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Acute effects of resveratrol to enhance cocaine-induced dopamine neurotransmission in the striatum

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HIGHLIGHTS

- Resveratrol enhanced cocaine-induced dopamine neurotransmission.
- The acute effect of resveratrol is similar to that of MAO inhibitors.
- An acute injection of resveratrol enhanced cocaine-induced locomotor activity.

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ABSTRACT

Resveratrol is known as an activator of SIRT1, which leads to the deacetylation of histone and non-histone protein substrates, but also has other pharmacological profiles such as the inhibition of monoamine oxidase (MAO)-A and MAO-B. Resveratrol was previously demonstrated to potentiate the rewarding effects of chronic cocaine via activation of SIRT1. However, the role of resveratrol in cocaine responses in the acute phase remains unexplored. Therefore, we investigated the acute effects of resveratrol on cocaine-stimulated dopamine neurotransmission by analyzing protein phosphorylation in neostriatal slices. Treatment with resveratrol (50 μM for 30 min) enhanced cocaine-induced increases in the phosphorylation of DARPP-32 at Thr34 and GluA1 at Ser845, postsynaptic substrates for dopamine/D1 receptor/PKA signaling, and a cocaine-induced decrease in the phosphorylation of tyrosine hydroxylase at Ser40, a presynaptic substrate for dopamine/D2 receptor signaling. The inhibition of both MAO-A and MAO-B by clorgyline and pargyline, respectively, enhanced the effects of cocaine on DARPP-32 phosphorylation. The acute effect of resveratrol on cocaine-induced DARPP-32 phosphorylation was occluded with inhibition of MAO-A and MAO-B. In behavioral studies, resveratrol (40 mg/kg, s.c.) enhanced the increase in locomotor activity induced by acute cocaine administration (10 mg/kg, i.p.). Thus, this study provides pharmacological evidence that acute resveratrol enhances cocaine-induced dopamine neurotransmission and behavioral responses, presumably via mechanisms involving the inhibition of dopamine catabolism by MAO-A and MAO-B. Resveratrol may be useful to treat dysregulated dopamine neurotransmission, but it may enhance the risk of developing drug addiction.

1. Introduction

Resveratrol, a nonflavonoid polyphenol naturally found in red wine and grapes, is an activator of silent information regulator of transcription 1 (SIRT1) [2], which is one of the Class III nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylases (HDACs), also known as sirtuin [2,3]. The activation of SIRT1 by resveratrol reduces histone acetylation, resulting in a compact chromatin conformation that favors transcriptional inactivation. SIRT1 also deacetylates non-histone protein substrates and plays a fundamental role in regulating cellular processes that are associated with neuronal function in addition to metabolism, inflammation and stress response, cardiovascular function, and cancer [8].

Abbreviations: AMPK, AMP-activated protein kinase; CamKKβ, calmodulin-dependent kinase kinase β (CamKKβ); DARPP-32, dopamine-and cAMP-regulated phosphoprotein of M_r 32 kDa; ERK, extracellular signal-regulated kinase; HDACs, histone deacetylases; MAO-A, monoamine oxidase-A; MAO-B, monoamine oxidase-B; NAD, nicotinamide adenine dinucleotide; PDE, phosphodiesterases; PKA, protein kinase A; SDS, sodium dodecyl sulfate; SIRT1, silent information regulator of transcription 1; SIRT2, silent information regulator of transcription 2; TH, tyrosine hydroxylase.

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SIRT1 plays a critical role in the brain. For example, SIRT1 promotes learning and memory [7] and was protective against Alzheimer's disease in a mouse model [5]. The SIRT1 activator, resveratrol, may offer a promising new treatment for neurodegenerative diseases, whereas the neuroprotective roles of HDAC inhibitors have also been reported [3]. Furthermore, SIRT1 is involved in drug addiction to psychostimulants [19]. Chronic cocaine induced SIRT1 and SIRT2 expression, which is associated with increased histone H3 acetylation in the nucleus accumbens, and the activation of SIRT1 by resveratrol enhanced the rewarding effects of cocaine [18]. The study demonstrates that resveratrol promotes drug addiction, although the precise molecular mechanisms underlying the action of resveratrol are not fully elucidated.

Resveratrol has been used as a SIRT1 activator, but the effect of resveratrol is now thought to be indirect [9]. In a recent study, resveratrol was shown to inhibit phosphodiesterases (PDEs) and to induce cAMP/Epac1 signaling, which activates SIRT1 via the calcium/calmodulin-dependent kinase kinase β (CamKKβ)/AMP-activated protein kinase (AMPK) pathway [17]. In addition, resveratrol has pharmacological profiles that inhibit the catabolism of monoamines by monoamine oxidase (MAO)-A and MAO-B and the re-uptake of noradrenaline and serotonin into pre-synaptic neurons [12,22,23], resulting in the facilitation of monoamine neurotransmission. This evidence suggests that resveratrol regulates neuronal function via mechanisms involving the facilitation of monoamine neurotransmission as well as SIRT1 activation.

Cocaine exerts psychostimulant action, mainly by inhibiting dopamine reuptake. We therefore hypothesized that resveratrol may facilitate cocaine-induced dopamine neurotransmission under acute experimental conditions. To evaluate this hypothesis, we investigated the phosphorylation-states of DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of M_r 32 kDa) as well as other protein kinase A (PKA)-substrates in the striatum. DARPP-32 is selectively enriched in medium spiny neurons in the striatum and is phosphorylated at Thr34 by PKA [21]. The phosphorylation-state of DARPP-32 is very sensitive to changes in cAMP/PKA signaling, and the ability of cocaine to activate D1 and D2 receptor signaling can be detected by analyzing DARPP-32 phosphorylation in striatal slices [6,16]. The present findings reveal that resveratrol enhances cocaine-induced dopamine neurotransmission, presumably by inhibiting MAO-A and MAO-B.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice at 6–8 weeks old were purchased from Japan SLC (Shizuoka, Japan). The mice were housed under a constant temperature (23 ± 2 °C) and a 12-h light/dark cycle (light period: 07:00–19:00 h). The mice were given free access to food and water throughout the experiments. All mice used in this study were handled in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the U.S. National Institutes of Health, and the specific protocols were approved by the Institutional Animal Care and Use Committee of Kurume University School of Medicine.

2.2. Drugs

Trans-resveratrol, clorgyline and pargyline were obtained from Sigma–Aldrich (St. Louis, MO, USA). Cocaine hydrochloride was obtained from Takeda Chemical Industries (Osaka, Japan).

2.3. Western blot assay

2.3.1. Incubation and processing of neostriatal slices

Neostriatal slices were prepared, as described [15]. Briefly, coronal brain slices (350 μm) were prepared using a vibrating blade microtome, VT1000S (Leica Microsystems, Nussloch, Germany). Striata were dissected from the slices, and the slices were pre-incubated in fresh Krebs-HCO_3^- buffer at 30 °C under constant oxygenation with 95% O_2/5% CO_2 for 60 min. The slices were treated with drugs as specified in each experiment. After drug treatment, the slices were transferred to Eppendorf tubes, frozen on dry ice, and stored at −80 °C until they were assayed.

The frozen tissue samples were sonicated in boiling 1% sodium dodecyl sulfate (SDS) containing 50 mM sodium fluoride. Equal amounts of protein (100 μg) were processed by using 10% polyacrylamide gels as described [15].

2.3.2. Immunoblotting

The membranes were immunoblotted using phosphorylation state-specific antibodies raised against phospho-peptides: phospho-Thr34 DARPP-32 (the PKA-site; CC500; 1:4000 dilution); phospho-Thr75 DARPP-32 (the Cdk5-site; 1:5000 dilution); phospho-Ser97 DARPP-32 (the CK2-site; 1:500 dilution); phospho-Ser845 GluA1 (the PKA-site; p1160-845; 1:250 dilution) (PhosphoSolutions, Aurora, CO, USA); phospho-Thr202/Tyr204 ERK (the MEK-site; 1:2000 dilution) (New England BioLabs, Beverly, MA, USA); phospho-Ser40 tyrosine hydroxylase (TH) (the PKA-site; AB5935; 1:1000 dilution) (Millipore, Billerica, MA, USA); acetyl-Lys9 Histone H3 (06-942; 1:1000 dilution) (Abcam, Cambridge, UK); phospho-Thr172 AMPKα (2535; 1:1000 dilution) (Cell Signaling Technology, Danvers, MA, USA). Antibodies generated against DARPP-32 (C24-5a; 1:40,000 dilution), GluA1 (E-6; 1:250 dilution) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), ERK (1:1000 dilution) (New England BioLabs), TH (TH-16; 1:10,000 dilution) (Sigma), Histone-H3 (05-499; 1:500–1000 dilution) (Millipore, Billerica, MA, USA), and AMPKα (2532; 1:1000 dilution) (Cell Signaling Technology) were used to determine the total amount of proteins.

The membrane was incubated with a goat anti-mouse or anti-rabbit Alexa 680-linked IgG (1:5000 dilution) (Molecular Probes, Eugene, OR, USA) or a goat anti-mouse or anti-rabbit IRDye™ 800-linked IgG (1:5000 dilution) (Rockland, Gilbertsville, PA, USA). Fluorescence at infrared wavelengths was detected by the Odyssey infrared imaging system (LI-COR, Lincoln, NE, USA) and quantified using Odyssey software.

2.4. Measurement of locomotor activity

Mice were habituated to activity cages for 60 min. After habituation, mice received pretreatment of either vehicle or resveratrol (40 mg/kg, s.c.). At 30 min after pretreatment, mice received cocaine (10 mg/kg, i.p.) or saline injections, and their locomotor activity was recorded for 90 min. The vehicle for resveratrol contained (final concentration) 5% DMSO + 5% Tween-20 + 5% polyethylene glycol 400 + 85% saline. The number of horizontal and vertical (or rearing) movements was determined as activity counts using an area sensor (NS-AS01; Neuroscience, Tokyo, Japan), and data were stored and analyzed with a computerized system (DAS system; Neuroscience, Tokyo, Japan).

2.5. Statistical analysis

The data were expressed as the mean ± SEM. Data were analyzed with one-way ANOVA followed by the Newman–Keuls test or two-way ANOVA followed by the Bonferroni test. The analyses were performed with Prism 4.0 software (GraphPad, San Diego, CA, USA).