Chronic cocaine enhances release of neuroprotective amino acid taurine: a microdialysis study

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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 transmission by activating dopamine D_1 receptors in different areas of the brain[5-7]. Increase in glutamatergic transmission mediated by the activation on 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 endogenous protective agents that may oppose the over activation of glutamatergic system. Here we show that repeated cocaine treatment increased synaptic levels of the neuroprotective amino acid taurine that opposes the excessive excitatory actions of the glutamatergic system in the rat brain. 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.

The effect of acute cocaine treatment on extracellular levels of glutamate and taurine are shown in Figure 1. Although, there were small decreases in interstitial striatal levels 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 Miguenes et al. who reported similar results after 1 mg/kg intravenous cocaine treatment in the nucleus accumbens[12]. Other investigators found higher concentrations of extracellular glutamate in the nucleus accumbens after a relatively high dose of 30 mg/kg intrperitoneal cocaine treatment[13]. Previously, a dose of 15 mg/kg of systemic cocaine was shown to release somatodendritic dopamine that stimulated dopamine D_1 receptors that in turn increased release of glutamate in the ventral tagmental area [14]. 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 in the synaptic cleft in the striatum (Fig. 1). Our microdialysis experiments show that basal synaptic levels 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 synaptic level 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[15]. 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 synaptic levels of glutamate after chronic cocaine treatment. First, repeated cocaine exposure may increase the number of dopamine D_1 receptors that could enhance glutamate release[14]. 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[16, 17] and subsequently enhance glutamate release (Fig. 3).

There are no previous reports on the release of endogenous taurine in the synaptic cleft after either acute or chronic cocaine treatment. Taurine has been shown to be a neuroprotective agent in numerous investigations[10] and several mechanisms for its neuroprotective effects have been proposed. Most of the studies have suggested antagonism of the glutamate-induced excitotoxicity. For example, it has been proposed that taurine may reduce glutamate-induced cell death by augmentation of mitotochondrial function and the regulation of intracellular (cytoplasmic and intramitochondrial) calcium homeostasis[18]. Taurine was also shown to decrease D-[^{3}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[19]. 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[20]. 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[20, 23, 24], no previous investigation has demonstrated spontaneous release of taurine in the mammalian brain by substances of abuse. We report spontaneous release of taurine after chronic, but not acute cocaine treatment (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 substances of abuse. 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, 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[20-22]. Future studies may determine if prior intake of taurine or acamprosate would be of preventive value in mitigating neurotoxicity and addictive sequela to exposure to drugs of abuse.

The extracellular levels of glutamate and taurine 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[25]. 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. 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.

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Figure Legends

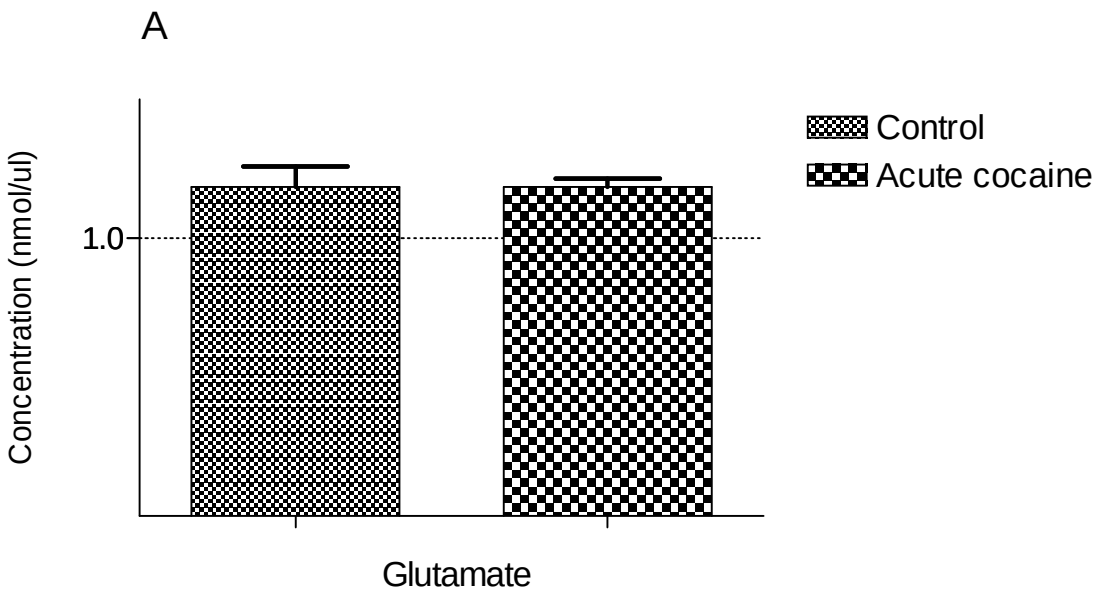
Fig. 1. Extracellular release of glutamate and taurine in striatum after acute cocaine treatment. 30-minutes before the microdialysis assay rats were injected intraperitoneally with 10mg/kg cocaine or an equal volume of saline (controls). There were no significant changes in synaptic levels of glutamate (**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**.

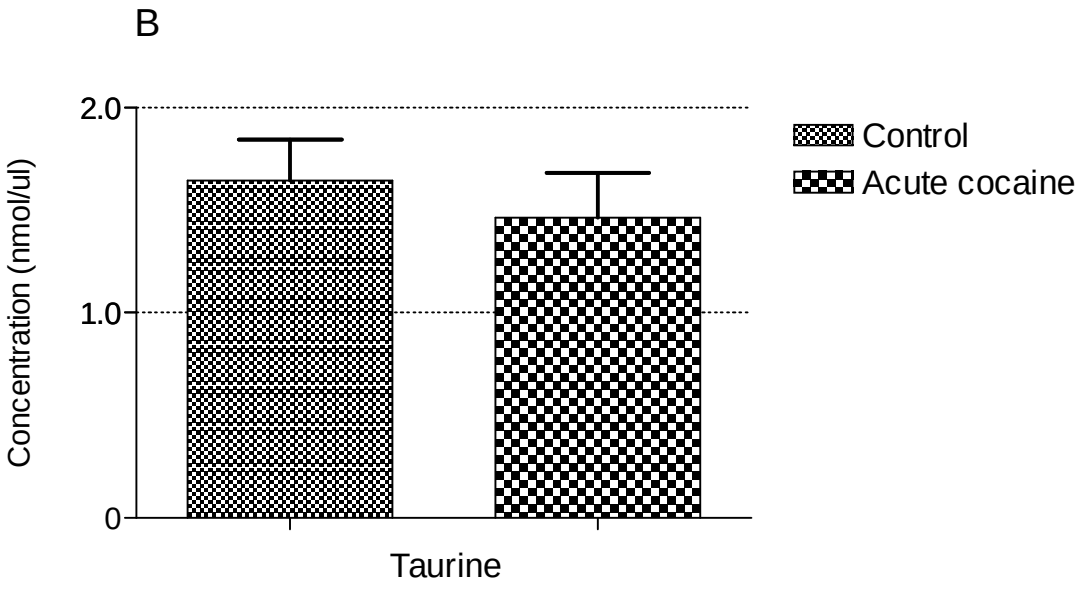
Fig. 2. Extracellular release of glutamate and taurine in striatum after chronic cocaine treatment. Two groups of rats were injected intraperitoneally with 10mg/kg cocaine or equal volume of saline (controls) six-days per week for three-weeks. Microdialysis was initiated 24-hours after last injection 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**.

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 coaine (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

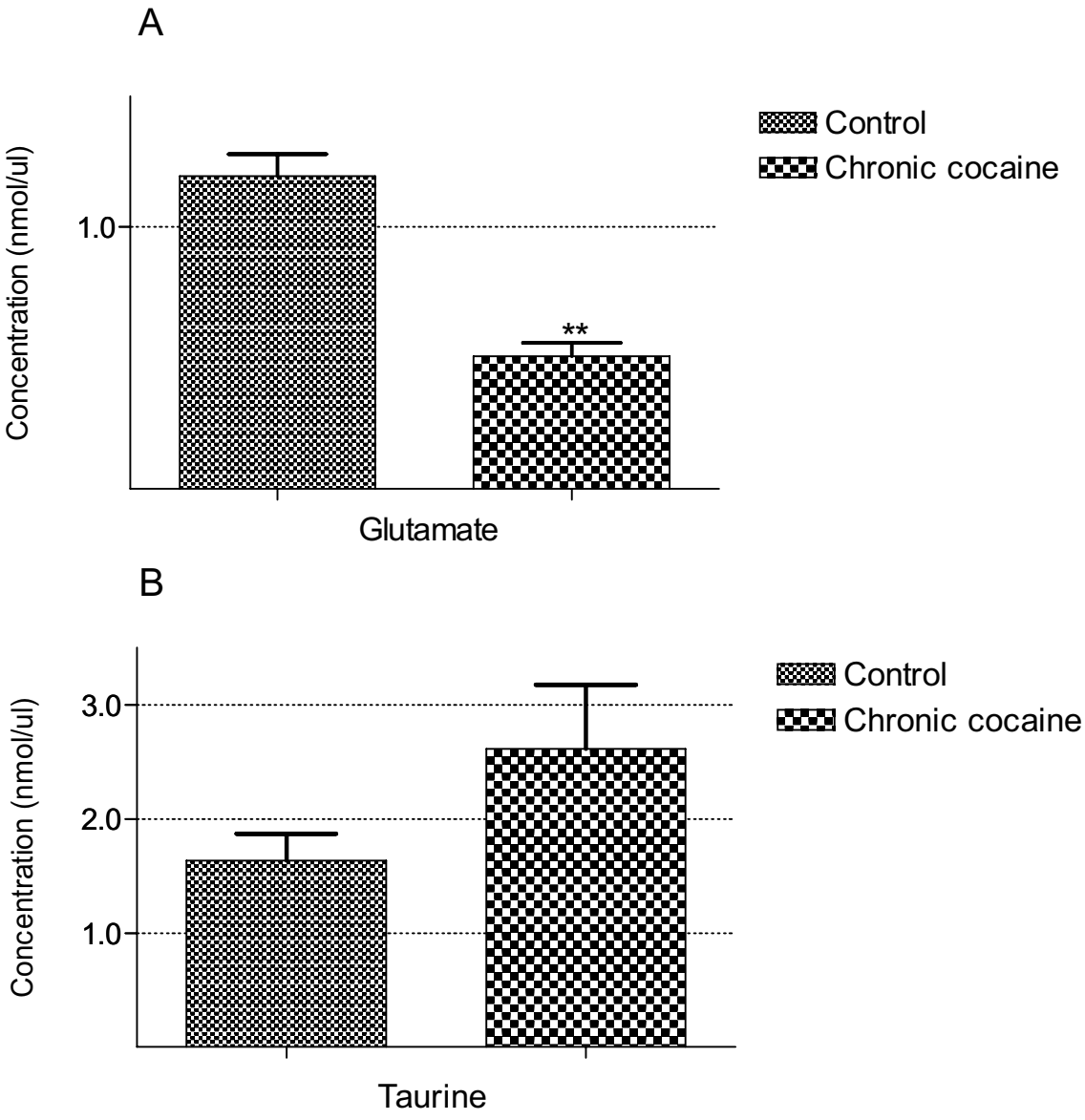
glutamate (**A**) (Two-tailed ***t*-test:** t=2.456 df=6; *P<0.05) and taurine (**B**) (Two-tailed ***t*-test:** t=4.469 df=6; **P<0.05). Error bars, s.e.m.; n=7 in **A** and **B**.

Acute Cocaine Effect





Basal Amino Acids at Synapse



Effect of Cocaine Challenge