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Short communication

Selective reduction of striatal type II glucocorticoid receptors in rats by 3,4-methylenedioxymethamphetamine (MDMA)

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A single 20 mg/kg dose of 3,4-methylenedioxymethamphetamine (MDMA) administered to rats markedly decreased serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels in hippocampus, frontal cortex and striatum seven days following injection. MDMA also significantly decreased type II glucocorticoid receptor levels in the striatum, but not in hippocampus or frontal cortex. Since no difference in basal serum corticosterone levels was observed between the two groups, MDMA may decrease striatal type II glucocorticoid receptors via a corticosterone-independent mechanism.

3,4-Methylenedioxymethamphetamine (MDMA); Glucocorticoid receptors; 5-Hydroxytryptamine; Striatum; Corticosterone; Neurotoxicity

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA) is a popular drug of abuse which has attracted considerable attention due to its neurotoxic effects on serotonergic neurons (O'Hearn et al., 1988). Many studies have documented that single or multiple doses of MDMA to rats can produce marked decreases in serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in several brain areas (Stone et al., 1987; Commins et al., 1987). The mechanism for MDMA-induced 5-HT terminal degeneration is not known. Recent work in this laboratory has demonstrated that MDMA produces dose- and time-dependent increases in serum corticosterone levels, possibly by a 5-HT_2 receptor mechanism (Nash et al., 1988).

Recently, adrenal steroid hormone receptors have been classified into the type I and II receptor subtypes (De Kloet and Reul, 1987). The type I receptor is located primarily in the hippocampus, has a very high affinity for corticosterone as well as aldosterone, and mediates the tonic effects of glucocorticoids on brain function. The type II receptor has a high affinity for synthetic glucocorticoids, such as dexamethasone and RU 28362, is widely distributed throughout the brain and is involved in the modulation of neuronal function due to elevated glucocorticoid levels. The type II glucocorticoid receptors are decreased by elevations in circulating corticosterone levels (De Kloet and Reul, 1987) and may mediate some of the neurotoxic effects of glucocorticoids (Sapolsky et al., 1986).

The present study was designed to determine if MDMA, in addition to its well-known effect on 5-HT systems, can also regulate neuronal type II glucocorticoid receptors labelled with the selective type II glucocorticoid receptor ligand [^{3}H]RU 28362.

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2. Materials and methods

Male Sprague-Dawley rats weighing 175-200 g upon delivery were purchased from Zivic Miller Laboratory (Hillson, PA). The animals were housed six per cage in a temperature-controlled room (22-24°C) with a light/dark cycle of 12/12 h (lights on at 06:00 h). Food (Wayne Lab Blox) and tap water were available ad lib.

Rats (N = 6/group) were randomly assigned to either the vehicle (distilled water) or MDMA (20 mg/kg) treatment groups. The racemic mixture of MDMA was generously provided by Dr. David E. Nichols, Purdue University. Rats were injected (1 ml/kg) s.c. between 08:00-09:00 h and returned to their home cages. Seven days later, rats were rapidly decapitated between 08:00-10:00 h and trunk blood collected for subsequent corticosterone determination. The hippocampus, frontal cortex and striatum were rapidly dissected on a chilled glass plate, frozen on dry ice and stored at −80°C until the time of assay.

Neuronal levels of 5-HT and 5-HIAA were quantitated by high performance liquid chromatography with electrochemical detection as previously described (Nash et al., 1988). Serum corticosterone levels were measured using a sensitive and specific radioimmunoassay procedure (Nash et al., 1988). For the type II glucocorticoid receptor measurements, tissues were homogenized in TEDGM buffer (10 mM Tris, 1 mM EDTA, 2.5 mM dithiothreitol, 10% glycerol, 20 mM sodium molybdate; pH = 7.4 at 4°C) and centrifuged at 250 000 × g for 30 min at 4°C. Two hundred microlitres of the resulting cytosol were incubated with 20 nM [^{3}H]RU 28362 ± a 500-fold excess of unlabelled RU 28362 (New England Nuclear) to define non-specific binding. The total incubation volume was 250 μl. Following a 24 h incubation period at 4°C, duplicate 100 μl cytosol aliquots were applied to Sephadex LH 20 minicolumns made out of disposable 1 ml pipette tips equilibrated with TEDGM buffer. One hundred microlitres of TEDGM were used to wash in the cytosol. Thirty minutes later 400 μl of TEDGM were used to elute the receptor-bound [^{3}H]RU 28362 into scintillation vials, which were then filled with 10 ml of Safety Solve (Research Products International) and counted in a scintillation counter at 30-35% efficiency. Cytosolic protein content was measured using the Lowry method. Receptor concentrations are expressed as fmol [^{3}H]RU 28362/mg protein. The 20 nM concentration of [^{3}H]RU 28362 measures 92% of the theoretical maximum number of receptors (B_{max}) as determined by Scatchard analysis and shows an excellent correlation ($r = 0.99$; $N = 14$) with the estimated B_{max} values (Lowy, in preparation).

All values are expressed as the means ± S.E. Student's two-tailed t-tests were used to assess significance between groups. Correlations were performed using the method of Spearman.

3. Results

As previously reported, MDMA administration reduced the concentration of 5-HT (30-50%) and

TABLE 1

The effect of MDMA (20 mg/kg s.c.) on brain 5-HT and 5-HIAA concentrations (ng/mg tissue) 7 days following acute administration. Each value is the mean ± S.E. of six rats. The numbers in the parentheses represent the percent decrease as compared to respective controls [a] $P < 0.1$; [b] $P < 0.05$.

	Frontal cortex		Hippocampus		Striatum	
	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA
Vehicle	0.20 ±0.01	0.17 ±0.01	0.18 ±0.01	0.20 ±0.01	0.25 ±0.01	0.41 ±0.02
MDMA (20 mg/kg)	0.14 [b] ±0.01 (−30%)	0.13 [b] ±0.01 (−24%)	0.09 [b] ±0.01 (−50%)	0.13 [b] ±0.02 (−35%)	0.15 [b] ±0.03 (−40%)	0.32 [a] ±0.04 (−22%)

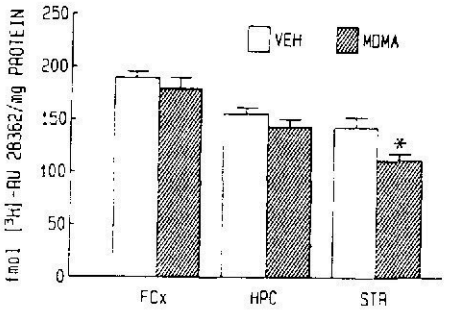

Fig. 1. The effect of MDMA (20 mg/kg s.c.) on the concentration of type II glucocorticoid receptors in the frontal cortex (FCx), hippocampus (HPC) and striatum (STR) 7 days following acute administration in vehicle (open bars)- and MDMA (hatched bars)-treated rats. Each value represents the mean ± S.E. of six rats. Significant differences ($P < 0.05$) from vehicle-treated rats are designated by an asterisk.

5-HIAA (22-35%) in all three brain areas examined seven days following a single administration (table 1).

MDMA administration significantly decreased (22%) type II glucocorticoid receptor content in the striatum, but had no effect on receptor levels in the frontal cortex or hippocampus (fig. 1). There was a significant positive correlation (rho = 0.69; $P < 0.05$; $N = 12$) between 5-HT levels and type II glucocorticoid receptor content in the striatum. No other significant correlations between type II glucocorticoid receptor and 5-HT or 5-HIAA levels in any of the other brain regions were observed.

Serum corticosterone levels at the time of killing were not significantly different between the vehicle (1.0 ± 0.4 μg/dl) and MDMA (1.6 ± 0.5 μg/dl) treated groups.

4. Discussion

In agreement with previous studies (Stone et al., 1987; Commins et al., 1987), a single dose of MDMA produced marked decreases in brain concentrations of 5-HT and 5-HIAA. In view of the ability of MDMA to increase serum corticosterone levels (Nash et al., 1988) and the involvement of glucocorticoid receptors in neurotoxicity (Sapolsky et al., 1986), type II glucocorticoid receptors were also quantitated in contralateral tissues. High levels of type II glucocorticoid receptors were found in all three brain areas examined, which is consistent with the postulated widespread neuronal distribution of this receptor subtype (De Kloet and Reul, 1987). MDMA selectively decreased striatal type II glucocorticoid receptors, while having no effect on receptor levels in the hippocampus or frontal cortex. The MDMA-induced decrease (22%) in striatal glucocorticoid receptors is similar in magnitude to the effect of a 3 week period of stress which produces a 25% decrease in hippocampal glucocorticoid receptor content (Sapolsky et al., 1984).

The mechanism responsible for the selective decrease in striatal type II glucocorticoid receptors produced by MDMA is not known, but appears to be independent of elevated serum corticosterone levels. Although acute administration of MDMA elevates serum corticosterone levels (Nash et al., 1988), no difference in basal serum corticosterone concentration was observed 24 h following administration of MDMA as compared to vehicle-treated rats (Nash, unpublished observations). Thus, it does not appear that MDMA produces chronic elevations in serum corticosterone levels which could subsequently decrease type II glucocorticoid receptor number. This is supported by the present results where serum corticosterone levels were not elevated 7 days following a single administration of MDMA. The equivalent basal serum corticosterone levels between the two groups also suggests that occupation of the type II glucocorticoid receptors by endogenous corticosterone leading to an apparent decrease in the number of receptors is not a factor contributing to the reduction in striatal type II glucocorticoid receptors. Depletion of striatal 5-HT or 5-HIAA levels by MDMA would not appear to be the sole mechanism responsible for the observed decrease in striatal type II glucocorticoid receptors, since 5-HT and 5-HIAA levels in the hippocampus and frontal cortex were decreased to a similar extent, but no decrease in glucocorticoid receptor levels was observed in these two brain areas. Since MDMA also alters striatal concentrations of dopamine (Commins et al., 1987; Stone et al., 1987), MDMA-induced changes in dopamine levels or in other as yet unidentified neuromodulators could also contribute to the

selective decrease in the number of striatal type II glucocorticoid receptors. It is of interest that MDMA produces a selective decrease in striatal type II glucocorticoid receptors and the striatum is one of the brain areas most sensitive to MDMA-induced neurotoxicity (O'Hearn et al., 1988). This raises the possibility that the MDMA-induced decrease in striatal type II glucocorticoid receptor number is related more to neuronal cell death than to 5-HT depletion alone. Previous studies have demonstrated that the decrease in hippocampal glucocorticoid receptor number due to aging and chronic glucocorticoid treatment is associated with a decreased number of hippocampal neurons (Sapolsky et al, 1986).

Previous studies also suggest that biogenic amines can regulate the number of glucocorticoid receptors. Injection of the 5-HT neurotoxin, 5,6-dihydroxytryptamine, into the dorsal raphe increases hippocampal cytosolic [^{3}H]corticosterone binding to type I and II adrenal steroid receptors (Angelucci et al., 1982). Another example of biogenic amine regulation of glucocorticoid receptor levels is the induction of glucocorticoid receptor immunoreactivity in the intermediate lobe of the pituitary (Seger et al., 1988). In this study, glucocorticoid receptor immunoreactivity was not detectable in the intermediate lobe of intact rats. Denervation of the intermediate lobe by frontal deafferentation of the medial basal hypothalamus depleted intermediate lobe dopamine levels by 95% and resulted in the expression of glucocorticoid receptor immunoreactivity (Seger et al., 1988). These studies indicate that 5-HT and dopamine may exert an inhibitory influence on glucocorticoid receptor number, whereas the present study suggests that 5-HT may exert a stimulatory effect on glucocorticoid receptor levels, at least in the striatum. It seems likely that the modulatory effect of neurotransmitters on glucocorticoid receptor regulation is complex with both stimulatory and inhibitory influences as well as regional differences in regulatory mechanisms.

In conclusion, a single injection of MDMA produced marked reductions in 5-HT and 5-HIAA levels in several brain areas, as well as a selective decrease in striatal type II glucocorticoid receptors. These results also indicate that type II glucocorticoid receptors can be regulated by a corticosterone-independent mechanism. There is extensive evidence that glucocorticoids produce complex effects on a variety of biogenic amine systems (McEwen, 1987). The present study indicates that this interaction is reciprocal in nature, since alterations in biogenic amine levels can also regulate the concentration of neuronal glucocorticoid receptors.

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