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Short communication

Differential trafficking of the vesicular monoamine transporter-2 by methamphetamine and cocaine

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Abstract

High-dose administration of cocaine or methamphetamine to rats acutely (≤24 h) alters vesicular dopamine transport. This study elucidates the nature of these changes. Results reveal a differential redistribution of the vesicular monoamine transporter-2 (VMAT-2) within striatal synaptic terminals after drug treatment. In particular, cocaine shifts VMAT-2 protein from a synaptosomal membrane fraction to a vesicle-enriched fraction, as assessed ex vivo in fractions prepared from treated rats. In contrast, methamphetamine treatment redistributes VMAT-2 from a vesicle-enriched fraction to a location that is not retained in a synaptosomal preparation. These data suggest that psychostimulants acutely and differentially affect VMAT-2 subcellular localization.

Keywords: VMAT-2 (vesicular monoamine transporter-2); Cocaine; Methamphetamine; Trafficking; (Rat)

1. Introduction

The vesicular monoamine transporter-2 (VMAT-2) is the sole transporter responsible for sequestration of intraneuronal monoamines. Amphetamines, presumably including methamphetamine, profoundly affect dopamine storage in synaptic vesicles (Sulzer et al., 1995; Cubells et al., 1994). It has been suggested that intraneuronal sequestration of dopamine protects against autooxidation of excess intraneuronal dopamine, which in turn may damage intracellular structures and compromise function (LaVoie and Hastings, 1999; Fleckenstein et al., 2000).

High-dose methamphetamine administration decreases VMAT-2 ligand binding in rodents, as assessed in whole tissue preparations days after drug administration (Hogan et al., 2000; Frey et al., 1997). These long-term reductions have been used as an index of neuronal cell loss. Until recently, psychostimulant-induced changes in VMAT-2 protein levels have not been reported at early time points (i.e. ≤24 h) after methamphetamine treatment. However, recent work employing subcellular fractionation has demonstrated changes in both VMAT-2 activity (assessed by measuring vesicular dopamine uptake) and dihydrotetrabenazine (a VMAT-2 ligand) binding in purified synaptic vesicles 1 h and 1 day after drug treatment (Brown et al., 2000; Hogan et al., 2000). For instance, our laboratory has demonstrated that in vivo administration of methamphetamine, as well as another psychostimulant, cocaine, decreases and increases dihydrotetrabenazine binding, respectively, in a purified vesicular preparation (Brown et al., 2001a,b).

Although the mechanism(s) by which cocaine and methamphetamine influence VMAT-2 is(are) not known, stimulant-induced redistribution of the VMAT-2-containing vesicles may provide an explanation. Hence, the purpose of the present study was to determine if the change in dihydrotetrabenazine binding caused by methamphetamine and cocaine treatment is due to changes in the subcellular distribution of the VMAT-2. The results suggest that cocaine and methamphetamine differentially affect VMAT-2 subcellular location and, presumably, synaptic vesicle distribution. Specifically, cocaine treatment redistributes VMAT-2 from a synaptosomal membrane to a vesicle-enriched fraction. In contrast, methamphetamine redistributes VMAT-2 from the

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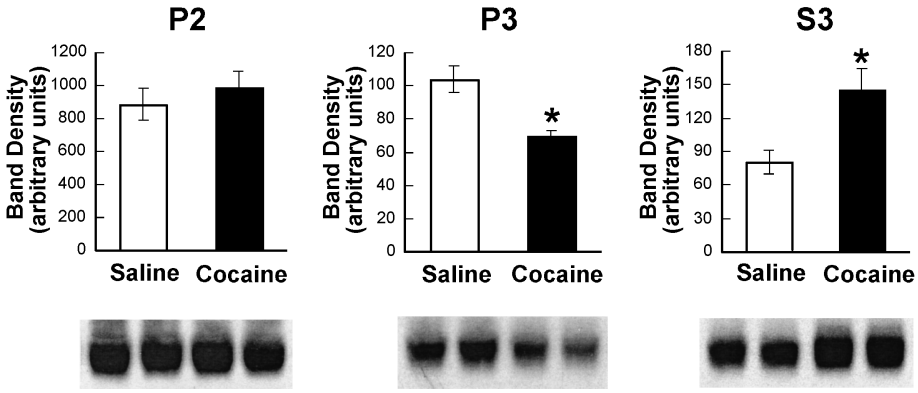

Fig. 1. Cocaine alters VMAT-2 immunoreactivity in subcellular fractions. Rats received a single administration of cocaine (30 mg/kg, i.p.) or saline vehicle (1 ml/kg, i.p.). All animals were sacrificed 1 h after the cocaine or saline injection. Columns represent the mean optic density, and error bars represent the S.E.M. of determinations in six treated rats. *Values for cocaine-treated rats that are significantly different from saline-treated controls ($P \leq 0.05$).

vesicle-enriched fraction without significantly altering synaptosomal membrane VMAT-2 protein levels. These differential patterns induced by cocaine and methamphetamine may underlie some of the different neurochemical and neurotoxic effects of these psychostimulants.

2. Materials and methods

All experiments were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. Where indicated, male Sprague–Dawley rats (weighing 280–330 g) received a single injection of cocaine (30 mg/kg i.p.), multiple high-dose injections of methamphetamine (4×10 mg/kg per injection, s.c., 2-h intervals), or saline vehicle (1 ml/kg per injection).

Striatal synaptosomes were prepared from rats decapitated 1 h after treatment as previously described (Fleckenstein et al., 1997). Briefly, striatal tissue was homogenized in cold 0.32 M sucrose and centrifuged (800×*g* for 12 min; 4 °C). The supernatant (S1) was then centrifuged (22,000×*g* for 15 min; 4 °C) and the resulting pellet (P2, synaptosomal fraction) was resuspended at 50 mg original wet weight/ml in cold water and a portion saved for Western blot analysis. The remainder of the synaptosomal sample was centrifuged for 20 min at 22,000×*g* (4 °C) to pellet lysed synaptosomal membranes (P3, synaptosomal membrane fraction), which were then resuspended at 50 mg original wet weight/ml and saved for Western blot analysis. Prior to resuspension of the plasmalemmal membrane fraction, the supernatant (S3, vesicle-enriched fraction) was removed and saved for Western blot analysis.

Binding of VMAT-2 antibody was performed using 60 μl aliquots of synaptosomal (P2), synaptosomal membrane (P3), or vesicle-enriched (S3) preparations. Each aliquot was added to 20 μl of loading buffer (final concentration: 2.25% sodium dodecyl sulfate, 18% glycerol, 180 mM Tris base (pH 6.8), 10% β-mercapto-ethanol and bromophenol blue), boiled for 10 min, and loaded on a 10% sodium dodecyl sulfate-polyacrylamide gel. Following electrophoresis, samples were transferred to polyvinylidene difluoride membrane, blocked with 5% nonfat dry milk in Tris-buffered saline with Tween (250 mM NaCl, 50 mM Tris pH 7.4 and 0.05% Tween 20), and probed with the VMAT-2

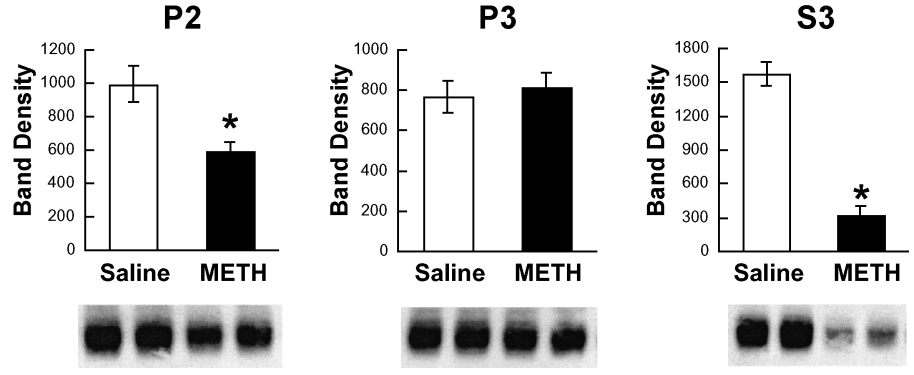

Fig. 2. Methamphetamine alters VMAT-2 immunoreactivity in subcellular fractions. Rats received multiple high-dose injections of methamphetamine (4×10 mg/kg per injection, s.c., 2-h intervals), or saline vehicle (1 ml/kg per injection). All animals were sacrificed 1 h after the final methamphetamine or saline injection. Columns represent the mean optic density, and error bars represent the S.E.M. of determinations in six treated rats. *Values for methamphetamine-treated rats that are significantly different from saline-treated controls ($P \leq 0.05$).

antibody (provided by J.W.H.). Bound antibody was visualized with HRP-conjugated goat anti-rabbit antibody, and antigen–antibody complexes were visualized by chemiluminescence. Multiple exposures of blots were obtained to ensure development within the linear range of the film. Bands on blots were quantified by densitometry using Kodak 1D image-analysis software.

3. Results

Results presented in Fig. 1 demonstrate that a single injection of cocaine (30 mg/kg; i.p.) increases VMAT-2 immunoreactivity by 80% in the S3 (vesicle-enriched) fraction prepared from the striata of rats sacrificed 1 h after treatment. This increase was concurrent with a 33% decrease in the associated P3 (synaptosomal membrane) fraction, with no difference between P2 (synaptosomal) fractions. Data presented in Fig. 2 demonstrate that 1 h after multiple high-dose administration of methamphetamine (4×10 mg/kg; s.c.), VMAT-2 immunoreactivity in the S3 fraction was decreased by 80% compared to saline-treated controls. This decrease was concurrent with a 40% decrease in the P2 fraction and no difference in the P3 fractions.

4. Discussion

Cocaine (Brown et al., 2001a,b) and methamphetamine (Brown et al., 2000; Hogan et al., 2000) increase and decrease, respectively, dihydrotetrabenazine binding and dopamine uptake as assessed in a purified vesicular preparation. Explanations that may underlie the psychostimulant-induced changes include: (1) conformational changes that alter VMAT-2 function and dihydrotetrabenazine binding; (2) changes in the turnover (degradation or synthesis) of the VMAT-2; and (3) redistribution (trafficking) of VMAT-2 and/or VMAT-2-containing synaptic vesicles. The possibility of conformational changes can be largely eliminated as antibody binding and Western blot analysis, using a denaturing gel, are not influenced by the conformational state of the VMAT-2, or by the internal vs. external expression of the protein (i.e. lipid membranes are dissociated and proteins are denatured). The possibility that VMAT-2 synthesis or degradation contributes to the present results also seems unlikely given the rapid nature of the effects (i.e. at 1 h after cocaine or methamphetamine treatment). This is supported by findings that the $t_{1/2}$ for monoamine transporter and receptor turnover is greater than 1 day (i.e. the $t_{1/2}$ for dopamine receptor and transporter are 2–3 and 6 days, respectively; Norman et al., 1987; Battaglia et al., 1988; Fleckenstein et al., 1996) and by findings in human and animal studies that there are no significant changes in total VMAT-2 protein levels measured 1 day after cocaine or methamphetamine exposure (Hogan et al., 2000; Wilson et al., 1996a,b).

Instead of alterations in conformation, synthesis, or degradation, results presented in this study suggest that redistribution of the VMAT-2 protein, and presumably synaptic vesicles, is the mechanism likely responsible for the changes in the VMAT-2 in the purified vesicular preparations after cocaine or methamphetamine treatment. In particular, cocaine administration causes a redistribution of VMAT-2 protein from the P3 to the S3 fraction. In these experiments, the total amount of VMAT-2 protein in the P3 in untreated animals is ≈70% of that found in the P2 fraction (data not shown). Hence, the relatively small decrease in P3 VMAT-2 immunoreactivity after cocaine treatment would be expected to result in a large increase in S3 (given that the total amount of protein in the P2 fraction is not altered by cocaine treatment). Previous studies demonstrated that total VMAT-2 levels are not changed by cocaine administration in brain homogenate or slice preparations. Our data are consistent with these previous data since no changes in total synaptosomal VMAT-2 were detected after cocaine treatment. Moreover, our data demonstrate that cocaine may redistribute VMAT-2: a phenomenon that would not be detected when assessing total VMAT-2 protein levels.

In contrast to the effects of cocaine on VMAT-2, results presented in Fig. 2 demonstrate that methamphetamine treatment largely decreased VMAT-2 immunoreactivity in the S3 fraction. This was concurrent with a moderate decrease in P2 VMAT-2 and no change in P3 VMAT-2 levels. This decrease in S3 and P2 VMAT-2 may suggest trafficking from the P2 fraction altogether (i.e. trafficking out of the portion of nerve terminal retained in a synaptosomal preparation) since decreases observed in the P2 and S3 fraction are not likely due to degradation of protein (Hogan et al., 2000; Wilson et al., 1996a). Interestingly, amphetamine increases the phosphorylation of synapsin, thereby dissociating vesicles from actin filaments (Iwata et al., 1996, 1997). Competitive inhibition of synapsin (a phenomenon that would presumably mimic synapsin phosphorylation) reduces the number of synaptic vesicles within the nerve terminal (Augustine et al., 1999). The location to where the VMAT-2 is distributed remains to be investigated.

In summary, results demonstrate that cocaine and methamphetamine differentially affect the subcellular distribution of VMAT-2, and presumably synaptic vesicles. These drugs differentially affect the trafficking of VMAT-2 with respect to the S3 fraction (methamphetamine *out of* and cocaine *into*), which suggests that these drugs differentially alter trafficking of vesicles between different cellular locations. It has been previously suggested that whole tissue VMAT-2 protein levels are a good indicator of neuron integrity; however, this does not imply that VMAT-2 is static and lacks intracellular regulation. In fact, the present data demonstrate that VMAT-2 can be differentially redistributed among subcellular fractions. These acute changes in VMAT-2 protein (that are not apparent in whole tissue preparations) may contribute to the differential behavioral profiles, neuro-

toxicity, and abuse patterns induced by methamphetamine and cocaine.

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