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Short communication

Modulation of extracellular neurotransmitter levels in the nucleus accumbens by a taurine uptake inhibitor

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Abstract

Using in vivo microdialysis, we examined the effect of local perfusion of the taurine uptake inhibitor guanidinoethyl sulfonate on extracellular levels of various neurotransmitters in the rat nucleus accumbens. Guanidinoethyl sulfonate (500 μM–50 mM) produced a concentration-dependent increase in extracellular taurine levels. While 500 μM and 5 mM concentrations of guanidinoethyl sulfonate were largely without effect, 50 mM guanidinoethyl sulfonate produced a significant decrease in extracellular levels of aspartate, glutamate and glycine, with no effect on extracellular dopamine levels. These results indicate that guanidinoethyl sulfonate can modulate extracellular amino acid levels in the nucleus accumbens.

Keywords: Amino acid; Dopamine; Guanidinoethyl sulfonate; Microdialysis; (Rat)

1. Introduction

The endogenous sulfonated β-amino acid taurine has numerous functions in the nervous system, including osmoregulation and neuromodulation. Taurine has potent inhibitory properties and can hyperpolarize neuronal membranes by increasing chloride influx, most likely via allosteric activation of $GABA_A$ or glycine receptor ion channels (Huxtable, 1989). As a result of these inhibitory actions, taurine has been shown to modulate the release of numerous classes of neurotransmitters, including amino acids and catecholamines (Huxtable, 1989; Oja and Saransaari, 1996).

Guanidinoethyl sulfonate is a taurine analogue that competitively antagonizes membrane taurine transporters (Huxtable et al., 1979; Huxtable, 1989). This ligand allows the possibility of pharmacologically manipulating brain extracellular levels of taurine. However, very few studies have examined the effects of guanidinoethyl sulfonate levels on the release of other neurotransmitters in vivo. The aim of the present study was to examine the effects of local perfusion of guanidinoethyl sulfonate on extracellular levels of dopamine and various amino acid neurotransmitters in the nucleus accumbens of the freely moving rat.

2. Materials and methods

Male Long Evans hooded rats ($n = 7$, 250–350 g, Charles River Laboratories, Wilmington, MA) were used. Animals were housed individually in a temperature-controlled facility under a 12:12 light–dark cycle with ad libitum access to food and water throughout all procedures. Extreme care was taken to minimize pain and discomfort to animals throughout all procedures. All experiments were carried out in accordance with institutional and National Institute of Health guidelines.

Animals were anesthesized and surgically implanted with guide cannula according to previously described methods (Olive and Maidment, 1998). Guide cannula (0.90 mm OD × 15 mm length, Plastics One, Roanoke, VA) were aimed at the nucleus accumbens (coordinates AP +1.7 mm, ML +1.0 mm, DV −5.0 mm from bregma) according to the atlas of Paxinos and Watson (1986). Coordinates were calculated so that the membrane of the microdialysis probe would extend into both the core and shell regions of the nucleus accumbens. The wound was closed with 3-0 silk, and topical 2% bacitracin and 5% xylocaine were applied to minimize post-surgical infection

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and discomfort. Animals were then allowed to recover for 5 days while individually housed in cylindrical plexiglass cages. During recovery, animals were inspected daily for general health and well-being. Following recovery, animals were lightly re-anesthesized with 2% halothane and implanted with microdialysis probes constructed from 24-gauge stainless steel thin-wall tubing and equipped with 2 mm cuprophane membranes (208 μm OD, Baxter Healthcare, McGaw Park, IL; final depth −8.0 mm from skull surface). Probes were perfused with artificial cerebrospinal fluid (aCSF) consisting of 125 mM NaCl, 2.5 mM KCl, 0.5 mM $NaH_2PO_4 \cdot H_2O$, 5 mM Na_2HPO_4, 1 mM $MgCl_2 \cdot 6H_2O$, 5 mM D-glucose, 1.2 mM $CaCl_2 \cdot 2H_2O$, pH = 7.2-7.5, at a rate of 0.5 μl/min using a syringe pump (Harvard Apparatus, Holliston, MA). Probes were then stabilized with dental cement and attached to dual channel liquid swivels (Instech Laboratories, Plymouth Meeting, PA) for freely moving microdialysis procedures as described elsewhere (Olive and Maidment, 1998).

Approximately 16 h following probe implantation, the aCSF flow rate was increased to 2.5 μl/min. Following a 30-min re-equilibration period, a 60-min baseline collection period commenced. The taurine uptake inhibitor guanidinoethyl sulfonate (Toronto Research Chemicals, North York, Ontario, Canada) was administered locally through the microdialysis probe via inclusion into the perfusion medium (i.e., reverse microdialysis) at concentrations of 500 μM, 5 mM and 50 mM for 40 min at each concentration. Microdialysis samples were collected every 10 min into polypropylene tubes in a refrigerated fraction collector (SciPro, North Tonawanda, USA). Samples were then split into two equal aliquots for separate high performance liquid chromatography (HPLC) analysis of dopamine and amino acid neurotransmitters (aspartate, glutamate, glycine and taurine) as described elsewhere (Olive et al., 2000). Two aliquots of aCSF as well as aCSF containing each concentration of guanidinoethyl sulfonate were also analyzed by HPLC as control blanks. The values of any resulting peaks in the chromatogram observed in aCSF or drug blanks were subtracted from the final neurotransmitter concentration. The absolute level of each neurotransmitter in the six baseline collection period samples was averaged and, subsequently, all neurotransmitter levels were converted to a percentage of their respective basal levels. Data points were individually compared against baseline using a one-way repeated measures analysis of variance (ANOVA) followed by a Tukey's post-hoc test.

Following microdialysis procedures, rats were deeply anesthesized with Nembutal (75 mg/kg i.p.) and perfused transcardially with 250 ml of 4% paraformaldehyde. Brains were then postfixed overnight at 4°C, cryoprotected in 30% sucrose for 48 h at 4°C, cut into 40 μm coronal sections on a cryostat (Leica, Deerfield, IL), mounted onto gelatin-coated slides, and stained with Cresyl violet (see Olive et al., 2000 for details). Verification of probe placement in the nucleus accumbens core and shell regions was performed under light microscopy.

In an attempt to determine the amount of guanidinoethyl sulfonate that diffused from the probe into the extracellular fluid, we performed an in vitro efflux assay. Two microdialysis probes (2 mm cuprophane membrane) were perfused with 50 mM (8.36 μg/μl) guanidinoethyl sulfonate in aCSF at 2.5 μl/min while being immersed in polypropylene microcentrifuge tubes containing 25 μl of drug-free aCSF. The aCSF-containing tubes in which the probes were immersed were changed every 10 min for 40 min to simulate the drug perfusion studies performed in awake animals. Following the in vitro perfusion, the aCSF in which the probes were immersed as well as the 50 mM drug-containing perfusate were then assayed for guanidinoethyl sulfonate content using HPLC with electrochemical detection (methodology identical to that described for amino acid detection in Olive et al. (2000) except that 8.36 and 4.18 μg/μl calibration standards were used for quantitation of aCSF guanidinoethyl sulfonate content). Drug-free aCSF blanks were assayed as controls.

3. Results

3.1. Effects of local perfusion of guanidinoethyl sulfonate on extracellular taurine levels in the nucleus accumbens

Local perfusion of the nucleus accumbens with guanidinoethyl sulfonate produced a concentration-dependent increase in extracellular levels of taurine. The lowest concentration tested (500 μM) was without effect. The 5 mM concentration of guanidinoethyl sulfonate produced a significant increase in extracellular levels of taurine (maximum 203 ± 39% of baseline, $P < 0.05$) during administration of the drug, which declined to basal levels following

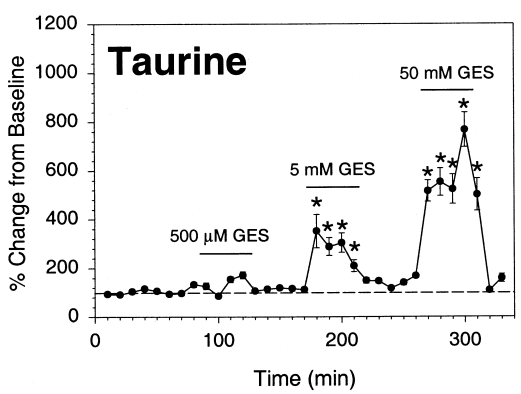

Fig. 1. Concentration-dependent increase in extracellular levels of taurine in the rat nucleus accumbens ($n = 7$) produced by local perfusion of the taurine uptake inhibitor guanidinoethyl sulfonate by reverse microdialysis. Drug perfusion is indicated by horizontal bar at stated concentrations. Data are expressed as a percent of baseline taurine levels (i.e., the absolute amount of taurine per sample during the first six time points), and represent mean ± S.E.M. * Indicates significantly different from baseline levels ($P < 0.05$).

washout (Fig. 1). Local perfusion of 50 mM guanidinoethyl sulfonate resulted in an even larger increase in extracellular levels of taurine (maximum 667 ± 71% of baseline, $P < 0.05$) during administration of the drug, which declined to basal levels following washout (Fig. 1).

3.2. Effects of local perfusion of guanidinoethyl sulfonate on extracellular dopamine and amino acid levels in the nucleus accumbens

The lowest concentration of guanidinoethyl sulfonate (500 μM) was without effect on extracellular levels of dopamine, aspartate, glutamate and glycine (Fig. 2a–d) in the nucleus accumbens. The 5 mM concentration of guanidinoethyl sulfonate also was without effect on extracellular levels of these neurotransmitters, except with regards to asparate, which showed a small decrease (39 ± 5% of baseline, $P < 0.05$) at only one time point during drug perfusion (Fig. 2b). While the highest concentration (50 mM) of guanidinoethyl sulfonate produced no effect on extracellular dopamine levels in the nucleus accumbens (Fig. 2a), this concentration did produce a decrease in extracellular levels of aspartate (maximum 49 ± 6% of baseline, $P < 0.05$, Fig 2b), glutamate (maximum 60 ± 5% of baseline, $P < 0.05$, Fig. 2c), and glycine (maximum 54 ± 4% of baseline, $P < 0.05$, Fig. 2d) during drug perfusion. All drug-induced changes in extracellular neurotransmitter levels returned to basal levels following removal of the drug.

3.3. In vitro estimation of guanidinoethyl sulfonate diffusion into the extracellular space

When 50 mM (8.36 μg/μl) guanidinoethyl sulfonate was perfused through microdialysis probes, it was found that a mean concentration of 0.50 ± 0.03 μg/μl (range 0.25–0.67 μg/μl) of guanidinoethyl sulfonate diffused into the aCSF in which the probe was immersed per 10-min sample. Thus, the amount of guanidinoethyl sulfonate that diffused out of the probe during each microdialysis sample was approximately 6.19% of the drug perfusate concentration (i.e., 3.1 mM when 50 mM was perfused).

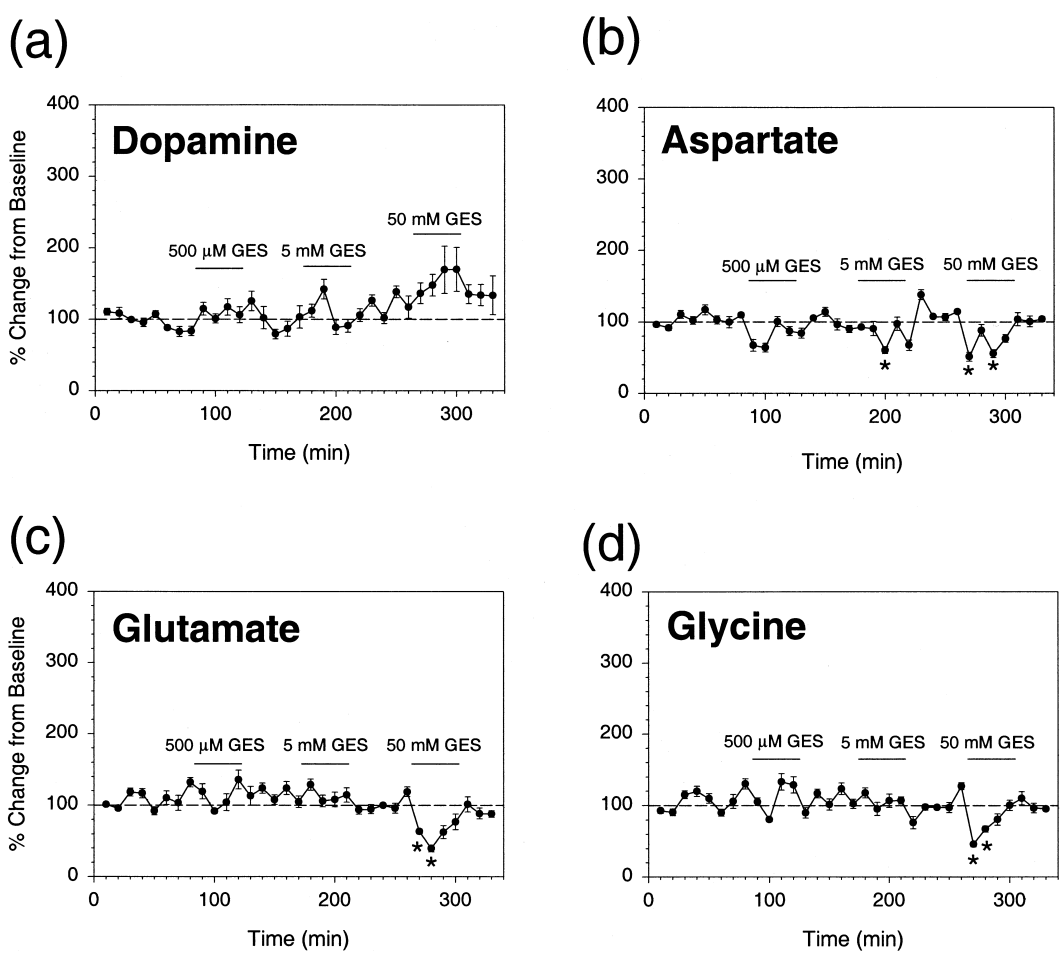

Fig. 2. Alterations in extracellular levels of (a) dopamine, (b) aspartate, (c) glutamate and (d) glycine in the rat nucleus accumbens ($n = 7$) produced by local perfusion of the taurine uptake inhibitor guanidinoethyl sulfonate by reverse microdialysis. Drug perfusion is indicated by horizontal bar at stated concentrations. Data are expressed as a percent of baseline levels (i.e., the absolute amount of neurotransmitter per sample during the first six time points), and represent mean ± S.E.M. * Indicates significantly different from baseline levels ($P < 0.05$).

4. Discussion

As expected, we found that local perfusion of the taurine uptake inhibitor guanidinoethyl sulfonate into the nucleus accumbens of rats produced concentration-dependent increases in extracellular taurine levels. These changes were accompanied by decrease in extracellular amino acid levels (i.e., aspartate, glutamate and glycine) at the highest dose tested (i.e., 50 mM). These observations are consistent with previous reports that taurine and related compounds can inhibit ionotropic glutamate receptor-mediated responses in neurons (Kurachi et al., 1983) and can inhibit ethanol withdrawal-associated increases in extracellular levels of glutamate in the nucleus accumbens (Dahchour and De Witte, 2000). The ability of guanidinoethyl sulfonate or increased taurine levels to decrease extracellular levels of excitatory amino acids may also be an important mechanism in the neuroprotective actions of taurine (Oja and Saransaari, 1996).

We found that guanidinoethyl sulfonate produced no changes in extracellular levels of dopamine in the nucleus accumbens. Thus, taurine-related mechanisms do not appear to influence basal release of dopamine in this region. While other investigators have found that local perfusion of taurine and related compounds can modulate extracellular dopamine levels in the dorsal striatum (Ruotsalainen and Ahtee, 1996; Ruotsalainen et al., 1996, 1998), these effects may reflect functional neurochemical differences between the nucleus accumbens and the dorsal striatum with regards to taurinergic regulation of basal dopamine release.

It is currently difficult to determine whether the observed effects of guanidinoethyl sulfonate on nucleus accumbens amino acid neurotransmission were due to elevations in extracellular taurine levels or by actions of the drug independent of extracellular taurine levels. Indeed, guanidinoethyl sulfonate alone has been demonstrated to possess pharmacological activity at $GABA_A$ receptors (Herranz et al., 1990; Mellor et al., 2000). Experiments comparing the effects observed in the present study with those examining the effects of exogenously administered taurine on amino acid transmission in the nucleus accumbens are necessary to address this issue. Alternatively, the development of selective taurine transporter inhibitors that are devoid of intrinsic biological activity would provide more useful pharmacological methods for investigating the neuromodulatory properties of taurine. Nonetheless, given the role of the nucleus accumbens in various neurobehavioral pathologies such as drug addiction and schizophrenia, further investigations into taurinergic modulation of neurotransmission in this region are clearly warranted.

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