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The Metabotropic Glutamate Receptor 7 (mGluR₇) Allosteric Agonist AMN082 Modulates Nucleus Accumbens GABA and Glutamate, but not Dopamine, in Rats

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Abstract

The group III metabotropic glutamate receptor 7 (mGluR₇) has been implicated in many neurological and psychiatric diseases, including drug addiction. However, it is unclear whether and how mGluR₇ modulates nucleus accumbens (NAc) dopamine (DA), *L*-glutamate or γ -aminobutyric acid (GABA), important neurotransmitters believed to be involved in such neuropsychiatric diseases. In the present study, we found that systemic or intra-NAc administration of the mGluR₇ allosteric agonist N,N'-dibenzylhydrazide-ethane-1,2-diamine dihydrochloride (AMN082) dose-dependently lowered NAc extracellular GABA and increased extracellular glutamate, but had no effect on extracellular DA levels. Such effects were blocked by (R,S)- α -methylserine-O-phosphate (MSOP), a group III mGluR antagonist. Intra-NAc perfusion of tetrodotoxin (TTX) blocked the AMN082-induced increases in glutamate, but failed to block the AMN082-induced reduction in GABA, suggesting vesicular glutamate and non-vesicular GABA origins for these effects. In addition, blockade of NAc GABA_B receptors by 2-hydroxy-saclofen itself elevated NAc extracellular glutamate. Intra-NAc perfusion of 2-hydroxy-saclofen not only abolished the enhanced extracellular glutamate normally produced by AMN082, but also decreased extracellular glutamate in a TTX-resistant manner. We interpret these findings to suggest that the increase in glutamate is secondary to the decrease in GABA, which overcomes mGluR₇ activation-induced inhibition of non-vesicular glutamate release. In contrast to its modulatory effect on GABA and glutamate, the mGluR₇ receptor does not appear to modulate NAc DA release.

Keywords

mGluR₇; AMN082; dopamine; GABA; glutamate; nucleus accumbens

L-glutamate is the major excitatory neurotransmitter in the central nervous system (CNS), and its functions are mediated by both ionotropic and metabotropic glutamate receptors. Metabotropic glutamate receptors (mGluRs) are divided into three groups: group I (mGluR₁ and mGluR₅), group II (mGluR₂ and mGluR₃) and group III (mGluR₄, mGluR₆, mGluR₇ and mGluR₈), based upon sequence homology, intracellular signal transduction mechanisms and pharmacological properties (Cartmell and Schoepp, 2000; for review).

The mGluRs, especially the group I and group II mGluRs, have recently become attractive therapeutic targets for drug development for the treatment of CNS diseases, including drug abuse (Spooren et al., 2003; Marek, 2004; Alexander and Godwin, 2006; Witkin et al., 2007),

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while group III mGluRs are the least studied because of lack of selective pharmacological tools. The recent development of N,N'-dibenzhydryl-ethane-1,2-diamine dihydrochloride (AMN082), a selective systemically active mGluR₇ allosteric agonist (Mitsukawa et al., 2005), has made neuropharmacological and neurochemical studies of mGluR₇ functions in the CNS feasible. *In vitro* cAMP and GTPγS binding assays in recombinant cells expressing mGluR₇ demonstrate that AMN082 is a potent (EC₅₀ 64-290 nM) and selective (at least 30-150-fold) allosteric agonist of mGluR₇ over other mGluR subtypes and some iGluRs (EC₅₀ >10 μM) (Mitsukawa et al., 2005). It was recently reported that systemic administration of AMN082 inhibits acquisition of Pavlovian fear conditioning (Fendt et al., 2007), susceptibility to seizures (Sansig et al., 2001), animal analogs of anxiety and depression (Callaerts-Vegh et al., 2006; Palucha et al., 2007), and both cocaine self-administration and reinstatement of drug-seeking behavior (Li et al., 2006). However, the cellular mechanisms underlying such actions are poorly understood.

Given the important role of brain DA, *l*-glutamate and GABA transmission in many neuropsychiatric diseases (Spooren et al., 2003; Marek, 2004; Wise, 2005; Alexander and Godwin, 2006; Witkin et al., 2007), studies of the possible modulation of such neurotransmission by mGluR₇ seem called for. Previous studies have shown that local perfusion of *l*-2-amino-4-phospho-nobutyrate (L-AP4), a non-selective group III mGluR (mGluR₄, mGluR₆, mGluR₇ and mGluR₈) agonist, *lowered* extracellular DA, glutamate, and GABA levels in many brain regions, including the NAc (Anwyl 1999; Hu et al., 1999; Panatier et al., 2004; Xi et al., 2003; Kogo et al., 2004; Cartmell and Schoepp, 2000 for review). In contrast, other reports indicated that activation of group III mGluRs by L-AP4 *increased* glutamatergic transmission in entorhinal cortex (Evans et al., 2000) and periaqueductal grey (Marabese et al., 2005). These data suggest that glutamate transmission in different brain loci may be modulated differently by group III mGluRs.

Anatomic evidence demonstrates that mGluR₇ has the highest CNS density of all group III mGluR subtypes (Shigemoto et al., 1997; Bradley et al., 1998; Corti et al., 1998; Kinoshita et al., 1998). The most intense expression is seen in the olfactory bulb, hippocampus, dorsal striatum and NAc (Ferraguti and Shigemoto, 2006). Further, immunohistochemical and electromicroscopic studies demonstrate that mGluR₇ is located predominantly on presynaptic terminals of glutamatergic and GABAergic neurons (Shigemoto et al., 1996; Bradley et al., 1996; Dalezios, 2002; Somogyi, 2003; Cartmell and Schoepp, 2000 for review). These data suggest that mGluR₇ may play an important role in modulating glutamate and GABA release in those brain regions. However, research on this topic has been hampered by lack of selective mGluR₇ agonists or antagonists. Given the important role of the NAc in emotion, motivation, brain reward, and drug addiction (Wise, 2005), we investigated the effects of AMN082, a selective mGluR₇ allosteric agonist (Mitsukawa et al., 2005), on extracellular DA, *l*-glutamate and GABA in the NAc by *in vivo* brain microdialysis. The research was undertaken in the hope that information on the role of AMN082 on NAc DA, *l*-glutamate and GABA may shed light on the potential utility of AMN082 in the treatment of neuropsychiatric diseases and/or drug addiction.

MATERIALS AND METHODS

Animals

Male Long-Evans rats (Charles River Laboratories, Raleigh, NC) weighing 250 to 300 g were used. They were housed individually in a climate-controlled animal room on a reversed light-dark cycle with free access to food and water. The animals were maintained in a facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals of the U.S. National Academy of Sciences* (National

Research Council, Commission on Life Sciences, Institute of Laboratory Animal Resources) and were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse of the U.S. National Institutes of Health.

Surgery

All rats were bilaterally implanted with intracranial guide cannulae (20 gauge, 14 mm; Plastics One, Roanoke, VA) into the NAc for *in vivo* microdialysis. The surgery was performed under sodium pentobarbital anesthesia (65 mg/kg i.p.) with standard aseptic surgical and stereotaxic technique. The stereotaxic coordinates for the NAc were +1.7 mm anterior to Bregma, ± 2.0 mm mediolateral, -4.0 mm ventral to the skull surface according to the atlas of Paxinos and Watson (1986) using an approach angle of 6° from vertical. The guide cannulae were fixed to the skull with 4 stainless steel skull screws (Small Parts Inc., Miami Lakes, FL) and dental acrylic.

In vivo Microdialysis

In vivo microdialysis was performed in freely moving animals 5-7 days after intracranial surgery. Dialysis probes were constructed as described before (Xi et al., 2003). The active region of the dialysis membrane was 1.0 mm in length, approximately 100 μ m in diameter. Probes were inserted into the NAc at least 12 hours before the experiments to minimize damage-induced DA, glutamate or GABA release during the experiment. On the day of the experiment, dialysis buffer (5 mM glucose, 2.5 mM KCl, 140 mM NaCl, 1.4 mM CaCl₂, 1.2 mM MgCl₂, 0.15% phosphate buffered saline, pH 7.4) was perfused through the probe (2.0 μ l/min) via syringe pump (Bioanalytical Systems, Inc. West Lafayette, IN) beginning at least 2 hr prior to sample collection. Dialysis samples were collected every 20 min into 10 μ l 0.5 M perchloric acid to prevent degradation of neurotransmitters. After 1 hr of baseline collection, various drugs were administered systemically or locally infused into the NAc by reverse microdialysis. After collection, all samples were frozen at -80°C until analyzed.

Quantification of DA

Dialysate DA was measured with the ESA electrochemical detection system (ESA Inc., Chelmsford, MA). The DA mobile phase contained 4.76 mM citric acid, 150 mM Na₂HPO₄, 3 mM sodium dodecyl sulfate, 50 mM EDTA, 10% methanol, and 15% acetonitrile, pH 5.6. DA was separated using an ESA MD-150 $\times$ 3.2 mm reverse phase column and oxidized/reduced using an ESA Coulochem III detector. Three electrodes were used: a pre-injection port guard cell (+0.25 V) to oxidize the mobile phase, an oxidation analytical electrode (E1, -0.1 V), and a reduction analytical electrode (E2, 0.2 V). The area under curve (AUC) of the DA peak was measured using an "EZChrom Elite" ESA chromatography data system. DA values were quantified with an external standard curve (1-1000 fM).

Quantification of Glutamate and GABA

Concentrations of glutamate and GABA in the dialysis samples were determined using high pressure liquid chromatography with fluorometric detection. The mobile phase consisted of 18% acetonitrile (v/v), 100 mM Na₂HPO₄, 0.1 mM EDTA, pH 6.04. A reverse-phase column (RP-18, 10 cm $\times$ 3 μ m ODS, Bioanalytical systems Inc., West Lafayette, IN) was used to separate the amino acids, and precolumn derivatization of amino acids with *o*-phthalaldehyde was performed using an ESA Model 542 autosampler (ESA Inc. Chelmsford, MA). Glutamate and GABA were detected by a fluorescence spectrophotometer (LC 530, from ESA Inc.). Two sets of different excitation wavelengths (Ex λ) and emission wavelengths (Em λ) were used to simultaneously measure glutamate (Ex λ , 314 nm; Em λ , 394 nm) and GABA (Ex λ , 336 nm; Em λ , 420 nm) levels from the same samples. The AUCs of the glutamate and GABA peaks were measured using an "EZChrom Elite" ESA chromatography data system. Glutamate and

GABA values were quantified with external standard curves (glutamate: 0.1-5 μ M; GABA: 0.01-1 μ M). The limits of detection for glutamate and GABA were 1-2 pM and 0.1-1 pM, respectively.

Drugs

AMN082 [N,N'-dibenzylhydrazyl-ethane-1,2-diamine dihydrochloride], MSOP [(R,S)- α -methylserine-o-phosphate], tetrodotoxin (TTX) and 2-hydroxy-saclofen were purchased from Tocris Bioscience (Ellisville, MO). For intraperitoneal (i.p.) injection, AMN082 was dissolved in 0.5% Tween-80 (Sigma-RBI, St. Louis, MO). The i.p. injection volumes of AMN082 solution were between 0.5-1.0 ml. For local NAc perfusion, all drugs were dissolved in the dialysis buffer described above.

Histology

Following microdialysis, rats were euthanized by pentobarbital overdose (>100mg/kg i.p.) and perfused transcardially with 0.9% saline followed by 10% formalin. Brains were removed and placed in 10% formalin for 1 week. The tissue was blocked around the NAc and coronal sections (100 μ m thick) made by vibratome. The sections were stained with cresyl violet and examined by light microscopy. Animals with cannula locations outside the NAc were excluded from data analysis.

Data analyses

Drug-induced changes in extracellular DA, glutamate or GABA levels are calculated and expressed as % changes of the mean values of 3 baselines immediately before drug administration. All data are presented as means $\pm$ S.E.M.. Two-way (treatment vs. time) analysis of variance (ANOVA) with repeated measures over time was used to analyze data reflecting time courses of neurochemical changes after drug administration (Figures 1, 2, 3, 4, 5). Post-ANOVA pre-planned individual group comparisons were carried out using the Bonferroni statistical procedure.

RESULTS

Effects of systemic administration of AMN082 on extracellular DA, GABA and glutamate in the NAc

Basal levels of NAc extracellular DA (0.37 ± 0.05 nM), *l*-glutamate (0.77 ± 0.11 μ M) and GABA (138.92 ± 15.75 nM) did not differ significantly among the different groups of rats. Systemic administration of AMN082 (3, 10, 20 mg/kg i.p.) failed to alter extracellular DA levels in the NAc (Figure 1A). Two-way ANOVA with repeated measurements over time revealed statistically a significant difference only in the time main effect ($F_{17,425}=2.39$, $p<0.01$), but not in the treatment main effect ($F_{3,25}=0.89$, $p>0.05$), or in the treatment $\times$ time interaction ($F_{51,425}=1.28$, $p>0.05$). In contrast, the same doses of AMN082 dose-dependently decreased extracellular GABA (Figure 1B) and increased extracellular glutamate levels in the NAc (Figure 1C). At the highest concentration (20 mg/kg, i.p.), AMN082 produced an approximate 50% reduction in extracellular GABA and an approximate 250% increase in extracellular glutamate in the NAc at 5 hr after AMN082 administration. Two-way ANOVA with repeated measurements over time for the data shown in Figure 1B revealed a statistically significant treatment main effect ($F_{3,22}=7.72$, $p<0.001$), time main effect ($F_{17,374}=6.65$, $p<0.001$), and treatment $\times$ time interaction ($F_{51,374}=1.75$, $p<0.001$). Individual group comparisons using the Bonferroni test revealed statistically significant decreases in GABA after administration of AMN082 at 10 mg/kg or 20 mg/kg, but not after 3 mg/kg (see significance levels for specific individual inter-group comparisons indicated on Figure 1B). Similarly, two-way ANOVA with repeated measurements over time for the glutamate data

shown in Figure 1C revealed a statistically significant treatment main effect ($F_{3,22}=3.29$, $p<0.05$), time main effect ($F_{17,374}=12.67$, $p<0.001$), and treatment $\times$ time interaction ($F_{51, 374}=2.69$, $p<0.001$). Individual group comparisons using the Bonferroni test revealed statistically significant increases in glutamate after 10 mg/kg, 20 mg/kg, but not 3 mg/kg AMN082 administration (see significance levels for specific individual inter-group comparisons indicated on Figure 1C).

Effects of local perfusion of AMN082 into the NAc on extracellular DA, GABA and glutamate

To determine whether the effects observed above were mediated by action on mGluR₇ receptors located in the NAc, different concentrations of AMN082 (1-100 μ M) were locally infused into the NAc. Consistent with the effects after systemic administration, local perfusion of AMN082 failed to alter NAc DA (Figure 2A), but significantly decreased extracellular GABA (Figure 2B) and increased extracellular glutamate (Figure 2C) in a concentration-dependent manner. Two-way ANOVA with repeated measurements over increasing concentrations of locally infused AMN082 for the DA data (Figure 2A) revealed no statistically significant treatment main effect ($F_{1,11}=0.76$, $p>0.05$). On the other hand, two-way ANOVA for the GABA data, shown in Figure 2B, revealed a statistically significant treatment main effect ($F_{1,9}=7.48$, $p<0.05$), time main effect ($F_{11,99}=2.4$, $p<0.05$), and treatment $\times$ time interaction ($F_{11,99}=2.54$, $p<0.01$). Post-ANOVA individual group comparisons using the Bonferroni test revealed statistically significant decreases in GABA after 10 μ M or 100 μ M (but not 1 μ M) AMN082, when compared with baseline before AMN082 administration. Two-way ANOVA with repeated measurements over time for the glutamate data, shown in Figure 2C, revealed a statistically significant main effect ($F_{1,11}=2.86$, $p<0.05$), time main effect ($F_{11,121}=1.88$, $p<0.05$), and treatment $\times$ time interaction ($F_{11, 121}=2.34$, $p<0.05$). Individual group comparisons revealed statistically significant increases in extracellular glutamate after 100 μ M (but not 1 or 10 μ M) AMN082, when compared with baseline before AMN082 administration.

Effects of the group III mGluR antagonist MSOP on AMN082-induced changes in extracellular GABA and glutamate

To determine whether the AMN082-induced changes in NAc GABA and glutamate are truly mediated by group III mGluR₇ action, we observed the effects of group III mGluR antagonism on AMN082-induced changes in GABA and glutamate. Local perfusion of MSOP (100 μ M) alone did not significantly alter extracellular NAc GABA or glutamate (Figure 3), suggesting the existence of low glutamate tone on NAc group III mGluRs. However, under continuous local perfusion conditions, MSOP completely blocked the 10 mg/kg AMN082-induced decrease in GABA (Figure 3A) and the AMN082-induced increase in glutamate (Figure 3B). Two-way ANOVA with repeated measurements over time for the data shown in Figure 3A revealed a statistically significant treatment main effect ($F_{2,13}=44.49$, $p<0.001$), time main effect ($F_{17,221}=2.79$, $p<0.001$), and treatment $\times$ time interaction ($F_{34,221}=2.70$, $p<0.001$). Two-way ANOVA for the glutamate data shown in Figure 3B revealed a statistically significant time main effect ($F_{17,238}=3.04$, $p<0.001$) and treatment $\times$ time interaction ($F_{34,238}=2.25$, $p<0.001$), but not treatment main effect ($F_{2,14}=2.71$, $p>0.05$). Individual treatment group comparisons using the Bonferroni test revealed that AMN082-induced changes in GABA and glutamate were significantly blocked by MSOP (see significance levels indicated directly on Figures 3A and 3B).

Effects of the GABA_B receptor antagonist 2-hydroxy-saclofen on AMN082-induced increases in glutamate

It is well documented that mGluR₇ is a G_i protein-coupled receptor and that its main effect is inhibition of neuronal activity or neurotransmitter release (Cartmell and Schoepp, 2000). We

hypothesized that AMN082-induced increases in glutamate may be secondary to a reduction in extracellular GABA levels, which leads to a disinhibition of glutamate release via presynaptic GABA_B receptors. To test this hypothesis, the GABA_B receptor antagonist 2-hydroxy-saclofen was locally perfused into the NAc to block NAc GABA_B receptors. We found that local perfusion of 2-hydroxy-saclofen (1-1000 μ M) alone significantly increased extracellular glutamate levels in a concentration-dependent manner (Figure 4A), suggesting the existence of inhibitory GABA tone on NAc glutamate release. Two-way ANOVA for the data shown in Figure 4A revealed a statistically significant treatment main effect ($F_{1,10}=11.32$, $p<0.01$), time main effect ($F_{14,140}=11.02$, $p<0.001$), and treatment $\times$ time interaction ($F_{14,140}=11.28$, $p<0.001$). However, in the presence of continuous local perfusion of 2-OH-saclofen, AMN082 (20 mg/kg i.p.) produced a significant reduction in extracellular glutamate, opposite to the increase observed in the absence of 2-hydroxy-saclofen (Figure 4B). Two-way ANOVA for the data shown in Figure 4B revealed a statistically significant treatment main effect ($F_{1,11}=12.61$, $p<0.01$) and treatment $\times$ time interaction ($F_{17,186}=8.08$, $p<0.001$), but not time main effect ($F_{17,186}=1.35$, $p>0.05$). Post-ANOVA individual group comparisons indicated that AMN082-induced increases or decreases in the absence or presence of 2-hydroxy-saclofen, respectively, were statistically significant when compared with the baseline before AMN082 administration (see significance levels indicated directly on Figures 4A and 4B).

Effects of the voltage-dependent Na⁺-channel blocker TTX on AMN082-induced changes in NAc GABA and glutamate

Finally, to determine vesicular and/or non-vesicular origins, we observed the effects of local perfusion of TTX on AMN082-induced changes in GABA and glutamate. TTX (1 μ M) alone did not significantly alter extracellular glutamate (Figure 5B), but decreased extracellular GABA (~30%, Figure 5A). However, local perfusion of TTX (1 μ M) failed to block AMN082 (10 mg/kg)-induced decreases in NAc GABA (Figure 5A), suggesting non-vesicular origins of the extracellular GABA. Two-way ANOVA with repeated measurements over time for the GABA data shown in Figure 5A revealed a statistically significant time main effect ($F_{17,340}=9.21$, $p<0.001$), but no significant treatment main effect ($F_{2,20}=0.16$, $p>0.05$) and no significant treatment $\times$ time interaction ($F_{34,340}=0.50$, $p>0.05$). In contrast to the effects on NAc GABA, intra-NAc TTX blocked AMN082-induced increases in glutamate observed in the absence of 2-hydroxy-saclofen, but failed to block the AMN082-induced decreases in glutamate observed in the presence of 2-hydroxy-saclofen (Figure 5B). Two-way ANOVA with repeated measurements over time for the glutamate data shown in Figure 5B revealed a statistically significant treatment main effect ($F_{3,18}=4.45$, $p<0.05$) and a significant treatment $\times$ time interaction ($F_{51,306}=3.88$, $p<0.001$). Individual group comparisons using the Bonferroni test revealed that both the AMN082-induced increase or decrease in NAc glutamate in the absence or presence of TTX or TTX plus 2-hydroxy-saclofen, respectively, were statistically significant, when compared with the baselines before AMN082 administration.

Histological examinations

Figure 6A shows a representative microdialysis probe position in rat brain, demonstrating that the active membrane portion of the probe, below the non-membrane (stainless steel) portion, was located in the NAc. Figure 6B shows a depiction of all microdialysis probe locations (active dialysis membranes) in the NAc. The active membranes of the dialysis probes were located within both the core and shell. There were no obvious differences in placements of the dialysis probes across the different experimental groups of rats.

DISCUSSION

The present study, for the first time, demonstrates that systemic or local administration of the selective mGluR₇ agonist AMN082 into the NAc dose-dependently decreases extracellular GABA, increases extracellular glutamate, but has no effect on extracellular DA in the NAc. The group III mGluR antagonist MSOP (Thomas et al., 1996), when locally perfused into the NAc, blocked AMN082-induced changes in NAc GABA and glutamate, suggesting effects mediated by activation of NAc group III mGluR₇ receptors. Further, the increase in NAc glutamate appears to be secondary to a reduction in NAc GABA levels. This is supported by the finding that local perfusion of 2-hydroxy-saclofen, a selective GABA_B receptor antagonist (Kerr et al., 1988), increased extracellular glutamate, while intra-NAc perfusion of 2-hydroxy-saclofen blocked AMN082-induced increases in NAc glutamate. Further, intra-NAc 2-hydroxy-saclofen reversed the action of AMN082, i.e., AMN082 lowered NAc glutamate in the presence of 2-hydroxy-saclofen. The voltage-dependent Na⁺ channel blocker TTX selectively blocked the AMN082-induced increase in glutamate, but not the decrease in GABA or glutamate. These data suggest that the AMN082-enhanced glutamate was derived from action potential-dependent vesicular sources, while the decreased GABA and glutamate (in the presence of 2-hydroxy-saclofen) were derived from action potential-independent non-vesicular sources. Together, these data suggest that mGluR₇ plays an important role in the modulation of NAc GABA and glutamate, but not of DA.

Strikingly, AMN082-induced changes in extracellular GABA and glutamate display a slow-onset and long-acting (at least 5 hr) profile. The underlying mechanisms remain unclear. It might be related to AMN082's binding and action on an allosteric mGluR₇ site and/or AMN082's pharmacokinetic properties. This slow-onset and long-acting property may also explain why AMN082-induced increases (~20%) in NAc glutamate observed during local perfusion (1 hr at each concentration) were much lower than those observed 5 hr after systemic administration (100-250%) (see Figures 1-2). Overall, these findings are consistent with the cellular distributions of mGluR₇ on presynaptic GABA and glutamate, but not DA, terminals in many brain regions (Bradley et al., 1996; Shigemoto et al., 1997; Wada et al., 1998; Kinoshita et al., 1998; Kosinski et al., 1999; Kogo et al., 2004). Moreover, mGluR₇ receptors have been shown to be located exclusively within active presynaptic zones of axon terminals (Shigemoto et al., 1996; Dalezios et al., 2002; Somogyi et al., 2003), suggesting that mGluR₇ may functionally act as an autoreceptor regulating glutamate release, or as a heteroreceptor regulating the release of GABA.

Previous *in vitro* electrophysiological studies have shown that activation of group III mGluRs by L-AP4 produces an inhibitory effect on GABA release in such brain regions as hippocampus (Desai et al., 1994; Gereau and Conn, 1995; Kogo et al., 2004), striatum (Lafon-Cazal et al., 1999), substantia nigra (Wittmann et al., 2001) and cerebral cortex (Schaffhauser et al., 1998). However, the receptor subtype mechanism has remained unclear because L-AP4 is a non-selective group III mGluR agonist. The present study demonstrates that activation of mGluR₇ by the selective agonist AMN082 produces a similar inhibitory effect on NAc GABA release, suggesting that mGluR₇ may be involved in L-AP4's action on striatal GABA release. Further, local perfusion of the group III mGluR antagonist MSOP completely blocked AMN082-induced reduction in NAc GABA levels, supporting an effect mediated by group III mGluR₇s. This is consistent with a recent finding that intra-periaqueductal gray perfusion of MSOP blocked AMN082-induced inhibition of neuronal and behavioral responses to nociceptive stimuli, as well as GABA and glutamate release in the ventromedial medulla (Marabese et al., 2007). We note the reports that MSOP does not block the actions of AMN082 in *in vitro* cell lines that express fragments of mGluR₇ (Mitsukawa et al., 2005; Pelkey et al., 2007). It seems not unlikely that such mGluR₇ fragments may have low sensitivity to MSOP due to loss of receptor integrity or altered receptor configuration.

Two mechanisms are believed to underlie extracellular GABA origins. One is Ca^{++} -dependent vesicular release evoked by depolarization-induced neuronal activation (Haugstad et al., 1992), and another is Ca^{++} -independent non-vesicular GABA release from neuronal and/or glial sources mediated by reversal of GABA transporters (Attwell et al., 1993; Levi and Raiteri, 1993). Further, it has been reported that glutamate may stimulate non-vesicular GABA release by activation of both N-methyl-D-aspartate (NMDA) and non-NMDA receptors (Belhage et al., 1993; Liu et al., 2006). To further explore the origins of the mGluR₇-modulated GABA, we perfused TTX, a voltage-dependent Na^+ channel blocker, locally into the NAc to block action potential propagation and subsequent activation of voltage-dependent Ca^{++} channels. We found that TTX failed to block the AMN082-induced decrease in extracellular GABA levels. These data suggest that mGluR₇-induced reduction in NAc GABA is derived predominantly from non-vesicular origins.

In contrast to the reduction in NAc GABA levels, systemic or local administration of AMN082 increased NAc extracellular glutamate. This is consistent with the finding that L-AP4 increases glutamate release in entorhinal cortex (Evans et al., 2000) and periaqueductal grey (Marabese et al., 2005), but conflicts with other findings that L-AP4 inhibits glutamate release in the NAc (Manzoni et al., 1997; Martin et al., 1997; Xi et al., 2003) and other brain regions (see review by Cartmell and Schoepp, 2000). Given that GABA exerts inhibitory control over NAc glutamate release via presynaptic GABA_B receptors (Takahashi et al., 1998; Lei et al., 2003; Xi et al., 2003; Porter et al., 2004; Sun et al., 2006), we hypothesized that AMN082-induced reduction in extracellular GABA levels may lower GABA tone on GABA_B receptors located on glutamatergic terminals, leading to an increase (i.e., disinhibition) in NAc extracellular glutamate. To test this hypothesis, 2-hydroxy-saclofen, a selective GABA_B receptor antagonist (Kerr et al., 1988), was locally infused into the NAc. We found that such local perfusion significantly increased NAc extracellular glutamate levels in a concentration-dependent manner, suggesting significant GABA tone on glutamate release mediated via presynaptic GABA_B receptors. Moreover, local perfusion of 2-hydroxy-saclofen not only completely blocked AMN082-induced glutamate enhancement, but also reversed AMN082's action, i.e., changed the glutamate enhancement to glutamate inhibition. These findings suggest that, first, AMN082-induced increases in NAc glutamate are mediated by AMN082-induced reduction in NAc GABA levels; and second, AMN082 actually produces two opposite actions on NAc extracellular glutamate, i.e., direct inhibition (observed in the presence of 2-hydroxy-saclofen) and an indirect disinhibition (observed in the absence of 2-hydroxy-saclofen). Thus, the observed increases in glutamate appear to be a final net effect of these two opposite actions resulting from mGluR₇ activation (Figure 7).

Another important finding is that the voltage-dependent Na^+ channel inhibitor TTX selectively blocked AMN082-induced increases in glutamate, but failed to block AMN082-induced reductions in NAc glutamate observed in the presence of 2-hydroxy-saclofen. The first finding suggests that the increased NAc glutamate, mediated by GABA_B receptor disinhibition, is derived from action potential-dependent vesicular glutamate sources. This is congruent with previous reports that GABA_B receptors inhibit vesicular glutamate release predominantly by inhibiting presynaptic calcium influx (Isaacson, 1998; Takahashi et al., 1998). The second finding suggests that mGluR₇ also inhibits action potential-independent, non-vesicular glutamate release. This is consistent with our finding that mGluR₇ activation inhibits non-vesicular GABA release (present study) and a previous finding that activation of group III mGluRs by L-AP4 inhibits non-vesicular glutamate release in the NAc (Xi et al., 2003). In addition, electrophysiological evidence shows that Ca^{++} channel blockers fail to block L-AP4-induced inhibition of excitatory synaptic transmission in the NAc (Manzoni et al., 1997).

Finally, we found that the mGluR₇ agonist AMN082 failed to alter NAc extracellular DA, which appears to conflict with previous reports that activation of group III mGluRs by L-AP4

inhibited DA release in the NAc (Hu et al., 1999) and dorsal striatum (Mao et al., 2000). Given that L-AP4 is a non-selective group III mGluR agonist and has highest binding affinity for mGluR₈ and lowest affinity for mGluR₇, it is likely that such a reduction in DA was mediated by activation of mGluR₈, rather than mGluR₇. However, as we have reported previously, NAc GABA also exerts inhibitory control on presynaptic DA release (Xi et al., 2003). Based on this, AMN082-induced reduction in GABA release should produce an increase in NAc DA, via GABA_B receptor-mediated disinhibition. However, in the present experiments we did not see a significant alteration in NAc DA after AMN082 administration. This is consistent with our recent finding that γ -vinyl GABA, a GABA degradation enzyme inhibitor, significantly elevated NAc extracellular GABA levels, but failed to alter extracellular NAc DA levels (Peng et al., 2007). Clearly, more studies are required to resolve these apparent inconsistencies and to further study whether and how non-vesicular GABA modulates DA release in the NAc.

In conclusion, the present *in vivo* microdialysis study demonstrates that mGluR₇ activation produces an inhibitory effect on non-vesicular GABA and glutamate release in the NAc and an indirect increase in vesicular glutamate release in the NAc via a GABAergic disinhibitory mechanism. Given that mGluR₇-knockout mice display increased seizure susceptibility (Sansig et al., 2001), impaired working memory and reduced anxiety-like behavior (Callaerts-Vegh et al., 2006), better understanding of mGluR₇ involvement in GABA or glutamate synaptic transmission may provide insight into the role of mGluR₇ receptors in neuropsychiatric diseases. Further, NAc GABA and glutamate transmission have been thought to play an important role both in drug reward and relapse to drug-seeking behavior (Kalivas, 2004; Everitt and Robbins, 2005; Xi et al., 2003). Thus, the present findings suggest that novel compounds that target the mGluR₇ may have pharmacotherapeutic potential in the treatment of both neuropsychiatric diseases and drug addiction.

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Figure 1.

Effects of systemic administration of AMN082 on extracellular DA, GABA and glutamate levels in the NAc. AMN082 (3-20 mg/kg, i.p.) had no effect on extracellular DA (Panel A), but dose-dependently decreased extracellular GABA (Panel B) and increased extracellular glutamate (Panel C) in the NAc. * $p < 0.05$, *** $p < 0.001$, compared with the baseline of each treatment group.

Figure 2.

Effects of local administration of AMN082 on extracellular DA, GABA and glutamate levels in the NAc. Local perfusion of AMN082 (1-100 μ M) into the NAc failed to alter extracellular DA levels (Panel A), but dose-dependently lowered extracellular GABA (Panel B) and increased extracellular glutamate (Panel C) in the NAc. * $p < 0.05$, ** $p < 0.01$, compared with the baseline of each treatment group.

Figure 3. Effects of the group III mGluR antagonist MSOP on AMN082-induced changes in NAc GABA and glutamate. Local perfusion of MSOP (100 μ M) into the NAc completely blocked AMN082 (10 mg/kg)-induced decreases in GABA (Panel A) and increases in glutamate (Panel B), while MSOP alone had no effect on NAc GABA and glutamate. * p <0.05, ** p <0.01, *** p <0.001, compared with the baseline of each treatment group.

Figure 4.

Effects of the GABA_B receptor antagonist 2-hydroxy-saclofen (2-OH-saclofen) on AMN082-induced increases in extracellular NAc glutamate. Local perfusion of 2-hydroxy-saclofen (1-1000 μ M) by itself into the NAc significantly increased basal levels of extracellular NAc glutamate (Panel A). However, in the presence of 2-hydroxy-saclofen, AMN082 produced a decrease in extracellular glutamate (Panel B). * p <0.05, ** p <0.01, *** p <0.001, compared with the baseline in each treatment group.

Figure 5.

Effects of the voltage-dependent Na^+ channel blocker tetrodotoxin (TTX) on AMN082-induced changes in NAc GABA and glutamate. Intra-NAc TTX (1 μM) alone significantly lowered extracellular GABA levels (Panel A), but failed to alter extracellular glutamate levels (Panel B). Intra-NAc TTX (1 μM) failed to alter AMN082 (10 mg/kg)-induced decreases in NAc GABA (Panel A) or glutamate in the presence of 2-hydroxy-saclofen (100 μM , Panel B), but blocked AMN082-induced increases in NAc glutamate seen in the absence of 2-hydroxy-saclofen. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, when compared with the baseline in each treatment group.

Figure 6.

Panel A: Photomicrograph of a stained coronal brain section demonstrating the position and extent of a representative guide cannula and representative microdialysis probe, including the non-membrane portion (~1 mm beyond the tip of the guide cannula) and the active membrane portion. Panel B: Schematic reconstructions of positions of active microdialysis membranes within the NAc. Dialysis membranes tended to span the length of the core and shell compartments of the NAc.

Figure 7.

Hypothetical mechanisms of mGluR₇ actions on NAc GABA and glutamate release. mGluR₇ has been shown to be located on glutamatergic and GABAergic terminals in many brain regions, including dorsal striatum and NAc. Activation of mGluR₇ located on GABAergic terminals inhibits non-vesicular GABA release via G_i-protein coupled mechanisms. Such a reduction in extracellular GABA levels lowers GABA tone on GABA_B receptors located on glutamatergic terminals, which leads to disinhibition of (i.e., an increase in) vesicular glutamate release. Similarly, activation of mGluR₇ located on glutamatergic terminals also inhibits non-vesicular glutamate release by a G_i-coupled intracellular mechanism. Thus, the increase in extracellular glutamate observed in the present study appears to be the final net effect of both direct inhibition and indirect disinhibition after mGluR₇ activation by AMN082 administration.