Behavioral/Systems/Cognitive

Neurotensin Activates GABAergic Interneurons in the Prefrontal Cortex

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Converging data suggest a dysfunction of prefrontal cortical GABAergic interneurons in schizophrenia. Morphological and physiological studies indicate that cortical GABA cells are modulated by a variety of afferents. The peptide transmitter neurotensin may be one such modulator of interneurons. In the rat prefrontal cortex (PFC), neurotensin is exclusively localized to dopamine axons and has been suggested to be decreased in schizophrenia. However, the effects of neurotensin on cortical interneurons are poorly understood. We used *in vivo* microdialysis in freely moving rats to assess whether neurotensin regulates PFC GABAergic interneurons. Intra-PFC administration of neurotensin concentration-dependently increased extracellular GABA levels; this effect was impulse dependent, being blocked by treatment with tetrodotoxin. The ability of neurotensin to increase GABA levels in the PFC was also blocked by pretreatment with 2-[1-(7-chloro-4-quinolinyl)-5-(2,6-dimethoxyphenyl)pyrazole-3-yl)carbonylamino]tricyclo(3.3.1.1.^{3,7})decan-2-carboxylic acid (SR48692), a high-affinity neurotensin receptor 1 (NTR1) antagonist. This finding is consistent with our observation that NTR1 was localized to GABAergic interneurons in the PFC, particularly parvalbumin-containing interneurons. Because neurotensin is exclusively localized to dopamine axons in the PFC, we also determined whether neurotensin plays a role in the ability of dopamine agonists to increase extracellular GABA levels. We found that D_2 agonist-elicited increases in PFC GABA levels were blocked by pretreatment with SR48692, consistent with data indicating that D_2 autoreceptor agonists increase neurotensin release from dopamine-neurotensin axons in the PFC. These findings suggest that neurotensin plays an important role in regulating prefrontal cortical interneurons and that it may be useful to consider neurotensin agonists as an adjunct in the treatment of schizophrenia.

Key words: **dopamine; GABA; interneuron; neurotensin; prefrontal cortex; schizophrenia**

Introduction

Alterations in the function of the prefrontal cortex (PFC) are thought to underlie the cognitive deficits and negative symptoms of schizophrenia. Neuropathological studies indicate a dysfunction of cortical GABAergic neurons in schizophrenia. Among the changes observed in postmortem studies of schizophrenia is a decrease in levels of the mRNA encoding GAD_{67}, the GABA synthetic enzyme (Akbarian et al., 1995; Volk et al., 2000; Volk and Lewis, 2002; Hashimoto et al., 2003). Other studies have uncovered a corresponding upregulation of prefrontal cortical $GABA_A$ receptor mRNA and binding (Benes et al., 1996; Ohnuma et al., 1999; Volk and Lewis, 2002) and reduced expression of the GABA transporter GAT-1 (Ohnuma et al., 1999; Pierri et al., 1999; Volk et al., 2001), which may be compensatory responses to decreased GABAergic tone. In addition, changes in the expression of calcium-binding proteins that are localized to different populations of cortical interneurons have been reported (Daviss and Lewis, 1995; Beasley and Reynolds, 1997; Reynolds and Beasley, 2001; Reynolds et al., 2002).

Because of the central role that interneurons play in cortical function and the posited dysfunction of GABAergic cells in schizophrenia, it is important to understand the regulatory mechanisms that govern the activity of GABAergic interneurons. In the rodent, the peptide transmitter neurotensin (NT) is found in a subpopulation of dopamine neurons that project from the ventral tegmental area (VTA) to the PFC (Hokfelt et al., 1984; Seroogy et al., 1987; Studler et al., 1988). There are no neurotensin-containing cell bodies in the rat PFC, and the only source of neurotensin in the PFC is in axons derived from VTA neurons that also contain tyrosine hydroxylase, the dopamine synthetic enzyme (Studler et al., 1988; Febvret et al., 1991). Thus, neurotensin in the rat PFC is exclusively localized to dopamine axons. Because an estimated 39% of synaptic contacts in the PFC that are made by dopamine terminals are with GABA-containing dendrites (Sesack et al., 1995a), neurotensin may be a critical regulator of GABAergic function.

Audinat et al. (1989) reported that neurotensin increases bicuculline-sensitive ($GABA_A$-mediated) IPSPs in PFC pyramidal cells. In addition, we found recently that systemic administration of the neurotensin agonist PD149163 increases Fos expression in prefrontal cortical GABA interneurons (Petrie et al.,

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2004). These studies suggest that neurotensin modulates the activity of GABAergic interneurons, but direct studies are lacking.

We explored the hypothesis that neurotensin activates prefrontal cortical GABA interneurons. Using fluorescent immunohistochemistry, we determined whether the high-affinity neurotensin receptor NTR1 is localized to GABAergic interneurons. We then used *in vivo* microdialysis to assess the effects of intracortical neurotensin administration on extracellular GABA levels. Finally, because neurotensin is colocalized with dopamine in the rat PFC, we determined whether cortical neurotensin release accounts for the paradoxical increase in extracellular GABA levels observed after administration of dopamine D_2-like agonists (Grobin and Deutch, 1998).

Materials and Methods

Subjects. Adult male Sprague Dawley rats weighing 275–345 g (Harlan, Birmingham, AL) were group housed on a 12 h light/dark cycle with lights on at 6:00 A.M. Food and water were available *ad libitum*. All studies were performed in accordance with the National Institutes of Health *Guide for Care and Use of Laboratory Animals* and under the oversight of the Vanderbilt University Animal Care and Use Committee.

NTR1 antibody generation. Rabbits were immunized with keyhole limpit hemocyanin-conjugated synthetic peptides corresponding to the N-terminal region (residues 1–18, 5–21, and 50–69) of NTR1. The antibodies generated were used to probe immunoblots of protein extracts from rat cortex, hypothalamus, liver, and lung. Tissues were homogenized, and P2 pellets were obtained as described previously (Carraway et al., 1993). Proteins were separated by SDS-PAGE on 10% gels. Equal amounts of protein were transferred to polyvinylidene difluoride membranes, and antibody specificity was assessed using immunoblots, as described previously (Carraway et al., 2003).

Immunohistochemistry. Rats were perfused with PBS followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Coronal 40 μm thick sections were cut on a freezing microtome. To determine whether NTR1 is localized to specific types of GABAergic interneurons, a dual immunofluorescence protocol (Fadel and Deutch, 2002) was used to reveal NTR1-like immunoreactivity and one of three calcium-binding proteins that define functionally distinct sets of interneurons (Kawaguchi and Kubota, 1993). Parvalbumin (PV)-, calbindin (CB)-, and calretinin (CR)-containing cells in the PFC together account for the vast majority of interneurons (Gabbott et al., 1997). Control procedures in immunohistochemistry experiments included preadsorption of the NTR1 antibody with the peptide fragment against which the antibody had been generated (10 μg/ml) and omission of the primary antibody. Sections were incubated in a mixture of primary antibodies directed against NTR1 (1:4000) and one of three calcium-binding proteins, including mouse anti-parvalbumin (1:1500; Sigma, St. Louis, MO), mouse anti-calbindin (1:2000; Sigma), and goat anti-calretinin (1:2500; Chemicon, Temecula, CA). Secondary antibodies were cyanine 3 (Cy3)-conjugated donkey anti-rabbit IgG (1:1500; Jackson ImmunoResearch Laboratories, West Grove, PA) and Cy2-conjugated donkey anti-mouse or anti-goat IgG (1:1250; Jackson ImmunoResearch Laboratories).

Sections were examined under epifluorescent illumination to determine the percentage of NTR1-like immunoreactive (-li) neurons that also were immunoreactive for one of the three calcium binding proteins. In each animal, at least 100 PV-, 100 CB-, and 50 CR-li neurons were counted in a "column" of the prelimbic cortex running from the white matter to the pial surface; the percentage of the calcium-binding protein expressing cells that were also immunoreactive for NTR1 was determined.

Surgical procedure. Animals were anesthetized and placed into a stereotaxic frame. A burr hole was drilled over the target area [anteroposterior, +2.8; lateral, +2.1; dorsoventral, −2.3 (Paxinos and Watson, 1986)], and a guide cannula (Bioanalytical Systems, West Lafayette, IN) was inserted into the brain at a 17° angle, just medial and parallel to the white matter of the forceps minor. The guide cannula was secured to the skull with dental acrylic, and ampicillin (150 mg/kg, s.c.; Henry Schein, Melville, NY) was administered prophylactically.

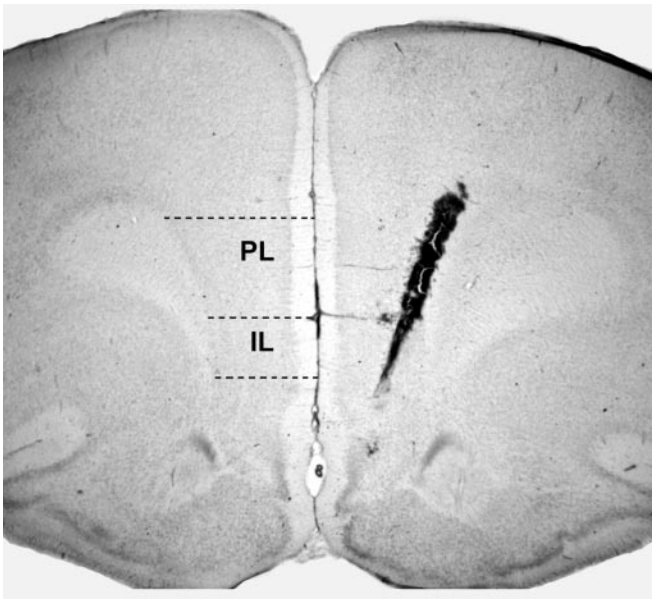

Figure 1. Typical localization of the microdialysis probe in the PFC. In this toluidine blue-stained section, the exchange portion of the probe can be seen in the deep layers of the prelimbic (PL) and infralimbic (IL) PFC.

Microdialysis. Four to six days after surgery, the animals were transferred to dialysis chambers. A microdialysis probe (260 μm outer diameter, with a 3.0 mm exchange length; Bioanalytical Systems) was inserted through the guide cannula. The probe was perfused overnight at a flow rate of 0.2 μl/min with artificial CSF (ACSF) containing (in mM): 1.25 $CaCl_2$, 0.83 $MgCl_2$, 120 NaCl, 20 $NaHCO_3$, 2.2 KCl, 0.5 Na_2SO_4, 0.5 KH_2PO_4, 4.9 D-glucose, and 0.2 ascorbic acid, pH = 6.9 ± 0.1. The next morning, the flow rate was increased to 2.0 μl/min. After a 90 min equilibration period, four baseline perfusate fractions were collected at 20 min intervals; basal extracellular GABA levels, uncorrected for probe recovery, averaged 61.3 ± 3.4 fmol/μl.

Neurotensin or quinpirole was administered through the dialysis probe during the next 20 min fraction; control animals received the ACSF vehicle. In some cases, animals were pretreated with the NTR1 antagonist 2-[1-(7-chloro-4-quinolinyl)-5-(2,6-dimethoxyphenyl)pyrazole-3-yl) carbonylamino]tricyclo(3.3.1.1.^{3,7})decan-2-carboxylic acid (SR48692) or vehicle (DMSO at a final concentration of 0.002% in ACSF) administered through the dialysis probe in the fraction before, during, and after administration of agonist. To control for variability in flow rate associated with syringe switching, the syringes containing ACSF were always changed in parallel for drug- and vehicle control-treated animals. Dialysates were collected for an additional 120–160 min and stored at −80°C until analyzed for amino acid levels by HPLC.

At the end of the dialysis session, the animals were anesthetized and perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed, and serial 100 μm coronal sections through the PFC were cut and stained with toluidine blue. A person unaware of the treatment condition of the animals evaluated the sections for acceptable probe placement, which met the following criteria: the exchange portion of the probe was in the deep layers of the prelimbic and infralimbic PFC and did not cross the midline, penetrate the white matter, or enter the anterior olfactory nucleus (Fig. 1).

Drug treatments. We determined the effects of intra-PFC delivery of neurotensin (100 nM and 1 μM; Sigma) on extracellular GABA levels in the PFC and determined whether NT-evoked changes in extracellular GABA levels were modified by pretreatment with the NTR1 antagonist SR48692 (100 or 500 nM; Sanofi Recherche, Toulouse, France) or its vehicle (DMSO at a final concentration of 0.002% in ACSF). We also determined whether changes in extracellular GABA levels required depolarization of GABA neurons by pretreating with the sodium channel blocker tetrodotoxin (TTX; 1 μM; Sigma).

In an experiment aimed at assessing the role of neurotensin in D_2

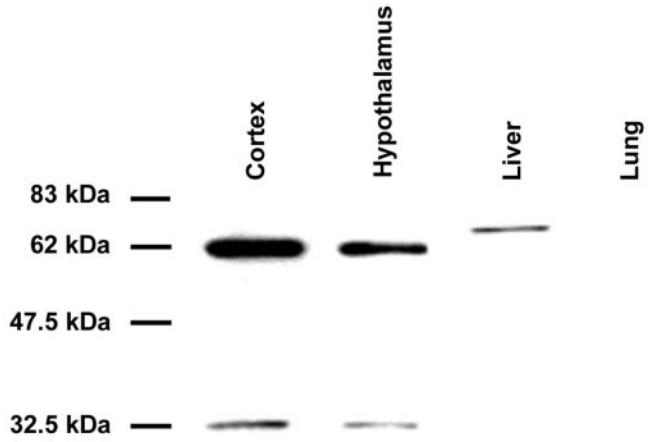

Figure 2. Immunoblot showing expression of NTR1 in rat brain and peripheral sites. Consistent with the known distribution of NTR1, a 60 kDa band representing glycosylated NTR1 is visible in protein samples from rat cortex, hypothalamus, and liver but not lung. The lower-molecular-weight band appears to be an NTR1 fragment.

agonist-evoked increases in extracellular GABA levels in the PFC, animals received either vehicle or quinpirole (100 μM; Sigma) through the dialysis probe; the active drug concentration was based on previous data from our laboratory indicating that this concentration of the D_2 agonist reliably increases prefrontal cortical extracellular GABA levels (Grobin and Deutch, 1998). Some quinpirole-infused animals were pretreated with the NTR1 antagonist SR48692.

SR48692 binding affinities for dopamine receptors. To determine whether SR48692 had any affinity for dopamine receptors, the NTR1 antagonists was screened against human D_1, $D_{2\ long}$, D_3, D_4, and D_5 receptors by the National Institute of Mental Health Psychoactive Drug Screening Program, as described previously (Shapiro et al., 2003) (a full description of assay conditions can be found at http://kidb.cwru.edu/nimh/binding.php).

HPLC. The levels of amino acids in the dialysates were determined using reverse-phase HPLC with electrochemical and fluorescent detection. GABA was added to dialysis samples as an internal standard. Samples were derivitized using *o*-pthalaldehyde and loaded into an autosampler for injection onto a 1.5 μm C18 column (Alltech Associates, Deerfield, IL). The mobile phase was 100 mM sodium phosphate buffer containing 10% methanol, pH 3.70, and the flow rate was set at 1.2 ml/min with the column temperature maintained at 40°C. The glutamate and GABA derivitization products were detected with an RF-10Axl fluorescence detector (Shimadzu, Kyoto, Japan) and an electrochemical detector (ESA, Chelmford, MA) placed in series.

Statistical analysis. Mean baseline levels of GABA and glutamate were determined by averaging the amino acid level in each of the four baseline fractions. If any baseline sample from an animal varied by >30% of the mean, it was eliminated; data from animals with less than three baseline samples were not included in the analysis. Data were analyzed using two-factor (time × treatment) ANOVA with repeated measures on the time factor. If a significant interaction was detected, Bonferroni *post hoc* tests were used to determine the source of the variation.

Results

Expression of NTR1 in the PFC

Extracts of rat brain membranes probed with the NTR1 antibody showed a major band at 60 kDa (Fig. 2), with a minor band present at ~33 kDa. The larger band represents glycosylated NTR1, whereas the 33 kDa band appears to be a neurotensin receptor fragment (Boudin et al., 1995). When large amounts of protein from liver and lung extracts were loaded onto the gel, a faint higher-mass band could be seen in liver but not lung samples, consistent with the known distribution of NTR1 (Mendez et al., 1997). Identical blots that were developed with the NTR1 antibody preadsorbed with the peptide antigen showed no specific staining (data not shown).

NTR1-like immunoreactive neurons were widely distributed in the PFC, including the infralimbic, prelimbic, and shoulder cortices. There was a sharp decrease in the density of NTR1-li neurons in the more lateral motor and somatosensory cortex. NTR1-li neurons in the PFC were mainly encountered in the deep layers, with fewer NTR1-li neurons present in the superficial layers. NTR1-like immunoreactivity appeared to be concentrated at the perimeter of cortical cells, suggesting that the receptor is predominantly membrane associated (Fig. 3). NTR1 immunoreactivity was not observed in sections incubated in antibody preadsorbed with NTR1 peptide nor in sections in which the primary antibody was omitted (data not shown).

Most prefrontal cortical NTR1-li neurons had a characteristic pyramidal cell morphology. However, smaller nonpyramidal cells also expressed NTR1. These cells were identified as interneurons on the basis of colocalization with calcium-binding proteins (Fig. 3). The majority of PV- and CB-containing interneurons expressed NTR1-like immunoreactivity (86 ± 2 and 74 ± 4%, respectively), whereas expression of NTR1-like immunoreactivity was observed in a minority (41 ± 3%) of CR-containing interneurons.

Effect of neurotensin on extracellular amino acid transmitter levels in the PFC

Intracortical administration of neurotensin increased extracellular GABA levels in a concentration-dependent manner. Administration of 100 nM neurotensin did not significantly increase extracellular GABA levels, although a trend toward an increase was noted, whereas 1 μM neurotensin significantly increased extracellular GABA levels (Fig. 4). Pretreatment with 500 nM SR48692 through the dialysis probe completely blocked the NT-evoked increase in extracellular GABA levels (Fig. 4). Local administration of either 100 nM SR48692 ($n = 3$) or 500 nM SR48692 ($n = 5$) did not change extracellular GABA levels when compared with vehicle (see Fig. 7). Two-way ANOVA (treatment × time) with repeated measures on the time factor revealed a significant time × treatment interaction ($F_{(5,30)} = 2.429$; $p \leq 0.001$) as well as significant time and treatment effects. Similarly, perfusion with the sodium channel blocker TTX blocked NT-evoked increases in GABA levels (Fig. 5). Despite the NT-evoked increase in extracellular GABA levels, neurotensin did not alter extracellular glutamate levels in the cortex (Fig. 6).

Intracortical quinpirole administration caused a sharp increase in extracellular GABA levels (Fig. 7). The effect of the D_2 agonist was temporally specific, being restricted to a single fraction, with GABA levels rapidly returning to baseline.

The ability of quinpirole to evoke an increase in extracellular GABA levels was completely blocked by intracortical delivery of the NTR1 antagonist SR48692 in the fraction before, during, and after administration of quinpirole (Fig. 7). ANOVA revealed a significant time × treatment interaction ($F_{(4,55)} = 6.78$; $p \leq 0.001$) as well as main effects of time and treatment. Subsequent *post hoc* analyses revealed that quinpirole-treated animals had significantly higher GABA levels than all other treatment groups in the first fraction after quinpirole challenge. The effects of SR48692 on D_2-elicited increases in extracellular GABA were not attributable to any direct effects of the NTR1 antagonist on dopamine receptors, because SR48692 did not display any significant affinity ($K_i > 10{,}000$ nM) for dopamine D_1, D_2, D_3, D_4, or D_5 receptors.

Discussion

Neurotensin increases GABA release in the PFC, as reflected by an impulse-dependent increase in extracellular GABA levels. Consistent with the localization of NTR1 to many PFC interneurons, pretreatment with an NTR1 antagonist blocked neurotensin-evoked increases in extracellular GABA levels in the PFC. Interestingly, the ability of a dopamine D_2 agonist to activate GABAergic interneurons was also blocked by SR48692 pretreatment, suggesting that D_2 agonist modulation of GABA neurons in the PFC may involve release of neurotensin from cortical axons that contain both neurotensin and dopamine.

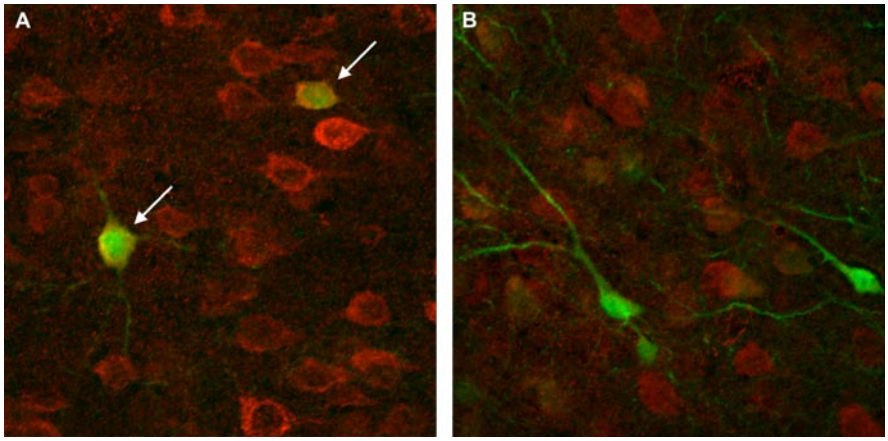

Figure 3. Expression of the high-affinity neurotensin receptor NTR1 in PFC neurons. ***A***, NTR1 (red) is expressed in interneurons that contain the calcium-binding protein parvalbumin (green; arrows) as well as neurons with a pyramidal cell morphology. NTR1 immunoreactivity appears to be concentrated at the perimeter of the cells. ***B***, NTR1 (red) is not expressed in this calbindin-immunoreactive interneurons (green), although many calbindin-immunoreactive cells are NTR1 positive.

Expression of NTR1 on GABA interneurons

Autoradiographic and *in situ* hybridization studies have concluded that PFC neurons express NTR1 (Nicot et al., 1994; Alexander and Leeman, 1998), but the phenotype of NTR1-positive neurons has not been determined (Boudin et al., 1996). We found that pyramidal cells are the major cortical cell type in which NTR1 is expressed but that many interneurons defined on the basis of calcium-binding protein expression also displayed NTR1 immunoreactivity. Almost all cells in the rat PFC that express PV, CR, or CB are interneurons (DeFelipe, 1997; Gabbott et al., 1997; Gonchar and Burkhalter, 1997). Although NTR1 was expressed to varying degrees by all types of interneurons, it was most often localized to PV cells.

Ultrastructural studies indicate that dopamine axons in the PFC form synapses with interneurons (Smiley and Goldman-Rakic, 1993; Sesack et al., 1995b), particularly PV-containing cells (Sesack et al., 1998). The finding that PV-containing interneurons are the major target of dopamine/neurotensin axons is consistent with our finding that NTR1 is mainly expressed by PV interneurons, although we also found that CB- and some CR-li cells express NTR1. Because dopamine axons do not synapse onto cortical CR neurons (Sesack et al., 1995a), the localization of NTR1 to CR neurons suggests that neurotensin effects on CR cells may occur by volume transmission.

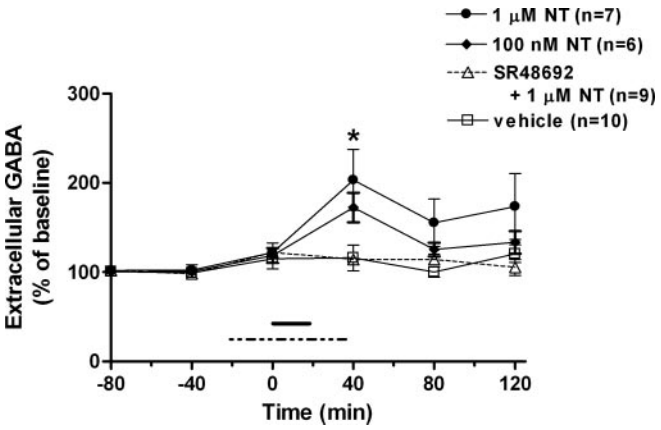

Figure 4. Effect of neurotensin on extracellular GABA levels in the PFC. Data are presented as the mean ± SEM percentage change from baseline. Local infusion of neurotensin (solid bars) caused a dose-related increase in extracellular GABA levels. Local infusion of SR48692 (500 nM; dashed bar) blocked the increase in extracellular GABA evoked by neurotensin. $*p \leq 0.01$ for 1 μM NT-treated animals compared with vehicle- and SR48692-treated animals.

Local administration of neurotensin increases extracellular GABA levels

Neurotensin dose-dependently increased extracellular GABA levels in the PFC. This is consistent with the finding of Audinat et al. (1989) that neurotensin enhances GABA-mediated IPSPs in rat PFC pyramidal cells *in vitro*. Because we administered neurotensin directly into the PFC and found that most interneurons express NTR1, the neurotensin-evoked increase in extracellular GABA probably reflects direct activation of GABAergic interneurons by the peptide. However, we cannot exclude the possibility that other (subcortical) sources of GABA may also contribute to neurotensin-evoked changes in GABA levels.

The neurotensin-elicited increase in extracellular GABA levels in the PFC is consistent with reports indicating that neurotensin increases extracellular GABA levels in subcortical structures, including the striatum (Tanganelli et al., 1994; Ferraro et al., 1997, 1998), globus pallidus (Ferraro et al., 1997), and hippocampus (Rakovska et al., 1998). We found that neurotensin evoked a significant increase in extracellular GABA levels, which was comparable in magnitude to neurotensin-induced changes in GABA levels in subcortical sites. The small magnitude of neurotensin-evoked GABA increases probably reflects metabolism of exogenously administered neurotensin by metalloendopeptidases (Woulfe et al., 1992), which play an important role in regulating neurotensin activity *in vivo* (Vincent et al., 1997a,b; O'Connor, 2001).

The neurotensin-induced increase in cortical extracellular GABA levels was blocked by pretreatment with SR48692. This observation is consistent with our previous data indicating that SR48692 blocked the ability of the NTR1 agonist PD149163 to increase Fos expression in PFC GABA interneurons (Petrie et al., 2004). Together, these data suggest a specific role for NTR1 in regulating GABA interneuron activity. Although all three neurotensin receptor subtypes are expressed in the cortex (Alexander and Leeman, 1998; Sarret et al., 2003a,b), neurotensin acts as an NTR2 antagonist in heterologous expression systems (Vita et al., 1998; Yamada et al., 1998), and NTR3 is primarily localized to intracellular compartments (Mazella et al., 1998; Sarret et al., 2003a), suggesting that NT-evoked increases in extracellular

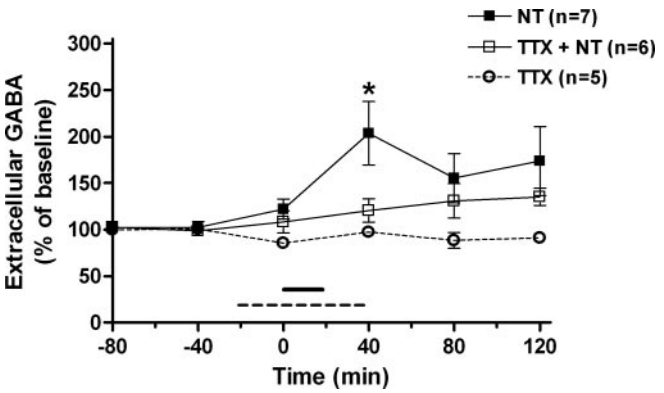

Figure 5. Effect of the sodium channel blocker tetrodoxin on NT-elicited increases in extracellular GABA levels in the PFC. Intracortical TTX (1 μM through the dialysis probe; dashed line) completely blocked the increase in extracellular GABA elicited by 1 μM NT (solid line). *$p \leq 0.05$ for NT-treated animals compared with all other treatment groups. Error bars represent SEM.

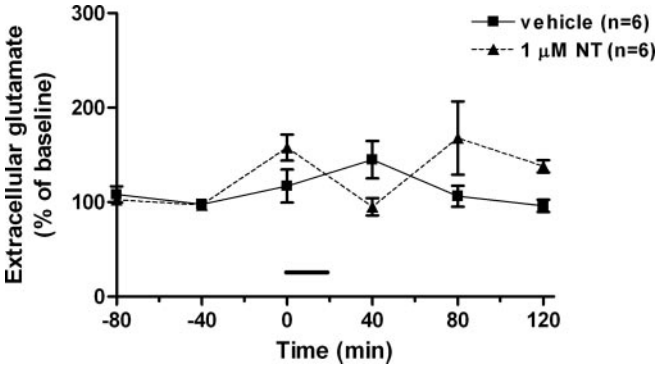

Figure 6. Effect of neurotensin on extracellular glutamate levels in the PFC. Local infusion of neurotensin (solid line) did not significantly change extracellular glutamate levels compared with vehicle-treated animals. Error bars represent SEM.

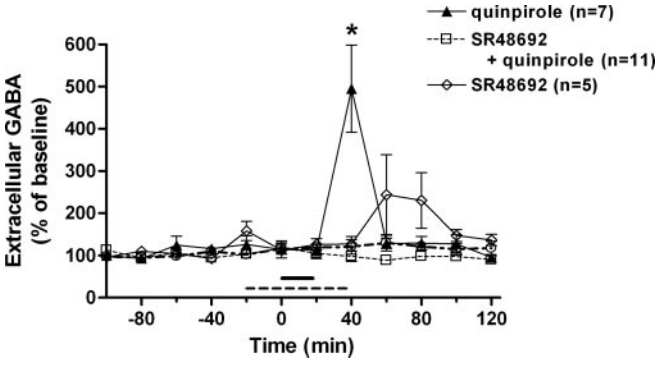

Figure 7. Effect of quinpirole on extracellular GABA levels in the PFC. Data are presented as the mean ± SEM percentage change from baseline. Local infusion of quinpirole (100 μM through the dialysis probe; solid bar) significantly increased extracellular GABA levels compared with vehicle-treated animals and to baseline values. Local infusion of SR48692 (500 nM; dashed bar) completely blocked quinpirole-elicited increases in extracellular GABA but had no significant effect when administered alone. *$p \leq 0.01$ for quinpirole-treated animals compared with all other treatment groups.

GABA levels are mediated by NTR1 but not NTR2 or NTR3 receptors.

NTR1 is localized to pyramidal cells as well as GABAergic interneurons. The axon collaterals of pyramidal cells synapse onto and functionally regulate interneurons (DeFelipe and Farinas, 1992; Staiger et al., 1996; Buhl et al., 1997), and recent reports suggest that glutamate agonists increase extracellular GABA in the rat PFC (Del Arco and Mora, 2000, 2002). It is therefore possible that neurotensin-induced increases in extracellular GABA levels are indirect, initiated by activation of NTR1 on pyramidal cells. However, we failed to detect a consistent increase in extracellular glutamate levels after local neurotensin administration, suggesting that neurotensin-evoked increases in extracellular GABA are not secondary to local changes in glutamate release. Because dialysis lacks the temporal resolution to define transient changes in glutamate, which is quickly cleared by glutamate transporters, we cannot rule out the possibility that a brief increase in glutamate release drives GABA neurons.

Dopamine D_2 agonist-evoked increases in extracellular GABA levels

We previously reported that systemic and local administration of D_2- but not D_1-like dopamine agonists increases extracellular GABA levels in the PFC (Grobin and Deutch, 1998). These data were puzzling because D_2 receptors are typically coupled to inhibitory signal transduction pathways (Missale et al., 1998). Dopamine D_2 receptors are expressed by interneurons and pyramidal cells in the PFC and are present on dopamine axons, where they function as release-modulating autoreceptors (Wolf and Roth, 1987). In a series of studies, Bean et al. (1990) and Bean and Roth (1991) demonstrated that activation of D_2 autoreceptors in the PFC decreases dopamine release but increases release of the colocalized peptide transmitter neurotensin.

Thus, D_2 agonists may alter the activity of PFC neurons that are postsynaptic to dopaminergic axons by promoting neurotensin release. Activation of NTR1 increases intracellular calcium (Hermans et al., 1994), stimulates cyclic nucleotide production (Yamada et al., 1993; Slusher et al., 1994), and promotes phospholipase C activity (Hermans et al., 1992; Watson et al., 1992), consistent with neurotensin-elicited excitatory effects occurring through activation of NTR1. These observations suggest that quinpirole may increase extracellular GABA levels in the PFC indirectly, by promoting neurotensin release from colocalized neurotensin-dopamine axons. The released neurotensin would in turn target NTR1 on GABAergic cells to activate interneurons.

Consistent with our hypothesis that D_2 agonist-evoked increases in extracellular GABA levels in the PFC are secondary to release of neurotensin, we found that SR48692 pretreatment completely blocked quinpirole-evoked increases in PFC GABA levels. However, the NTR1 antagonist alone did not alter GABA levels, suggesting that neurotensin does not play a significant role in regulating basal GABA release. This agrees well with observations that peptide transmitters in colocalized peptide-monoamine neurons are released during high-frequency firing rates or under burst firing conditions, but under basal conditions, peptide release is low.

The SR48692-mediated disruption of D_2-elicited increases in GABA levels is likely attributable to actions at NTR1. The affinity of SR48692 for NTR1 (~3 nM) is two orders of magnitude greater than that observed at NTR2 (Labbe-Jullie et al., 1995; Mazella et al., 1996). Consistent with previous data, we estimate that the concentration of SR48692 delivered across the dialysis membrane is <20% of the 500 nM SR48692 infused through the probe, and thus the effective concentration of SR48692 is consistent with actions at NTR1. As noted previously, the efficient metabolism of exogenous neurotensin by metalloendopeptidases probably accounts for the difference in magnitude between neurotensin- and quinpirole-evoked changes in GABA levels. Moreover, synaptically released neurotensin is more likely to act on postsynaptic

(GABAergic) targets than exogenous peptide administered by reverse dialysis into the extracellular space.

Implications

Neurotensin has been suggested to act as an endogenous antipsychotic drug (APD) (Nemeroff, 1980) based on the similarities of the behavioral effects observed in animals treated with APDs or injected centrally with neurotensin (Kinkead et al., 1999). Several studies have reported that CSF neurotensin levels are reduced in schizophrenic patients, especially those with prominent negative symptoms (Widerlov et al., 1982; Garver et al., 1991), and normalize after APD treatment (Breslin et al., 1994).

Our studies were performed in the rat. The laminar distributions of dopamine- and neurotensin-li axons in the PFC of primate species differ from those seen in the rat (Gaspar et al., 1990), suggesting that neurotensin may not be colocalized with dopamine in humans. However, it is possible that neurotensin may not be detectable in cortical dopamine axons under basal conditions but may be rapidly induced and become apparent after appropriate challenges, as is the case in striatal neurons (Merchant et al., 1991; Deutch and Zahm, 1992). Furthermore, tyrosine hydroxylase and neurotensin mRNAs are colocalized in some human ventral tegmental area neurons, although the projection target(s) of these neurons remains unknown (Bean et al., 1992). Even if neurotensin and dopamine are not colocalized in the primate PFC, activation of D_2 heteroreceptors on neurotensin axons may promote neurotensin release.

Dysfunction of the prefrontal cortex has been suggested to underlie the cognitive deficits and negative symptoms of schizophrenia (Weinberger et al., 1986; Goldman-Rakic and Selemon, 1997; Volk and Lewis, 2002). These features of the illness are relatively resistant to treatment with typical APDs, which are potent D_2 receptor antagonists. If increases in cortical neurotensin contribute to the reduction of negative symptoms and cognitive deficits in schizophrenia, and neurotensin release is promoted by D_2 receptor agonists, this may explain why typical APDs do not treat primary negative symptoms and cognitive deficits effectively. Our data suggest that agents that increase cortical neurotensin may be a useful adjunctive treatment for schizophrenia.

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