Effects of p-Chloroamphetamine on Brain Serotonin Neurons*

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p-Chloroamphetamine (PCA) is a useful pharmacologic tool for selectively increasing brain serotonin function acutely by release of serotonin into the synaptic cleft. PCA produces behavioral, neurochemical and neuroendocrine effects believed due to serotonin release after doses in the range of 0.5–5 mg/kg. At higher doses and at longer times, PCA causes depletion of brain serotonin. The mechanisms of this depletion are not well understood but require the serotonin uptake carrier. Antagonism of PCA-induced depletion of brain serotonin is a useful means of assessing the ability of a compound to block the serotonin uptake carrier on brain serotonin neurons. PCA can also be used as a neurotoxic agent to deplete brain serotonin in functional studies, apparently by destroying some serotonergic nerve terminals. Used in this way, PCA has an advantage over 5,6- and 5,7-dihydroxytryptamines in being effective by systemic injection, and it affects brain serotonergic projections with a different neuroanatomic specificity than the dihydroxytryptamines.

KEY WORDS: p-Chloroamphetamine; serotonin; neurotoxicity; dihydroxytryptamines.

INTRODUCTION

When I joined the Lilly Research Laboratories in 1963, two of my colleagues there—Drs. Jack Mills and Irwin Slater—introduced me to Professor Morris H. Aprison at Indiana University Medical Center as a local scientist who could help me learn about neurochemistry and about brain serotonin as a neurotransmitter. That same year, after I had begun experiments on brain serotonin, Dr. Mills suggested I investigate some halogenated amphetamines that he had synthesized, after Pletscher et al. (1) reported that p-chloromethamphetamine (Figure 1) depleted serotonin in rat brain. Because my acquaintance with halogenated amphetamines began the same year as my acquaintance with Professor Aprison, and I have enjoyed both acquaintances, I chose this topic for a minireview in the volume honoring Professor Aprison.

HISTORY OF p-CHLOROAMPHETAMINE

When Pletscher and colleagues (1) first reported that p-chloromethamphetamine selectively depleted serotonin but not norepinephrine in rat brain, the compound was of interest because it did not directly inhibit either of the two enzymes in serotonin biosynthesis. Numerous chlorinated analogs of amphetamine had been synthesized and examined in the Lilly Research Laboratories as anorectic agents (2,3). When we studied those compounds, p-chloroamphetamine (PCA) emerged as the most potent serotonin depletor in the series (4,5). Following the important contributions of Sanders-Bush, Sulser and their co-workers (6-8), PCA became a much-used research tool for selective modification of serotonergic function in brain. Acutely, PCA releases serotonin and

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enhances serotonergic function, but depletes serotonin stores. The longer-term effect of high doses of PCA is depletion of serotonin and apparent diminution of serotonergic function.

### Structure-Activity Relationships

Several of the structural analogs of PCA that we have studied are shown in Figure 1. Our early studies (4,5) revealed that PCA was slightly more potent as a serotonin depletor than was p-chloromethamphetamine, the compound studied by Pletscher et al. (1). In fact, p-chloromethamphetamine is rapidly and extensively metabolized to PCA in rats (9). Other N-alkyl analogs of PCA deplete serotonin, at least in part because they are metabolized to PCA (9,10). Huang et al. (11) reported that the α-ethyl homolog of PCA was less potent than PCA in depleting brain serotonin in rats. Longer and shorter side chain homologs were inactive or less potent than PCA in depleting brain serotonin, as were analogs with the chloro substituent in other positions on the phenyl ring (12). As we began our studies, we were surprised to see Pletscher et al. (13) report that p-chloro-N-methylphenylethylamine depleted brain serotonin, for we found that the α-methyl branch was essential. I wrote Professor Pletscher to point out the discrepancy and suggest we might exchange compound samples, but he replied that an error had been made in the paper and that the structure of the active compound should have had the methyl on the α-carbon not on the nitrogen; in other words, it was PCA not p-chloro-N-methyl-phenylethylamine. Thus our structure-activity studies agreed well.

An especially interesting analog of PCA was the β,β-difluoro compound. Fluorine is the smallest of the halogens. The two fluorine substituents on the β-carbon change the shape of the PCA molecule very little, i.e., have minimum steric influence. But they greatly reduce the basicity of the nitrogen (its ability to accept a proton), reducing the pKa value (negative logarithm of the ionization constant) from above 9 to about 6.8 (14). At physiological pH, β,β-difluoro-PCA exists mainly as an uncharged molecule, whereas PCA is almost entirely protonated (cationic). β,β-Difluoro-PCA is very different from PCA in the rate of its metabolism and in its tissue distribution, illustrating the importance of ionization in drug metabolism and localization. PCA has a relatively long half-life in rats and localizes preferentially in tissues such as lung, kidney and brain. The β,β-difluoro analog was rapidly metabolized (by oxidative deamination) and localized preferentially in fat, the tissue containing least amounts of PCA. The β,β-difluoro compound caused short-term depletion of brain serotonin when given at high doses in rats to produce equivalent brain levels as those obtained with PCA but did not cause long-term depletion of brain serotonin, apparently because the drug did not persist long enough in brain.

### Characteristics of Serotonin Depletion

**Acute.** When PCA is administered to rats, brain serotonin content is decreased within one or two hours and remains reduced as shown in Figure 2. Accompanying the acute decrease in serotonin content is a decrease in 5-hydroxyindoleacetic acid content (15) and a decrease in tryptophan hydroxylase activity measured in brain homogenates (7). Tryptophan hydroxylation in vivo measured by the accumulation of 5-hydroxytryptophan after decarboxylase inhibition is also decreased (16). Although total serotonin content is decreased, extracellular concentrations of serotonin measured by push-pull cannula techniques (17), by in vivo voltammetry (18,19) or by brain microdialysis (20) are increased. Adell et al. (21) reported that PCA still increased serotonin concentration in the microdialysis fluid even after reserpine treatment of the rats which markedly decreased basal concentrations of serotonin. They suggested that PCA...