Effect of Acute and Daily Neurotensin and Enkephalin Treatments on Extracellular Dopamine in the Nucleus Accumbens

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The injection of neurotensin or the enkephalin analog Tyr-D-Ala-Gly-MePhe-Gly(ol) (DAMGO) into the A10 region of rats produces a motor stimulant effect that is associated with an increase in the postmortem levels of dopamine metabolites in the nucleus accumbens. These behavioral and neurochemical effects are augmented following daily administration. *In vivo* dialysis in the nucleus accumbens of conscious rats was used to determine if the acute and augmented behavioral responses following neurotensin or DAMGO administration are associated with an increase in extracellular dopamine concentrations. The acute injection of DAMGO produced a dose-dependent (0.03–3.3 nmol) elevation in extracellular dopamine and its metabolites in the nucleus accumbens. Neurotensin also produced a dose-related (0.1–3.3 nmol) increase in dopamine and its metabolites. The elevation in extracellular dopamine produced by DAMGO, but not by neurotensin, was positively correlated with the increase in motor activity. Following daily treatment of either neurotensin (1.0 nmol $\times$ 4 d) or DAMGO (0.03 nmol $\times$ 4 d), a significant elevation in extracellular dopamine levels occurred in the nucleus accumbens compared to an acute injection. The time course of the change in extracellular dopamine after daily injections was similar to the time course of the behavioral stimulation for both compounds. These data demonstrate that enhanced dopamine release in the nucleus accumbens mediates the acute behavioral effect of DAMGO but does not entirely explain the motor effects of neurotensin. However, enhanced dopamine release may mediate the behavioral sensitization produced by daily injection of both peptides into the A10 region.

Neurotensin and Met-enkephalin are neuropeptides that modulate the activity of dopamine neurons in the ventromedial mesencephalon. Neurotensin receptors are located directly on dopaminergic perikarya (Palacios and Kuhar, 1981; Quirion et al., 1985; Dils and Kalivas, 1989; Dana et al., 1989), and activation of these receptors by neurotensin produces an increase in impulse generation, both directly and indirectly by inhibiting autoreceptor-induced hyperpolarization (Innis et al., 1985; Pin-

nock, 1985; Shi and Bunney, 1988; Seutin et al., 1989). Supporting an excitatory modulation of dopamine cells by neurotensin, microinjection of neurotensin into the A10 dopamine region elicits a motor stimulant response that is blocked by pretreatment with dopamine antagonists (Kalivas et al., 1983a; Cador et al., 1985) and increases measures of dopamine turnover in limbic dopamine axonal terminal fields (Kalivas et al., 1983a; Phillips et al., 1988; Cador et al., 1989).

Enkephalin acts on the μ -opioid receptor subtype (Latimer et al., 1987) to indirectly activate dopamine neurons. Thus, unlike neurotensin receptors, μ -opioid receptors are located on non-dopaminergic neurons in the ventromedial mesencephalon (Dils and Kalivas, 1989). While activation of μ -receptors inhibits the activity of the nondopaminergic neurons, it indirectly stimulates the dopamine perikarya (Gysling and Wang, 1983; Matthews and German, 1984; Lacey et al., 1989). Injection of enkephalin analogs into the A10 region increases locomotor activity by a dopamine-dependent mechanism (Broekkamp et al., 1979; Kelley et al., 1980; Joyce et al., 1981; Kalivas et al., 1983b). Intra-A10 injection of enkephalin analogs also increases the levels of dopamine metabolites in A10 dopamine terminal fields (Kalivas et al., 1983b; Latimer et al., 1987; Cador et al., 1989). Based upon these findings, it has been postulated that enkephalin may stimulate dopamine cells via disinhibition of GABAergic afferents (Kelley et al., 1980; Kalivas et al., 1990), and it was recently shown that pretreatment with the GABA_A agonist baclofen abolished the capacity of intra-A10 injection of enkephalin analog to increase motor activity and dopamine metabolites in the nucleus accumbens (Kalivas et al., 1990).

When either neurotensin or μ -opioids are microinjected daily into the A10 dopamine region, the acute motor stimulant effect of these compounds is augmented (Joyce and Iversen, 1979; Vezina and Stewart, 1984; Kalivas, 1985; Kalivas and Taylor, 1985; Elliot and Nemeroff, 1986). Furthermore, the increase in postmortem levels of dopamine metabolites in the nucleus accumbens by microinjected neurotensin or enkephalin analog is augmented following daily administration (Kalivas, 1985; Kalivas and Taylor, 1985). The augmented behavioral response appears to be analogous to the behavioral sensitization produced by daily administration of the indirect dopamine agonists amphetamine and cocaine (Robinson and Becker, 1986; Kuzcenski and Segal, 1988; Post and Weiss, 1988). Recently it has been demonstrated using *in vivo* dialysis in the nucleus accumbens or striatum of the conscious rat that the behavioral sensitization produced by daily amphetamine or cocaine administration is associated with an augmentation in the extracellular concentrations of dopamine (Robinson et al., 1988; Kazahaya et al., Kalivas and Duffy, 1990).

Received Dec. 7, 1989; revised Mar. 12, 1990; accepted Apr. 9, 1990.

This work was supported in part by USPHS Grants MH-40817, DA-03906, and NS-24388. We wish to thank Jenny Baylon for assistance in preparing the manuscript.

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In the present study, *in vivo* dialysis in the nucleus accumbens of the conscious rat was used to determine if the behavioral stimulation following acute administration of neurotensin or the μ -opioid enkephalin analog Tyr-D-Ala-Gly-MePhe-Gly(ol) (DAMGO) into the A10 region is associated with an increase in extracellular dopamine levels. Furthermore, the peptides were administered daily to investigate whether the augmented motor stimulant response was associated with an augmented elevation in extracellular dopamine concentration.

Materials and Methods

Animal housing, surgery, drugs, and microinjection procedure. Male Sprague-Dawley rats (Laboratory Animal Research Facility, Pullman, WA) were individually housed in a temperature- and humidity-controlled environment with food and water made available *ad libitum*. Rats weighing 250–350 gm were anesthetized with Equithesin, i.p., mounted in a stereotaxic apparatus, and were implanted with a unilateral cannula (20 gauge stainless steel; Small Parts, Miami, FL) 3 mm over the nucleus accumbens (A/P 9.1 mm; M/L 1.7 mm; D/V 0.0 mm; relative to the interaural line; Pellegrino et al., 1979) for later insertion of a dialysis probe. A stainless steel obturator (25 gauge; Small Parts) was inserted into the chronic cannula. In addition, the rats were implanted with bilateral stainless steel guide cannulae (26 gauge; Small Parts) 1 mm dorsal to the A10 dopamine region (A/P 2.6 mm; M/L 0.6 mm; D/V –2.5 mm). The cannulae were angled (6°) to avoid penetration of the midsagittal sinus or cerebral aqueduct. Obturators (33 gauge stainless steel; Small Parts) were placed in the guide cannulae, and the rats were allowed a minimum 7 d recovery period prior to behavioral testing.

Microinjection of sterile, isotonic saline vehicle (0.5 μ l/side), neurotensin (Bachem, Inc., Torrance, CA) or DAMGO (Bachem) were made into the A10 region over 60 sec by inserting an injection needle (33 gauge stainless steel tubing; Small Parts) 1 mm below the end of the chronic guide cannula. Simultaneous bilateral infusions were made into the unrestrained rat using 1 μ l syringes (Hamilton, Reno, NV) mounted in an infusion pump (Sage, USA) that was connected to the injection needle via PE-10 tubing. Twenty seconds after completing the infusion, the injection needles were removed and the obturators replaced.

Behavioral measurements. Rat motor behavior was monitored in 4 photocell chambers (Omnitech, Columbus, OH) located inside individual wooden boxes with separate air supply and light source (see Latimer et al., 1987, for details). At least 24 hr prior to beginning behavioral studies, the rats were placed in the photocell cage for 60 min, and patency of the dialysis cannula was verified, and a sham injection was made that consisted of inserting a 33 gauge needle into the A10 region. On the day of a behavioral trial or the night prior to the trial, the rats were placed into a photocell cage and a dialysis probe was inserted into the nucleus accumbens. Following a minimum 120 min or overnight equilibration period, baseline samples of dialysate (20 min each) were collected for 60 or 80 min. The rats then received an intra-A10 microinjection of saline, neurotensin, or DAMGO, and samples of dialysate were obtained every 20 min for 120 or 180 min. Following the experiment, the dialysis probe was removed, the obturator replaced, and the rat returned to its home cage. For the dose-response study, each rat was tested a maximum of 3 times with a different dose of neurotensin or DAMGO separated by a minimum 72 hr intertrial interval. Previous studies demonstrate that separation of acute DAMGO or neurotensin microinjections by 72 hr prevents the development of behavioral sensitization observed after daily injections (Kalivas and Taylor, 1985; Kalivas, 1985; Elliot and Nemeroff, 1986). For the sensitization study, following the first injection in the dialysis chamber with saline, neurotensin (1.0 nmol) or DAMGO (0.03 nmol), the rats received a daily injection of the same treatment for the next 3 d in the home cage. The doses of peptide used in the daily treatment study were based upon published or preliminary data demonstrating behavioral sensitization after daily administration (Kalivas and Taylor, 1985; Elliot and Nemeroff, 1986). Three to six days later, the rats were returned to the photocell cage, and dialysis and drug injections performed as described above for the first day of the experiment.

Dialysis and neurochemical measurements. The dialysis probe was constructed as described by Robinson and Whishaw (1988). Two to 3 mm of dialysis tubing was exposed at the tip of the probe. The probe

was inserted into a connector that allowed control of the depth of penetration and fixation onto the chronically implanted guide cannula. The connector was attached to a liquid swivel, which was located on a balance beam to minimize discomfort to the rat. In 56 of the 95 experiments, either prior to inserting the probe or after removing the probe from the rat, recovery was determined by placing the probe for 20 min in the dialysate buffer (5 mM KCl, 120 mM NaCl, 1.8 mM CaCl_2 , 1.2 mM MgCl_2 plus 0.2 mM PBS to give pH 7.4) containing dopamine (10^{-7} M), 3,4-dihydroxyphenylacetic acid (DOPAC; 10^{-6} M), homovanillic acid (HVA; 10^{-6} M), and 5-hydroxyindoleacetic acid (5-HIAA; 10^{-6} M). The mean $\pm$ SEM percent recoveries at room temperature were as follows: dopamine, 9.3 ± 0.6 ; DOPAC, 20.4 ± 1.4 ; HVA, 13.0 ± 1.2 ; and 5-HIAA, 8.1 ± 0.4 . The flow rate was 2.13 μ l/min (Harvard Instruments) throughout the experiment, and the concentrations of dopamine, DOPAC, HVA, and 5-HIAA were determined using HPLC-EC. The dialysis samples were collected into 20 μ l of mobile phase [0.1 M citric acid, 75 mM Na_2HPO_4 , 0.6–1.0 mM heptane sulfonic acid, 0.1 mM EDTA, 13% methanol (vol/vol), pH 3.8–4.2, with 2.0 pmol dihydroxybenzylamine as the internal standard], and the entire sample was injected into the HPLC system. A reversed-phase column (C-18, 25 cm; Bioanalytical, Indianapolis, IN) was used to separate the biogenic amines, and a coulometric electrochemical detection system (ESA, Inc., Bedford, MA) utilizing 3 electrodes (preinjection port guard electrode = +0.4 V, preoxidation electrode = +0.02 V, working electrode = +0.35 V) was used to oxidize the amines. Standard curves ranging from 0.01 to 10 pmol were used to quantify each compound.

Histology and statistics. Rats were killed with an overdose of pentobarbital, and their brains were perfused via intracardiac infusion with PBS (60 ml) followed by 10% paraformaldehyde-saline (60 ml). The brains were stored in 10% formaldehyde for at least a week. Coronal sections (100 μ m thick) were made of the cannulae tracks with a vibratome, mounted on gelatin-coated slides, and stained with cresyl violet. The location of the dialysis probe track or tip of the microinjection cannulae was determined by an individual unaware of the animal's behavioral or neurochemical response. The time-course behavioral and neurochemical data were statistically evaluated using a 2-way repeated-measures analysis of variance (ANOVA), followed by the least-significant difference test for multiple comparisons, as described by Milliken and Johnson (1984). The cumulative behavioral and neurochemical data were analyzed using a 1-way ANOVA, followed by a Dunnett's test for comparison to saline. Prior to data analysis and presentation, the neurochemical data were converted to percent change from the average of the 3 baseline measurements. Table 1 shows the concentration of each amine not corrected for probe recovery. Correlation coefficients for comparing amine levels to locomotor behavior were obtained using linear-regression analysis.

Results

Effect of acute DAMGO and neurotensin on extracellular dopamine

Figure 1A shows that DAMGO injection into the A10 region produced a dose-dependent increase in horizontal photocell counts. A dose of 0.03 nmol elicited a significant elevation during the first 20 min after injection, and the largest dose (3.3 nmol) produced a maximal response between 80 and 120 min after injection. A latency to maximal response following injection of relatively large doses of μ -opioid agonists into the A10 region is consistent with previous reports (Joyce et al., 1981; Kalivas et al., 1983b). Figure 1B shows that DAMGO produced a dose-dependent elevation in the extracellular concentration of dopamine in the nucleus accumbens. The increase was statistically significant after the 2 largest doses of DAMGO (0.3 and 3.3 nmol) between 40 and 100 min after injection. Figure 2 shows that DAMGO also elevated the levels of the dopamine metabolites, DOPAC and HVA, and the serotonin metabolite, 5-HIAA, in the extracellular fluid in the nucleus accumbens. The increase in DOPAC was dose-dependent, and significant elevations began at 40 min after the injection of all doses. All doses of DAMGO significantly elevated the level of HVA by 40 min after injection, and the levels remained enhanced for

Table 1. Raw baseline values for the biogenic amines recovered from the dialysis probe

Compound	Dose-response study		Sensitization study		
	Neurotensin (34)	DAMGO (26)	Saline (9)	DAMGO (14)	Neurotensin (12)
Dopamine	6.9 ± 1.0	6.8 ± 1.1	3.4 ± 0.8	5.6 ± 1.1	3.3 ± 1.4
DOPAC	444.9 ± 83.7	321.0 ± 64.2	349.8 ± 137.2	467.4 ± 35.2	387.5 ± 59.5
HVA	227.9 ± 42.8	196.8 ± 46.2	172.4 ± 29.2	177.5 ± 30.9	294.0 ± 82.3
5-HIAA	66.3 ± 13.2	68.1 ± 14.1	—	—	—

Data shown are mean ± SEM fmol/min of the average of the 3 baseline samples collected prior to microinjection of peptide or saline. The data are not corrected for probe recovery. The number in parentheses refers to the number of rats used in each experiment.

the duration of the experiment. The 2 largest doses of DAMGO were found to increase the concentration of 5-HIAA from 40 min to the end of the experiment, and at the lowest dose statistical significance was observed at 40 and 120 min after injection. Figures 1 and 2 also show the cumulative behavioral and neurochemical responses for 120 min following DAMGO microinjection. All doses of DAMGO significantly elevated photocell counts, DOPAC, HVA, and 5-HIAA, while only the 2 largest doses increased extracellular dopamine levels.

Figure 3 shows that neurotensin significantly increased horizontal photocell counts and the extracellular level of dopamine in the nucleus accumbens. The highest dose of neurotensin (3.3 nmol) significantly increased dopamine between 40 and 120 min after injection, while the next highest dose (1.0 nmol) elevated dopamine between 60 and 120 min. Behavioral activity was elevated by the 3 highest doses of neurotensin. However, the time course of the behavioral response was different depending upon the dose. At the 2 highest doses (1.0 and 3.3 nmol), the majority of the hyperactivity occurred beyond 40 min after injection, while at a lower dose (0.3 nmol), the increase occurred at 20 min after injection. Figure 4 demonstrates that all doses of neurotensin significantly elevated DOPAC by 60 min after injection, with the 3 highest doses producing an effect by 40 min after injection. HVA was also increased after all doses of neurotensin by 60 min after injection. The effect of neurotensin on extracellular 5-HIAA was not dose-dependent. The most marked effects were produced by the lowest and highest doses. Figures 3 and 4 also show the cumulative behavioral and neurochemical responses. By this measure, all doses of neurotensin employed significantly elevated DOPAC and HVA. The 2 highest doses of neurotensin increased dopamine, while the lowest and highest doses elevated 5-HIAA. Only the largest dose of neurotensin significantly elevated horizontal photocell counts.

Effect of daily DAMGO and neurotensin injection into the A10 region

DAMGO (0.03 nmol), neurotensin (1.0 nmol), or saline was administered into the A10 region once a day for 4 d. Figure 5 shows that after daily neurotensin administration, an acute injection of neurotensin given 4–6 d later produced a significant elevation in both photocell counts and extracellular dopamine levels in the nucleus accumbens compared to the first day of administration. The time course for both effects was similar, with a peak effect after daily administration occurring at 40 min after injection. The increases in DOPAC or HVA were not significantly augmented over that produced by a single acute injection of neurotensin (Fig. 6). Figure 5 also demonstrates that

the effect of DAMGO on both horizontal photocell counts and extracellular dopamine was augmented in rats pretreated with daily DAMGO. The time course of the increase in dopamine after DAMGO injection was similar to the motor response in that levels were elevated 20 min after injection and remained elevated for 120 min. However, motor activity showed a maximum at 20 min after injection that was not reflected in extracellular dopamine. No augmentation was observed in the increase in DOPAC or HVA produced by an acute injection of DAMGO in daily DAMGO pretreated rats (Fig. 6). Figures 5 and 6 also show that daily administration of saline did not alter the effect of a subsequent acute injection of saline on horizontal photocell counts, dopamine, DOPAC, or HVA.

Correlations between behavior and dopamine

From the data in Figures 1–6 it appears that either dopamine and/or its metabolites could be significantly correlated with the behavioral response following the acute or daily injection of DAMGO or neurotensin into the A10 region. When the cumulative changes in photocell counts were compared to the cumulative changes in dopamine, a significant correlation was observed after acute and daily injection of DAMGO, and after daily administration of neurotensin (Fig. 7). In contrast, dopamine and behavior were not correlated after acute administration of neurotensin. Behavior was not found to be significantly correlated with DOPAC, HVA, or 5-HIAA in any treatment group (Table 2). The concentration of dopamine was not correlated with any of its metabolites, except after daily neurotensin, for which case dopamine was positively correlated with the level of DOPAC. In all treatment groups, the level of DOPAC was positively correlated with the level of HVA.

Histology

Figure 8 illustrates the location of the dialysis probe in the nucleus accumbens and of the injection cannulae in the A10 dopamine region. Of the 55 rats used in this study, 47 were found to have both injection cannulae in the A10 region (including the nucleus fascicularis, nucleus linearis, nucleus paraventricularis, and nucleus parabrachialis pigmentosus), and all but 2 rats had at least 50% of the dialysis probe in the nucleus accumbens. The remaining portion of the probe was located dorsally in the mesolimbic striatum or ventrally in the olfactory tubercle.

Discussion

These data demonstrate that the motor stimulant response resulting from injection of the μ -opioid agonist DAMGO, but not

Figure 1. Effect of acute DAMGO injection into the A10 region on motor activity and extracellular dopamine in the nucleus accumbens. **A**, Dopamine in the nucleus accumbens is shown as percent $\pm$ SEM change from the average of the 3 dialysis samples obtained prior to injection of saline or DAMGO. Dialysis samples were collected for 120 min following saline injection and for 180 min following DAMGO. Thus, statistical analysis was performed only out to 120 min after injection. The baseline values in this experiment are shown in Table 1. At right, the data are shown as the means $\pm$ SEM average percent change over the 120 min after injection. This value was calculated by dividing the average of the values obtained after microinjection by the average of the baseline values obtained prior to injection. **B**, Horizontal photocell counts are shown as means $\pm$ SEM. At right, the data are shown as the mean $\pm$ SEM cumulative photocell counts over 120 min after injection. The arrow indicates the time of injection after the last baseline sample was collected at time = 0. Saline, $N = 9$; 0.03 nmol, $N = 8$; 0.3 nmol, $N = 5$; 3.3 nmol, $N = 4$. The time course data were statistically evaluated with a 2-way repeated measures ANOVA followed by a least-significant difference test for comparison of DAMGO to saline at a given time interval. $p < 0.05$ for dopamine, 0.3 and 3.3 nmol between 40 and 120 min treatment $F(3, 22) = 4.794$, $p = 0.010$; time $F(8, 176) = 3.651$, $p = 0.001$; interaction $F(24, 176) = 3.156$, $p < 0.001$. $p < 0.05$ for photocell counts, 0.03 nmol at 20 min, 0.3 nmol at 100 and 120 min, 3.3 nmol at 80–120 min, treatment $F(3, 22) = 8.607$, $p = 0.001$; time $F(8, 176) = 10.524$, $p < 0.001$; interaction $F(24, 176) = 5.839$, $p < 0.001$. * $p < 0.05$ for the cumulative data compared to saline injection using a 1-way repeated-measures ANOVA, followed by a Dunnett's test for multiple comparisons. For dopamine, $F(3, 25) = 4.800$, $p = 0.010$, and for behavior, $F(3, 25) = 9.075$, $p = 0.004$.

neurotensin, into the A10 region is positively correlated with an increase in extracellular dopamine content in the nucleus accumbens. Furthermore, when the behavioral stimulation response to these peptides was augmented by daily administration, the concentration of extracellular dopamine was found to increase in parallel.

The increase in extracellular dopamine in the nucleus accumbens

Figure 2. Effect of acute injection of DAMGO into the A10 region on extracellular levels of DOPAC, HVA, and 5-HIAA in the nucleus accumbens. The data were obtained simultaneously with the dopamine levels and behavior shown in Figure 1. **A**, DOPAC; **B**, HVA; **C**, 5-HIAA. Symbols: $\square$, saline; $\blacklozenge$, 0.03 nmol; $\diamond$, 0.3 nmol; $\blacksquare$, 3.3 nmol. See legend to Figure 1 for further details. For the time course data, a least-significant difference test was used. For DOPAC, $p < 0.05$ difference from saline was observed at all doses from 40–120 min; treatment $F(3, 22) = 17.552$, $p < 0.001$; time $F(8, 176) = 30.094$, $p < 0.001$; interaction $F(24, 176) = 8.360$, $p < 0.001$; for HVA, $p < 0.05$ difference from saline was observed at all doses from 40–120 min; treatment $F(3, 22) = 7.935$, $p = 0.001$; time $F(8, 176) = 15.086$, $p < 0.001$; interaction $F(24, 176) = 5.606$, $p < 0.001$; for 5-HIAA, $p < 0.05$ difference from saline was observed at 0.3 and 3.3 nmol from 40 to 120 min, at 0.03 nmol at 40 and 120 min; treatment $F(3, 22) = 7.780$, $p < 0.001$; time $F(8, 176) = 4.748$, $p < 0.001$; interaction $F(24, 176) = 4.293$, $p < 0.001$. * $p < 0.05$ for the cumulative data compared to saline injection using a 1-way ANOVA followed by a Dunnett's test. For DOPAC, $F(4, 33) = 6.492$, $p = 0.001$; for HVA, $F(4, 33) = 7.623$, $p < 0.001$; for 5-HIAA, $F(4, 33) = 5.610$, $p = 0.002$.

by acute injection of DAMGO is consistent with previous studies showing that the motor stimulant response produced by μ -opioid agonist administration into the A10 region is blocked by intra-accumbens administration of dopamine antagonist or depletion of dopamine in the nucleus accumbens by pretreatment with 6-hydroxydopamine (Kelley et al., 1980; Kalivas et al., 1983b). Also, the administration of μ -opioids into the A10 region produces a dose-dependent elevation in the concentration of dopamine metabolites in postmortem nucleus accumbens tissue (Kalivas et al., 1983b; Latimer et al., 1987; Cador et al., 1989). Likewise, the fact that neurotensin elicited a dose-de-

Figure 3. Effect of acute injection of neurotensin into the A10 region on motor activity and extracellular levels of dopamine in the nucleus accumbens. *A*, Dopamine in the nucleus accumbens is shown as percent change $\pm$ SEM from the average of the 3 dialysis samples obtained prior to injection of saline or neurotensin. The baseline values in this experiment are shown in Table 1. At right, the data are shown as the mean $\pm$ SEM average percent change over the 120 min after injection. This value was calculated by dividing the average of the values obtained after microinjection by the average of the values obtained prior to injection. *B*, Horizontal photocell counts are shown as means $\pm$ SEM. At right, the data are shown as the mean $\pm$ SEM cumulative photocell counts over 120 min after injection. The arrow indicates the time of injection after the last baseline sample collected at time = 0. Saline, $N = 12$; 0.1 nmol, $N = 6$; 0.3 nmol, $N = 5$; 1.0 nmol, $N = 5$; 3.3 nmol, $N = 6$. The time-course data were statistically evaluated using a 2-way repeated-measures analysis of variance followed by a least-significant difference multiple comparisons test for comparing neurotensin to saline treatment within a given time interval. $p < 0.05$ for dopamine, 0.3 nmol at 120 min, 1.0 nmol at 40–120 min, 3.3 nmol at 20, 60, 100 and 120 min, treatment $F(4, 29) = 4.557$, $p = 0.006$; time $F(8, 232) = 1.639$, $p = 0.115$; interaction $F(32, 232) = 1.894$, $p = 0.004$. $p < 0.05$ for photocell counts, 0.3 nmol at 20, 1.0 nmol at 60 min, 3.3 nmol at 40–120 min, treatment $F(4, 29) = 4.661$, $p = 0.005$; time $F(8, 232) = 12.855$, $p < 0.001$; interaction $F(32, 232) = 3.952$, $p < 0.001$. $*p < 0.05$ for cumulative data compared to saline injection at each time point using a 1-way repeated-measures ANOVA, followed by a Dunnett's test for multiple comparisons. For dopamine, $F(4, 33) = 4.366$, $p = 0.007$, and for behavior, $F(4, 33) = 4.914$, $p = 0.004$.

pendent increase in extracellular dopamine in the nucleus accumbens corroborates a number of previous investigations indicating that neurotensin activates A10 dopamine neurons. Intra-A10 injection of neurotensin increases motor activity (Kalivas et al., 1983b; Cador et al., 1985; Elliot and Nemeroff, 1986), and pretreatment with dopamine antagonist or destruction of dopamine in the nucleus accumbens with 6-hydroxydopamine abolishes this effect. Furthermore, the increase in motor activity

Figure 4. Effect of acute injection of neurotensin into the A10 region on extracellular levels of DOPAC, HVA, and 5-HIAA in the nucleus accumbens. The data were obtained simultaneously with the dopamine levels and behavior shown in Figure 3. *A*, DOPAC; *B*, HVA; *C*, 5-HIAA. Symbols: $\square$, saline; $\diamond$, 0.1 nmol; $\triangle$, 0.3 nmol; $\diamond$, 1.0 nmol; $\blacksquare$, 3.3 nmol. See figure legend for Figure 3 for further details. $*p < 0.05$, compared to saline injection using a 1-way ANOVA followed by a Dunnett's test (cumulative data). For the cumulative data, for DOPAC, $F(4, 33) = 6.492$, $p = 0.001$; for HVA, $F(4, 33) = 7.623$, $p < 0.001$; for 5-HIAA, $F(4, 33) = 5.610$, $p = 0.002$. For the time-course data, a least-significant difference test was used, and for DOPAC, $p < 0.05$ difference from saline at 0.1, 0.3, and 1.0 nmol from 40–120 min, and at 3.3 nmol from 60–120 min; treatment $F(4, 29) = 6.492$, $p = 0.001$; time $F(3, 32) = 21.906$, $p < 0.001$; interaction $F(32, 232) = 4.615$, $p < 0.001$; for HVA, $p < 0.05$ difference from saline at 0.3, 1.0, and 3.3 nmol from 40–120 min, and at 0.1 nmol from 60–120 min; treatment $F(4, 29) = 7.624$, $p < 0.001$; time $F(8, 232) = 21.136$, $p < 0.001$; interaction $F(32, 232) = 5.032$, $p < 0.001$; for 5-HIAA, $p < 0.05$ difference from saline at 0.1 nmol from 40–120 min, at 0.3 nmol at 60, 100, and 120 min, at 1.0 and 3.3 nmol from 100–120 min; treatment $F(4, 29) = 5.610$, $p = 0.002$; time $F(8, 232) = 4.414$, $p < 0.001$; interaction $F(32, 232) = 3.619$, $p < 0.001$.

by neurotensin has been shown to be associated with an increase in the level of dopamine metabolites in a number of brain regions, including the nucleus accumbens (Kalivas et al., 1983a; Cador et al., 1989). Finally, neurotensin produces an increase in the firing frequency of A10 and A9 dopamine neurons (Innis et al., 1985; Pinnock, 1985; Shi and Bunney, 1988; Seutin et al., 1989).

Considering the numerous convergent data supporting activation of A10 dopamine neurons by neurotensin, it was sur-

Figure 5. Effect of daily microinjection of neurotensin (1.0 nmol) or DAMGO (0.03 nmol) into the A10 region on motor activity and extracellular dopamine levels in the nucleus accumbens. The dopamine data are shown as the mean $\pm$ SEM percent change from the average of the 3 baseline measurements obtained prior to injection of peptide or saline. The baseline values are shown in Table 1. The photocell data are shown as mean $\pm$ SEM horizontal photocell counts. Rats received 4 daily injections of saline (A, $N = 5$ first day; $N = 4$ last day), neurotensin (B, $N = 6$ both days) or DAMGO (C, $N = 7$ both days) into the A10 region, followed 4–6 d later by a subsequent injection. The first and last injections were made in the photocell cage with a dialysis probe in the nucleus accumbens. The data were statistically analyzed using a 2-way repeated-measures ANOVA followed by a least-significant difference test for comparisons between the first and last day within a given time bin. For the dopamine data: for saline, treatment $F(1, 7) = 0.173$, $p = 0.690$; time $F(8, 56) = 0.759$, $p = 0.640$; interaction $F(8, 56) = 1.511$, $p = 0.174$; $p < 0.05$ for neurotensin at 28–80 min, treatment $F(1, 10) = 11.652$, $p = 0.007$; time $F(8, 80) = 5.865$, $p < 0.001$; interaction $F(8, 80) = 4.208$, $p < 0.001$; for DAMGO, treatment $F(1, 12) = 5.101$, $p = 0.043$; time $F(8, 96) = 4.66$, $p < 0.001$; interaction $F(8, 96) = 1.885$, $p = 0.071$. For behavioral data, for saline, treatment $F(1, 7) = 0.455$, $p = 0.522$; time $F(8, 56) = 5.298$, $p < 0.001$; interaction $F(8, 56) = 0.86$, $p = 0.556$; for neurotensin, treatment $F(1, 10) = 4.973$, $p = 0.050$; time $F(8, 80) = 5.754$, $p < 0.001$; interaction $F(8, 80) = 2.022$, $p = 0.054$; $p < 0.05$ for DAMGO at 40 and 120 min, treatment $F(1, 12) = 12.350$, $p = 0.004$; time $F(8, 96) = 7.063$, $p < 0.001$; interaction $F(8, 96) = 1.28$, $p = 0.263$.

prising that unlike DAMGO, the behavioral stimulant effect of neurotensin was not correlated with the elevation in extracellular dopamine in the nucleus accumbens. This indicates that the enhanced release of dopamine in the nucleus accumbens by neurotensin is not mediating the behavioral response. Post-mortem neurochemical measurements of dopamine metabolites reveal that neurotensin injection into the A10 region increases dopamine transmission in many limbic and cortical dopamine

Figure 6. The cumulative data for the effect of daily neurotensin and DAMGO administration into the A10 region. * $p < 0.05$ for comparison of first day of injection to the last day using a 1-way ANOVA. For the dopamine data, for saline $F(1, 8) = 0.540$, $p = 0.824$; for neurotensin $F(1, 11) = 3.414$, $p = 0.007$; for DAMGO $F(1, 13) = 2.681$, $p = 0.020$. For the behavioral data, for saline $F(1, 7) = 0.540$, $p = 0.824$; for neurotensin $F(1, 11) = 6.165$, $p < 0.001$; for DAMGO $F(1, 13) = 9.781$, $p = 0.009$. For the DOPAC data, for saline $F(1, 7) = 0.719$, $p = 0.4251$; for neurotensin $F(1, 11) = 1.142$, $p = 0.310$; for DAMGO $F(1, 13) = 0.335$, $p = 0.563$. For the HVA data, for saline $F(1, 8) = 1.232$, $p = 0.304$; for neurotensin $F(1, 11) = 0.493$, $p = 0.499$; for DAMGO $F(1, 13) = 1.463$, $p = 0.252$.

terminal fields (Kalivas et al., 1983b; Cador et al., 1989), and perhaps extracellular dopamine levels in one of these nuclei would be better correlated with neurotensin-induced motor stimulation. Alternatively, dopamine may not be the sole determinant of neurotensin-induced behavior. Even the highest dose of neurotensin produced a maximal increase in extracellular dopamine of only 35%, while the highest dose of DAMGO elicited a 110% increase. Thus, dopamine may be a more critical determinant of the motor response to DAMGO since it elicits a quantitatively greater increase in dopamine transmission. This is supported by the fact that daily neurotensin produced a greater increase in extracellular dopamine (up to a 150% increase), and under these conditions the behavioral response was significantly correlated with extracellular dopamine.

Previous studies have noted a difference between the behavioral effects of intra-A10 administration of neurotensin and opioids. While opioids produce a general increase in exploratory behaviors, neurotensin produces a preferential elevation in rearing behavior (Kelley et al., 1980; Kalivas et al., 1983b; Cador et al., 1985). Also, neurotensin produces a decrease, while opioids enhance feeding behavior (Cador et al., 1986), and neurotensin elicits a rate-decreasing effect, while opioids produce a rate-increasing effect in an operant task (Kelley et al., 1989). The differences between neurotensin and DAMGO observed in these studies and the present report may be a reflection of differences in the mechanisms by which these peptides activate dopamine

Figure 7. Plots of the correlation between behavior and extracellular levels of dopamine and its metabolites in the nucleus accumbens. These data were derived from Figures 1–6. Behavior refers to the total horizontal photocell counts measured for 120 min after injection. The dopamine values were obtained by summing the percent changes in dopamine measured for 120 min after injection, then dividing by 6 (the number of dialysis samples collected over 120 min) to yield the average percent change. The line was drawn by linear-regression analysis. See Table 2 for the correlation coefficients.

perikarya. Using light and electron microscopic receptor autoradiography, neurotensin receptors have been localized almost exclusively on dopamine neurons in the A10 region (Palacios and Kuhar, 1981; Quirion et al., 1985; Woulfe and Beaudet, 1989; Dana et al., 1989), while μ -opioid receptors are on non-dopaminergic neurons (Dilts and Kalivas, 1989). These anatomical data are consistent with the electrophysiological studies showing that neurotensin activates dopamine neurons both by direct stimulation and by disinhibition from dopamine D_2 au-

receptor activation (Pinnock et al., 1985; Shi and Bunney, 1988; Seutin et al., 1989), and that μ -opioids act on nondopaminergic perikarya to disinhibit dopamine neuronal activity (Gysling and Wang, 1983; Matthews and German, 1984; Lacey et al., 1989). Based upon the fact that coadministration of the GABA_B agonist, baclofen, with DAMGO into the A10 region abolishes DAMGO-induced motor activity, it has been postulated that activation of μ -opioid receptors may inhibit GABA release in the A10 region, thereby disinhibiting dopamine neuronal activity (Kalivas et al., 1990). In addition to these receptor-mediated effects, it was found that neurotensin binds with a relatively low affinity (400 nM) to the apamin binding site on a Ca^{2+} -activated potassium channel to inhibit potassium flux (Hugues et al., 1982). It is possible that the concentrations of neurotensin achieved by microinjection could significantly inhibit this K^+ flux, which would activate dopamine neurons (Shepard and Bunney, 1988). Although many behavioral, neurochemical, and electrophysiological distinctions can be made regarding the actions of neurotensin and DAMGO in the A10 region, it is of interest that both neurotensin and opioids are self-administered into the A10 region (Bozarth and Wise, 1980; Glimcher et al., 1987), indicating that both peptides have similar reinforcing effects.

In addition to dopamine, an elevation in extracellular dopamine metabolites in the nucleus accumbens was also produced by intra-A10 administration of neurotensin or DAMGO. In general, the levels of DOPAC and HVA were not significantly correlated with either photocell counts or the concentration of dopamine. The lack of correlation between dopamine and its metabolites indicates that the extracellular levels of DOPAC and HVA may not directly reflect dopamine release. Since DOPAC is derived partly from the intracellular metabolism of released dopamine that has been taken back into the dopamine terminal (Chang and Ramirez, 1989), in postmortem tissue it has often been used as an index of dopamine neurotransmission (Bannon and Roth, 1983; Kalivas et al., 1983a, b; Kato et al., 1984; Commissiong, 1985; Latimer et al., 1987). However, it has also been argued that extracellular DOPAC results primarily from the degradation of intracellular pools of newly synthesized dopamine, and not of recently released dopamine (Zetterstrom et al., 1988). With regard to the present study, it is possible that dopamine metabolites may directly reflect dopamine release, but that the increase in dopamine release in response to neurotensin or DAMGO may be occurring more extensively in a

Table 2. Correlations between locomotion and extracellular amines in the nucleus accumbens

Comparison	Dose-response study		Sensitization study	
	DAMGO (15)	Neurotensin (20)	DAMGO (13)	Neurotensin (11)
Locomotion vs dopamine	0.735 ^b	0.028	0.573 ^a	0.661 ^a
Locomotion vs DOPAC	0.162	0.159	0.132	0.462
Locomotion vs HVA	0.090	0.293	0.472	0.261
Locomotion vs 5-HIAA	0.043	0.220	—	—
Dopamine vs DOPAC	0.128	0.070	0.215	0.718 ^a
Dopamine vs HVA	0.034	0.191	0.170	0.527
DOPAC vs HVA	0.868 ^b	0.475 ^a	0.665 ^a	0.724 ^a

Data show the correlation coefficient (r). The number in parentheses refers to the degrees of freedom in each group.

^a $p < 0.05$, using regression analysis.

^b $p < 0.005$.

Figure 8. Location of dialysis probes in the nucleus accumbens and injection cannula tips in the A10 region of all rats used in this study. In the *upper* charting, the location of each probe is indicated by a *vertical line* corresponding to a 2 mm length of the exposed dialysis membrane. The *dashed line* indicates the 2 probes that did not have at least 50% of the dialysis membrane in the nucleus accumbens. In the *lower* charting, the *filled circles* indicate cannulae placement from data used in the study, while the *open circles* indicate cannulae placement determined to be outside the A10 dopamine region. Although the cannulae were bilaterally implanted, for illustrative purposes, the bilateral cannulae tips are both drawn on the same side when their relative location differed. Distances given refer to distance from the interaural line according to the atlas of Paxinos and Watson (1986).

terminal field outside the nucleus accumbens. It would be expected that if not released in the immediate vicinity of the dialysis probe, dopamine would be rapidly metabolized and appear as DOPAC and HVA in the extracellular fluid adjacent to the probe (Rice et al., 1985).

The extracellular levels of the serotonin metabolite 5-HIAA were elevated by acute administration of both neurotensin and DAMGO. Although the increase in 5-HIAA following DAMGO was dose dependent, the increase after neurotensin was not over the dose range examined, with the lowest and highest doses of neurotensin producing statistically significant elevations. Previous studies have demonstrated an elevation (Garcia-Sevilla et al., 1978b), inhibition (Long et al., 1984), or no change (Widerlov et al., 1982) in postmortem serotonin synthesis or tissue concentrations of 5-HIAA after intracerebroventricular administration of neurotensin. In contrast, the majority of studies have found that activation of opioid receptors increases serotonin turnover in the brain (Yarbrough et al., 1973; Garcia-Sevilla et al., 1978a). Thus, the elevation in extracellular 5-HIAA is consistent with both peptides increasing serotonin transmission in the nucleus accumbens (however, see the discussion above regarding the physiological significance of monoamine metabolite measures). There are 3 possible explanations for the observed changes in 5-HIAA. (1) There are serotonin perikarya in the A10 region (Steinbusch, 1981), and activation of these neurons could enhance serotonin transmission in the nucleus accumbens. (2) The A10 region projects to the dorsal raphe (Domesick,

1988), and modulation of this projection could stimulate serotonin efferents to the nucleus accumbens. (3) There is behavioral and neurochemical evidence that serotonin transmission in the nucleus accumbens or striatum may regulate dopamine neurotransmission (Carter and Pycock, 1979; Kuczenski and Segal, 1989). Thus, feedback onto serotonergic neurons in the dorsal raphe and/or terminals in the nucleus accumbens resulting from peptide-induced activation of the mesoaccumbens dopamine projection may elicit the elevation in extracellular 5-HIAA.

The augmentation in extracellular dopamine following daily administration of neurotensin or DAMGO into the A10 region is consistent with previous studies. Daily administration of μ -opioids into the A10 region has been shown to produce an augmented behavioral response upon subsequent administration (Joyce and Iversen, 1979; Vezina and Stewart, 1984; Kalivas et al., 1985). The augmented behavioral response was found to be associated with an augmented increase in the postmortem concentration of DOPAC and HVA in the nucleus accumbens (Kalivas, 1985). Likewise, the behavioral response to neurotensin has been shown to augment with daily administration and to be associated with an enhancement in the postmortem concentration of DOPAC in the nucleus accumbens (Elliot and Nemeroff, 1985; Kalivas and Taylor, 1985). While the present data indicate that enhanced dopamine release in the nucleus accumbens may mediate the behavioral augmentation produced by daily peptide injection into the A10 region, they do not indicate the site of cellular alteration allowing for the enhanced

release of dopamine. Three possibilities exist: (1) a change in axon terminal regulation of dopamine release, (2) a change in somatodendritic regulation, making the neurons more easily stimulated, or (3) an alteration in the afferent regulation of the neuron promoting increased excitability. The present study does not distinguish between these mechanisms.

In summary, these data demonstrate acute microinjection of the μ -agonist, DAMGO, and neurotensin into the A10 region elicits an increase in extracellular dopamine in the nucleus accumbens that is associated with the motor stimulant response. However, only after DAMGO was the increase in dopamine significantly correlated with behavior. The data also demonstrate that behavioral sensitization to either daily injection of neurotensin or DAMGO is associated with increased extracellular levels of dopamine in the nucleus accumbens, supporting the contention that behavioral sensitization to these peptides may be mediated by enhanced dopamine release in the nucleus accumbens.

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