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COMBINED ADMINISTRATION OF A NON-NEUROTOXIC 3,4-METHYLENEDIOXYMETHAMPHETAMINE ANALOGUE WITH AMPHETAMINE PRODUCES SEROTONIN NEUROTOXICITY IN RATS.

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SUMMARY

In the present study, a central serotonin neurotoxicity was induced by combining a non-neurotoxic 3,4-methylenedioxymethamphetamine analogue, 5-methoxy-6-methyl-2-aminoindan (MMAI), with the non-vesicular dopamine (DA) releaser, *S*-(+)-amphetamine (Amp). With the multiple dosing regimen utilized neither drug alone resulted in any changes in serotonergic parameters, including 5-HT, 5-HIAA and the number of 5-HT uptake sites. However, MMAI (10 mg/kg) in combination with Amp (2x2.5 mg/kg) did result in a long-term 20% decrease in cortical serotonergic parameters. The same dose of Amp plus 20 mg/kg MMAI resulted in a 50 to 60% reduction. Effects in the hippocampus and caudate nucleus were similar. These data support the hypothesis that DA release plays a critical role in the serotonin neurotoxicity of substituted amphetamines.

Key Words - MDMA, neurotoxicity, dopamine, serotonin, amphetamine

Much of the recent study of 3,4-methylenedioxymethamphetamine (MDMA) has implicated the involvement of dopamine (DA) in the selective serotonin neurotoxicity that is seen after single or multiple doses. For example, pretreatment with agents that decrease the levels of DA, such as α-methyl-*p*-tyrosine, reserpine, monofluoromethyl-DOPA, or lesions with 6-hydroxydopamine (6-OH-DA), blocked or attenuated the apparent toxic actions of MDMA (Stone, Johnson, Hanson and Gibb, 1988; Schmidt, Black and Taylor, 1990). In addition, serotonergic agents, such as ketanserin and fluoxetine, also affect dopaminergic responses following high doses of MDMA (Schmidt et al., 1990; Nash, Meltzer and Gudelsky, 1990; Schmidt, 1987; Callaway, Johnson, Gold, Nichols and Geyer, in press). Furthermore, there appears to be a relationship between the relative ability of MDMA-like drugs to induce neurotoxicity and their potency as DA releasers. For example, *S*-(+)-MDMA is the more potent enantiomer for releasing DA and for induction of neurotoxicity (Johnson, Hoffman and Nichols, 1986; Schmidt, Levin and Lovenberg, 1987).

We have recently identified several analogues of MDMA that retained the MDMA-like discriminative cue in drug discrimination experiments but apparently lacked the long-term neurotoxic actions of MDMA. One of these analogues, 5-methoxy-6-methyl-2-aminoindan (MMAI) is of particular interest since it is very selective for synaptosomal 5-HT release (100- and 50-fold selectivity over DA and NE, respectively; Johnson, Conarty and Nichols, unpublished results) but does not induce any long-term changes in serotonergic parameters even after a multiple dosing regimen (20 mg/kg every 12 h for 4 days; Johnson, Frescas, Oberlender and Nichols, in press). However, MMAI acutely depletes 5-HT and 5-HIAA (at 3 h) after a high dose, an action similar to that seen with MDMA (Johnson et al., in press).

The aim of the present study was to test the hypothesis that non-vesicular DA release plays a critical role in the neurotoxicity of some MDMA-like drugs. This was approached by attempting to induce a serotonin neurotoxic response by combining MMAI treatment with relatively low doses of *S*-(+)-amphetamine sulfate (Amp).

METHODS

Rats (male Sprague-Dawley rats weighing 175-199 g; Harlan, Indianapolis, IN) were treated with either saline, 2.5 mg/kg Amp (twice), 10 mg/kg MMAI·HCl, or a combination of MMAI and Amp. Preliminary results indicated the following treatment regimen was most effective for induction of persistent monoamine changes: Amp (i.p.) was given 15 min before and 2 h after (total dose 5 mg/kg) MMAI (s.c.). Animals in the other three control groups were given only one of the drugs alone or saline at each time of injection. This regimen was repeated every 12 h for 4 days. In a second experiment, the same dosing regimen was employed with the same dose of Amp but the dose of MMAI was increased to 20 mg/kg. In both studies the rats were sacrificed 1 week after the last dose and the frontal cortex, hippocampus and caudate nucleus were rapidly removed and frozen in liquid nitrogen. The levels of 5-HT, DA, NE and their major metabolites were quantitated using standard HPLC-EC techniques (Johnson, Huang, Oberlender, Nash and Nichols, 1990). The number of serotonin uptake sites remaining was analyzed utilizing the uptake inhibitor [^{3}H]paroxetine as previously described (Johnson et al., 1990) with only minor modifications. Specifically, 150 µl of tissue homogenate (1.5 to 3 mg wet wt.) was added to each tube to give a final incubation volume of 1.65 ml. It has previously been shown that the long-term decrease in [^{3}H]paroxetine binding with MDMA treatments is associated with a decreased number of uptake sites, linked to a degeneration of certain serotonin axons (Battaglia, Yeh, O'Hearn, Molliver, Kuher and De Souza, 1987). The means (N=6) for raw data values were compared using an analysis of variance followed by a modified t-test post hoc comparison as embodied in the computer program EPISTAT (EPISTAT Services, Richardson, TX)

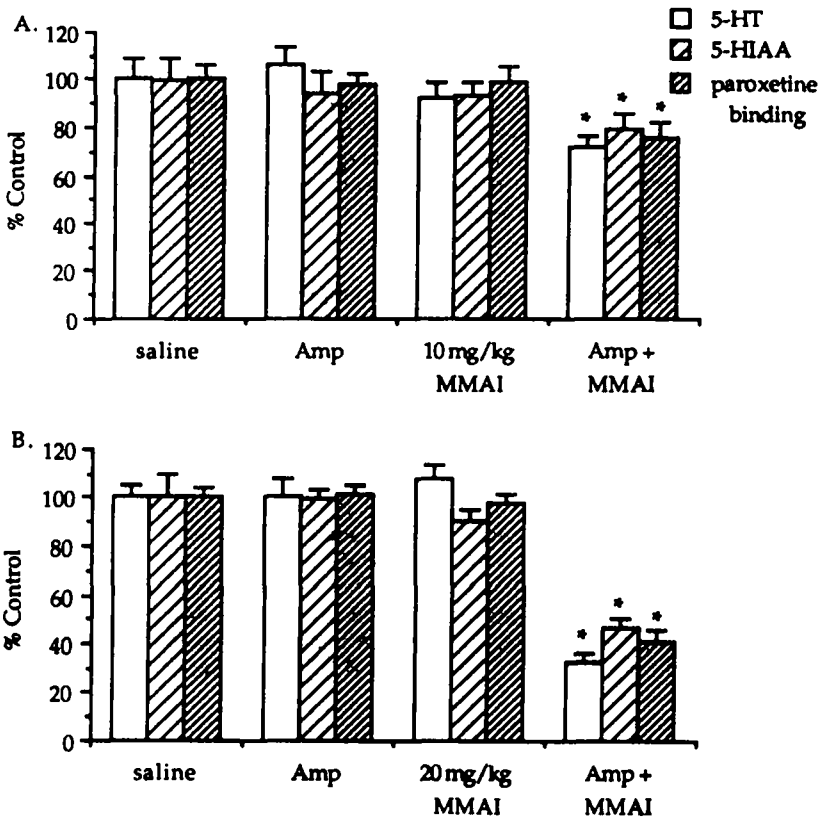

FIGURE 1. Cortical levels of 5-HT, 5-HIAA and serotonin uptake sites one week following the last of 8 doses. Rats were injected with either 10 mg/kg (A) or 20 mg/kg (B) of MMAI and/or 2x2.5 mg/kg *S*-(+)-amphetamine sulfate as described in the text. Values are expressed as a percent of the saline control value. Control levels for A are as follows (mean ± S.E.): 5-HT, 474 ± 40 pg/mg wet wt.; 5-HIAA, 551 ± 46 pg/mg wet wt.; paroxetine binding sites, 19.0 ± 1.2 fmol/mg wet wt. Control levels for B are as follows: 5-HT, 344 ± 19 pg/mg wet wt.; 5-HIAA, 352 ± 34 pg/mg wet wt.; paroxetine binding sites, 17.3 ± 0.7 fmol/mg wet wt. *Indicates significant decrease from saline control ($p < 0.05$, ANOVA followed by post hoc comparison).

RESULTS AND DISCUSSION

As seen in Fig. 1, neither Amp nor MMAI treatment alone resulted in any significant changes in cortical serotonergic parameters 1 week after dosing. However, when the Amp and 10 mg/kg MMAI treatments were combined, an approximately 20% decrease in cortical serotonergic parameters was seen (Fig. 1A). When the dose of MMAI was increased to 20 mg/kg, a 50 to 60% decrease was observed (Fig. 1B). Similar decreases in serotonergic parameters occurred in the caudate nucleus. In addition, significant decreases in caudate DOPAC levels (to approximately 55% of control) were seen with the latter treament. However, no persistant changes were seen in cortical NE, DA, DOPAC or HVA; hippocampal NE; or striatal NE, DA or HVA levels. Furthermore, a long-term decrease in caudate DOPAC levels is also seen after neurotoxic doses of (+)-MDMA which may reflect changes in DA turnover rate consequent to serotonergic neurotoxicity (Schmidt et al., 1987).

The results from the hippocampal region are similar to those seen in the frontal cortex and caudate nucleus (data not shown). While none of the single drug treatments caused any long-term changes in serotonergic parameters, MMAI and Amp in combination significantly decreased all the serotonergic parameters. With 10 mg/kg MMAI plus Amp an approximate 20% decrease was seen, while doses of 20 mg/kg MMAI and Amp gave a 60% reduction in serotonergic parameters. It is interesting to note that Amp was able to induce this response with MMAI despite the relative lack of dopaminergic innervation in the hippocampus.

This might suggest that there is a link between the toxicity induced in the hippocampus and that seen in brain regions where the dopamine levels are higher, such as the caudate nucleus. The nature of this apparent link is unclear, although it has been noted that 6-OH-DA lesions in the striatum block the hippocampal serotonin neurotoxicity induced by MDMA (Schmidt et al., 1990). Alternatively, it is possible that Amp is inducing neurotoxicity in the hippocampus by releasing norepinephrine (NE). However, results from much of the earlier work mentioned above seem to argue against this. For example, selective lesioning of DA axons by bilateral injection of 6-OH-DA in the substania nigra or pretreatment with the selective DA uptake inhibitor GBR-12909 are able to block MDMA's neurotoxicity in several brain areas including the hippocampus (Stone et al., 1988; Schmidt et al., 1990).

The present data support the hypothesis that DA, and more specifically non-vesicular DA release, plays a critical role in the serotonergic neurotoxicity of some MDMA-like drugs. The fact that the dose-toxicity relationship is dependent upon the dose of the serotonergic agent (MMAI) suggests that this toxicity is also dependent upon the extent of depletion of 5-HT and/or the degree of drug induced 5-HT release. Most importantly, the results to date suggest that MDMA-like drugs that retain the behavioral effects of MDMA but lack neurotoxicity may be designed by structural modifications that significantly reduce the ability of the compound to release non-vesicular DA.

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