Short communication

Reduction in brain serotonin markers by α-ethyltryptamine (Monase)

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α-Ethyltryptamine (Monase) is both an inhibitor of monoamine oxidase and a monoamine releasing agent. To determine whether α-ethyltryptamine induces serotonergic deficits similar to that of other monoamine releasing agents such as 3,4-methylenedioxymethamphetamine (MDMA) and para-chloroamphetamine (PCA), rats were given multiple doses (8 × 30 mg/kg s.c.) of α-ethyltryptamine acetate. Serotonin and 5-hydroxyindole acetic acid (5-HIAA) levels and the number of 5-HT uptake sites (determined from [^{3}H]paroxetine binding) were significantly decreased in frontal cortex samples at one week killing. A significant decrease in 5-HT and 5-HIAA levels was also observed in rat hippocampal samples. The results provide evidence that α-ethyltryptamine may induce serotonin neurotoxicity similar to that of MDMA and PCA.

α-Ethyltryptamine (Monase); 5-HT neurotoxicity; MDMA (3,4-methylenedioxymethamphetamine); p-Chloroamphetamine (PCA)

1. Introduction

α-Ethyltryptamine, a non-hydrazine reversible monoamine oxidase inhibitor (Greig et al., 1959) was registered by the Upjohn Company under the trade name Monase. It was put into clinical trials in 1957 and later was introduced into medical practice as a new and safer monoamine oxidase inhibitor type of antidepressant (Theodore, 1961). However, it was withdrawn from the market in 1962 because a serious blood disorder occurred in several patients taking the drug (Upjohn annual report 1962). There has apparently been little interest in Monase since that time. However, in a recent note, a German group reported a death due to ingestion of 700 mg of α-ethyltryptamine; the authors referred to Monase as a 'designer drug' (Daldrup et al., 1986).

In addition to its monoamine oxidase inhibitory activity, it has been shown that α-ethyltryptamine is also a monoamine releasing agent. As a serotonin releasing agent, it is equipotent to (±)-amphetamine, but its dopamine releasing potency is less than that of amphetamine (Baker et al., 1980). It is known that the monoamine releasing agents 3,4-methylenedioxymethamphetamine (MDMA) and para-chloroamphetamine (PCA) are serotonin neurotoxins (eg. see Schmidt et al., 1987). Both MDMA and PCA have potent effects on the serotonergic system, but are less potent as dopamine releasing agents (Schmidt et al., 1987). The similarities between the neurotoxic actions of MDMA and PCA have led to speculation that the mechanisms of neurotoxicity may be related for the two compounds (Schmidt, 1987).

From these known neurochemical effects and its mood elevating properties it was reasoned that α-ethyltryptamine might have pharmacological and toxicologic properties similar to MDMA and PCA. In particular, it was of interest to determine whether α-ethyltryptamine would induce serotonergic deficits similar to those produced by MDMA and PCA. In this report, a study based on multiple dosing of rats with α-ethyltryptamine is presented.

2. Materials and methods

2.1. Materials

α-Ethyltryptamine acetate was purchased from Aldrich Chemical Co. [^{3}H]Paroxetine (20.5 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Fluoxetine was kindly provided by Eli Lilly Laboratories (Indianapolis, IN). HPLC standards were purchased from Sigma Chemical Co. (St Louis, MO).

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2.2. Drug administration

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 175–200 g were individually caged in a temperature controlled room with a 12/12 h lighting schedule. One week after the last of eight (every 12 h) (s.c.) injections of 30 mg/kg α-ethyltryptamine acetate or 1 ml/kg saline, rats were killed by decapitation. The brains were removed and rapidly dissected on ice. The hemispheres were separated and the frontal cortex and hippocampal regions frozen with liquid nitrogen. The samples were stored separately at −70°C until assay using the [^{3}H]paroxetine binding or HPLC-EC methods.

2.3. Determination of [^{3}H]paroxetine binding sites in frontal cortex

Measurement of [^{3}H]paroxetine binding was performed using a modified procedure of Battaglia et al. (1988). Brain frontal cortex from one hemisphere was thawed, weighed and homogenized in 5 ml TNC buffer (Tris HCl 50 mM, NaCl 120 mM, KCl 5 mM) with a Brinkman polytron (setting 6, 2 × 20 s). The homogenates were centrifuged twice at 30000 × *g* for 10 min with an intermittent buffer wash and were then resuspended in the same volume of assay buffer. As described by Battaglia et al. (1988), the ability of a single saturating concentration (1 nM) of [^{3}H]paroxetine to bind to tissue homogenates was examined. Non-specific binding was defined as that displaceable with 1 μM fluoxetine. Incubations were initiated by the addition of 150 μl of tissue to 1.50 ml of the TNC buffer containing [^{3}H]paroxetine to give a total volume of 1.65 ml. The final [^{3}H]paroxetine concentration was 1 nM. The tubes were allowed to equilibrate at 24°C for 1 h before adding 4 ml of ice-old TNC buffer and filtration with a Brandel Cell Harvestor through Whatman GF/C filters presoaked in double distilled water. The tubes and filters were then washed twice with 4 ml of ice-cold buffer and the filters were allowed to air dry before placing them in scintillation vials. ACS scintillation cocktail, 10 ml (Amersham, Arlington Heights, IL) was added and the vials were allowed to stand overnight. The samples were then counted at an efficiency of 54%.

2.4. Determination of monoamine levels in frontal cortex and hippocampus

High pressure liquid chromatography (HPLC) with electrochemical detection was used to determine biogenic amines and metabolite levels. A mobile phase containing 50 mM NaH_2PO_4, 30 mM citric acid, 0.1 mM Na_2EDTA, 0.034% sodium octyl sulfate and 25% v/v methanol was used. The cortical and hippocampal samples were prepared by homogenizing the weighed brain areas from one hemisphere in 0.5 ml of HPLC mobile phase without sodium octyl sulfate, using a motor-driven Teflon pestle and Eppendorf 1.5 ml centrifuge tube. An internal standard of 100 ng/ml of 3-(3,4-dihydroxyphenyl)propionic acid (DHPPA) was used. The samples were then centrifuged at 14000 × *g* for 4 min with a table top centrifuge. The supernatant was assayed for monoamines and their metabolites by injection of 50 μl onto a Brownlee C18 analytical cartridge column (Anspec, Ann Arbor, MI) with a flow rate of 1 ml/min. The HPLC-EC system consisted of a refrigerated autosampler (TosoHaas, Philadelphia, PA), and a model 400 EG&G Princeton electrochemical detector (Princeton, NJ) with a dual electrode potential set at $E_1 = -200$ mV and $E_2 = 850$ mV versus the Ag/AgCl reference electrode.

2.5. Data analysis

The concentrations of norepinephrine (NE), dopamine (DA), homovanillic acid (HVA), DOPAC, 5-HT and 5-HIAA were determined using the Dynamax Method Manager software (Rainin, Woburn, MA) implemented on an Apple Macintosh SE computer. Values from treated animals were compared to those of control animals using Student's t-test. The total number of rats tested in each group was eight (N = 8).

3. Results

Treatment with α-ethyltryptamine had no significant effect on NE, DA, DOPAC, or HVA levels either in cortex or hippocampus samples one week following four days of α-ethyltryptamine (data not shown). But as seen in fig. 1, α-ethyltryptamine produced a significant decrease in all serotonergic markers (5-HT, 5-HIAA and the number of 5-HT uptake sites) in frontal cortex. Similarly, both 5-HT and 5-HIAA were significantly decreased in the hippocampus at one week (fig. 2).

4. Discussion

The results of the present work show that α-ethyltryptamine can significantly decrease 5-HT and 5-HIAA levels and the number of 5-HT uptake sites. The decrease is persistent since it lasts at least one week after the last dose of α-ethyltryptamine. It has been shown that MDMA and PCA also result in persistent decrease in these serotonergic markers (for see Schmidt et al., 1987). Recently, histological studies

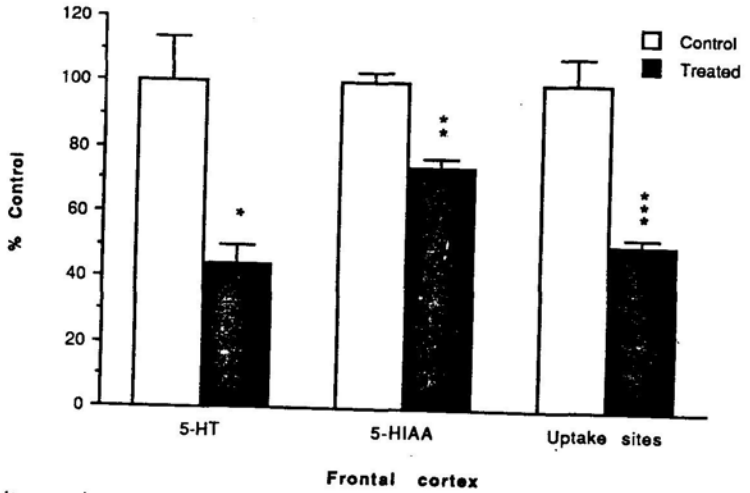
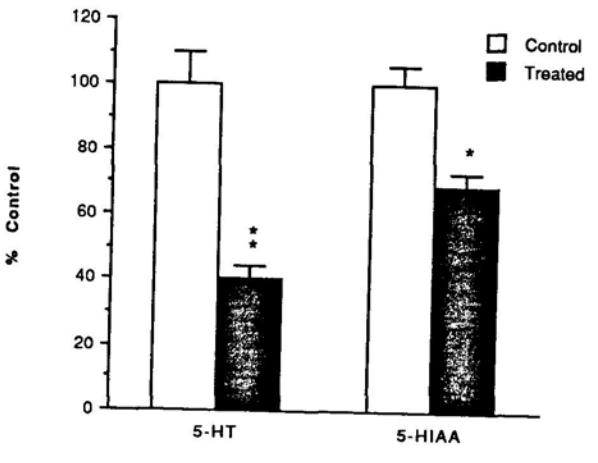


Fig. 1. Saline (1 ml/kg) or α-ethyltryptamine acetate (30 mg/kg) was injected s.c. twice a day for four consecutive days and rats were killed one week later. 5-HT and 5-HIAA levels were determined in frontal cortex using HPLC-EC techniques. The number of uptake sites remaining was examined by determining the ability of a saturating concentration of [^{3}H]paroxetine to bind to tissue homogenates, as described in the methods section. Values are presented as a percent of their saline control, given as means ± S.E.M. for N = 8. Saline control levels were as follows: cortical 5-HT, 356 ± 49 pg/mg wet weight; cortical 5-HIAA, 354 ± 11 pg/mg wet weight; cortical uptake sites, 22.7 ± 1.9 fmol/g wet weight. N = total number of rats tested in one group. * Indicates significantly less than control ($P < 0.01$ Student's t-test). ** Indicates significantly less than control ($P < 0.001$ Student's t-test). *** Indicates significantly less than control ($P < 0.0001$ Student's t-test).

have shown that high doses of MDMA or PCA lead to a selective degeneration of the fine axon serotonergic projections ascending from the dorsal raphe nucleus (e.g. see O'Hearn et al., 1988). Since α-ethyltryptamine causes the same type of decrease in serotonin markers as MDMA and PCA, one might infer that Monase may be inducing a similar neurotoxic response.

The similarities between the neurochemical effects of MDMA and those reported for PCA have led some investigators to suggest that the two might also have a similar mechanism of neurotoxicity (Schmidt, 1987). Recent work has implicated dopamine in the expression of serotonin neurotoxicity (e.g. see Stone et al., 1988; Schmidt et al., 1990). However, the exact mechanism of the neurotoxicity is not understood. Most recently, we have shown that serotonin neurotoxicity is produced by the combined administration of a non-neurotoxic serotonin-releasing agent and the dopamine releasing agent S-amphetamine (unpublished results). Further research may prove that agents with the potency to release non-vesicular stores of both catecholamines and serotonin can induce serotonin neurotoxicity.



g. 2. Saline (1 ml/kg) or α-ethyltryptamine acetate (30 mg/kg) was jected s.c. twice a day for four consecutive days and rats were killed ie week later. 5-HT and 5-HIAA levels were determined in hip-campus using HPLC-EC techniques. Values are represented as ans ± S.E.M. for N = 8. Saline control levels were as follows: HT, 310 ± 32 pg/mg wet weight; 5-HIAA, 386 ± 24 pg/mg wet ight. N = total number of rats tested in one group. * Indicates nificantly less than control ($P < 0.01$ Student's t-test). ** Indicates significantly less than control ($P < 0.001$ Student's t-test).

Although in this report we present no direct evidence to support speculation as to the mechanism of α-ethyltryptamine induced serotonergic deficits, it is not unreasonable to suppose that PCA, MDMA and α-ethyltryptamine may act by similar mechanisms. This premise is supported by the similar long-term effects of all these drugs. Furthermore, the similar monoamine releasing properties of MDMA, PCA and α-ethyltryptamine, and the apparent link between these actions and serotonin neurotoxicity argue for a similar neurotoxic mechanism. In either case, the present report does indicate that α-ethyltryptamine results in persistent changes in serotonergic markers after subacute dosing. If actual serotonin neuron degeneration can be verified histologically, this observation will add a new chemical structure type to the known amphetamine neurotoxins (i.e. PCA and MDMA) and further study of its effects may help to elucidate the neurotoxic mechanisms of similar agents.

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