Published in final edited form as:

Synapse. 2011 May ; 65(5): 449–451. doi:10.1002/syn.20896.

Ephedrine Decreases Vesicular Monoamine Transporter-2 Function

Jonathan D. Ellis, Christopher L. German, Elisabeth Birdsall, Jarom E. Hanson, Marcus A. Crosby, Shane D. Rowley, Nicole A. Sawada, Jeremiah N. West, Glen R. Hanson, and Annette E. Fleckenstein

Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112, USA

Keywords

dopamine; VMAT2; fenfluramine; methamphetamine

Ephedrine, which has been used as a decongestant and weight loss agent, causes both central serotonin and dopamine (DA) release (Bowyer et al., 2000). Ephedrine-like compounds: 1) are substrates for norepinephrine transporters; 2) have some affinity for DA transporters; and 3) have low and/or negligible affinity for adrenergic receptors (Rothman et al., 2003).

Many DA-releasing agents, including methamphetamine (METH) and amphetamine (AMPH), cause persistent dopaminergic (DAergic) deficits (for review, Brown and Yamamoto, 2003; Fleckenstein et al., 2009). In contrast, ephedrine damages these neurons to a lesser degree. For example, Bowyer et al. (2000) reported that 7 d after treatment, multiple ephedrine injections (4×25 mg/kg/injection, 2-h intervals) reduced striatal DA levels by 26%, whereas multiple injections of a lesser dose of AMPH (4×5 mg/kg/injection, 2-h intervals) reduced striatal DA levels by 72%. AMPH-induced extracellular striatal DA levels in that report were twice those after ephedrine over the first 3 injections. Thus, the authors suggested that ephedrine may be less toxic to DA neurons because it does not sufficiently increase DA levels within the extracellular space or nerve terminals.

It has been suggested that persistent (e.g., effects lasting longer than 7 days) DAergic deficits caused by AMPH and METH are a consequence of long-lasting (e.g., greater than 24 – 48 h) impairment of vesicular monoamine transporter-2 (VMAT2) function that leads to aberrant DA accumulation and DA-associated reactive species formation (Chu et al., 2008; for review, see also Brown and Yamamoto, 2003; Fleckenstein et al., 2009; Yamamoto and Bankson, 2005). To date, the effects of ephedrine upon VMAT2 function have not been evaluated. Thus, the present study examined the effects of ephedrine on vesicular DA uptake. Both acute (e.g., 1-h) and persistent (e.g., 7-d) effects on VMAT-2 were assessed as contributors to and indicators of persistent DAergic deficits, respectively. For comparison, effects of the serotonin-releasing agent fenfluramine were also assessed.

Male Sprague Dawley rats (averaging 315 – 415 g; Charles River; Raleigh NC) were maintained under conditions of controlled lighting with food and water provided *ad libitum*. Rats were housed in a warm environment (25 – 27°C) during treatment, unless otherwise indicated, and sacrificed by decapitation. Studies were approved by the Institutional Animal Care and Use Committee at the University of Utah and conducted in accordance with the

National Institutes of Health Guidelines. (-)-Ephedrine hydrochloride and fenfluramine were obtained from Sigma-Aldrich, St. Louis, MO and A.H. Robins, Richmond, VA, respectively. Doses were calculated as the free base. 3,4-[Ring-2,5,6- ^3H]DA was purchased from Perkin Elmer (Boston, MA). Vesicular [^3H]DA uptake was assessed essentially as described previously (Brown et al., 2000, 2001). Comparisons among three or more groups were conducted using analysis of variance followed by Newman-Keuls *post hoc* comparisons, or by two-way ANOVA.

Results presented in Fig. 1A demonstrate the dose-response effects of multiple injections of ephedrine ($3 \times 0 - 25$ mg/kg/injection, s.c., 2-h intervals, $n = 5-10$), as assessed in striatal cytoplasmic vesicles prepared from treated rats 1 h after the final injection. The decrease caused by 3 repeated injections of 25 mg/kg/injection was reversed 24 – 48 h after drug treatment (Fig. 1B), the apparent time period of recovery from ephedrine-induced deficits. This same regimen was without persistent effect on VMAT2 function, as assessed 168 h later (Fig. 1C). Ephedrine effects were not restricted to the striatum, as repeated injections of the stimulant also routinely decreased hippocampal VMAT2 function (data not shown). This lack of specificity is not unique to ephedrine, as repeated injections of other DA-releasing agents such as METH likewise decrease both striatal and hippocampal VMAT2 function (Brown et al., 2000, 2001, 2002; Rau et al., 2006). In contrast to DA-releasing agents, repeated injections of fenfluramine (4×12.5 mg/kg/injection, s.c. 2-h intervals) did not affect either striatal (70.94 ± 7.66 and 64.62 ± 3.89 fmol/ μg protein for saline and fenfluramine, respectively, $n = 8$) or hippocampal (3.140 ± 0.155 and 3.509 ± 0.333 fmol/ μg protein for saline and fenfluramine, respectively, $n = 8$) VMAT2 function.

Because temperature can contribute to the effects of stimulants on DAergic neurons (Bowyer et al., 2000), the impact of ephedrine-induced hyperthermia upon VMAT2 function was investigated. Ephedrine (3 injections, s.c., 25 mg/kg/injection, 2-h intervals) increased core body temperature (e.g., from 37.5 ± 0.1 to 40.0 ± 0.2 °C in rats maintained in a warm environment as assessed 30 min prior to the first injection and decapitation, respectively). Attenuation of ephedrine-induced hyperthermia (e.g., from 37.6 ± 0.2 to 38.8 ± 0.1 °C in rats maintained in a cooler environment (19°C) as assessed 30 min prior to the first injection and decapitation, respectively) did not alter the concurrent decrease in vesicular DA uptake (94.0 ± 8.8 vs. 79.0 ± 4.5 fmol/ μg protein for saline- and ephedrine-treated rats maintained in a warmer environment, respectively, and 105.0 ± 7.4 vs. 66.3 ± 2.1 fmol/ μg protein for saline- and ephedrine-treated rats maintained in a cool environment, respectively, $n = 5-6$). Two-way ANOVA demonstrated that ephedrine decreased uptake regardless of temperature (e.g., there was a main effect of ephedrine; temperature overall had no effect on uptake nor did it interact with ephedrine; $p = 0.05$).

The effects of repeated ephedrine injections resembled those of multiple methylenedioxymethamphetamine (MDMA) injections (4×10 mg/kg/injection, s.c., 2-h intervals; Hansen et al., 2002) and/or a single, high-dose injection of METH (15 mg/kg, s.c., Brown et al., 2002); that is, these treatments rapidly decreased VMAT2 function with at least a partial recovery 24 h after treatment. Further, multiple MDMA injections (e.g., 3 injections, i.p., 9.2 or 13.8 mg/kg/injection, 2-h intervals; Nash and Yamamoto, 1992) or a single, high-dose METH injection (17.5 mg/kg, i.p.; Peat et al., 1983) do not cause persistent DAergic deficits as determined by assessing tyrosine hydroxylase activity and/or striatal DA content. Since ephedrine is a sympathomimetic amine similar to METH, weak base proton-gradient disruption may contribute to this rapid, reversible VMAT2 inhibition.

In contrast to these reversible effects, repeated high-dose METH injections cause both acute decreases in VMAT2 function that persist at least 48 h (Brown et al., 2000; Chu et al., 2008) and persistent DAergic deficits (see Brown and Yamamoto, 2003; Fleckenstein et al., 2008).

The persistent damage was likely attributable to prolonged, elevated intracellular DA levels within the striatum consequent to the decrease in VMAT-2 function that presumably increases and perpetuates oxidative stress. Depletion of terminal DA content prior to METH administration by Thomas et. al. (2008) led these authors to propose METH disruption of striatal DA homeostasis as an initiating event in persistent toxicity. The transient disruption of VMAT2 function by ephedrine, however, did not lead to persistent striatal deficits (Fig. 1C). Thus the reversibility of the effect of ephedrine on VMAT2, in addition to its lesser effects on DA levels as suggested by Bowyer et al. (2000), may contribute to its lack of persistent DAergic decreases.

Of note, attenuation of the hyperthermia induced by a multiple ephedrine injections did not prevent the decrease in VMAT2 function caused by the stimulant. Similarly, attenuation of hyperthermia does not prevent the decrease in VMAT2 function caused by a single METH injection (Brown et al., 2002), demonstrating another common feature of these treatments.

As mentioned above, administration of either the releasing agents METH or MDMA decreases striatal cytoplasmic VMAT2 function. In contrast, DA reuptake inhibitors such as cocaine and methylphenidate increase striatal vesicular DA uptake in this tissue fraction (Brown et al., 2001; Sandoval et al., 2002). Thus, the effects of ephedrine reported herein more closely resemble the acute effects of a releasing agent than a reuptake inhibitor. Similarly, Rothman et al. (2003) reported that, with the exception of (-) pseudoephedrine, all of the ephedrine-like compounds that these authors examined *in vitro* were devoid of reuptake inhibitor properties and acted as substrates for catecholamine transporters.

In summary, the present study demonstrates the impact of ephedrine on VMAT2 function; an effect resembling that of other catecholamine-releasing agents. This effect is transient, as is likely its impact on intra- and extra-neuronal DA levels, and thus may explain the lack of persistent neurotoxic effects on DAergic neurons caused by the stimulant.

Acknowledgments

This work was supported by grants DA 00869, DA 04222, DA 13367, DA 11389, DA 019447, and DA 00378 from the National Institute on Drug Abuse.

References

- Bowyer JF, Newport GD, Slikker W Jr, Gough B, Ferguson SA, Tor-Agbidye J. An evaluation of l-ephedrine neurotoxicity with respect to hyperthermia and caudate/putamen microdialysate levels of ephedrine, dopamine, serotonin, and glutamate. *Toxicol. Sci.* 2000; 55:133–142. [PubMed: 10788568]
- Brown JM, Hanson GR, Fleckenstein AE. Methamphetamine rapidly decreases vesicular dopamine uptake. *J. Neurochem.* 2000; 74:2221–2223. [PubMed: 10800970]
- Brown JM, Hanson GR, Fleckenstein AE. Cocaine-induced increases in vesicular dopamine uptake: role of dopamine receptors. *J. Pharmacol. Exp. Ther.* 2001; 298:1150–1153. [PubMed: 11504813]
- Brown JM, Riddle EL, Sandoval V, Weston RK, Hanson JE, Crosby MJ, Ugarte YV, Gibb JW, Hanson GR, Fleckenstein AE. A single methamphetamine administration rapidly decreases vesicular dopamine uptake. *J. Pharmacol. Exp. Ther.* 2002; 302:497–501. [PubMed: 12130707]
- Brown JM, Yamamoto BK. Effects of amphetamines on mitochondrial function: role of free radicals and oxidative stress. *Pharmacol. Ther.* 2003; 99:45–53. [PubMed: 12804698]
- Chu PW, Seferian KS, Birdsall E, Truong JG, Riordan JA, Metcalf CS, Hanson GR, Fleckenstein AE. Differential regional effects of methamphetamine on dopamine transport. *Eur. J. Pharmacol.* 2008; 590:105–110. [PubMed: 18599036]

- Fleckenstein AE, Volz TJ, Hanson GR. Psychostimulant-induced alterations in vesicular monoamine transporter-2 function: Neurotoxic and therapeutic implications. *Neuropharmacology*. 2009; 56:133–138. [PubMed: 18662707]
- Hansen JP, Riddle EL, Sandoval V, Brown JM, Gibb JW, Hanson GR, Fleckenstein AE. Methylenedioxymethamphetamine decreases plasmalemmal and vesicular dopamine transport: mechanisms and implications for neurotoxicity. *J. Pharmacol. Exp. Ther.* 2002; 300:1093–1100. [PubMed: 11861820]
- Nash JF, Yamamoto BK. Methamphetamine neurotoxicity and striatal glutamate release: comparison to 3,4-methylenedioxymethamphetamine. *Brain Res.* 1992; 581:237–243. [PubMed: 1356579]
- Peat MA, Warren PF, Gibb JW. Effects of a single dose of methamphetamine and iprindole on the serotonergic and dopaminergic system of the rat brain. *J. Pharmacol. Exp. Ther.* 1983; 225:126–131. [PubMed: 6187915]
- Rau KS, Birdsall E, Volz TJ, Riordan JA, Baucum AJ, Adair BP, Bitter R, Gibb JW, Hanson GR, Fleckenstein AE. Methamphetamine administration reduces hippocampal vesicular monoamine transporter-2 uptake. *J. Pharmacol. Exp. Ther.* 2006; 318:676–682. [PubMed: 16687477]
- Rothman RB, Vu N, Partilla JS, Roth BJ, Hufeisen SJ, Compton-Toth BA, Birkes J, Young R, Glennon RA. In vitro characterization of ephedrine-related stereoisomers at biogenic amine transporters and the receptorome reveals selective actions as norepinephrine transporter substrates. *J. Pharmacol. Exp. Ther.* 2003; 307:138–145. [PubMed: 12954796]
- Sandoval V, Riddle EL, Hanson GR, Fleckenstein AE. Methylphenidate redistributes vesicular monoamine transporter-2: role of dopamine receptors. *J. Neurosci.* 2002; 22:8705–8710. [PubMed: 12351745]
- Thomas DM, Francescutti-Verbeem DM, Kuhn DM. The newly synthesized pool of dopamine determines the severity of methamphetamine-induced neurotoxicity. *J. Neurochem.* 2008; 105(3): 605–616. [PubMed: 18088364]
- Yamamoto BK, Bankson MG. Amphetamine neurotoxicity: cause and consequence of oxidative stress. *Crit Rev Neurobiol.* 2005; 17:87–117. [PubMed: 16808729]

Fig. 1.

Panel A: Rats received ephedrine (3 injections, 5 – 25 mg/kg/injection, s.c., 2-h intervals, n = 6) or saline vehicle (3 injections, 1 ml/kg/injection, s.c., 2-h intervals, n = 6) and were sacrificed 1 h later. Panels B and C: Rats received ephedrine (3 injections, 25 mg/kg/injection, s.c., 2-h intervals, n = 5–10) or saline vehicle (zero-time controls; 3 injections, 1 ml/kg/injection, s.c., 2-h intervals, n=5–10) and were sacrificed 1 – 168 h later. Values represent the means $\pm$ SEM. * Value significantly different from saline-treated controls (p 0.05).