μm C_{18} column RCM 100 (inner diameter 8 mm) protected by a Guard Pak precolumn module (Waters) was used. The mobile phase, consisting of 0.1 *M* sodium acetate, 0.1 *M* citric acid, 15% (vol/vol) methanol, and 500 μl/L *N*-dibutylamine (pH 3.5), was pumped at flow rate of 0.6 ml/min. The detector potential was set at 0.70 V vs. Ag/AgCl reference electrode. The standard stock solution contained 100 μg/ml H_2O of the following: 5-hydroxytryptamine hydrochloride, 5-hydroxyindoleacetic acid hydrochloride, and DHBA (internal standard), all from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The stock solution was stored at −80°C. Aliquots were thawed and diluted to 1:1,000 daily.

This procedure gave excellent resolution of the 5-HT, 5-HIAA, and DHBA peaks in all samples and of NE in supernatants from raphe nucleus. For determination of NE in cerebral cortex, the run was repeated with a modified mobile phase (5% methanol, 350 μl *N*-dibutylamine/L) to allow better separation of the NE peak from the solvent front peak.

Correlation between cortical glucose utilization and 5-HT content of raphe nucleus in lesioned brain

In 10 animals given different doses (50–300 mg/kg) of PCPA 24 h before the lesion was made, a DG study was performed on the third postlesion day and determination of glucose utilization was made autoradiographically in cortex regions after removal of brainstem areas. Raphe area was dissected out as described above and extracted for determination of 5-HT, 5-HIAA, and NE.

LCGU results in the lesioned hemisphere for five cor-

tical areas in each animal (visual, auditory, parietal, sensorimotor, and frontal) were expressed as percentage of normal and averaged to give cortical glucose utilization in Fig. 2.

Statistical analysis

The Bonferroni procedure (two tailed) for simultaneous multiple comparisons was used for determination of statistical significance between groups (Wallenstein et al., 1980).

In Table 1 PCPA-treated groups were compared with normal; in Table 2 lesioned treated groups were compared with both the normal and the lesioned untreated groups. In Tables 3, 4, and 5 intact treated groups and the lesioned untreated group were compared with the normal group, while lesioned treated groups were compared only with the corresponding PCPA controls.

RESULTS

Effect of PCPA on LCGU in unlesioned brain

PCPA in doses of 100, 200, and 300 mg given 4 days earlier had little, if any effect, on LCGU in unlesioned animals, the slight decrease in cortical areas reaching significance in only two regions with the 200-mg/kg dose (Table 1).

Effect of PCPA on LCGU in lesioned brain

As shown previously (Pappius, 1981), 3 days following a focal freezing lesion, LCGU was severely depressed in all cortical areas of the lesioned hemisphere and to a much lesser extent in some, but not all, subcortical structures (Table 2). Some decrease in LCGU was also seen in the cortical and subcortical areas of the hemisphere contralateral to the lesion. Brainstem structures and white matter regions were unaffected.

PCPA in doses of 50 and 100 mg/kg given 24 h before the lesion was made had no effect on cortical glucose utilization throughout the lesioned brain. In marked contrast, PCPA in doses of 200 and 300 mg/kg selectively increased glucose utilization in all cortical areas of the lesioned hemsiphere, whereas in untreated animals it was depressed following injury. However, normal levels were not reached. The two doses were equally effective in elevating the cortical LCGU on the side of the lesion. There was also some effect of the two higher doses of PCPA in subcortical areas of the lesioned hemisphere, but with one exception (substantia nigra) not in the contralateral hemisphere.

Effect of PCPA on 5-HT and 5-HIAA content of cerebral cortex in intact and lesioned brain

In intact brain 24 h after an intraperitoneal injection of PCPA (day 0), there was a dose-dependent decrease in cortical content of 5-HT and 5-HIAA (Table 3). Following 100-, 200-, and 300-mg injections, there was further depletion up to day 3. Similar results were obtained in the hemisphere contralateral to the lesion in the traumatized brain.

TABLE 1. *Local cerebral glucose utilization (LCGU) in selected areas of intact brain after treatment with* p-*chlorophenylalanine (PCPA)*

Brain area or structure	Normal (n = 18)	PCPA controls		
		100 mg/kg (n = 6)	200 mg/kg (n = 7)	300 mg/kg (n = 5)
Cortical areas				
Visual	115 ± 3	110 ± 5	109 ± 7	106 ± 5
Auditory	162 ± 6	155 ± 11	145 ± 9	149 ± 6
Parietal	110 ± 3	104 ± 8	93 ± 3[a]	106 ± 9
Sensorimotor	121 ± 4	116 ± 6	102 ± 5[b]	115 ± 8
Frontal	120 ± 4	114 ± 10	107 ± 8	104 ± 12
Subcortical structures				
Substantia nigra	71 ± 3	74 ± 5	69 ± 4	76 ± 6
Medial geniculate	129 ± 5	122 ± 8	114 ± 4	128 ± 5
Hypothalamus	54 ± 2	58 ± 5	61 ± 4	44 ± 3
Globus pallidus	62 ± 2	63 ± 4	62 ± 3	63 ± 4
Caudate	102 ± 3	102 ± 8	91 ± 6	102 ± 5
Brainstem structures				
Inferior colliculus	198 ± 8	182 ± 10	182 ± 6	192 ± 11
Lateral lemniscus	128 ± 5	123 ± 10	120 ± 9	124 ± 7
White matter				
Corpus callosum	44 ± 3	41 ± 3	44 ± 3	41 ± 3
Internal capsule	35 ± 2	37 ± 2	37 ± 2	37 ± 2

PCPA was given as a single dose 4 days before LCGU determination. Values are means ± SE, expressed as μmol/100 g/min, n = no. of animals.

[a] Significantly different from normal, $p < 0.01$.

[b] Significantly different from normal, $p < 0.05$.

TABLE 2. *Local cerebral glucose utilization (LCGU) 3 days after freezing lesion in selected areas of traumatized rat brain after pretreatment with* p-*chlorophenylalanine (PCPA)*

Brain site	Untreated (n = 15)	PCPA treated 50 mg/kg (n = 5)	100 mg/kg (n = 7)	200 mg/kg (n = 11)	300 mg/kg (n = 11)
Lesioned hemisphere					
Cortical areas					
Visual	56 ± 2[a]	67 ± 10[a]	70 ± 11[a]	89 ± 6[a,b]	84 ± 7[a,b]
Auditory	67 ± 4[a]	73 ± 12[a]	84 ± 16[a]	118 ± 8[a,b]	115 ± 10[a,b]
Parietal	56 ± 4[a]	55 ± 4[a]	62 ± 6[a]	67 ± 5[a]	80 ± 8[a,c]
Sensorimotor	58 ± 4[a]	68 ± 10[a]	66 ± 8[a]	95 ± 6[a,b]	97 ± 9[b,d]
Frontal	59 ± 2[a]	74 ± 9[a]	70 ± 9[a]	101 ± 6[b]	82 ± 6[a,b]
Subcortical structures					
Substantia nigra	51 ± 3[a]	56 ± 7	55 ± 5	64 ± 2[c]	56 ± 4[d]
Medial geniculate	86 ± 4[a]	89 ± 9[a]	93 ± 14[d]	107 ± 7[c]	105 ± 7[d]
Hypothalamus	53 ± 4	46 ± 6	47 ± 3	53 ± 3	50 ± 5
Globus pallidus	49 ± 3[a]	47 ± 4[d]	45 ± 7[d]	58 ± 4	53 ± 3
Caudate	78 ± 4[a]	72 ± 3[a]	67 ± 10[a]	92 ± 4	90 ± 5
Brainstem structures					
Inferior colliculus	183 ± 10	169 ± 9	167 ± 12	192 ± 12	177 ± 8
Lateral lemniscus	111 ± 7	106 ± 12	95 ± 11	110 ± 5	101 ± 8[d]
White matter					
Corpus callosum	38 ± 2	35 ± 4	31 ± 4	40 ± 3	43 ± 4
Internal capsule	31 ± 2	33 ± 4	28 ± 2	29 ± 3	30 ± 3
Hemisphere contralateral to lesion					
Cortical areas					
Visual	100 ± 5[d]	100 ± 8	105 ± 10	102 ± 5[d]	99 ± 6[d]
Auditory	142 ± 9	119 ± 12[a]	144 ± 15	139 ± 7	134 ± 8[d]
Parietal	95 ± 4[d]	90 ± 3[a]	94 ± 10	94 ± 5[a]	96 ± 6
Sensorimotor	104 ± 4[a]	94 ± 5[a]	104 ± 10	105 ± 6	105 ± 6
Frontal	103 ± 4	98 ± 5	103 ± 10	107 ± 7	97 ± 5[a]
Subcortical structures					
Substantia nigra	58 ± 2[d]	65 ± 3	58 ± 7	68 ± 3[c]	61 ± 4
Medial geniculate	107 ± 4[a]	105 ± 6	103 ± 13	111 ± 4	112 ± 6
Hypothalamus	52 ± 3	47 ± 5	48 ± 3	54 ± 4	46 ± 4
Globus pallidus	50 ± 3[a]	48 ± 5[d]	44 ± 6[a]	55 ± 3	56 ± 4
Caudate	94 ± 4	85 ± 4[d]	71 ± 11[a]	93 ± 4	90 ± 6
Brainstem structures					
Inferior colliculus	180 ± 7	170 ± 10	191 ± 29	186 ± 8	179 ± 10
Lateral lemniscus	115 ± 5	106 ± 9	96 ± 15	110 ± 3	107 ± 6
White matter					
Corpus callosum	36 ± 2	34 ± 2	34 ± 4	38 ± 3	37 ± 3
Internal capsule	32 ± 2	31 ± 1	27 ± 3	32 ± 2	27 ± 3

PCPA was given 24 h before lesion (i.e., 4 days before LCGU determination). Values are means ± SE, expressed as μmol/100 g/min, n = no. of animals.

[a] Significantly different from normal (data in Table 1), $p < 0.01$.

[b] Significantly different from untreated, $p < 0.01$.

[c] Significantly different from untreated, $p < 0.05$.

[d] Significantly different from normal, $p < 0.05$.

As previously reported (Pappius and Dadoun, 1987), in the lesioned hemisphere of untreated animals, there was a significant decrease in 5-HT content of the cortex on postlesion day 1 and a highly significant increase in 5-HIAA on both days 1 and 3, indicative of an increased turnover in the serotonergic system. In animals pretreated with 50 and 100 mg PCPA/kg, doses without effect on glucose utilization (see above), no decrease in 5-HT content due to the lesion was demonstrated, but 5-HIAA was significantly increased on both days 1 and 3 as compared with PCPA controls. These results indicate that injury still induced an increase in 5-HT turnover despite lowering by these doses of the drug of the absolute levels of both the neurotransmitter and its metabolite.

In contrast, in animals pretreated with 200 and 300 mg PCPA/kg, doses that, as indicated above, significantly ameliorated the metabolic depression in traumatized brain, no changes in cortical 5-HT and 5-HIAA levels were demonstrated when compared with corresponding PCPA controls, with both indoleamines depleted to <10% of normal cortical levels.

TABLE 3. *Effect of* p-*chlorophenylalanine (PCPA) on serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) content of cerebral cortex in intact and lesioned rat brain*

	5-HT (pmol/g)			5-HIAA (pmol/g)		
Postlesion day	0	1	3	0	1	3
Intact brain						
Normal	1,840 ± 62 (25)			794 ± 38 (25)		
PCPA controls						
50 mg/kg	1,157 ± 155 (5)[a]	1,165 ± 139 (5)[a]	1,191 ± 122 (6)[a]	360 ± 42 (5)[a]	335 ± 47 (5)[a]	355 ± 53 (6)[a]
100 mg/kg	862 ± 144 (4)[a]	604 ± 109 (7)[a]	350 ± 96 (5)[a]	274 ± 36 (4)[a]	164 ± 25 (7)[a]	105 ± 11 (5)[a]
200 mg/kg	581 ± 89 (3)[a]	215 ± 10 (3)[a]	326 ± 114 (2)[a]	196 ± 1 (3)[a]	105 ± 19 (3)[a]	118 ± 23 (3)[a]
300 mg/kg	418 ± 14 (3)[a]	170 ± 23 (3)[a]	202 ± 36 (3)[a]	192 ± 8 (3)[a]	44 ± 5 (3)[a]	73 ± 5 (3)[a]
Lesioned hemisphere						
Untreated		1,516 ± 54 (21)[a]	1,982 ± 113 (18)		1,491 ± 110 (21)[a]	1,425 ± 106 (18)[a]
PCPA treated						
50 mg/kg		1,202 ± 54 (5)	1,015 ± 78 (8)		680 ± 131 (5)[b]	645 ± 80 (8)[b]
100 mg/kg		1,069 ± 163 (5)	693 ± 51 (7)[b]		580 ± 59 (5)[b]	323 ± 32 (7)[b]
200 mg/kg		281 ± 56 (6)	130 ± 16 (4)		92 ± 13 (6)	57 ± 6 (4)
300 mg/kg		166 ± 14 (4)	113 ± 17 (4)		57 ± 5 (4)	65 ± 12 (3)
Contralateral to lesion						
Untreated		1,907 ± 60 (21)	2,114 ± 121 (18)		907 ± 48 (21)	883 ± 49 (18)
PCPA treated						
50 mg/kg		1,088 ± 77 (5)	1,085 ± 66 (8)		327 ± 47 (5)	326 ± 25 (8)
100 mg/kg		823 ± 101 (5)	717 ± 100 (5)[c]		318 ± 66 (5)	196 ± 29 (7)
200 mg/kg		255 ± 25 (6)	182 ± 32 (4)		73 ± 3 (6)	73 ± 16 (4)
300 mg/kg		127 ± 9 (4)	133 ± 60 (3)		41 ± 6 (4)	90 ± 10 (3)

PCPA was given as a single dose 24 h before day 0, thus 24 h before lesion in traumatized animals. Values are means ± SE, with no. of animals in parentheses.

[a] Significantly different from normal, $p < 0.01$.

[b] Significantly different from corresponding PCPA control, $p < 0.01$.

[c] Significantly different from corresponding PCPA control, $p < 0.05$.

Effect of PCPA on 5-HT and 5-HIAA content of raphe area in intact and lesioned brain

The effect of a freezing lesion on the content of 5-HT and 5-HIAA in the raphe area was not determined previously. Data summarized in Table 4 show a general tendency of both 5-HT and 5-HIAA to increase bilaterally in the raphe with time after a unilateral freezing lesion in untreated animals and those pretreated with 50 and 100 mg PCPA/kg, but not in the 200- and 300-mg PCPA/kg groups. However, because of considerable variability in the results, the differences from appropriate controls, when they occurred, were not always statistically significant.

On the other hand, when the results were expressed as a sum of the neurotransmitter and its metabolite (5-HT + 5-HIAA), as in Fig. 1, highly statistically significant increases indicative of activated 5-HT metabolism in the raphe area were demonstrated on postlesion day 1 and, and to a lesser extent, on day 3 in lesioned animals, untreated or pretreated with 50 or 100 mg PCPA/kg. In contrast to findings in the cortex where such changes were restricted to the lesioned hemisphere, in the raphe unequivocally bilateral effects were induced by the injury. As in the cortex, at doses of PCPA that ameliorated the depression in glucose utilization in the lesioned hemisphere (200 and 300 mg/kg), no effect of injury on 5-HT metabolism in the raphe area was noted, although neither 5-HT nor 5-HIAA was as depleted as in the cortex.

Effect of PCPA on NE content of cerebral cortex and raphe nucleus in intact and lesioned brain

It will be seen from Table 5 that in traumatized brain, bilateral decreases in cortical NE level were confirmed (Pappius and Dadoun, 1986). PCPA tended to decrease transiently the NE content of intact brain, with the effect being statistically significant only at the higher doses of PCPA on day 1. In PCPA-treated animals the effect of injury on cortical NE was statistically significant only in the lesioned hemsiphere at 300 mg/kg on day 3. However, when the PCPA-treated groups were pooled, the differences in NE levels between control and lesioned brain were statistically significant (control: $n = 21$, NE 1,636 ± 63 pmol/g; day 3 lesioned hemisphere; $n = 20$, NE 1,314 ± 40 pmol/g, $p < 0.01$; hemisphere contralateral to lesion: NE 1,468 ± 30 pmol/g, $p < 0.05$). The NE content of the raphe area was unaffected by injury or PCPA.

Correlation between cortical glucose utilization and 5-HT content of raphe nucleus in traumatized brain

In Fig. 2 cortical glucose utilization on the side of the lesion was plotted against 5-HT content of the raphe area in the lesioned hemisphere of the same animals. A highly statistically significant correlation was found ($r = 0.82$, $p < 0.01$). A similar correlation was noted with 5-HT content in the contralateral raphe area ($r = 0.76$, $p < 0.01$). Cortical 5-HT content could not be determined in these an-

TABLE 4. *Effect of* p-*chlorophenylalanine (PCPA) on serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) content of raphe nucleus in intact and lesioned rat brain*

	5-HT (pmol/g)			5-HIAA (pmol/g)		
Postlesion day	0	1	3	0	1	3
Intact brain						
Normal	3,684 ± 112 (11)			2,251 ± 110 (11)		
PCPA controls						
50 mg/kg	3,398 ± 197 (7)	2,270 ± 109 (5)[a]	3,321 ± 190 (7)	2,271 ± 120 (7)	1,383 ± 180 (5)[a]	2,194 ± 125 (7)
100 mg/kg	2,020 ± 202 (6)[a]	1,280 ± 108 (7)[a]	1,340 ± 187 (5)[a]	1,262 ± 139 (6)[a]	1,016 ± 141 (7)[a]	1,303 ± 81 (5)[a]
200 mg/kg	1,479 ± 86 (6)[a]	650 ± 22 (5)[a]	1,246 ± 116 (4)[b]	814 ± 52 (6)[a]	650 ± 22 (5)[a]	1,089 ± 27 (4)[a]
300 mg/kg	1,853 ± 194 (3)[a]	717 ± 69 (3)[a]	1,190 ± 241 (3)[a]	970 ± 172 (6)[a]	621 ± 65 (3)[a]	1,474 ± 337 (3)[a]
Lesioned hemisphere						
Untreated		3,952 ± 268 (7)	4,532 ± 141 (9)		2,721 ± 271 (7)	2,915 ± 206 (9)
PCPA treated						
50 mg/kg		3,737 ± 273 (5)[c]	3,622 ± 202 (7)		2,984 ± 321 (5)[c]	2,717 ± 192 (7)
100 mg/kg		2,521 ± 215 (7)[c]	1,889 ± 145 (12)		2,080 ± 189 (7)[c]	1,574 ± 104 (12)
200 mg/kg		920 ± 92 (8)	885 ± 130 (8)		841 ± 74 (8)	933 ± 100 (8)
300 mg/kg		702 ± 23 (4)	1,031 ± 78 (6)		573 ± 34 (4)	1,215 ± 71 (6)
Contralateral to lesion						
Untreated		3,908 ± 182 (7)	4,188 ± 239 (8)		2,705 ± 185 (7)	3,230 ± 306 (8)
PCPA treated						
50 mg/kg		3,977 ± 251 (5)[c]	3,681 ± 226 (7)		2,567 ± 351 (5)[d]	2,533 ± 225 (7)
100 mg/kg		2,526 ± 149 (7)[c]	2,098 ± 143 (12)		1,876 ± 173 (7)[c]	1,667 ± 140 (12)
200 mg/kg		713 ± 97 (6)	1,050 ± 103 (8)		791 ± 80 (8)	1,083 ± 67 (8)
300 mg/kg		966 ± 64 (4)	1,371 ± 163 (6)		828 ± 75 (4)	1,720 ± 229 (6)

PCPA was given as a single dose 24 h before day 0, thus 24 h before lesion in traumatized animals. Values are means ± SE, with no. of animals in parentheses.

[a] Significantly different from normal, $p < 0.01$.

[b] Significantly different from normal, $p < 0.05$.

[c] Significantly different from corresponding PCPA control, $p < 0.01$.

[d] Significantly different from corresponding PCPA control, $p < 0.05$.

imals since the tissue was used for autoradiography. In other PCPA-treated animals, parallel dose-dependent changes in 5-HT content of the raphe area and the frontoparietal cortical regions were found (Tables 3 and 4).

DISCUSSION

The major conclusion from the results summarized in this article is that the depressed functional state of a lesioned brain, as assessed by metabolic mapping with the DG method, can be ameliorated by inhibition of 5-HT synthesis. These findings provide compelling evidence that the increased turnover of 5-HT throughout the cortex of focally lesioned hemisphere is of functional significance. This implies that alterations in the levels of indoleamines in injured brain do reflect increased release of this transmitter. Such a conclusion is compatible with the postulated inhibitory nature of 5-HT action on cortical neuronal activity (Bloom et al., 1972; Sastry and Phillis, 1977; Taylor and Stone, 1981).

It is unlikely that blood elements were the source of the increased 5-HT turnover found in the cortical areas of injured brain. Release of 5-HT from platelets in the lesion area has been demonstrated 6 h after lesioning in the cat (Costa et al., 1974). However, rat platelets contain little 5-HT (Costa et al., 1974), and the lesion area was always excluded from the tissues analyzed in the present experiments. Persistence of the increased 5-HT metabolism for 6 days after injury is a further argument against it being of blood origin. Similarly, the increase in release of 5-HT in injured brain cannot be attributed to stress (Joseph and Kennett, 1983) as there was no physiological evidence (e.g., increased blood pressure or elevated serum glucose levels) that the animals were chronically stressed and such effects would be expected to occur bilaterally.

It might have been anticipated that in intact animals decreased 5-HT synthesis would lead to increased glucose utilization, particularly in the cortex, where this neurotransmitter is thought to be inhibitory. This was not the case in PCPA-treated unlesioned animals where with the higher doses of the drug, if anything, lower rates of LCGU were found. Such negative results with the DG method must be interpreted with caution since they presumably reflect the overall functional state of the brain rather than the lack of involvement of a specific pathway (McCulloch et al., 1982). Under normal conditions changes in one neuronal system may be counterbalanced by those in another, and in the case of 5-HT complex reciprocal interrelationships with other neurotransmitters have been well documented (Soubrié et al., 1984; Vanderwolf and Baker, 1986). Furthermore, in the cortex the exact function of 5-HT remains to be elucidated (Emson and Hunt, 1979; Soubrié et al., 1984; Jones, 1986),

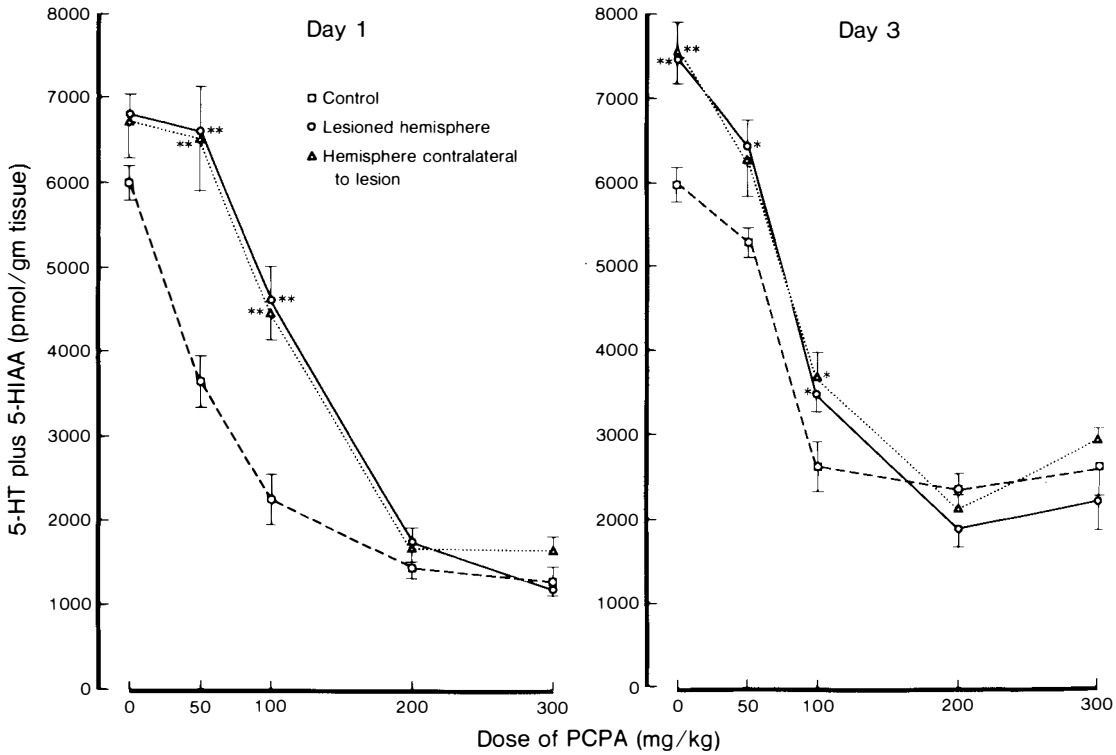

FIG. 1. Indoleamine content [serotonin (5-HT) plus 5-hydroxyindoleacetic acid (5-HIAA)] of the raphe area following a freezing lesion in *p*-chlorophenylalanine (PCPA)–treated rats on days 1 and 3 postlesion. (Squares), control animals; (circles), lesioned hemisphere; (triangles), hemisphere contralateral to lesion. Averages ± SE are given. No. of animals as in Table 4. **Statistically significantly different from control, $p < 0.01$; *statistically significantly different from control, $p < 0.05$.

but like that of NE (Cedarbaum and Aghajanian, 1978; Waterhouse and Woodward, 1980; Aston-Jones, 1985) it appears to involve the modulation of the activity of other transmitters, at least to some degree. Under essentially resting conditions, it may be necessary to challenge the overall system before general functional effects can be demonstrated. Brain injury may be one such situation where activation of the serotonergic system by trauma results in functional alterations in the cortex that can then be selectively affected by inhibition of 5-HT synthesis. It is also possible that with finer resolution than was available in the present studies, alterations in glucose utilization of discrete structures such as the raphe nuclei could be demonstrated in intact PCPA-treated animals.

The lack of effect of the lower doses of PCPA in the freezing lesion model of injury is in agreement with electrophysiological data showing that under normal conditions the amount of 5-HT synthesized is in excess of what is needed to meet functional requirements and that brain 5-HT must fall to extremely low levels before function is impaired (Vanderwolf and Baker, 1986). With doses of PCPA of 50 or 100 mg/kg in lesioned brain, apparently enough 5-HT can still be mobilized (Fig. 1) to maintain the functional depression associated with injury. Only when the increase in 5-HT turnover as a result of injury is completely prevented at doses of PCPA exceeding 200 mg/kg is the functional depression affected. Thus, despite highly significant correlation between the cortical glucose utilization and the 5-HT content of the raphe area (Fig. 2) and by inference of the cortex, since the two change in parallel under these conditions, the capacity for increased turnover is probably more important than the 5-HT level per se in determining the effectiveness of PCPA doses in injured brain.

The pathologically induced increase in 5-HT turnover was shown in the present studies to be the cause of depressed LCGU. A similar effect was demonstrated as a result of pharmacologically raised 5-HT levels. Thus, intracarotid administration of 5-HT in the presence of monoamine oxidase inhibition has been shown to acutely decrease cortical LCGU (Grome and Harper, 1985). In contrast, there is a recent report that electrical stimulation of the raphe increased glucose use in a number of thalamic structures and in highly circumscribed frontal regions of the cortex (Cudennec et al., 1987). Monoamine oxidase inhibition followed by 5-HT has also been shown to decrease cerebral blood flow (Eidelman et al., 1978). This, however, was not the case in lesioned brain in which uncoupling

TABLE 5. *Norepinephrine content of cerebral cortex and raphe nucleus in intact and lesioned rat brain after treatment with* p-*chlorophenylalanine (PCPA)*

	Cerebral cortex			Raphe nucleus		
Postlesion day	0	1	3	0	1	3
Intact brain						
Normal	1,713 ± 69 (21)			3,226 ± 258 (11)		
PCPA controls						
50 mg/kg	1,522 ± 105 (7)	1,316 ± 112 (5)	1,381 ± 124 (6)	4,284 ± 219 (7)	3,091 ± 297 (5)	4,083 ± 196 (6)
100 mg/kg	1,513 ± 146 (4)	1,372 ± 53 (7)	1,785 ± 105 (6)	3,641 ± 363 (6)	3,119 ± 270 (7)	4,344 ± 379 (5)
200 mg/kg	1,464 ± 113 (3)	1,325 ± 37 (8)[a]	1,626 ± 151 (4)	3,578 ± 106 (6)	4,209 ± 128 (5)	3,522 ± 291 (5)
300 mg/kg	1,432 ± 90 (3)	975 ± 68 (3)[a]	1,705 ± 80 (6)	3,407 ± 296 (3)	3,414 ± 121 (3)	4,728 ± 462 (3)
Lesioned hemisphere						
Untreated		1,044 ± 47 (13)[a]	1,330 ± 46 (12)[a]		3,030 ± 167 (7)	3,596 ± 222 (9)
PCPA treated						
50 mg/kg		1,493 ± 170 (5)	1,243 ± 41 (7)		3,787 ± 227 (5)	3,969 ± 119 (7)
100 mg/kg		1,156 ± 98 (5)	1,473 ± 86 (5)		3,920 ± 218 (7)	3,322 ± 154 (12)
200 mg/kg		1,069 ± 91 (6)	1,355 ± 80 (3)		3,252 ± 201 (8)	3,193 ± 136 (8)
300 mg/kg		872 ± 60 (4)	1,175 ± 74 (3)[b]		3,374 ± 369 (4)	4,036 ± 275 (6)
Contralateral to lesion						
Untreated		1,221 ± 68 (13)[a]	1,465 ± 75 (12)		2,935 ± 225 (7)	3,554 ± 202 (8)
PCPA treated						
50 mg/kg		1,450 ± 142 (5)	1,456 ± 48 (7)		3,756 ± 224 (5)	4,025 ± 149 (7)
100 mg/kg		1,308 ± 114 (5)	1,449 ± 90 (5)		3,664 ± 255 (5)	3,424 ± 123 (12)
200 mg/kg		1,113 ± 129 (6)	1,522 ± 31 (3)		3,250 ± 210 (8)	3,075 ± 134 (8)
300 mg/kg		1,056 ± 83 (3)	1,485 ± 37 (3)		3,167 ± 305 (4)	4,272 ± 321 (6)

PCPA was given as a single dose 24 h before day 0, thus 24 h before lesion in traumatized animals. Values are means ± SE, expressed as pmol/g, with no. of animals in parentheses.

[a] Significantly different from normal, $p < 0.01$.

[b] Significantly different from corresponding PCPA control, $p < 0.01$.

of LCGU and local cerebral blood flow was clearly demonstrated (Pappius, 1981). The mechanisms responsible for the uncoupling of cerebral metabolism and blood flow in injured brain, confirmed recently by Gaab et al. (1987), are unknown. Whether cerebral vessels are less responsive to 5-HT under these conditions or whether the relative vasodilatation is due to other factors remains to be elucidated.

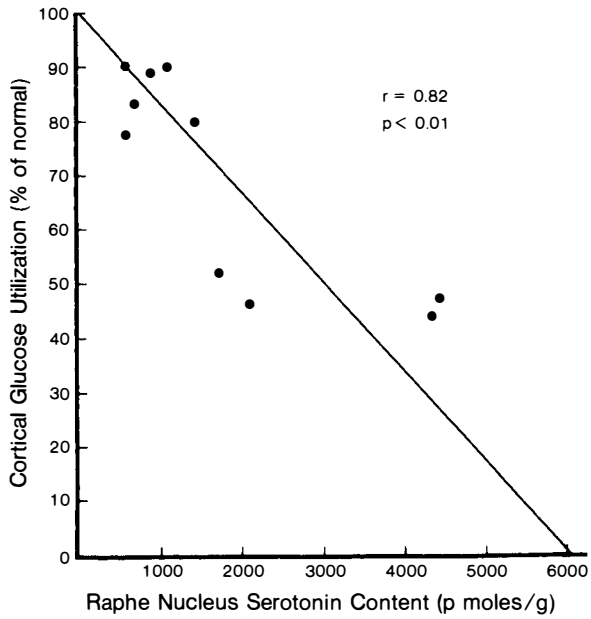

FIG. 2. Correlation between cortical glucose utilization and raphe area serotonin content of traumatized brain in *p*-chlorophenylalanine-treated rats.

The effects of PCPA in the freezing lesion model of brain injury do not appear to be mediated by changes in NE. Biochemical and functional interactions between the noradrenergic locus ceruleus and serotonergic raphe systems are well documented (e.g., Kostowski et al., 1974; Saavedra et al., 1976; Pujol, 1979; Pujol et al., 1981; Agren et al., 1986; Brassard et al., 1987) and both systems are affected by brain injury (Pappius and Dadoun, 1986, 1987). PCPA has been reported to affect NE levels in brain under some conditions (Miller et al., 1970; Rebec et al., 1981; Reader and Gauthier, 1984; Reader et al., 1986; Vanderwolf and Baker, 1986); in all cases, however, following higher doses than used in the present experiments. The decrease in cortical NE resulting from traumatization was statistically significant when pooled groups were compared with pooled controls. Thus, PCPA did not prevent the alteration of NE content resulting from injury.

The pathways involved in inducing changes in the serotonergic system in injured brain remain obscure, but are most likely polysynaptic. In the cat, the nucleus raphe dorsalis has been shown to receive afferent projections from the locus ceruleus complex and several other areas (Sakai et al., 1977), but to date no direct projections from cortical areas have been demonstrated. Widespread ascending projections from the midbrain raphe to all cortical areas are well documented (Beaudet and Descarries, 1976; Moore et al., 1978; Lidov et al., 1980; Parent et al., 1981) and have been reported to

project unilaterally (either ipsilaterally or contralaterally) from the dorsal raphe (van der Kooy and Hattori, 1980) and bilaterally or contralaterally from the median raphe (Jacobs et al., 1978). In view of these findings, unilateral effects of a focal cortical injury on the serotonergic system in the cortex with bilateral changes in the raphe area are particularly difficult to explain in terms of known anatomical connections. In keeping with our working hypothesis (Pappius and Wolfe, 1983*a*, 1984, 1986), other mechanisms are most likely involved in the overall response of the brain to injury mediated by a variety of other processes such as the arachidonic acid cascade and possibly also other transmitter systems. Recently evidence has been obtained that prostaglandin D_2, the major eicosanoid in the rat, functions as a modulator of serotonergic activity in this species (Bhattacharya et al., 1985), while prostaglandin formation has also been implicated as one of the underlying mechanisms in the widespread depression in the functional state of the rat brain that develops in response to a freezing lesion (Pappius and Wolfe, 1983*b*).

Finally, the release of NE has been considered as protective for brain recovery from ischemia by some (Koide et al., 1986) and as deleterious by others (Busto et al., 1985). The present findings that inhibition of 5-HT synthesis in traumatized brain leads to normalization of cortical function, as determined by metabolic mapping, suggests that blockage of this neurotransmitter system may have a potential in the development of novel therapeutic approaches to brain injury and perhaps other conditions associated with depressed cerebral function, such as stroke.

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