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Short communication

Oxygen radicals diminish dopamine transporter function in rat striatum

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Abstract

Incubation of striatal synaptosomes with the oxygen radical generating enzyme, xanthine oxidase, decreased [^{3}H]dopamine uptake: an effect attributable to a decreased V_{max}. Concurrent incubation with the superoxide radical scavenger, superoxide dismutase, abolished the xanthine oxidase-induced decrease. These results indicate that, like methamphetamine administration in vivo, reactive oxygen species diminish dopamine transporter function in vitro. The significance of these findings to mechanisms responsible for effects of methamphetamine is discussed. © 1997 Elsevier Science B.V.

Keywords: Oxygen radical; Dopamine transporter; Methamphetamine

1. Introduction

Administration of the amphetamine analog, methamphetamine, causes both oxygen radical formation (Kondo et al., 1994; Giovanni et al., 1995; Fleckenstein et al., 1997a) and a rapid and reversible decrease in dopamine transporter function (Fleckenstein et al., 1997b; Fleckenstein et al., 1997c). An association between these phenomena is suggested by findings that reactive oxygen species may alter the activity of a variety of aminergic transporters (Braughler, 1985; Debler et al., 1986; Volterra et al., 1994; Tarrant and Williams, 1995), including the dopamine transporter (Pögün et al., 1994; Berman et al., 1996). Because of its potential role in mediating methamphetamine-induced decreases in dopamine uptake, the purpose of this study was to characterize the effect of reactive oxygen species on dopamine transporter function. To elucidate the direct effect of oxygen radicals on dopamine transport, xanthine oxidase was used to generate reactive oxygen species as described previously (Braughler, 1985; Debler et al., 1986; Berman et al., 1996). The results reveal that oxygen radicals, like methamphetamine administration (Fleckenstein et al., 1997c), diminish dopamine transporter V_{max}. Consistent with a role in effecting methamphetamine-induced dopaminergic neuronal impairment (Hirata et al., 1996), superoxide radicals contribute to this decrease. The significance of these findings to the effects of methamphetamine, and for the physiological regulation of monoaminergic systems, is discussed.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (200–300 g; Simonsen laboratories, Gilroy, CA) were maintained under conditions of controlled temperature and lighting, with food and water provided ad libitum. Rats were killed by decapitation. All procedures were conducted in accordance with approved national institutes of health guidelines.

2.2. Drugs and chemicals

(−)cocaine hydrochloride and (±)methamphetamine hydrochloride were supplied by the national institute on drug abuse (USA). Pargyline hydrochloride was supplied by Abbott Laboratories (North Chicago, IL). [7,8-^{3}H]dopamine (43 Ci/mmol) was purchased from Amersham Life Sciences (Arlington Heights, IL). Unless otherwise noted, xanthine oxidase (from buttermilk), superoxide dismutase (from bovine liver), catalase (from bovine liver) and all other reagents were purchased from Sigma (St. Louis, MO). Xanthine oxidase from sigma was employed at a concentration of 3 mU/ml (final concentration in

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incubation mixture) except in the kinetic experiments wherein xanthine oxidase obtained from Fluka (Ronkonkoma, NY) was used at a concentration of 10 mU/ml since its efficacy tested comparable to 3 mU/ml obtained from Sigma.

2.3. Assays

Fresh striatal tissue was homogenized in ice-cold 0.32 M sucrose and centrifuged ($800 \times g$ for 12 min; 4°C). Where indicated, xanthine oxidase, xanthine, superoxide dismutase, catalase, mannitol, deferoxamine, ascorbate, histidine or melatonin was added to the S1 fractions as described in text (total incubation volume of 1 ml). Control and 'treated' S1 fractions were incubated (37°C, 10 min). The resulting supernatant (S1) was then centrifuged ($22{,}000 \times g$ for 10 min; 4°C), and the resulting pellets (P2) resuspended in ice cold 0.32 M sucrose. [^{3}H]dopamine uptake (0.5 nM, except in Eadie–Hofstee analysis wherein 0.5 to 1000 nM was employed) in the P2 fraction was determined as described previously (Boja et al., 1992). Lactate dehydrogenase (LDH) activity was determined in the P2 fraction using the method of Wroblewski and LaDue (1955) and the protocol/reagents of Sigma Chemical with occluded LDH determined by addition of 2% Triton-X (1% final concentration) as described by Marchbanks (1967).

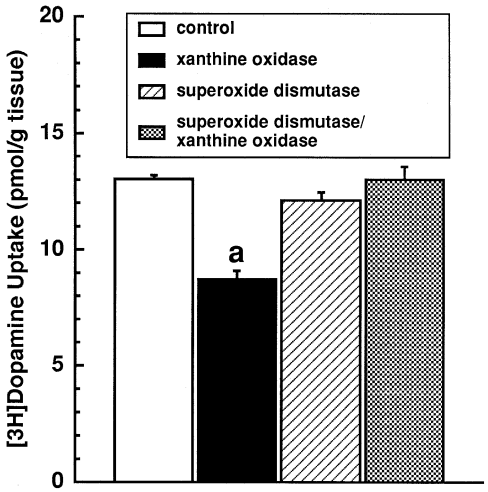

Fig. 2. Effects of superoxide dismutase on xanthine oxidase-induced decreases in [^{3}H]dopamine uptake in striatal synaptosomal preparations. In these experiments, striatal tissue was homogenized and centrifuged. The resulting supernatant (S1) was incubated with xanthine oxidase (3 mU/ml), superoxide dismutase (100 U/ml) or both enzymes. Enzyme concentrations reflect final concentrations in incubation mixtures. The treated S1 fraction was then centrifuged, and [^{3}H]dopamine uptake was assessed in the resulting P2 fraction. See Section 2 for details. Values represent means and vertical lines 1 S.E.M. of determinations from 3 independent experiments, with samples in each experiment run in triplicate. [a] Value for xanthine oxidase-treated synaptosomes that is significantly different from control synaptosomes (ANOVA with Fisher's post hoc comparison; $P \leq 0.05$).

3. Results

Results presented in Fig. 1 demonstrate that [^{3}H]dopamine uptake into striatal synaptosomes, prepared from rats decapitated 1 h after methamphetamine administration (15 mg/kg, s.c.), was decreased by 52% relative to saline controls. This effect is unrelated to residual levels of methamphetamine introduced by the in vivo treatment, as evidenced, in part, by the finding that methamphetamine concentrations in the synaptosomal preparations are less than 1% of the concentration required to decrease synaptosomal [^{3}H]dopamine accumulation by 50% (Fleckenstein et al., 1997b; Fleckenstein et al., 1997c).

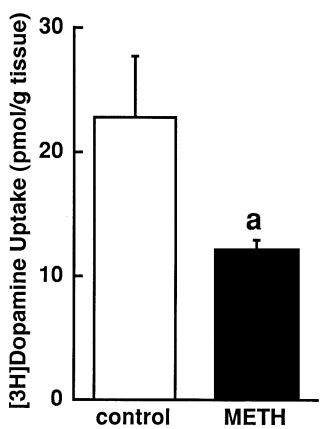

Fig. 1. Effects of methamphetamine administration on [^{3}H]dopamine uptake in rat striatum. Rats received methamphetamine (15 mg/kg, s.c.) or saline vehicle (1 ml/kg, s.c.) 1 h prior to decapitation. Columns represent the means and vertical lines 1 S.E.M. of determinations in 6 rats. [a] Value for methamphetamine-treated rats that is significantly different from saline-treated rats (Student's *t*-test; $P < 0.05$).

Like methamphetamine treatment in vivo, pre-incubation with xanthine oxidase decreased [^{3}H]dopamine uptake in rat striatal synaptosomes (Fig. 2): a decrease reflecting a reduction in V_{max} (3506 ± 783 and 1256 ± 165 pmol/g wet weight tissue/3 min for control and xanthine oxidase-treated synaptosomes, respectively; $P < 0.05$, paired Student's *t*-test), and no effect on K_m (102 ± 6 and 123 ± 7 nM for control and xanthine oxidase-treated synaptosomes, respectively). Preincubation with heat-inactivated xanthine oxidase did not affect [^{3}H]dopamine transport, suggesting that the decrease was not due to non-specific effects of the xanthine oxidase protein. Co-incubation with xanthine did not enhance the xanthine oxidase-mediated decrease in [^{3}H]dopamine transport, presumably because the enzyme utilized purines and aldehydes endogenous to the tissue preparation as its substrate. Xanthine per se, at concentrations of up to 100 μM, was without effect on [^{3}H]dopamine uptake. Xanthine oxidase was without effect on either occluded (i.e., intra-synaptosomal; Marchbanks, 1967) or total (intra- plus extra-synaptosomal) LDH activity in the P2 synaptosomal fraction suggesting that, at this

enzyme concentration, its ability to decrease [^{3}H]dopamine uptake did not reflect a loss of synaptosomal integrity: similar findings have been reported by Berman et al. (1996).

Several scavengers and antioxidants were evaluated for the ability to attenuate the xanthine oxidase-induced decrease in synaptosomal [^{3}H]dopamine uptake: catalase (10^3–10^6 U/ml), ascorbate (10 nM–10 mM), mannitol (100 nM–100 mM), deferoxamine (10 nM–1 mM), histidine (10 nM–1 mM) and melatonin (10 nM–1 mM) did not prevent the xanthine–oxidase effect. In general, these compounds were also without effect on [^{3}H]dopamine uptake per se. Only preincubation with superoxide dismutase (100 U/ml) prevented completely the xanthine oxidase-induced decrease in [^{3}H]dopamine uptake. Superoxide dismutase alone was without effect on uptake of [^{3}H]dopamine (Fig. 2).

4. Discussion

Previous studies have demonstrated that methamphetamine administration causes both oxygen radical formation in vivo (Kondo et al., 1994; Giovanni et al., 1995; Fleckenstein et al., 1997a), and a rapid and reversible decrease in dopamine transporter function (Fleckenstein et al., 1997b; Fleckenstein et al., 1997c). By demonstrating an oxygen radical-mediated impairment of dopamine transporters, this study demonstrates a possible association between these phenomena. Results reveal that preincubation with xanthine oxidase decreased the V_{max} of [^{3}H]dopamine uptake: similarly, a decrease in V_{max} of this transporter occurs after a single methamphetamine administration (Fleckenstein et al., 1997c).

To establish a causal role for reactive oxygen species, several antioxidants or radical scavengers were tested for their ability to attenuate the xanthine oxidase-induced decrease in dopamine transporter activity. Neither catalase (to scavenge hydrogen peroxide), mannitol (to scavenge hydroxyl radicals), histidine (to scavenge singlet oxygen), ascorbate nor melatonin prevented the enzyme-decreased [^{3}H]dopamine transport. The iron chelator deferoxamine similarly was without protective effect. Only concurrent incubation with the superoxide radical scavenger, superoxide dismutase, prevented the xanthine oxidase-induced decrease in uptake of [^{3}H]dopamine, thereby establishing that reactive oxygen species mediate the xanthine–oxidase induced effect on dopamine transporters. These data also suggest superoxide radicals impair dopamine transporter function. Recently, evidence for superoxide radicals in the long-term neurotoxic effects of methamphetamine has been described by Hirata et al. (1996) suggesting there may be similar causative factors for both phenomena.

These findings suggest a possible pathological and physiological significance of oxygen radical-mediated effects on transporter molecules. In the pathogenesis of methamphetamine neurotoxicity, changes in transporter function may be an early event in a cascade ultimately leading to neuronal damage. Since dopamine transporters are the primary means whereby dopamine is cleared from the synaptic cleft, disruption could lead to increased extracellular dopamine and ultimately the production of highly destructive reactive oxygen species. On the other hand, given that methamphetamine causes the formation of reactive neurotoxic substances, disruption of transporter function might actually serve a protective role by preventing neuronal uptake of such toxic species. As mentioned previously, transporter inhibitors such as amfonelic acid attenuate methamphetamine-neurotoxicity (Hotchkiss and Gibb, 1980; Schmidt and Gibb, 1985). A reactive oxygen species-mediated shutdown of transporters might, in essence, mimic transporter inhibitors and thereby afford neuroprotection. Further experiments into the possibility that oxygen radicals might play an important physiological role in the modulation of transporter function are warranted.

In conclusion, the findings that methamphetamine increases both oxygen radical formation and decreases dopamine transport, and that reactive oxygen species diminish dopamine transporter function, suggest that acute administration of methamphetamine may diminish transporter activity through an oxidative effect on the dopamine transporter. Future studies are necessary to establish that the methamphetamine-induced effects on dopamine transporters are reactive oxygen species-mediated. These findings may have important implications regarding mechanisms responsible for methamphetamine neurotoxicity as well as for the physiological regulation of these monoaminergic systems through oxidation of transporters by reactive oxygen species.

Acknowledgements

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