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Short Communication

PERSISTENCE OF LONG-LASTING SEROTONIN DEPLETION BY *p*-CHLOROAMPHETAMINE IN RAT BRAIN AFTER 6-HYDROXYDOPAMINE LESIONING OF DOPAMINE NEURONS

KENNETH W. PERRY, RICHARD M. KOSTRZEWA* and RAY W. FULLER†

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46185; and *East Tennessee State University School of Medicine, Johnson City, TN 37614, U.S.A.

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Abstract—In rats that had been treated neonatally with 6-hydroxydopamine (6OHDA) to deplete striatal dopamine more than 95%, a single injection of *p*-chloroamphetamine (pCA) (5 or 10 mg/kg, i.p.) resulted in depletion of striatal and hippocampal serotonin at 1 week to a similar extent as in control rats. These findings suggest that striatal dopamine is not essential to the long-lasting depletion of brain serotonin by pCA in rats.

Key words: p-chloroamphetamine; serotonin; dopamine; 6-hydroxydopamine; striatum; hippocampus

A single, high dose of pCA‡ given to rats causes depletion of brain serotonin that lasts up to several months [1, 2]. The serotonin-depleting effects of pCA are prevented by pretreatment with selective or nonselective inhibitors of that uptake carrier [3, 4], although the precise molecular mechanisms of the long-lasting depletion of brain serotonin are still unknown. Some recent evidence has indicated that dopamine is involved in the long-lasting depletion of serotonin by a related compound, MDMA [4–6]. A smaller amount of available evidence ([5, 7, 8]; discussed below) suggests that dopamine may also be involved in the neurotoxic effect of pCA toward brain serotonin neurons. To investigate that possibility, we studied rats treated neonatally with 6OHDA, a treatment resulting in nearly complete depletion of striatal dopamine, to determine if pCA could cause long-lasting depletion of brain serotonin in these rats.

Materials and Methods

Pregnant female Sprague-Dawley albino rats (Charles River Laboratories, Research Triangle Park, NC) were housed individually in Perspex cages in a room at 22 ± 1° with a 12:12 hr light–dark cycle (on at 7:00 a.m.). Food and water were freely available. At birth, litters were reassigned, so that each dam had rats from several litters, with each reconstituted litter consisting of 11 or 12 pups. Three days after birth, rats received desipramine (20 mg/kg, i.p., given as the hydrochloride salt) 1 h before 6OHDA hydrobromide (200 μg, half in each lateral ventricle) or the vehicle saline (0.85%) containing ascorbic acid (0.1%). This procedure has been described in detail by Kostrzewa and Gong [9]. When these treated rats were 7 weeks old (body weight 180–214 g), they were given a single i.p. injection of pCA hydrochloride (5 or 10 mg/kg, i.p.). One week later, the rats were killed, and their brains were quickly removed and dissected. Brain regions were frozen immediately on dry ice and were stored frozen at −60° prior to analysis. 6OHDA hydrobromide and (±)-pCA hydrochloride were purchased from the Regis Chemical Co. (Chicago, IL).

Serotonin, 5HIAA, dopamine, DOPAC and HVA were measured by liquid chromatography with electrochemical detection. The brain regions were sonicated in 1 mL of 0.10 M trichloroacetic acid containing 0.10 mg/mL of cysteine as a stabilizing agent and an internal standard that was 0.20 nmol/mL of 5-hydroxyindole carboxylic acid. The resulting homogenate was centrifuged at 12,000 *g* for 5 min. Thirty microliters of the supernatant fluid was injected directly onto the analytical column. The analytical column was an Econosphere C18 (5 μm, 4.6 × 150 mm) from Alltech Associates (Deerfield, IL). The mobile phase was 0.10 M monochloroacetic acid, 1 mM EDTA, 220 mg/L of sodium octanesulfonic acid, 8% acetonitrile, pH 2.6, at a flow rate of 1.0 mL/min and a temperature of 40°. An EG&G Applied Research model 400 LCEC Analyzer was used with an electrochemical detector and a Spectraphysics AS 3000 automatic sample injector with a refrigerated sample compartment (samples kept at 5°). The potential of the glassy carbon electrode was +0.75 V. Peak heights and sample concentrations were calculated with a Hewlett–Packard HP1000 chromatography data system.

Results

Table 1 shows that 5 and 10 mg/kg, i.p., doses of pCA hydrochloride caused pronounced and dose-related depletion of serotonin and 5HIAA concentrations in two brain regions—hippocampus and striatum. In rats treated neonatally with 6OHDA, the concentrations of serotonin and 5HIAA were significantly higher in the striatum. In the hippocampus, serotonin and 5HIAA concentrations were numerically higher in 6OHDA-treated rats than in the control rats, but this difference was statistically significant ($P < 0.05$) only in the case of serotonin, not with 5HIAA. pCA depleted serotonin and 5HIAA in both brain regions in 6OHDA-treated rats. The percent depletion of serotonin in 6OHDA-treated rats was only slightly less than in control rats in the hippocampus for both doses of pCA. The percent depletion of 5HIAA in 6OHDA-treated rats was slightly less than in control rats at the lower dose of pCA but was identical to that in control rats at the higher dose of pCA in the hippocampus. In the striatum, the percent depletion of both 5-hydroxyindoles was less at both doses of pCA in 6OHDA-treated rats than in controls. However, this difference was largely due to the higher control values in 6OHDA-treated rats. The absolute change in serotonin concentration, in nmol/g, was 1.24 and 1.50 at the low dose of pCA and 1.53 and 2.26 at the high dose of pCA in control and 6OHDA-treated rats, respec-

† Corresponding author. Tel. (317)276-4164; FAX (317)276-5546.

‡ Abbreviations: pCA, *p*-chloroamphetamine; 6OHDA, 6-hydroxydopamine; MDMA, 3,4-methylenedioxymethamphetamine; 5HIAA, 5-hydroxyindoleacetic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; and HVA, homovanillic acid.

Table 1. Depletion of brain 5-hydroxyindoles (serotonin and 5HIAA) by *p*-chloroamphetamine (pCA) in 6-hydroxydopamine (6OHDA)-treated and control rats

Dose of pCA (mg/kg, i.p.)	5-Hydroxyindole concentration (nmol/g)			
	Serotonin		5HIAA	
	Vehicle-treated	6OHDA-treated	Vehicle-treated	6OHDA-treated
	Hippocampus			
0	1.75 ± 0.08	2.01 ± 0.09	1.96 ± 0.06	2.11 ± 0.11
5	0.55 ± 0.03* (–69%)	0.83 ± 0.03* (–59%)	0.61 ± 0.05* (–69%)	0.88 ± 0.05* (–58%)
10	0.41 ± 0.04* (–76%)	0.62 ± 0.04* (–69%)	0.47 ± 0.06* (–76%)	0.52 ± 0.02* (–76%)
	Striatum			
0	2.24 ± 0.14	3.87 ± 0.17	2.70 ± 0.13	4.14 ± 0.08
5	1.00 ± 0.12* (–55%)	2.37 ± 0.12* (–39%)	1.33 ± 0.15* (–51%)	2.77 ± 0.11* (–33%)
10	0.71 ± 0.10* (–68%)	1.61 ± 0.08* (–58%)	1.03 ± 0.15* (–62%)	1.90 ± 0.09* (–54%)

Rats that were 7 weeks old were given a single i.p. injection of *p*-chloroamphetamine hydrochloride (5 or 10 mg/kg) and were killed 1 week later. Some rats had received 6-hydroxydopamine 3 days after birth. Values are means ± SEM, N = 5–6.

* Significant depletion vs control (0 dose) ($P < 0.05$).

tively. The absolute change in 5HIAA, in nmol/g, was 1.37 and 1.37 at the low dose of pCA and 1.67 and 2.24 at the high dose of pCA in control and 6OHDA-treated rats, respectively. Thus, in both parameters measured at both doses of pCA, there was at least as large an absolute change in 6OHDA-pretreated rats as in controls. Table 2 shows that neonatal treatment with 6OHDA resulted in nearly complete (95–97%) depletion of dopamine and of its two metabolites, DOPAC and HVA, in the striatum. Concentrations of these substances in the hippocampus were too low for accurate measurement by the procedures used.

Discussion

Dopamine has been implicated in the neurotoxic effects of amphetamine and methamphetamine toward nigrostriatal dopamine neurons in rats [10–12], in the neurotoxic effects of methamphetamine and MDMA toward serotonin neurons in rat brain [4, 12], and in the etiology of Parkinson's disease [13]. At least three different mechanisms by which dopamine might cause neurotoxicity have been proposed. First, the formation of hydrogen peroxide during the oxidation of dopamine by monoamine oxidase has been suggested as a cause of potential cytotoxicity [14]. Second, dopamine has been reported [15] to undergo oxidative conversion to 6OHDA, a known neurotoxic substance, after the injection of a large dose of methamphetamine into rats. Third, dopamine has been shown to undergo auto-oxidation analogous to that known to be involved in 6OHDA neurotoxicity, products of the auto-oxidation including quinones, superoxide anions and hydroxyl radicals [13].

Table 2. Depletion of striatal dopamine and its metabolites by 6-hydroxydopamine

Treatment	Concentration (nmol/g)		
	Dopamine	DOPAC	HVA
Vehicle	43.4 ± 1.4	7.69 ± 0.38	3.87 ± 0.33
6OHDA	1.2 ± 0.5* (–97%)	0.29 ± 0.16* (–96%)	0.18 ± 0.13* (–95%)

Rats were killed at 8 weeks of age after receiving 6-hydroxydopamine 3 days after birth. Values are means ± SEM, N = 6. Abbreviations: DOPAC, 3,4-dihydroxyphenylacetic acid; and HVA, homovanillic acid.

* Significant depletion from vehicle-treated ($P < 0.05$).

A possible role of dopamine in the neurotoxic effect of pCA toward brain serotonin neurons was raised by the evidence for dopamine involvement in similar neurotoxic effects of MDMA [4, 6]. There has been limited direct evidence to support a role of dopamine in the neurotoxic effects of pCA. Schmidt *et al.* [6] reported that L-dopa injection potentiated the long-lasting depletion of brain serotonin by pCA (and by MDMA or methamphetamine). Axt and Seiden [7] reported partial but significant protection by α-methyltyrosine pretreatment against brain serotonin depletion by pCA. Henderson *et al.* [8] presented circumstantial evidence that dopamine, which is released acutely by pCA, might be involved in the long-term depletion of brain serotonin by pCA. Our results show that nearly complete lesioning of dopamine nerve terminals in rat striatum did not prevent the long-lasting (1 week) depletion of striatal serotonin by pCA. These results with pCA seem at variance with data reported by Stone *et al.* [4], who studied MDMA. In their experiments, MDMA-induced losses of tryptophan hydroxylase in neostriatum, frontal cortex and hippocampus were attenuated, though not completely prevented, by 6OHDA injected into the substantia nigra. This difference in findings may indicate that dopamine plays a more prominent role in brain serotonin depletion by MDMA than in brain serotonin depletion by pCA. On the other hand, other experimental differences may be important. Dopamine in the striatum (a terminal area) was depleted almost completely in our experiment by 6-hydroxydopamine injected intraventricularly, but dopamine content in midbrain and brainstem regions may not have been depleted as completely [16, 17]. Because serotonergic projections from raphe nuclei pass near dopaminergic cells in the midbrain, the possibility that dopamine content in that region and not just in the terminal field is important must be considered. In any case, our findings that pCA caused long-lasting depletion of serotonin in rat striatum, even in rats whose striatal dopamine terminals were almost completely destroyed, indicate that striatal dopamine stores are not essential for the neurotoxic effects of pCA.

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