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Cocaine challenge enhances release of neuroprotective amino acid taurine in the striatum of chronic cocaine treated rats: a microdialysis study

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Abstract

Drug addiction is a serious public health problem. There is increasing evidence on the involvement of augmented glutamatergic transmission in cocaine-induced addiction and neurotoxicity. We investigated effects of acute or chronic cocaine administration and cocaine challenge following chronic cocaine exposure on the release of excitotoxic glutamate and neuroprotective taurine in the rat striatum by microdialysis. Cocaine challenge, following withdrawal after repeated cocaine exposure markedly increased the release of glutamate, which may cause neurotoxicity. Simultaneously, cocaine challenge after withdrawal also significantly increased the release of taurine, which counteracts glutamate-mediated excitotoxicity and possibly cell death. Thus, the mammalian brain has an endogenous self-protective mechanism against cocaine-mediated neurotoxicity and potentially addiction.

Keywords

Cocaine; glutamate; taurine; neuroprotection

1. Introduction

Cocaine inhibits high-affinity neurotransmitter uptake at the presynaptic nerve terminals to increase synaptic levels of dopamine, norepinephrine and serotonin [1]. This increase of synaptic dopamine may cause neurotoxicity [2,3]. At least two different mechanisms have been proposed for the development of dopamine-related neurotoxicity: 1) dopamine produces a free radical that may induce cell toxicity [2,3] and 2) dopamine reduces glutamate transport at its presynaptic sites to increase synaptic levels of this amino acid [4] and augments glutamate

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transmission by activating dopamine D1 receptors in different areas of the brain [5,6,7]. Increase in glutamatergic transmission mediated by the activation of N-methyl dextro-aspartate (NMDA) receptors has been shown to cause excitotoxicity and neuro-degeneration [8]. Others and we have reported protection against different psychotropic drug-induced neurotoxicity that may be achieved by prior or simultaneous administration of various pharmacological agents. For example, repeated treatment of rats with haloperidol induced neuronal damage that is ameliorated by prior administration of either G_{M1} ganglioside [9] or the endogenous amino acid, taurine [10]. Similarly, chronic gestational cocaine exposure causes neurotoxicity that could be prevented by co-administration of clozapine [11]. To our knowledge, there is no information if chronic cocaine would enhance release of the endogenous protective agent taurine that may oppose the over activation of glutamatergic system. Here we show that cocaine challenge following repeated cocaine treatment increased synaptic levels of the neuroprotective amino acid taurine that oppose the excessive excitatory actions of the glutamatergic system in the rat brain [10,12]. Thus, mammalian brain has an auto-protective mechanism to counter excitotoxicity and taurine or its synthetic derivative may be useful in the management and treatment of cocaine addiction and its neurotoxic effect.

2. Materials and Methods

The extracellular levels of amino acids were measured by microdialysis followed by high-pressure-liquid-chromatography (HPLC) in striata of male CD rats after acute and chronic administrations of cocaine. Rats were anesthetized with 60 mg/kg intraperitoneal pentobarbital and a microdialysis guide cannula was surgically implanted in the striatum using these coordinates: AP, +0.7 mm; ML, +2.7 mm; DV -6.0 mm [13]. Use of pentobarbital as an anesthetic agent has been shown to decrease dopamine release in striatum and nucleus accumbens [14,15]. Studies in our laboratory have shown that cortico-striatal glutamatergic neuronal firing rates are similar with either pentobarbital or urethane anesthesia [16]. Furthermore, the synaptic level of glutamate in the striatum under pentobarbital anesthesia was found to be similar as compared to the levels seen in the nucleus accumbens of un-anesthetized rats (Fig. 1) and [17]. Microdialysis probes (CMA/12; Bioanalytical Systems, West Lafayette, IN) were inserted into the guide cannulae and the probes were continuously perfused with artificial cerebrospinal fluid at a flow rate of 2 µl/min, and samples were collected every 10 min via a refrigerated fraction collector. Samples were analyzed using HPLC with electrochemical detection. Twenty-four CD rats were divided into four groups consisting of a control group, acute cocaine treated group, chronic cocaine treated group, and chronic cocaine group challenged with a single dose of cocaine. Acute cocaine treated rats received 10 mg/kg of intraperitoneal cocaine and the control group received equal volume of saline 30 min before microdialysis sample collection. Chronic cocaine treated group received 10 mg/kg of intraperitoneal cocaine six days each week for three weeks and were anesthetized for microdialysis 24 hours after the last dose of cocaine. In the cocaine challenge group, previously chronic cocaine treated rats (24 hours after the last dose of chronic cocaine treatment) received a single dose of 10 mg/kg intraperitoneal cocaine 30 min before microdialysis sample collection.

Measurement of amino acids by HPLC: A derivatation solution was prepared by dissolving 27mg o-phthal-aldehyde (OPA) in 1ml methanol (MeOH) to which 5µl beta-mercapto-ethanol and 9ml of 0.1M Na-tetraborate was added. Two mobile phases were used (pH 5.1). Mobile phase A: 20% MeOH and 80% 1M di-sodium hydrogen phosphate. Mobile phase B: 80% MeOH and 20% 1M di-sodium hydrogen phosphate. Gradient solution was: (1) linear addition of B to A over 4 min for a final ration 25% A to 75%B (v/v); (2) step-wise change to 100% mobile phase A for 8 min. The OPA solution (10 µl) was mixed and reacted for 2 min with 20 µl standards or samples and 20 µl of the solution was injected onto a column (ODS, 3×100 mm). Samples were quantified with three external standards (1, 10, and 100 pmol). Goodness

of fit for all standards was 0.97–0.99. Typical retention times were: Glu 3.2 min; Gln 3.8 min; Gly 5.9 min; Tau 9.4 min. The lower limit of sensitivity was at least 1 pmol/sample.

All animal procedures were in compliance with the Animal Welfare Act, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the City University of New York (CUNY) institutional guidelines and were approved by the CCNY Animal Care and Use Committee.

3. Results

The effect of acute cocaine treatment on extracellular levels of glutamate and taurine are shown in Figure 1. Although, there were small decreases in extracellular striatal efflux of glutamate and taurine in the acute cocaine treated rats compared to control group, these differences did not reach statistical significance. This observation regarding extracellular concentrations of glutamate following acute cocaine administration is in agreement with Miguénes et al. who reported similar results in the nucleus accumbens after 1 mg/kg intravenous cocaine treatment [17]. Other investigators found higher concentrations of extracellular glutamate in the nucleus accumbens after a relatively high dose of 30 mg/kg intraperitoneal cocaine treatment [18]. Previously, a dose of 15 mg/kg of cocaine was shown to release somatodendritic dopamine that stimulated dopamine D₁ receptors that in turn increased release of glutamate in the ventral tegmental area [19]. In contrast, it appears that lower doses of cocaine do not increase the levels of dopamine to a sufficient degree to activate release of glutamate to the synaptic cleft in the striatum (Fig. 1).

Our microdialysis experiments show that basal extracellular release of striatal glutamate in rats receiving chronic cocaine decreased significantly compared to the control group (Fig. 2). Interestingly, in the chronic cocaine treated group, the extracellular efflux of taurine increased by about 38% compared to the control group; but this difference did not reach statistical significance (Fig. 2). The mechanism for the reduction of extracellular glutamate following repeated cocaine administration may be due to a decrease in the activity of cysteine/glutamate exchange that was reported in the nucleus accumbens following long-term neuroadaptation induced by chronic cocaine exposure [20].

When our chronically cocaine treated group was challenged by an intraperitoneal administration of 10 mg/kg cocaine, a significant increase in extracellular concentrations of both glutamate and taurine were noted (Fig. 3). There are two possible mechanisms that may be involved in the dramatic increase of the extracellular release of glutamate after cocaine challenge following chronic cocaine treatment. First, repeated cocaine exposure may increase the number of dopamine D₁ receptors that could enhance glutamate release [19]. Such observation would suggest that chronic cocaine might, at least in part, cause neurotoxicity mediated by the release of glutamate. Second, repeated cocaine administration may desensitize group II metabotropic glutamate receptors in several brain areas [21,22] and subsequently enhance glutamate release following cocaine challenge after chronic cocaine treatment and withdrawal (Fig. 3). The mechanism for the release of taurine following cocaine challenge is not known. Excessive exposure to another neurotoxin (i.e. ammonia) may increase mRNA levels of taurine transporter [29]. This, however, does not explain how cocaine challenge increases extracellular taurine release.

4. Discussion

Sulfur-containing amino acids, such as taurine and homocysteine exhibit opposite effects in neuronal cells. The former is a neuroprotective agent [12] while the latter is an independent risk factor for cognitive dysfunction [30]. There are no previous reports on the release of

endogenous taurine or homocysteine in the extracellular fluid of striatum after acute or chronic cocaine treatment. There are some reports on effect of treatment with various drugs on the release or levels of taurine. Acute administration of D-cycloserine has been shown to increase extracellular concentration of D-serine without significant effect on taurine in the medial frontal cortex [31]. Moreover, repeated morphine exposure significantly reduced the endogenous level of hippocampal taurine [32]. Taurine has been shown to be a neuroprotective agent in numerous investigations and several mechanisms for its neuroprotective effects have been proposed. Most of the studies have suggested that the taurine-induced neuroprotective effect may be mediated by its antagonism of the glutamate-induced excitotoxicity. For example, it has been proposed that taurine may reduce glutamate-induced cell death by augmentation of mitochondrial function and the regulation of intracellular (cytoplasmic and intramitochondrial) calcium homeostasis [12]. In another study, Chen et al. [33] showed that taurine exerts its neuroprotective effect by a reduction of glutamate-induced elevation of intracellular Ca^{2+} by inhibiting the reverse mode of $\text{Na}^+/\text{Ca}^{2+}$ exchange in cultured neurons. Also, taurine was reported to inhibit glutamate-mediated calcium influx through L-, P/Q-, N-type voltage gated calcium channels and NMDA receptor calcium channel in whole brain primary neuronal cell cultures obtained from rat embryos [34]. Since taurine was reported to hyperpolarize neurons in the cerebellum [35] and hippocampus [36] by activating chloride channel, it appears likely that taurine may inhibit glutamate-induced neuronal depolarization through its action on opening the chloride channels. Taurine was also shown to decrease D- $[\text{}^3\text{H}]$ aspartate (a non-metabolized analog of glutamate) release from mouse corticostriatal slices by the activation of a chloride channel that is insensitive to regulation by GABA and/or strychnine-sensitive glycine receptors [23]. Moreover, acamprosate (calcium acetylhomotaurine), a synthetic analog of taurine has been reported to reduce NMDA receptor activation either through partial agonistic activity at the spermidine site or through its action at the metabotropic glutamate receptors [24]. Thus, multitude of mechanisms may be involved in the diminution of glutamate-induced excitotoxicity by taurine making it a good neuroprotective agent.

Although, taurine and its synthetic analog acamprosate are known to be neuroprotective agents that are used in the treatment of drug addiction [24,25,26] no previous investigation has demonstrated spontaneous release of taurine in the mammalian brain by substances of abuse. We report spontaneous increased release of taurine following cocaine challenge after chronic, but not acute cocaine treatment in striatum (Figs. 1 and 3). The amount of taurine released following acute administration of cocaine was not significantly different from control. This may be related to the dose of the drug or the duration between drug administration and collection of samples for microdialysis. Nevertheless, the results of our study suggest that the mammalian brain has a unique ability to counteract insult to neuronal tissue caused by glutamate in response to chronic exposure to substances of abuse by releasing taurine. Despite a substantial release of taurine following cocaine challenge (Fig. 3), chronic cocaine ingestion may cause neurotoxicity in the mammalian brain. For example pre- and post-natal exposure to cocaine has been reported to induce neurotoxicity [11]. In addition, cocaine addiction has been shown to cause dysregulation of prefrontal cortex-accumbens synaptic glutamate transmission that underlies the high motivation to seek drugs [37]. Clearly, released taurine is not sufficient to completely neutralize glutamate-induced excitotoxicity in all cases. Consequently, excessive intake of substances of abuse would lead to the development of drug addiction despite mammalian brains' effort to mitigate these adverse effects by the release of endogenous taurine. Nonetheless, high pharmacological doses of taurine and/or acamprosate have been found to be of therapeutic value in neuronal protection and in the management of cocaine, alcohol, and morphine addiction [24,27,28]. Future studies may determine if prior intake of pharmacological dosages of taurine or acamprosate would be of preventive value in mitigating neurotoxicity and addictive sequel to exposure to drugs of abuse.

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Acute Cocaine Effect

Fig. 1. Extracellular release of glutamate and taurine in striatum after acute cocaine treatment 30-minutes before the microdialysis assay rats were injected intraperitoneally with 10mg/kg cocaine or an equal volume of saline (controls). There were no significant changes in synaptic levels of glutamate (A) or taurine (B) by the Two-tailed *t*-test: (A) $t=0.004904$ $df=7$; $P>0.05$; (B) $t=0.9954$ $df=7$; $P>0.05$. Error bars, s.e.m.; $n=8$ in A and B.

Basal Amino Acids at Synapse

Fig. 2. Extracellular release of glutamate and taurine in striatum after chronic cocaine treatment Two groups of rats were injected intraperitoneally with 10mg/kg cocaine or equal volume of saline (controls) six-days per week for three-weeks. Microdialysis was initiated 24-hours after last injection of cocaine. **(A)** Basal level of glutamate in the cocaine group was significantly decreased compared to controls (Two-tailed *t*-test: $t=5.588$ $df=6$; $**P<0.05$). **(B)** Basal taurine levels were increased by 37% compared to controls, however, this change was not statistically significant (Two-tailed *t*-test: $t=1.370$ $df=6$; $P>0.05$). Error bars, s.e.m.; $n=7$ in **A** and **B**.

Effect of Cocaine Challenge

Fig. 3. Extracellular release of glutamate and taurine in striatum of chronic cocaine-treated rats measured after a cocaine “challenge”

Rats received 10 mg/kg cocaine (i.p.) for six days per week for three weeks. 24 hours after the last chronic cocaine dose, rats were challenged with 10 mg/kg cocaine (i.p.) or an equal volume of saline (i.p.) 30 minutes before microdialysis. There was a significant increase in both extracellular glutamate (A) (Two-tailed t -test: $t=2.456$ $df=6$; $*P<0.05$) and taurine (B) (Two-tailed t -test: $t=4.469$ $df=6$; $**P<0.05$). Error bars, s.e.m.; $n=7$ in **A** and **B**.