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BRIEF COMMUNICATION

α-Methyl-p-Tyrosine Partially Attenuates p-Chloroamphetamine-Induced 5-Hydroxytryptamine Depletions in the Rat Brain

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AXT, K. J. AND L. S. SEIDEN. *α-Methyl-p-tyrosine partially attenuates p-chloroamphetamine-induced 5-hydroxytryptamine depletions in the rat brain.* PHARMACOL BIOCHEM BEHAV **35**(4) 995–997, 1990.—α-Methyl-p-tyrosine (AMT) partially attenuates the long-term p-chloroamphetamine (pCA)-induced 5-hydroxytryptamine (5-HT) depletions. Pretreatment of rats with the tyrosine hydroxylase inhibitor AMT before treatment with the serotonin neurotoxin pCA decreased the extent of 5-HT depletion in the two brain regions examined. In these experiments, rats were administered AMT (150 mg/kg) 1 and 5 hours prior to an injection of pCA (5, 10, or 15 mg/kg). AMT reduced the pCA-induced 5-HT depletions in the striatum and to a lesser extent in the hippocampus. Furthermore, the attenuation of neurotoxicity was dependent on dose of pCA, with greater AMT effects at higher doses of pCA. AMT-pretreated rats were still significantly depleted of brain 5-HT following all doses of pCA. However, at the higher doses of pCA, the AMT-pretreated rats were significantly less depleted than saline-pretreated, pCA-treated rats. These results suggest that the neurotoxic effects of high doses of pCA on 5-HT-containing nerve terminals may be in part dependent on the availability of newly synthesized dopamine (DA).

Neurotoxicity p-Chloroamphetamine Serotonin Brain Rat Dopamine

p-CHLOROAMPHETAMINE (pCA) produces long-term neurochemical deficits in serotonergic (5-HT) nerve terminals. Levels of 5-HT and 5-HIAA are decreased, as is tryptophan hydroxylase (TPH) activity (22). The number of high affinity uptake sites is decreased and there is morphologic evidence of nerve terminal degeneration (14, 15, 21). The neurotoxic effects of pCA have been observed primarily in the serotonergic system; pCA does not have long-lasting effects on parameters of catecholamine function (9,22) or on the activities of glutamate decarboxylase or choline acetyltransferase (10).

The neurotoxic effects of the substituted amphetamines methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) (5, 17, 18, 23), methamphetamine (MA) (19), and fenfluramine (2, 16, 29) are well documented and are qualitatively comparable to pCA. Although pCA has been widely used as a tool for lesioning serotonergic pathways, the mechanism of its neurotoxicity, as well as that of the other amphetamine-related compounds, is not completely understood. However, we have reported that the 5-HT neurotoxin 5,6-dihydroxytryptamine (5,6-DHT) is formed in the rat brain shortly after a single neurotoxic dose of MA or pCA (3,4). We postulated that 5,6-DHT or radical oxygen species (produced during the formation of 5,6-DHT or upon its oxidation to quinones) mediate the neurotoxic effects of MA and pCA, and possibly other substituted amphetamines.

Administration of the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine (AMT) attenuates the long-term neurotoxic effects of MA on 5-HT nerve terminals (1, 6, 12). A functioning dopaminergic system has been suggested to be necessary for the neurotoxic effects of both MA (13,26) and MDMA (30). To elucidate further the mechanism of neurotoxicity of pCA and determine whether DA is involved in the neurotoxic process, it was of interest to determine whether AMT pretreatment protects against pCA neurotoxicity.

AMT (150 mg/kg, SC) was administered 5 and 1 hour prior to pCA. pCA was administered IP at doses of 5, 10, or 15 mg/kg, as

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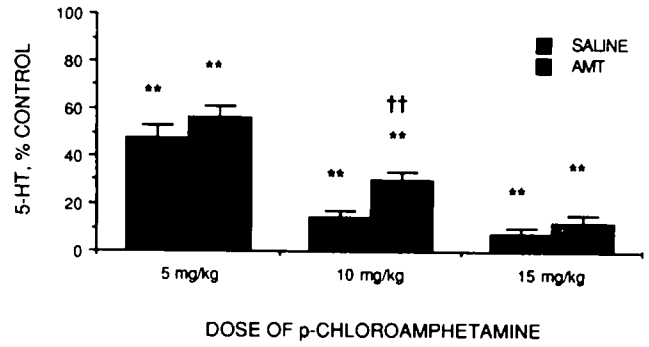

FIG. 1. Effect of pretreatment with saline or AMT on depletions of hippocampal 5-HT induced by various doses of p-chloroamphetamine (pCA). AMT (150 mg/kg) was administered 5 and 1 hour prior to pCA. Rats were sacrificed 2 weeks after pCA administration. Values are expressed as mean % control ± SEM; for all pCA values, n≥6. Control values (ng/mg wet tissue ± SEM are: (5 mg/kg group) (pooled saline and AMT): 0.350 ± 0.010, n = 5; (10 mg/kg group) saline: 0.408 ± 0.036; n = 6; AMT: 0.413 ± 0.038, n = 6; (15 mg/kg group) saline: 0.319 ± 0.028, n = 6; AMT: 0.297 ± 0.016, n = 6. 5-HT levels in saline and AMT controls were comparable, and, therefore, were pooled in the 5 mg/kg group (n = 2 and 3, respectively). $^{**}p<0.01$ compared to control, Newman-Keuls; $^{††}p<0.005$ compared to saline + pCA (10 mg/kg), Student $t = 3.80$.

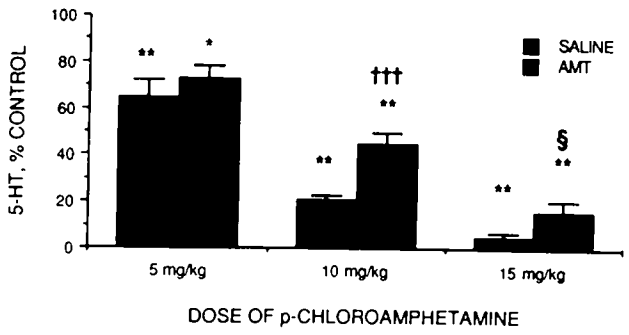

FIG. 2. Effect of pretreatment with saline or AMT on depletions of striatal 5-HT induced by various doses of p-chloroamphetamine (pCA). AMT (150 mg/kg) was administered 5 and 1 hour prior to pCA. Rats were sacrificed 2 weeks after pCA administration. Values are expressed as mean % control ± SEM; for all pCA values, n≥6. Control values (ng/mg wet tissue ± SEM) are: (5 mg/kg group) (pooled saline and AMT): 0.467 ± 0.045, n = 5; (10 mg/kg group) saline: 0.527 ± 0.034, n = 6; AMT: 0.573 ± 0.023, n = 6; (15 mg/kg group) saline: 0.224 ± 0.021, n = 6; AMT: 0.264 ± 0.033, n = 6. 5-HT levels in saline and AMT controls were comparable, and, therefore, were pooled in the 5 mg/kg group (n = 2 and 3, respectively). $^{*}p<0.05$, $^{**}p<0.01$ compared to control, Newman-Keuls; $^{†††}p<0.001$ compared to saline + pCA (10 mg/kg), Student $t = 4.58$; $^{§}p<0.05$ compared to saline + pCA (15 mg/kg), Student $t = 2.25$.

the free base. The highest dose (15 mg/kg) is the same dose after which 5,6-DHT is detectable in the hippocampus (4). Rats were sacrificed 2 weeks after drug administration. Brain regions of interest were dissected as previously described (11). Levels of 5-HT were determined using high performance liquid chromatography (3). Indole levels in pCA-treated groups were compared to their respective controls using ANOVA and the Newman-Keuls post hoc test. Two-tailed Student t-tests were used to compare 5-HT depletions in saline + pCA to AMT + pCA (each expressed as percent of their respective control).

AMT partially reduced the neurotoxic effects of 15 mg/kg pCA on 5-HT nerve terminals (Figs. 1 and 2). Levels of 5-HT in the striatum of rats treated with AMT + 15 mg/kg pCA were approximately three times those of rats treated with saline + 15 mg/kg pCA; 16.2 ± 4.6% (AMT + 15 mg/kg pCA) compared to 5.0 ± 1.9% (15 mg/kg pCA) of control ($p<0.05$, Student $t = 2.25$, Fig. 2). However, levels of 5-HT in the hippocampus of rats treated with AMT + 15 mg/kg pCA were not significantly different from those of rats treated with saline + 15 mg/kg pCA; 12.7 ± 3.5% of control (AMT + 15 mg/kg pCA) compared to 7.5 ± 2.7% of control (saline + 15 mg/kg pCA) (Fig.1). 5-HIAA levels were not determined for these samples.

AMT pretreatment also partially attenuated 5-HT depletions produced by a dose of 10 mg/kg pCA. The depletions of 5-HT after treatment with AMT + 10 mg/kg pCA were significantly less than those observed after treatment with saline + 10 mg/kg pCA both in the hippocampus (30.2 ± 3.4% compared to 14.5 ± 2.4% of control, $p<0.005$, Student $t = 3.80$, Fig. 1) and striatum (45.3 ± 5.0% compared to 20.9 ± 1.7% of control, $p<0.001$, Student $t = 4.58$, Fig. 2). 5-HIAA levels were also significantly less depleted in AMT-pretreated rats in both hippocampus (AMT + 10 mg/kg pCA: 30.5 ± 5.3%, saline + 10 mg/kg pCA: 15.0 ± 2.0%; $p<0.05$, Student $t = 2.72$) and striatum (AMT + 10 mg/kg pCA: 39.3 ± 4.1%, saline + 10 mg/kg pCA: 19.1 ± 2.7%; $p<0.005$, Student $t = 4.09$).

In contrast to the partial attenuation of pCA-induced (10 or 15 mg/kg) 5-HT depletions observed in AMT-pretreated rats, AMT had no significant effect on depletions of 5-HT produced by 5 mg/kg pCA in either hippocampus or striatum (Figs. 1 and 2). Hippocampal 5-HT was 56.5 ± 4.7% of control in AMT + 5 mg/kg pCA-treated and 47.4 ± 5.9% of control in saline + 5 mg/kg pCA-treated rats, $p = 0.34$. Striatal 5-HT was 73.2 ± 5.2% of control in AMT + 5 mg/kg pCA-treated and 64.3 ± 7.6% of control in saline + 5 mg/kg pCA-treated rats, $p = 0.25$. There was also no effect on 5-HIAA depletions. Levels of 5-HIAA in hippocampus were 50.0 ± 2.5% of control (AMT + 5 mg/kg pCA) compared to 44.3 ± 3.3% of control (saline + 5 mg/kg pCA), $p = 0.19$, and in striatum were 63.6 ± 5.7% of control (AMT + 5 mg/kg pCA) compared to 56.6 ± 5.0% of control (saline + 5 mg/kg pCA), $p = 0.38$. Depletions of striatal dopamine or DOPAC were not observed at any of the doses of pCA employed (data not shown).

AMT pretreatment, therefore, does not fully protect rats against pCA-induced depletions of 5-HT in the hippocampus or striatum. Although neostriatal 5-HT is significantly less depleted when rats treated with 10 or 15 mg/kg pCA are pretreated with AMT, this apparent attenuation does not occur in rats treated with 5 mg/kg pCA. Since pCA has been shown to cause release of DA (28), it is possible that a component of pCA-induced serotonergic neurotoxicity is mediated by the actions of released DA on the serotonergic terminals. At higher doses of pCA, the DA component may be significant, and thus can be eliminated by AMT-pretreatment. Note that the slight attenuation of 15 mg/kg pCA-induced depletions resulting from AMT pretreatment is significant in striatum (Fig. 2), but not in hippocampus (Fig. 1), where there is very little DA.

Since the pharmacologic and neurotoxic effects of substituted amphetamines on serotonergic neurons are similar (7, 8, 20, 24, 25, 31), albeit with differing potencies, it is probable that the mechanism of toxicity is identical for this group of drugs. It has been suggested that DA may mediate the neurotoxic effects of several substituted amphetamines on serotonergic neurons (13, 28, 30). The role of DA, or catechols, in amphetamine-induced serotonergic neurodegeneration is not yet clear, however, because of discrepant observations. AMT pretreatment has been shown to protect against the neurotoxic effects of MA (1, 6, 12). However, pretreatment with AMT does not completely prevent the MA-induced formation of 5,6-DHT in the rat hippocampus (1).

Endogenously produced 5,6-DHT has been hypothesized to be responsible for the neurotoxic effects of both MA and pCA (3,4). Schmidt and Taylor (27) have shown that AMT pretreatment does not protect serotonergic neurons from the acute pharmacologic effects of MDMA. However, more recent reports indicate that AMT protects against decreases in TPH activity and 5-HT levels measured 1 week (32) or 3 days (30) after drug administration. In addition, Stone *et al.* (30) demonstrated that bilateral 6-hydroxydopamine (6-OHDA) lesions of substantia nigra attenuated MDMA-induced decreases in neostriatal TPH activity, but not hippocampal or neocortical TPH activity. In contrast, Taylor and Schmidt (32) reported that unilateral nigral lesions with 6-OHDA had no effect on MDMA-induced reductions in TPH activity or 5-HT levels in either neostriatum or neocortex measured 3 hours or 1 week after drug administration.

In conclusion, a role for DA in the neurotoxic effects of substituted amphetamines on serotonergic neurons cannot be definitively assigned. Specifically in the case of pCA-induced neurotoxicity, DA availability does not appear to play a direct role in the neurotoxic actions of pCA, especially at lower doses of the drug. It has been suggested that AMT may prevent MA-induced neurotoxicity in part by scavenging reactive oxygen species (1). However, in light of the present observation that AMT is unable to fully protect serotonergic nerve terminals from the neurotoxic effects of pCA, this mechanism of protection by AMT seems less likely.

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