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European Journal of Pharmacology 456 (2002) 39–43

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Inhibitory effect of reserpine on dopamine transporter function

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Received 15 October 2002; accepted 22 October 2002

Abstract

Previous studies indicate that reserpine may disrupt dopamine transporter activity. Results presented herein reveal that it also inhibits potently synaptosomal [^{3}H]dopamine uptake. In addition, reserpine administration to rats decreased the V_{max} of synaptosomal dopamine transport, as assessed ex vivo 12 h after treatment. This decrease appeared, at least in part, dissociated from concurrent inhibition of the vesicular monoamine transporter-2 (VMAT-2). In separate experiments, synaptosomal dopamine uptake did not differ between wild-type and heterozygous VMAT-2 knockout mice, and reserpine treatment did not inhibit [^{3}H]dopamine uptake into cells heterogously expressing the human dopamine transporter. Taken together, these data suggest that reserpine may transiently alter dopamine transporter function in a noncompetitive, indirect manner.

Keywords: Reserpine; Dopamine transporter; VMAT-2 (vesicular monoamine transporter-2); Striatum

1. Introduction

Reserpine binds to the vesicular monoamine transporter-2 (VMAT-2) and thereby impairs vesicular catecholamine storage within nerve terminals. In addition, a few reports suggest that reserpine may bind to sites other than VMAT-2 (Norn and Shore, 1971; Blaschke et al., 1980). For instance, Bowyer et al. (1984) reported decreased synaptosomal [^{3}H]dopamine uptake following reserpine application in vitro. Other investigators have reported decreased synaptosomal dopamine uptake following a single administration (Harris and Baldessarini, 1973; Ross and Kelder, 1979). Still, it is assumed commonly that reserpine acts selectively on VMAT-2 and has little effect on plasmalemmal dopamine transporter activity. Since reserpine is used widely as a pharmacological tool to modulate catecholaminergic neuronal function, the purpose of this study was to investigate further the effects of reserpine on dopamine transporter activity.

2. Materials and methods

2.1. Materials

(−)-Cocaine hydrochloride was supplied generously by the National Institute on Drug Abuse (Bethesda, MD). Reserpine, dimethylsulfoxide (DMSO) and nomifensine were purchased from Sigma (St. Louis, MO). Tetrabenazine was purchased from Fluka (Sigma) or kindly provided by Drs. Jeffrey Erickson and Helene Varoqui (LSU Health Sciences Center, New Orleans, LA). [7,8-^{3}H]Dopamine (52 Ci/mmol) and 1-[2,3-^{3}H]glutamic acid (22.5 Ci/mmol) were purchased from Amersham Life Sciences (Arlington Heights, IL) and New England Nuclear (Boston, MA), respectively.

2.2. Animals

Male Sprague–Dawley rats (250–350 g; Simonsen Laboratories, Gilroy, CA) were maintained under conditions of controlled temperature and lighting, with food and water provided ad libitum. Rats were sacrificed by decapitation. Wild-type and heterozygous VMAT-2 knockout mice (Wang et al., 1997) were provided generously by Marc Caron

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0014-2999/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S0014-2999(02)02647-X

(Duke University Medical Center, Durham, NC). Reserpine was administered to rats in a vehicle of polyethylene glycol-300 and 1% citric acid (1:4 v/v). All procedures were conducted in accordance with approved National Institutes of Health guidelines.

2.3. Synaptosomal [^{3}H]dopamine and [^{3}H]glutamate uptake, and vesicular [^{3}H]dopamine uptake

Uptake of tritiated neurotransmitters into striatal synaptosomes was determined as described by Fleckenstein et al. (1999). Briefly, synaptosomes were incubated for 3 min in the presence of 0.5 nM [^{3}H]dopamine or 10 nM [^{3}H]glutamate (final concentrations) at 37 °C. Nonspecific values for the [^{3}H]glutamate assay were determined in the presence of 1 mM D-aspartate. [^{3}H]Dopamine uptake into vesicles isolated from striatal synaptosomes was determined as described by Brown et al. (2001). In these experiments, vesicles were incubated for 3 min in the presence of 30 nM [^{3}H]dopamine (final concentration) at 30 °C.

For the synaptosomal uptake assays, reserpine was dissolved in a 25% solution of DMSO (75% assay buffer), then diluted further when added to assay solution. The final concentration of DMSO in assay was 2.5% (for all concentrations of reserpine).

2.4. Plasma membrane and vesicular [^{3}H]dopamine uptake in SK-N-MC cells

SK-N-MC cells were stably transfected with either human plasma membrane dopamine transporter cDNA (Ciliax et al., 1995) or human VMAT-2 cDNA (Miller et al., 1999) inserted into pCDNA 3.1 (Invitrogen). Plasmalemmal [^{3}H]dopamine uptake was assessed as previously described (Pfil et al., 1996). Briefly, cells were incubated for 10 min in the presence of 50 nM [^{3}H]dopamine (final concentration) at 37 °C. Vesicular dopamine uptake was conducted as described by Erickson et al. (1992). Briefly, digitonin-permeabilized cells were incubated for 10 min in the presence of 50 nM [^{3}H]dopamine (final concentration) at 37 °C.

Reserpine was prepared in ethanol, then diluted to a final concentration of 100 nM using assay buffer. In a control experiment (see Results), reserpine was dissolved in either 100% DMSO or glacial acetic acid, then diluted to a final concentration of 0.01–10 μM using assay buffer. The final concentration of DMSO in the assay was 0.5%, and the concentration of acetic acid was essentially negligible.

2.5. Data analysis

Statistical analyses between two groups were conducted using two-tailed, unpaired Student's *t*-test. Analyses among multigroup data were conducted using analysis of variance, followed by a Fisher least significant difference (LSD) test. Differences among groups were considered significant if the probability of error was less than 5%.

3. Results

Reserpine has little ability to inhibit plasmalemmal dopamine uptake in striatal synaptosomal preparations when this relatively insoluble compound is suspended in an aqueous vehicle (assay buffer), as its estimated IC_{50} for [^{3}H]dopamine uptake (a value confounded by the lack of solubility) was 35.2 ± 9.4 μM ($n = 3$ determinations). However, upon dissolving in 25% DMSO (final concentration of DMSO in assay was 2.5%), reserpine inhibited [^{3}H]dopamine uptake with an IC_{50} of 5.1 ± 1.9 nM ($n = 4$ determinations). The 2.5% DMSO per se decreased plasmalemmal [^{3}H]dopamine uptake by approximately 20%. To preclude the possibility that the effect of reserpine was due to a nonspecific effect on synaptosomal function caused by the hydrophobic reserpine in DMSO, effects on transport of another substrate ([^{3}H]glutamate) were tested. Reserpine (in the presence of 2.5% DMSO in assay) at concentrations ranging from 100 fM–1 mM was without effect on [^{3}H]glutamate uptake relative to 2.5% DMSO control (data not shown). Because these data suggest the possibility that reserpine, in addition to its well-established ability to inhibit VMAT-2 function, may alter plasmalemmal dopamine transporter function, the effect of reserpine on plasmalemmal and vesicular dopamine uptake was assessed after administration in vivo.

Results presented in Fig. 1 demonstrate that a single administration of reserpine (5 mg/kg, i.p.) decreased [^{3}H]dopamine uptake into striatal synaptosomes prepared 12 h after reserpine treatment. In separate experiments, it was determined that this decrease in dopamine transporter function was due principally to a decrease in V_{max} of uptake (1918 fmol/mg tissue/3 min versus 650 fmol/mg tissue/3 min), and not K_m (42 nM versus 35 nM). Vesicular [^{3}H]dopamine uptake was diminished by 99% 12 h after this same reserpine treatment. The decrease in dopamine transporter function was largely recovered by 48 h and

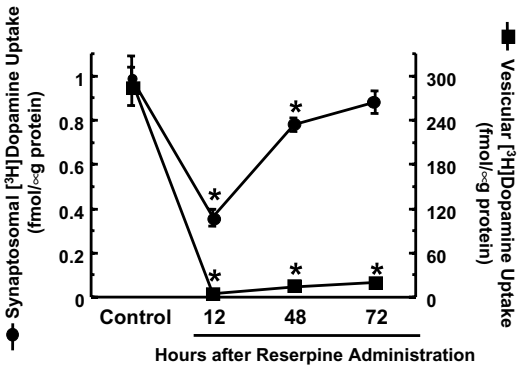

Fig. 1. Time–response effect of reserpine on [^{3}H]dopamine uptake into rat striatal synaptosomes and vesicles. Rats received reserpine (5 mg/kg, i.p.) and were decapitated 12, 48, or 72 h after treatment. Control rats received vehicle (1 ml/kg, i.p.) and were decapitated 12 h after vehicle injection. Values represent means, and vertical lines 1 S.E.M., of determinations in 6 rats/group. *Values different from the vehicle treatment group ($P < 0.05$).

completely by 72 h after treatment; however, vesicular [^{3}H]dopamine uptake was still inhibited by 95% and 93%, respectively (Fig. 1).

To examine the relationship between plasmalemmal and vesicular dopamine uptake, striatal synaptosomal dopamine uptake was assessed in wild-type and heterozygous VMAT-2 knockout mice (where approximately 50% of VMAT-2 was expressed (Wang et al., 1997)). Dopamine uptake did not differ between these two groups (44.1 ± 5.1 fmol/mg/3 min versus 48.8 ± 4.0 fmol/mg/3 min tissue for wild-type and heterozygous knockout mice, respectively; $n = 9–10$). These results are in agreement with a previous study that found no difference in synaptosomal dopamine uptake between these genotypes, nor a difference in dopamine transporter protein levels in striatal homogenates (Gainetdinov et al., 1998).

To further investigate whether or not reserpine directly inhibits the dopamine transporter, plasmalemmal [^{3}H]dopamine uptake was measured in cell lines (SK-N-MC) that expressed the human plasma membrane dopamine transporter, but did not express VMAT-2 nor contain synaptic vesicles. As shown in Fig. 2A, acute treatment with 100 nM reserpine (prepared in ethanol) did not inhibit [^{3}H]dopamine uptake into these cells. This was in contrast to the inhibitory effect of nomifensine (10 μM), a well-characterized dopamine transporter inhibitor. In a control experiment (data not shown), reserpine, at concentrations ranging from 0.01 to 10 μM, was without effect on dopamine uptake whether dissolved in DMSO or acetic acid (see Materials and methods).

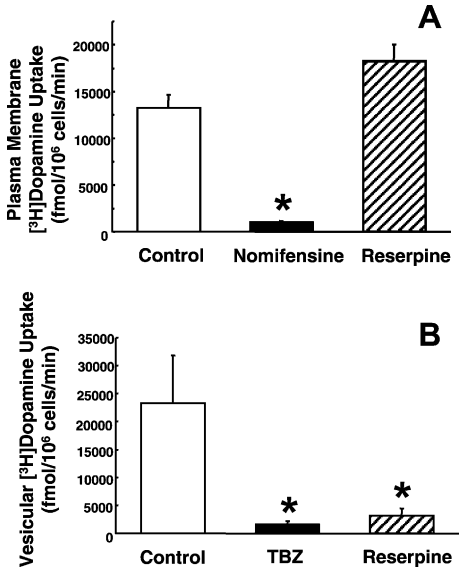

Fig. 2. Effects of reserpine on plasma membrane and vesicular [^{3}H]dopamine transport. (A) Plasma membrane [^{3}H]dopamine uptake in dopamine transporter-expressing cells exposed to reserpine (100 nM) or nomifensine (10 μM). (B) Vesicular [^{3}H]dopamine uptake in digitonin-permeablized VMAT-2-expressing cells exposed to tetrabenazine (10 μM) or reserpine (100 nM). Values represent means, and vertical lines 1 S.E.M. of three to four determinations. *Values different from control ($P < 0.05$).

To ensure that the concentration of reserpine used in Fig. 2A was indeed an effective concentration in this preparation, a parallel experiment was conducted (Fig. 2B). In this experiment, vesicular [^{3}H]dopamine uptake was measured in permeabilized SK-N-MC cells that expressed the human VMAT-2 only (localized to endoplasmic reticulum and other intracellular compartments). Acute treatment with reserpine (100 nM), as well as tetrabenazine (10 μM), inhibited vesicular [^{3}H]dopamine uptake by 86.7% and 93.2%, respectively. For control purposes (i.e., in order to assess [^{3}H]dopamine uptake in the absence of dopamine transporter or VMAT-2), both experiments were conducted with SK-N-MC cells that were not transfected with human dopamine transporter nor VMAT-2 cDNA. [^{3}H]Dopamine was not taken up by these cells at the plasma membrane nor the vesicular membrane, and there were no significant differences between treatment groups (data not shown).

4. Discussion

It is well established that reserpine causes a persistent inhibition of VMAT-2 function. It is believed commonly that this inhibitory effect is selective for the vesicular transporter, and that reserpine is without effect on plasmalemmal dopamine transporter function. However, the IC_{50} of reserpine (when dissolved in a DMSO-containing vehicle) for synaptosomal dopamine uptake in vitro was less than one-tenth the IC_{50} for cocaine-inhibiting dopamine uptake in this synaptosomal preparation (i.e., 153 nM; Fleckenstein et al., 1996). Based on these data and the finding that reserpine persists in rat brain tissue for at least 12 h after administration (Manara and Garattini, 1967) reserpine administration in vivo would presumably decrease both vesicular and plasmalemmal dopamine uptake. Accordingly, results presented in Fig. 1 demonstrate that a single injection of reserpine decreased vesicular and plasmalemmal dopamine uptake in vivo by approximately 99% and 60%, respectively, as assessed ex vivo in vesicles and synaptosomes, respectively, prepared 12 h after reserpine treatment.

One possible explanation for the decrease in synaptosomal dopamine uptake caused by reserpine is that altering vesicular dopamine uptake indirectly affects plasmalemmal dopamine transporter function. However, results presented in Fig. 1 demonstrate that the reserpine-induced decrease in vesicular dopamine uptake is not sufficient to decrease the activity of the plasmalemmal transporter, since the two phenomena were dissociated at 48 and 72 h after treatment (i.e., plasmalemmal dopamine uptake was decreased by 15–20%, whereas vesicular dopamine uptake was still decreased by greater than 90%). In another experiment, a dissociation between VMAT-2 function and dopamine transporter function was further demonstrated since synaptosomal dopamine uptake did not differ between wild-type mice and heterozygous VMAT-2 knockout mice; although it should be noted that a 50% reduction in VMAT-2 expression

may not be sufficient to simulate the effect on VMAT-2 induced by reserpine.

The decrease in dopamine transporter function observed following reserpine administration in vivo did not reflect a direct competitive interaction between reserpine and the dopamine transporter, as it was attributable to a decrease in V_{max} of uptake and not K_m. This issue was examined further by assessing the effect of reserpine on dopamine transporter activity in cells expressing the human dopamine transporter, but not VMAT-2 nor synaptic vesicles (Fig. 2A). In this experiment, reserpine treatment did not alter plasmalemmal dopamine uptake. Hence, it appears that mechanisms other than a direct inhibition of the carrier protein per se likely contribute to the diminution in dopamine transporter function caused by reserpine.

One possible explanation for the inhibitory effect of reserpine may be a loss of radioactivity from synaptosomes in the absence of vesicles that normally sequester radioactive dopamine and prevent diffusion of radioactive molecules, especially lipophilic metabolites formed by enzymatic or autooxidative degradation in the extracellular compartment. Arguing against this possibility are the ex vivo data demonstrating that at 72 h after reserpine treatment, plasmalemmal dopamine uptake is normal despite the near absence of vesicular dopamine uptake. Hence, another mechanism likely accounts for the inhibitory effect of reserpine on synaptosomal dopamine uptake.

Findings that a decrease in vesicular dopamine uptake per se is not sufficient to alter dopamine transporter function do not preclude the possibility that alterations in vesicular dopamine sequestration might contribute to the decrease in dopamine transporter function caused by reserpine treatment. For example, it is likely that a decrease in vesicular dopamine uptake induces a transient increase in intraneuronal dopamine concentrations. This effect on dopamine distribution might alter plasmalemmal dopamine uptake. Moreover, it is possible that if VMAT-2 is functionally in great excess in synaptosomes, then the slight (5–7%) recovery of VMAT-2 activity seen 48 and 72 h after treatment (Fig. 1) may be sufficient to alter intraneuronal dopamine sequestration and thereby reinstate dopamine transporter function. Further studies of the interaction between VMAT-2, the dopamine transporter and dopamine per se are warranted, especially since these transporters are the principal regulators of intra- and extracellular dopamine concentrations. Furthermore, high concentrations of dopamine per se have been demonstrated to decrease dopamine transporter function in vitro (Berman et al., 1996). Moreover, it has been demonstrated recently that direct application of dopamine causes internalization of human dopamine transporters in human embryonic kidney (HEK) 293 cells, thereby reducing transport capacity (Saunders et al., 2000), a phenomenon that would present itself as a decrease in V_{max} of plasmalemmal dopamine transport in the present synaptosomal model. One difficulty in integrating these previous studies with the hypothesis that intraneuronal dopamine might alter dopamine transporter function is that it cannot be determined from these previous studies whether intraneuronal or extraneuronal dopamine or both mediated the effects. Further studies assessing the interactions between dopamine, VMAT-2 and the plasmalemmal dopamine transporter are warranted.

In summary, results presented herein reveal that reserpine decreases plasmalemmal dopamine transporter function after direct application to synaptosomes, as well as ex vivo 12 h after drug treatment. In contrast, reserpine is without effect on plasmalemmal dopamine transport in cells lacking VMAT-2 and synaptic vesicles. The mechanism for this decrease remains unknown, but appears to be a noncompetitive inhibitory effect. The present findings suggest that inhibitory effects on the plasmalemmal dopamine transporter should be considered in future studies involving reserpine.

Acknowledgements

We acknowledge gratefully Marc Caron and Yan-Min Wang for their generosity and assistance in providing wild-type and heterozygous VMAT-2 knockout mice. We also thank Yvette V. Ugarte for her excellent technical assistance. This work was supported by USPHS grants DA00869, DA04222, DA00378, DA11389, NS09930 and ES09248.

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