ELSEVIER

European Journal of Pharmacology 411 (2001) 129–134

ejp

www.elsevier.nl/locate/ejphar

Short communication

The effects of glutamate receptor agonists on neurotensin release using in vivo microdialysis

James M. Radke, Michael J. Owens, Charles B. Nemeroff *

Laboratory of Neuropsychopharmacology, Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, P.O. Box AF, 1639 Pierce Drive, Atlanta, GA 30322-4990, USA

Received 15 November 2000; accepted 28 November 2000

Abstract

In the present study, extracellular concentrations of neurotensin were measured from the striatum, nucleus accumbens and the medial prefrontal cortex in the awake, freely moving rat. Using a highly sensitive solid phase radioimmunoassay, basal concentrations of neurotensin were 2–5 pg/sample. In each region, glutamate receptor agonists, *N*-methyl-D-aspartate (NMDA) and kainic acid, increased neurotensin release 2–3-fold. Preincubation with the Na^+ channel blocker tetrodotoxin abolished the glutamate receptor agonist-induced increases except in the striatum following kainic acid infusion. These findings indicate that activation of glutamate receptors may indirectly stimulate neurotensin release.

Keywords: NMDA (*N*-methyl-D-aspartate); Kainic acid; Striatum; Nucleus accumbens; Medial prefrontal cortex

1. Introduction

Neurotensin is a 13 amino acid neuropeptide distributed throughout the brain and periphery. Since its isolation (Carraway and Leeman, 1973), neurotensin has been extensively studied with much of the focus on its interaction with the dopaminergic system and its "antipsychotic-like" properties. In dopamine-rich brain regions, (nucleus accumbens, striatum, medial prefrontal cortex), neurotensin is present in a variety of neurons (Jennes et al., 1982; Quiron et al., 1992). Whether neurotensin interacts with neurotransmitter systems, other than dopamine however, remains relatively obscure. In vivo microdialysis has recently been used to examine the dopamine–neurotensin interactions by examining the effects of antipsychotic drugs on neurotensin release (Radke et al., 1998). Because the dopamine pathways and the glutamatergic pathways are two of the major inputs to the basal ganglia, it was of interest to examine the interaction of glutamate and neurotensin in these regions. Previous studies have shown that infusions of neurotensin increase glutamate release in the medial prefrontal cortex (Ferraro et al., 1995), and that repeated administration of the glutamate receptor agonist, *N*-methyl-D-aspartate (NMDA), increases the tissue content of neurotensin (Hanson et al., 1995a,b; Sanz et al., 1993).

One of the more useful and sensitive techniques to examine neurotransmitter interactions is in vivo microdialysis. Microdialysis, in combination with radioimmunoassay, has been used in several studies to measure the extracellular concentration of neurotensin (Bean et al., 1989; During et al., 1992; Radke et al., 1998; Wagstaff et al., 1997). The purpose of the following set of experiments was to examine the effects of activation of glutamatergic receptors on extracellular neurotensin in vivo. Therefore, the effects of NMDA and kainic acid infusions on the extracellular neurotensin in the rat striatum, nucleus accumbens and the medial prefrontal cortex were assessed. Finally, additional experiments were performed using the Na^+ channel antagonist, tetrodotoxin, to determine whether the glutamate receptor agonists were acting directly on neurotensin-containing neurons.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (300–325 g) were used in all experiments. Rats were group-housed (2–3 per

* Corresponding author. Tel.: +1-404-727-5881; fax: +1-404-727-3233.

E-mail address: cnemero@emory.edu (C.B. Nemeroff).

0014-2999/01/$ - see front matter
PII: S0014-2999(00)00912-2

cage) in a temperature-controlled room with a 12 h light–dark cycle. Food and water were provided ad libitum.

2.2. Surgery and microdialysis

Microdialysis probes were conical in design and constructed using a PAN dialysis membrane that was secured to a 26-gauge stainless steel tube with epoxy glue. Input to the probe was through polyethylene 50 tubing attached to the probe and the output was capillary tubing extending from the tip of the membrane, through the polyethylene 50 tubing and ending outside the microdialysis cage where samples could be collected. For probes inserted into the striatum, 4 mm of exposed membrane was used (4.5 mm total length). For probes inserted into the medial prefrontal cortex or the nucleus accumbens, a 2.0 mm length (2.5 mm total) was used. All connections were secured with epoxy and probes flushed with water to ensure no leakage. Prior to surgery, artificial cerebrospinal fluid was perfused through the probe. For the surgery, rats were anaesthetized with a mixture containing ketamine (37.5 mg/kg), xylamine (50 mg/kg) and acepromazine (1.5 mg/kg) and secured to a stereotaxic apparatus using the coordinate of Paxinos and Watson (1982); microdialysis probes were inserted into the striatum (AP +0.2, ML +1.5, DV −7.0 mm), the nucleus accumbens (AP +1.8, ML +1.0, DV −7.0 mm), and medial prefrontal cortex (AP +2.5, ML +0.3, DV −4.0 mm). Probes were secured to the skull with dental cement. Following surgery, rats were placed in CMA microdialysis cages, probes were attached to a pump and the animal was allowed to recover overnight as artificial cerebrospinal fluid was perfused through the probe.

All experiments were performed 1 day following surgery. Studies in our laboratory have shown that this time period allows the animal to fully recover from surgery and it still allows for the collection of extracellular neurotensin from neural tissue. For each experiment, three (basal) samples were collected before any pharmaceutical manipulation began. For rats receiving tetrodotoxin, artificial cerebrospinal fluid with tetrodotoxin (0.1 μmol/l) was perfused from samples 4 through 10, inclusively. All agonists were infused for 10 μl and represented by sample 8. For the striatum, a flowrate of 1.0 μl/min was used (30 μl samples) while the flowrate for the nucleus accumbens and medial prefrontal cortex was 0.5 μl/min (15 μl) due to the lower concentrations of neurotensin. For the latter two sets of samples, 15 μl of artificial cerebrospinal fluid were added for continuity in the radioimmunoassay. All samples were collected and stored at −70°C until assayed for neurotensin immunoreactivity.

2.3. Radioimmunoassay

The solid-phase radioimmunoassay is similar to those used previously by others (Maidment et al., 1991; Wagstaff et al., 1997). Removable 96-well immunoplates (NUNC) were first incubated with protein G (500 ng/ml in 0.1 M $NaCO_3$, pH 9.0) overnight. The following day, the solution was aspirated; the wells washed three times with a 'wash' buffer (0.15 K_2HPO_4 mmol/l, 0.02 M NaH_2PO_4 mmol/l, 0.2 mmol/l ascorbic acid, 0.2% Tween 20, 0.1% sodium azide, pH 7.5). Next, the neurotensin antibody (1:200,000 antirat antisera was a gift from Dr. C.D. Kilts, Emory University) in 25 μl of assay buffer (wash buffer + 0.1% gelatin) was placed in the wells and allowed to incubate for 2–4 h at room temperature. Following aspiration and washings, the wells were incubated with 25 μl of samples or standards and allowed to incubate overnight (4°C). The following day, ^{125}I-[tyr^1]-neurotensin was added (NEN/Dupont; 25 μl, 7000 cpm) and allowed to incubate for 2 h at room temperature. Finally, the wells were aspirated, washed and the wells were individually counted on a gamma counter at 85% efficiency. Using this procedure, neurotensin levels as low as 0.5 pg per well were detectable.

2.4. Data analysis

Neurotensin concentrations were grouped according to experimental group and time and subjected to analysis of variance with repeated measures. Where appropriate, further post hoc analysis was performed using a Student–Newman–Keuls test.

3. Results

3.1. Drug administration

Using an infusion of 10 μl, concentrations of 10^{-5} M for both NMDA and kainic acid were necessary to observe any changes in extracellular neurotensin in the striatum. Preliminary results using 10 μl (total flow) infusions of 10^{-6} M for either NMDA or kainic acid resulted in no significant changes in extracellular neurotensin in the striatum. Preliminary studies utilizing 10^{-4} mol/l kainic acid and NMDA in the caudate-putamen resulted in seizure-like activity in some animals (data not shown). To ensure that we were only assessing the interaction of these drugs on neurotensin release and not confounding effects of behaviors/seizures on neurotensin release, 10^{-5} M was chosen for our studies. Although no quantitative analyses of the rats' behaviors were performed, animals given an infusion of 10^{-5} M exhibited some hyperactivity but showed no signs of discomfort or seizure-like activity associated with high doses of these agonists (Sperk et al., 1986).

3.2. Effects of kainic acid on extracellular neurotensin

As shown in Fig. 1, infusion of kainic acid (10^{-5} M) produced a clear stimulatory effect on extracellular neurotensin in all three regions studied. Analysis of variance

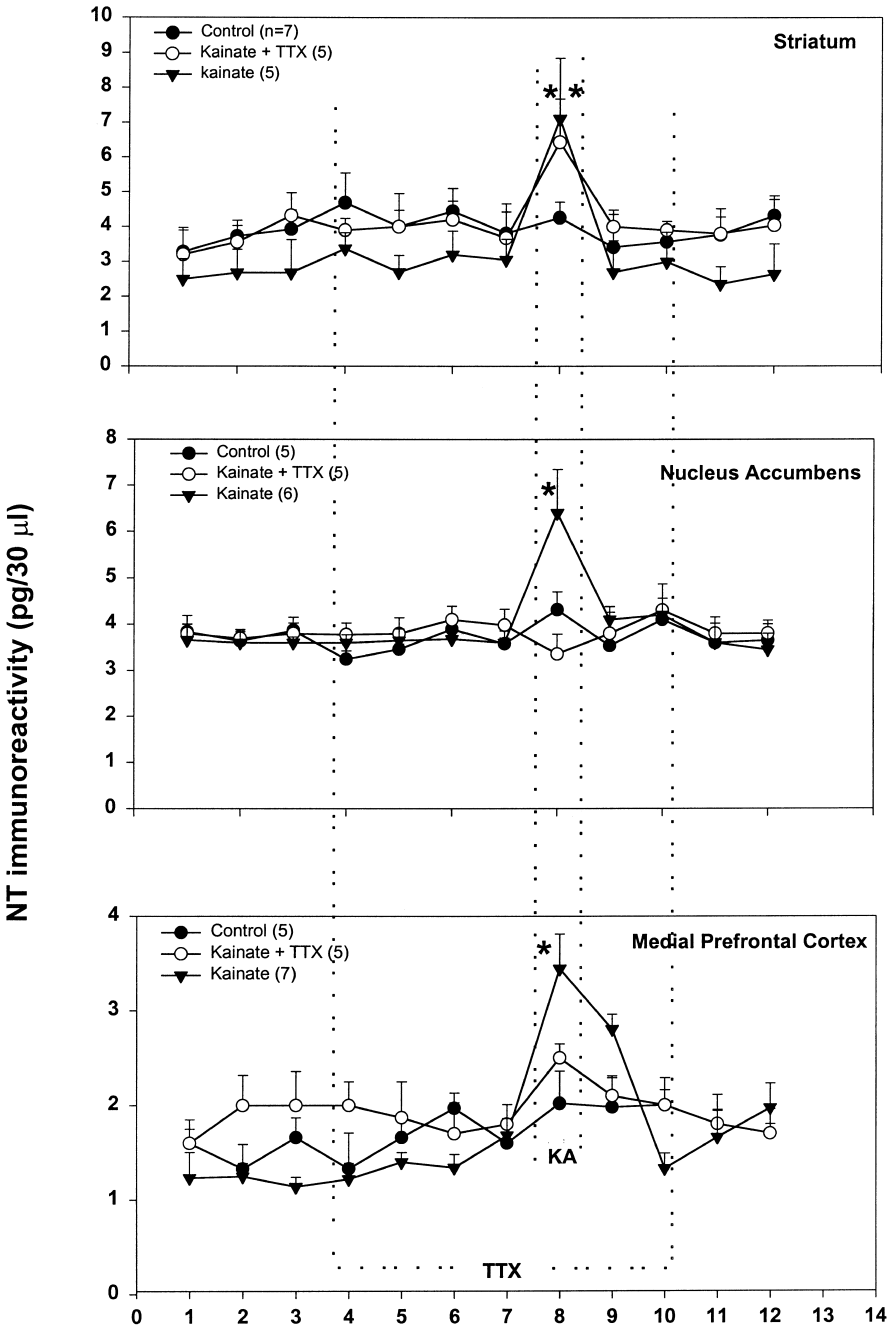

Fig. 1. The effects of kainic acid on neurotensin release in the striatum in conscious, freely moving rat. Microdialysis probes were placed in the rat striatum, nucleus accumbens or the medial prefrontal cortex. The following day, artificial cerebrospinal fluid at a flowrate of 1.0 (striatum) or 0.5 μl/min (nucleus accumbens, medial prefrontal cortex) was infused through the probes and collected and 30-min aliquots were assayed for neurotensin immunoreactivity. Vertical bars represent the infusion of artificial cerebrospinal fluid with tetrodotoxin (or artificial cerebrospinal fluid without tetrodotoxin) during samples 4 through 10 inclusively and the infusion of artificial cerebrospinal fluid with or without kainic acid during sample 8. Data presented as mean ± SEM. $^{*}P < 0.05$.

revealed that all three regions showed significant increases in extracellular neurotensin [striatum: $F(4,11) = 2.2$, $P < 0.05$, $n = 5$; nucleus accumbens, $F(6,11) = 23.8$, $P < 0.05$, $n = 7$; medial prefrontal cortex, $F(6,11) = 18.06$, $P < 0.05$, $n = 7$]. Closer examination revealed that in each region, the samples representing the kainic acid infusion (sample 8) were significantly different than all other samples, using post hoc analysis. In the striatum, the increase was 167% above basal (average of first three samples) levels; in the nucleus accumbens, the increase was 77%; in the medial prefrontal cortex, an increase of 190% above basal was observed.

Control groups for all three regions failed to show any significant differences in extracellular neurotensin over time. In rats receiving preincubation and concomitant infusion of tetrodotoxin (0.1 μM), no changes in basal extracellular neurotensin were observed. Furthermore, tetrodotoxin exposure did not block the kainic acid-induced increase in the striatum ($F(6,11) = 4.7$, $P < 0.05$, $n = 7$] but did block the kainic acid effect in both the nucleus accumbens and the prefrontal cortex.

3.3. Effects of NMDA on extracellular neurotensin

As shown in Fig. 2, infusion of NMDA, like kainic acid also produced a stimulatory effect on extracellular neu-

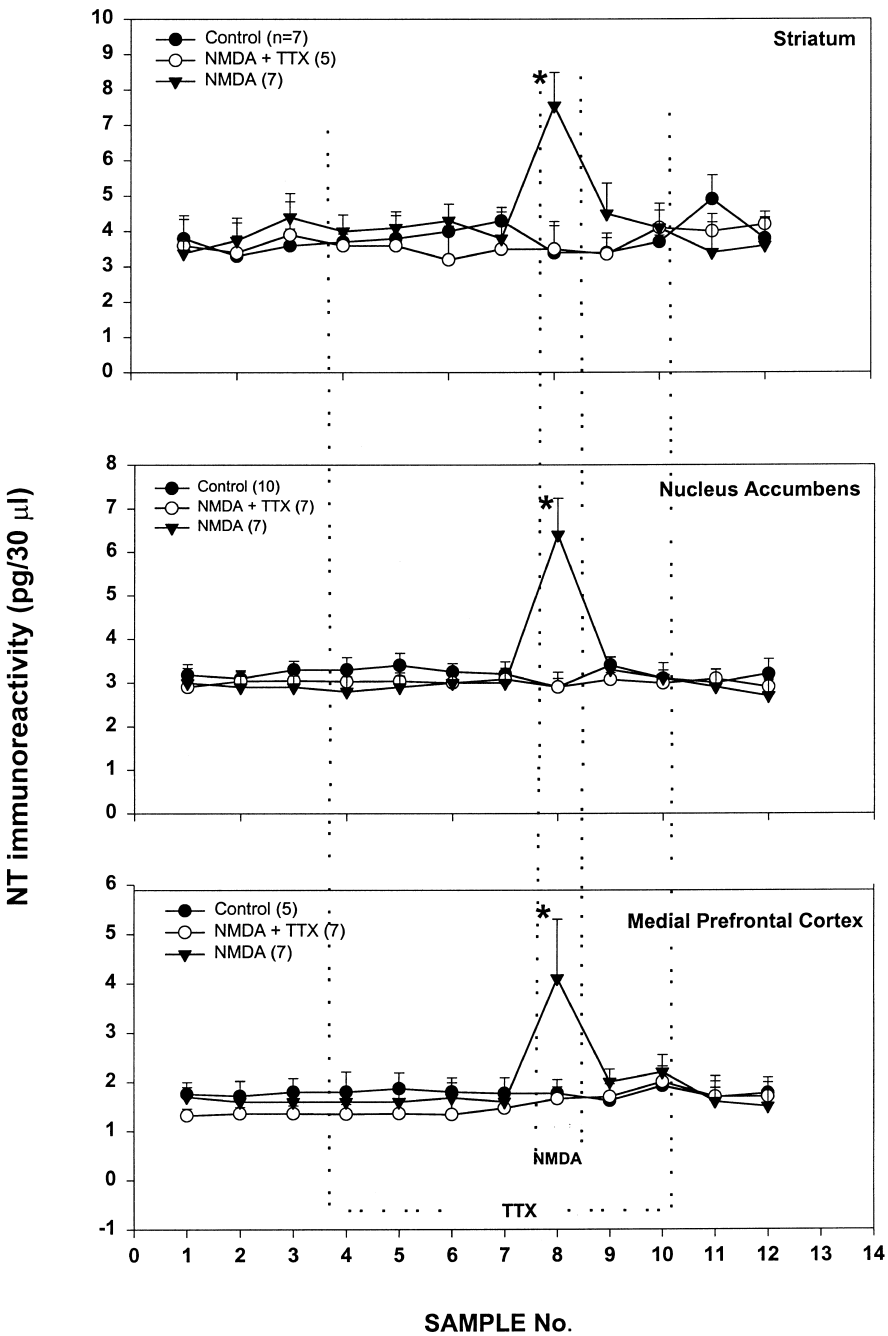

Fig. 2. The effects of NMDA on neurotensin release in the striatum in conscious, freely moving rat. Microdialysis probes were placed in the rat striatum, nucleus accumbens or the medial prefrontal cortex. The following day, artificial cerebrospinal fluid at a flowrate of 1.0 (striatum) or 0.5 μl/min (nucleus accumbens, medial prefrontal cortex) was infused through the probes and collected and 30-min aliquots were assayed for neurotensin immunoreactivity. Vertical bars represent the infusion of artificial cerebrospinal fluid with tetrodotoxin (or artificial cerebrospinal fluid without tetrodotoxin) during samples 4 through 10 inclusively and the infusion of artificial cerebrospinal fluid with or without NMDA during sample 8. Data presented as mean ± SEM. * $P < 0.05$.

rotensin. Analysis of variance revealed significant increases in extracellular neurotensin in all regions studied [striatum: $F(6,11) = 6.95$, $P < 0.05$, $n = 7$; nucleus accumbens, $F(6,11) = 24.9$, $P < 0.05$, $n = 7$; medial prefrontal cortex, $F(6,11) = 20.1$, $P < 0.05$, $n = 7$]. Also similar to the kainic acid results, post hoc analysis showed that only the sample containing the NMDA (10^{-5} mol/l) infusion, was significantly different than the other samples within each group.

The magnitude of the NMDA-induced increases above basal levels (average of first three samples) were 96%, 118% and 151% in the striatum, nucleus accumbens and medial prefrontal cortex, respectively.

Control groups for all three regions failed to show any significant differences in extracellular concentrations of neurotensin over time. In rats receiving a preincubation of tetrodotoxin (0.1 μmol/l), no changes in basal concentrations of neurotensin were observed. Furthermore, tetrodotoxin preincubation blocked the NMDA-induced increases in extracellular neurotensin in all three regions.

4. Discussion

Recently, the method of in vivo microdialysis has been used by several groups to measure extracellular concentrations of neurotensin from neural tissue (During et al., 1992; Maidment et al., 1991; Radke et al., 1998; Wagstaff et al., 1997). Such studies, along with in vitro preparations (Iversen et al., 1978), have established neurotensin as a Ca^{2+}-dependent releasable peptide that interacts with other neurotransmitter systems. Neurotensin has been shown to interact with several neurotransmitter systems including glutamate (Sanz et al., 1993), GABA [γ-aminobutyric acid] (O'Connor et al., 1992), acetylcholine (O'Connor et al., 1992; Tanganelli et al., 1994) and dopamine (Blaha et al., 1990; Chapman et al., 1992). The interaction between neurotensin and dopamine has been well documented. However, very few studies have examined how neurotensin interacts with the other major inputs to the rat basal ganglia, the glutamatergic pathways. In the present study, we report that glutamate receptor agonists significantly increased extracellular concentrations of neurotensin in all brain regions examined. The measurement of extracellular neurotensin, in our experiments, was performed using microdialysis, in combination with solid phase radioimmunoassay, which has proven to be a useful tool to measure the in vivo concentration of extracellular neurotensin in various regions of the brain (Maidment et al., 1991; Radke et al., 1998; Wagstaff et al., 1997). In the present study, we noted that depolarizing agents increased extracellular neurotensin differentially in the various brain regions examined. Generally, glutamate receptor agonists increased extracellular neurotensin in the medial prefrontal cortex and the nucleus accumbens to a greater extent than that observed in the striatum, suggesting that these regions have a greater pool of neurotensin available for immediate release, or a greater density of glutamate receptors.

More specifically, the effects of NMDA revealed that, in each of the regions examined, a significant increase of extracellular neurotensin was observed, and moreover, that the increases were blocked by tetrodotoxin. These results may indicate that the NMDA receptors responsible for neurotensin release are not present on neurotensin-containing terminals. Recently, Hanson et al. have demonstrated an interesting relationship between neurotensin, NMDA and dopamine. They reported that activation of dopamine D_1 receptors increases the tissue concentration of neurotensin and that the NMDA receptor antagonist MK-801 (Wagstaff et al., 1997) attenuated this increase. Further, MK801 alone appeared to have no effect on neurotensin concentrations (Hanson et al., 1995a). These data would suggest that NMDA receptors modulate neurotensin-containing cells indirectly through another neurotransmitter system, possibly the dopaminergic system (Wagstaff et al., 1997).

Kainic acid infusions resulted in similar increases of extracellular neurotensin. Contrasting the results with NMDA, however, was the finding that after kainic acid administration tetrodotoxin blocked the increase in extracellular neurotensin in the nucleus accumbens and medial prefrontal cortex, but not in the striatum. The latter results may indicate that some kainic acid-sensitive receptors (kainate and/or AMPA) are present on neurotensin-containing terminals. To date very few studies have examined the relationship that exists between kainic acid-sensitive receptors and neurotensin. Systemic injections of kainic acid (Sperk et al., 1986) have been shown to decrease neurotensin concentrations in the hippocampus and cortex. Others have examined neurotensin following specific kainic acid lesions in which temporary decreases in both neurotensin receptor density and neurotensin concentrations are observed (Schotte et al., 1988; Sugimoto et al., 1987). The effects of kainic acid and other glutamate receptor agonists on neurotensin release were assessed using an in vitro slice preparation from spinal cord; no significant changes in neurotensin release were observed (Gamse and Heuberger, 1983).

One interesting observation repeated throughout our experiments was that preincubation with tetrodotoxin failed to alter basal concentrations of extracellular neurotensin. Such observations have been seen with other neurotransmitters using in vivo microdialysis and may reflect a limiting factor in using microdialysis to measure small changes in neuropeptide release in vivo. In vitro studies have shown tetrodotoxin to reduce neurotensin release (Iversen et al., 1978; Maeda and Frohman, 1981) or have no effect on basal release (Micevych et al., 1982), indicating the limitation of using tetrodotoxin as a tool to analyze the voltage dependent release of neurotransmitters.

Overall, our results provide further novel information on the interaction between glutamate and neurotensin sys-

tems in the rat brain. Future studies examining the relationship between these two neurochemical systems in these and other brain regions should prove useful in understanding their respective roles in the central nervous system.

Acknowledgements

Supported by grant MH 39415 from the National Institutes of Health.

References

Bean, A.J., During, M.J., Roth, R.H., 1989. Stimulation-induced release of coexistent transmitters in the prefrontal cortex: an in vivo microdialysis study of dopamine and neurotensin release. J. Neurochem. 53, 655–657.

Blaha, C.D., Coury, A., Fibiger, H.C., Phillips, A.G., 1990. Effects of neurotensin on dopamine release and metabolism in the rat striatum and nucleus accumbens: cross validation using in vivo voltammetry and microdialysis. Neuroscience 34, 699–704.

Carraway, R.E., Leeman, S.E., 1973. The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. J. Biol. Chem. 248, 6854–6861.

Chapman, M.A., See, R.E., Bissette, G., 1992. Neurotensin increases extracellular striatal dopamine levels in vivo. Neuropeptides 22, 175–183.

During, M.J., Bean, A.J., Roth, R.H., 1992. Effects of CNS stimulants on the in vivo release of colocalized transmitters, dopamine and neurotensin, from rat prefrontal cortex. Neurosci. Lett. 140, 129–133.

Ferraro, L., Tanganelli, S., O'Conner, W.T., Bianchi, C., Ungerstedt, U., Fuxe, K., 1995. Neurotensin increases endogenous glutamate release in the neostriatum of the wake rat. Synapse 20, 363–364.

Gamse, R., Heuberger, S., 1983. Release of neurotensin from the rat spinal cord. Neurosci. Lett. 36, 87–91.

Hanson, G.R., Midgley, L.P., Bush, L.G., Gibb, J.W., 1995a. Response of extrapyramidal and limbic neurotensin systems to phencyclidine treatment. Eur. J. Pharmacol. 278, 167–178.

Hanson, G.R., Singh, N., Merchant, K., Johnson, M., Gibb, J.W., 1995b. The role of NMDA receptor systems in neuropeptide responses to stimulants of abuse. Drug Alcohol Depend. 37, 107–110.

Iversen, L.L., Iversen, S.D., Bloom, F., Douglas, C., Brown, M., Vale, W., 1978. Calcium-dependent release of somatostatin and neurotensin from rat brain in vitro. Nature 273, 161–163.

Jennes, L., Stumpf, W.E., Kalivas, P., 1982. Neurotensin: topographical distribution in rat brain by immunohistochemistry. J. Comp. Neurol. 210, 211–224.

Maeda, K., Frohman, L.A., 1981. Neurotensin release by rat hypothalamic fragments in vitro. Brain Res. 210, 261–269.

Maidment, N.T., Siddall, B.J., Rudolph, V.R., Erelyi, E., Evans, C.J., 1991. Dual determination of extracellular cholecystokinin and neurotensin fragments in rat forebrain: microdialysis combined with a sequential multiple antigen radioimmunoassay. Neuroscience 45, 81–93.

Micevych, P.E., Yaksh, T.L., Go, V.L., 1982. Opiate-mediated inhibition of the release of cholecystokinin and substance P but not neurotensin from cat hypothalamic slices. Brain Res. 250, 283–289.

O'Connor, W.T., Tanganelli, S., Ungerstedt, U., Fuxe, K., 1992. The effects of neurotensin on GABA and acetylcholine release in the dorsal striatum of the rat: an in vivo microdialysis study. Brain Res. 573, 209–216.

Paxinos, G., Watson, C., 1982. The Rat Brain Stereotaxic Coordinates. Academic Press, Australia.

Quiron, R., Rowe, W.B., Lapchak, P.A., Araujo, D.M., Beaudette, A., 1992. Distribution of neurotensin receptors in mammalian brain: what it is telling us about its interactions with other neurotransmitter systems. Ann. N. Y. Acad. Sci. 668, 109–119.

Radke, J.M., Owens, M.J., Nemeroff, C.B., 1998. Atypical antipsychotic drugs selectively increase neurotensin efflux in dopamine terminal regions. Proc. Natl. Acad. Sci. 95, 11462–11464.

Sanz, B., Exposito, I., Mora, F., 1993. Effect of neorotensin on the release of glutamic acid in the prefrontal cortex and striatum of the rat. NeuroReport 4, 1194–1196.

Schotte, A., Rostene, W., Laduron, P.M., 1988. Different subcellular localization of neurotensin–receptor and neurotensin–acceptor sites in the rat brain dopaminergic system. J. Neurochem. 40, 1026–1031.

Sperk, G., Wieser, R., Widmann, R., Singer, E.A., 1986. Kainic acid induced seizures: changes in somatostatin, substance P and neurotensin. Neuroscience 17, 1117–1126.

Sugimoto, T., Itoh, K., Yasui, Y., Kamiya, H., Uemura, Y., Mizuno, N., 1987. Changes of neurotensin-like immunoreactivity in the striatum of the cat after intrastriatal injection of kainic acid. J. Comp. Neurol. 263, 607–612.

Tanganelli, S., O'Connor, W.T., Ferraro, L., Bianchi, C., Beani, L., Ungerstedt, U., Fuxe, F., 1994. Facilitation of GABA release by neurotensin is associated with a reduction of dopamine release in rat nucleus accumbens. Neuroscience 60, 649–657.

Wagstaff, J.D., Gibb, J.W., Hanson, G.R., 1997. Role of dopamine D1- and NMDA-receptors in regulating neurotensin release in the striatum and nucleus accumbens. Brain Res. 748, 241–244.