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Evidence for a substrate of neuronal plasticity based on pre- and postsynaptic neurotensin–dopamine receptor interactions in the neostriatum

(microdialysis/D_2 receptors/dopamine release/γ-aminobutyric acid release)

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ABSTRACT **The major mechanism underlying the neuroleptic action of the tridecapeptide neurotensin (NT) appears to be an interaction with dopamine receptor mechanisms based on biochemical binding and behavioral experiments. *In vivo* microdialysis was used in conscious rats to investigate the effects of local perfusion with NT on the sensitivity of striatal dopamine D_1 and D_2 receptors for their selective agonists by monitoring extracellular dopamine, 3,4-dihydroxyphenylacetic acid, homovanilic acid, and γ-aminobutyric acid levels in the awake unrestrained male rat. Perfusion with NT (10 nM) counteracted the inhibitory effects of the dopamine D_2 agonist pergolide (500 nM) on extracellular levels of dopamine and γ-aminobutyric acid. In contrast, NT (10 nM) significantly enhanced the reduction of extracellular striatal levels of dopamine after perfusion with the D_1 agonist SKF 38393 (5 μM), and this combined treatment also resulted in a significant increase in the extracellular striatal levels of γ-aminobutyric acid. These results provide *in vivo* evidence that NT regulates central dopamine transmission by reducing pre- and postsynaptic dopamine D_2 and enhancing D_1 receptor sensitivity possibly through an antagonistic NT receptor–D_2 receptor interaction. This heteroregulation has the potential to substantially increase the plasticity within the dopamine synapse.**

The dopaminergic system in mammalian forebrain has been implicated in disorders such as schizophrenia and Parkinson disease. Therefore, the regulation of this system may have important implications for the pathophysiology and development of treatments of these disorders. The tridecapeptide neurotensin (NT) has been shown to be closely associated with this dopamine system and to display a neuroleptic or "antidopamine"-like profile; however, the nature of this "regulation" remains unclear (1–4). Several lines of evidence suggest that dopamine is involved in the control of γ-aminobutyric acid (GABA) neurons in the striatum. Thus, it has been shown that dopaminergic axons have direct synaptic connections with medium-sized spiny GABAergic neurons in rat striatum (5, 6) and that dopamine D_1 and D_2 receptors differentially regulate striatal GABA release and are stimulatory and inhibitory, respectively (7, 8). Binding and lesion studies have suggested that dopamine terminals and soma in the neostriatum contain NT receptors (9). NT reduces the affinity of dopamine D_2 but not dopamine D_1 receptors in membrane preparations from the nucleus accumbens, tuberculum olfactorium, and neostriatum (10–14). Thus, it has been postulated that the antagonism of dopamine function by NT is related to the existence of an antagonistic interaction within the nerve cell membrane between NT and dopamine D_2 receptors (10–12).

In the present study we tested the hypothesis that in the neostriatum of the awake unrestrained rat (15), NT selectively reduces dopamine D_2 receptor control of dopamine release, which leads to a switching of dopamine transmission toward dopamine D_1 receptor-mediated responses. Toward this aim we investigated the effects of local perfusion with NT on the sensitivity of dopamine D_1 and D_2 receptors for their selective agonists by monitoring extracellular dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanilic acid (HVA), and GABA levels. Thus, we evaluated the effects of local perfusion of the neostriatum with NT alone (a high and a low dose) and with pergolide (a preferential dopamine D_2 receptor agonist) or SKF 38393 (a preferential dopamine D_1 receptor agonist) (16, 17) by measuring extracellular levels of dopamine, DOPAC, HVA (18), and GABA (19) from a microdialysis probe (20, 21) permanently implanted into the neostriatum of the awake unrestrained male rat. Basal extracellular striatal dopamine and GABA levels measured using this technique have been shown (22–24) to be both voltage- and calcium-sensitive and as such can be considered to be derived predominantly from neuronal origin.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (Alab, Stockholm) weighing 350–400 g were used in this study. Animals were maintained on a standard light/dark cycle and allowed free access to food and water.

Surgery. During surgery the animals were mounted into a Kopf stereotaxic frame, and body temperature was continuously maintained at 37°C with a temperature controller (CMA 150, Carnegie Medicin, Stockholm). The animals were maintained under 1.5% halothane/98.5% air anaesthesia (delivered at 1.4 liters/min). After exposure of the skull and drilling of a burr hole, a microdialysis probe was stereotaxically implanted into the neostriatum at AP = 0.7 mm, L = 2.5 mm, DV = −5.5 mm; relative to bregma and dural surface; incisor bar −2.5 mm (25). The probe was permanently secured with methacrylic cement to two stainless steel screws that were implanted onto the skull.

Microdialysis Probes. The microdialysis probe used in the present study (CMA 10, Carnegie Medicin) was of concentric design. It consists of a tubular semipermeable membrane (polycarbonate; M_r 1500 cut-off; o.d., 0.5 mm; i.d., 0.4 mm; length, 2 mm) glued to a cannula (o.d., 0.6 mm) and sealed

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Abbreviations: NT, neurotensin; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanilic acid; GABA, γ-aminobutyric acid; TTX, tetrodotoxin.

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with glue at the tip. The perfusion medium is carried to the tip of the microdialysis probe (2 μl/min) by a fine inner barrel. The microdialysis membrane functions as an osmotic barrier so that compounds flow down their concentration gradient from the extracellular fluid into the continuous-flowing perfusion medium or vice versa.

Microdialysis Procedure. The animals were allowed to recover for 48 h after probe implantation. To prevent induction of adaptive mechanisms, the experiments were performed in a random order on either the second or third day after surgery. On the day of each experiment, the rat was placed in a modified activity bowl. The inlet tubing of the probe was connected to a liquid swivel and perfused with Ringer solution (147 mM Na^+/4 mM K^+/2.4 mM Ca^{2+}/154 mM Cl^-, pH 6.0) at a flow rate of 2 μl/min. In a set of separate experiments, the Na^+ channel antagonist tetrodotoxin (TTX; 1 μM) was added to the perfusion medium to determine if the NT-induced increase in striatal dopamine release was voltage-dependent. The doses of pergolide (500 nM) and SKF 38393 (5 μM) employed in this study are based on published dopamine dose–response curves (8, 26). We recognize that the 5 μM dose of SKF 38393 appears rather high compared with that administered for pergolide. However, preliminary data had shown the lower 500 nM dose of SKF 38393 was without effect on dopamine levels. In addition, the higher 5 μM dose of SKF 38393 failed to effect extracellular DOPAC and HVA levels. Finally, a previous microdialysis study has reported that SKF 38393 will selectively abolish the effects of SCH 23390 on striatal dopamine release and that a 1000-fold higher dose of SKF 38393 was necessary to obtain comparable effects with other selective D_1 agonists (27). Dialysates were collected every 30 min during the experiment. The dialysate collected during the first 30 min on both days served as a "washout fraction" before stable baseline levels were achieved and was thus discarded. At the end of each experiment the animal was disconnected from the swivel, the inlet and outlet tubings were cut and sealed, and the animal was returned to its home cage. At the end of the study rats were killed with an overdose of Mebumal (120 mg/kg i.p., Nord Vacc, Stockholm). The brain was removed from the skull, and the position of the microdialysis probe was verified by sectioning in a cryostat and microscopic examination.

Dopamine, DOPAC, HVA, and GABA Analysis. Forty microliters of each dialysate sample (60 μl) was assayed for dopamine, DOPAC, and HVA by HPLC separation followed by electrochemical detection. The HPLC apparatus consisted of two 100 × 3 mm reversed-phase columns packed with Spherisorb 5C18, a Walters dual piston pump, a LCD/Milton Roy CI-10 integrator, and a BAS LC 4B detector. The mobile phase consisted of 0.15 M sodium phosphate/0.1 mM EDTA/0.6 mM sodium octanesulfonate/17% (vol/vol) methanol, adjusted to pH 3.8. The flow rate of the mobile phase was 0.5 ml/min. The limit of detection of this assay was 0.02 pmol of dopamine. Ten microliters of each dialysate sample was assayed for GABA. The GABA assay has been described in detail (19). This assay is based on precolumn derivatization with an *o*-phthaldialdehyde/*t*-butyl thiol reagent, separation by reverse-phase HPLC on a column (4 mm in diameter) packed with Neucleosil 3 (C_{18}) resin, followed by electrochemical detection. Microdialysis perfusate samples containing 10 μl were placed in a refrigerated autoinjector (CMA 200, Carnegie Medicin) and injected directly onto this system. The mobile phase consisted of 0.15 M sodium acetate/1 mM EDTA, pH 5.4/50% (vol/vol) acetonitrile and was run under isocratic conditions. The flow rate was 0.8 ml/min. The limit of detection for this assay was 0.05 pmol of GABA.

RESULTS

Studies on Striatal Dopamine Release and Extracellular Dopamine Metabolite Levels. As shown in Fig. 1*A*, perfusion with the highest dose of NT (1 μM) was associated with a significant increase in striatal dopamine release (35%). The maximal effect was observed 90–120 min after the onset of perfusion with the peptide. The enchancement in striatal dopamine release after perfusion with the 1 μM dose of the peptide was completely counteracted when TTX (1 μM) was included in the perfusion medium (Fig. 1*B*). Extracellular HVA, but not DOPAC levels, were also increased in this brain region with respect to the control animals (data not shown) after perfusion with the 1 μM dose of NT. In contrast, perfusion with a low dose of NT (10 nM) had no effect on extracellular levels of dopamine or dopamine metabolites. Perfusion with pergolide (500 nM) produced a long-lasting inhibition of the extracellular striatal levels of dopamine (down to 60% of control values). However, perfusion with

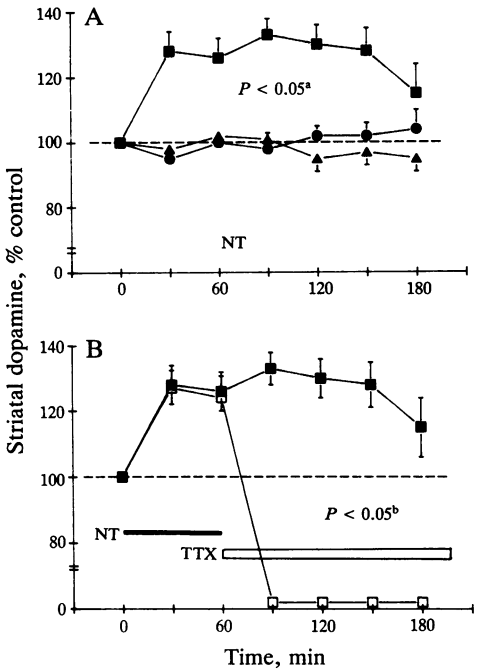

FIG. 1. (*A*) Time–response curves showing the effects of NT on extracellular dopamine levels in the neostriatum of awake freely moving rats. NT at 1 μM (■) and 10 nM (●) in the perfusion medium was perfused locally in the neostriatum for 60 min (solid bar in *B*). Control rats (▲) were not perfused with the peptide. Only the highest dose of NT increased striatal extracellular dopamine levels. The changes in extracellular dopamine levels are expressed as percent of three basal values. The baseline was calculated by averaging the three consecutive basal dopamine values collected immediately before local perfusion with NT. Basal dopamine level measured in 30-min perfusate fractions was 0.226 ± 0.03 pmol/40 μl of perfusate (mean ± SEM; 16 rats; 5 or 6 rats in each group). The maximum peak values are as follows: 7 ± 2%, 9 ± 4%, and 43 ± 6% (significantly different from control; $P < 0.05$) for the control and 10 nM and 1 μM NT groups, respectively. (*B*) The effects of perfusion with TTX (1 μM) and NT (1 μM) on extracellular dopamine levels. TTX (□) abolished the NT (■)-induced increase in extracellular dopamine (3 rats). Samples were collected and analyzed as in *A*. The solid bar indicated the period of perfusion with NT alone. The open bar indicated the period of perfusion with the TTX solution. The maximum and minimum peak values are as follows: for 1 μM NT, 27 ± 5.5%; for 1 μM TTX, −95 ± 5% (significantly different from NT; $P < 0.05$). NT was directly dissolved in the perfusion medium (Ringer solution). $P < 0.05^a$, significantly different from control; $P < 0.05^b$, significantly different from NT, where the superscripts a and b indicate significance for area under the curve. One-way ANOVA with the least square difference test (28).

the low concentration of NT (10 nM), which by itself had no effect on extracellular dopamine levels, plus pergolide (500 nM) significantly and markedly reduced the inhibitory effects of this dopamine D_2 agonist on extracellular levels of dopamine (Fig. 2*B*). Perfusion with SKF 38393 (5 μM) alone induced a reduction of extracellular striatal dopamine levels. In contrast, this decrease was significantly enhanced by simultaneous perfusion with NT (10 nM), as evaluated from the overall effects (area values) (Fig. 2*A*). With regard to the dopamine metabolites, this low dose of NT also significantly counteracted the inhibitory effects of pergolide on the striatal extracellular levels of DOPAC and elevated extracellular levels of HVA (Fig. 3 *A* and *B*, respectively). In contrast, perfusion with SKF 38393 (data not shown) had no effect on

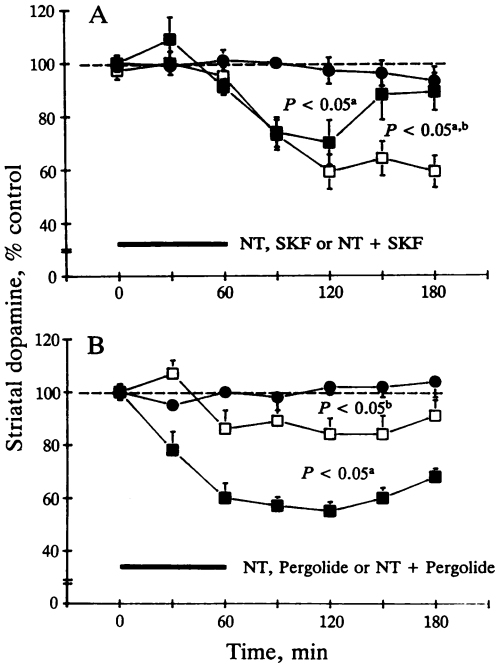

FIG. 2. Effect of NT on SKF 38393 (D_1)- and pergolide (D_2)-induced inhibition of dopamine release in the neostriatum of awake freely moving rats. The effects of local perfusion with either SKF 38393 (SKF; 5 μM) (*A*) or pergolide (500 nM) (*B*) on extracellular dopamine levels were estimated in 30-min perfusate fractions. SKF 38393 (D_1) or pergolide (D_2) were administered alone (■) or with 10 nM NT [agonist plus NT (□)] for 60 min (solid bars). NT alone (10 nM), which by itself has no effect on dopamine levels, was employed as the control (●). NT significantly counteracted the inhibitory effects of pergolide and enhanced the inhibitory effect of SKF 38393 on dopamine release in the striatum. The dose of SKF 38393 administered here is 100 times greater than that for pergolide (*A*). A lower (500 nM) dose of SKF 38393 had no effect on dopamine release. The baseline for statistical analysis was calculated by averaging the three consecutive basal dopamine values collected immediately before local perfusion with NT and/or agonist. Each data point represents the mean ± SEM (bars) value (5 or 6 rats in each group). The minimum peak values in *A* were −10 ± 4%, −34 ± 4%, and −47 ± 5% for control (10 nM NT), SKF 38393 (5 μM), and SKF 38393 plus NT, respectively, where values for SKF 38393 and SKF 38393 plus NT are significantly different from controls ($P < 0.05$). The minimum peak values in *B* were −11 ± 3%, −46 ± 4%, and −20 ± 5% for control, pergolide (500 nM), and pergolide plus NT, respectively, where the value for pergolide is significantly different from control ($P < 0.05$) and the value for pergolide plus NT is significantly different from agonist alone ($P < 0.05$). One-way ANOVA with the least square difference test (28). Pergolide and SKF 38393 were directly dissolved in the perfusion medium (Ringer solution). $P < 0.05^a$, significantly different from control; $P < 0.05^b$, significantly different from agonist alone; $P < 0.05^{a,b}$, significantly different from both control and agonist alone; superscripts a and b indicate significance for area under the curve.

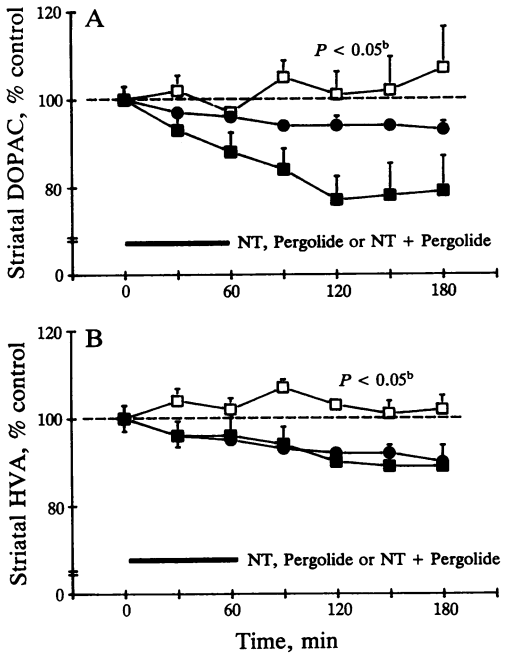

FIG. 3. Effect of NT on the pergolide-induced inhibition of extracellular DOPAC and HVA levels in the neostriatum of awake freely moving rats. The effects of local perfusion with pergolide (500 nM) alone or plus NT (10 nM) on extracellular DOPAC (*A*) and HVA (*B*) levels were estimated in 30-min perfusate fractions. Pergolide was administered alone (■) or in the presence of NT (□) for 60 min (solid bar). The dose of NT (10 nM), which by itself had no effect on DOPAC or HVA levels, was employed as the control (●). NT significantly counteracted the inhibitory effects of pergolide on striatal extracellular DOPAC and HVA levels. Basal DOPAC and HVA levels measured in 30-min perfusate fractions were 17.32 ± 2.2 pmol/40 μl for DOPAC and 11.17 ± 1.35 pmol/40 μl for HVA (mean ± SEM; 15 rats; 5 or 6 rats in each group). (*A*) The minimum peak values for DOPAC were −15 ± 4%, −26 ± 7%, and −6 ± 2% for control (10 nM NT), pergolide (500 nM), and pergolide plus NT, respectively. (*B*) The minimum peak values for HVA were −12 ± 3%, −13 ± 3%, and −4 ± 1% for control, pergolide, and pergolide plus NT, respectively, where the value for pergolide plus NT is significantly different from pergolide alone ($P < 0.05$). One-way ANOVA with the least square difference test (28) was used. $P < 0.05^b$, significantly different from pergolide alone; the superscript b indicates significance for area under the curve.

extracellular striatal DOPAC and HVA levels. Furthermore, perfusion with NT (10 nM) and SKF 38393 failed to influence striatal extracellular levels of DOPAC and HVA.

Studies on Striatal GABA Release. The same experiments as in Fig. 2 were done. The effects of NT, agonist, or both on extracellular striatal GABA release in the 30-min perfusate after the onset of perfusion are shown in Fig. 4. Perfusion with the low dose of NT (10 nM) had no effect on extracellular GABA levels. However, perfusion with pergolide (500 nM) produced inhibition of extracellular striatal GABA levels (down to 77% of control values; Fig. 4*B*). This inhibition of GABA release by pergolide was fully antagonized by NT (10 nM).

In contrast, combined treatment with SKF 38393 (5 μM) and NT (10 nM) resulted in a significant increase in GABA release (17%) compared with SKF 38393 alone (Fig. 4*A*). This increase was maintained for the entire experimental period (3 h, data not shown), whereas the ability of NT to reduce the inhibitory effect of pergolide on GABA release was maximal 30 min after the onset of perfusion and lasted 90 min.

DISCUSSION

The major findings in this study are that NT in a concentration (10 nM) that does not modulate extracellular striatal

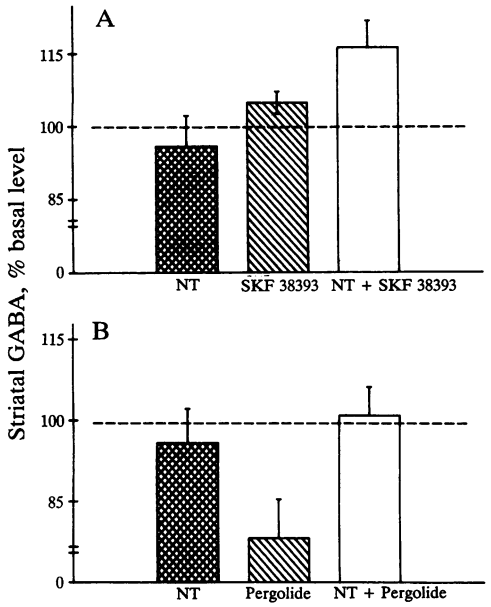

FIG. 4. Effect of NT on SKF 38393 (D_1)- and pergolide (D_2)-induced effects on GABA release in the neostriatum of awake freely moving rats. The effects of local perfusion with either SKF 38393 (5 μM) (*A*) or pergolide (500 nM) (*B*) on extracellular GABA levels were estimated in the 30-min perfusate after the onset of perfusion with NT and/or NT plus agonist. SKF 38393 (D_1) or pergolide (D_2) were administered alone or with 10 nM NT for 60 min. NT (10 nM) alone, which has no effect on GABA levels, was used as the control. NT significantly counteracted the inhibitory effects of pergolide and enhanced the excitatory effect of SKF 38393 on GABA release in the striatum. The dose of SKF 38393 administered is 100 times greater than that for pergolide. Basal GABA levels measured in 30-min perfusate fractions were 0.152 ± 0.07 nmol/10 μl of perfusate (mean ± SEM; 6 rats; 3 or 4 rats in each group). The baseline for statistical analysis was calculated by averaging the three consecutive basal GABA values collected immediately before local perfusion with NT and/or agonist. The minimum peak values in *A* were 96 ± 6%, 105 ± 2%, and 116 ± 5% for control (10 nM NT), SKF 38393 (5 μM), and SKF 38393 plus NT, respectively, where the value for SKF 38393 plus NT is significantly different from agonist alone ($P < 0.05$). The minimum peak values in *B* were 96 ± 6%, 78 ± 7%, and 100 ± 5% for control, pergolide (500 nM), and pergolide plus NT, respectively, where the value for pergolide plus NT is significantly different from agonist alone ($P < 0.025$) by the Student *t* test. Pergolide and SKF 38393 were directly dissolved in the perfusion medium (Ringer solution).

dopamine, DOPAC, HVA, and GABA levels, not only specifically counteracts the inhibitory effects of the preferential dopamine D_2 receptor agonist pergolide on dopamine release and metabolism (DOPAC and HVA) but also selectively reduces the inhibitory effect of dopamine D_2 receptor activation on GABA release in the striatum of the awake unrestrained male rat. It is probable that, at the low concentration used (500 nM), pergolide acts to reduce dopamine release by activation of inhibitory dopamine D_2 autoreceptors located on the striatal dopamine nerve terminals (29). In view of the existence of NT receptors located presynaptically on the striatal dopamine nerve terminals (9) and since NT reduces the affinity of D_2 receptors in striatal membranes (10–14), it is probable that activation of the NT receptor reduces the function of the adjacent dopamine D_2 autoreceptors (30). This interaction has been suggested to control the sensitivity of dopamine D_2 autoreceptors by a receptor–receptor interaction (10–12, 14, 31). It seems unlikely that a direct binding between NT and pergolide could explain the present findings, since pergolide lacks the catechol hydroxyl groups necessary for such a binding to occur (32). Thus, we provide *in vivo* evidence that dopamine–NT interactions regulate central dopamine transmission in the awake unrestrained animal at the dopamine terminal through a receptor–receptor interaction. An effect of NT on striatal acetylcholine may not be involved in increasing dopamine release, since perfusion with NT even in concentrations up to 1 μM fails to influence acetylcholine release in the striatum (33). However, these studies are complicated by the necessity to use cholinesterase inhibition. The dose of the inhibitor (34) and the ionic composition of the perfusion medium (35) have been shown to influence the responsiveness of striatal acetylcholine release. The increase in dopamine release induced by the high dose of NT (1 μM) that reflects an extracellular NT concentration $\approx$100 times above the K_d value of local NT receptors is instead postulated to be caused by a direct action of NT on voltage-dependent Ca^{2+} channels, since this effect is blocked by TTX and has been shown to be insensitive to dopamine autoreceptor activation (31).

Postsynaptic striatal D_2 receptors have been shown (7) to inhibit GABA release. Based on the present observations that NT (10 nM) can counteract the D_2 agonist-induced inhibition of striatal GABA release, it seems possible that upon activation, NT receptors *in vivo* can also reduce the transduction of postsynaptic D_2 receptors located on the GABA-containing medium-sized striatal neurons. Biochemical binding experiments on crude membrane preparations and behavioral evidence (3, 10, 36) also suggest that an antagonistic NT–D_2 receptor interaction in fact can occur also between NT and postsynaptic dopamine D_2 receptors. Finally, in the halothane-anaesthetized rat, NT (10 nM) by itself can increase GABA release in the dorsal striatum (33), which is in line with the above results, although such an action by NT could not be found in the present study on the awake unrestrained male rat. Thus, the consequence of the pre- and postsynaptic antagonistic NT–D_2 receptor interaction is to favor a reduction of both pre- and postsynaptic D_2 receptor function.

In contrast, NT (10 nM) actually enhances the dopamine D_1 receptor agonist SKF 38393-induced inhibition of striatal dopamine release. In addition, the observation that SKF 38393 either in the absence or presence of NT (10 nM) fails to influence striatal dopamine metabolism suggests that a mechanism other than a direct inhibition of tyrosine hydroxylase most likely explains the inhibitory effects of this drug on dopamine release in the present study. The present findings suggest that striatal GABA release may not be directly involved in the mediation of SKF 38393-induced inhibition of dopamine release since, at the dose employed in the present study, SKF 38393 alone failed to influence striatal GABA release. However, GABA inhibits dopamine release in the neostriatum by presynaptic actions on dopamine nerve terminals (37, 38), and in the present study we report that the NT enhancement of the inhibitory action of SKF 38393 on dopamine release parallels an increase in striatal GABA release. It is probable that the released GABA may act directly on the dopamine nerve terminal to further reduce dopamine release but GABA could also act by relieving the inhibition of the inhibitory striatonigral GABA pathways to the nigral dopamine cells (8, 39). Since NT (10 nM) does not directly modulate dopamine D_1 receptors (14), the NT-induced enhancement of the dopamine D_1-mediated inhibition of dopamine release may be secondary to the corresponding reduction in postsynaptic dopamine D_2 receptor function. Thus, dopamine D_2 receptor activation leads to a reduction of striatal GABA release (8, 40) and antagonistic dopamine D_1–D_2 receptor interactions have been shown to exist (41, 42).

In conclusion, the present results suggest that NT may switch dopamine transmission from the dopamine D_2 toward the D_1 receptor subtype (Fig. 5). We also provide strong evidence that this switching mechanism may reflect an an-

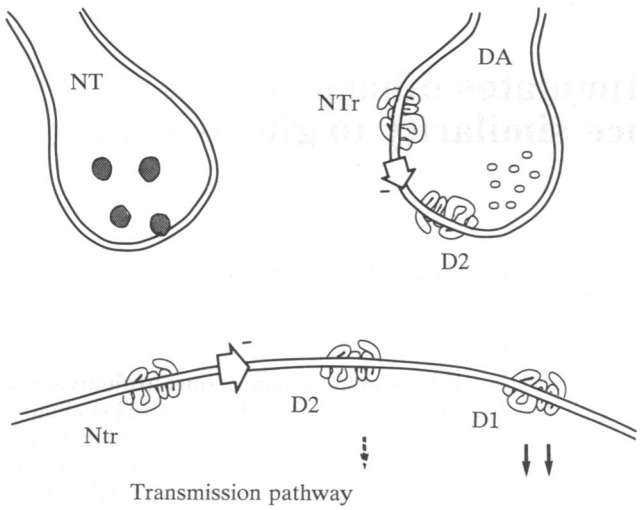

FIG. 5. Possible pre- and postsynaptic intramembrane interactions between NT receptors (NTr) and dopamine (DA) D_2 receptors in the neostriatum. Activation of NT receptors causes a reduced affinity in D_2 agonist binding sites and an enhanced effect of D_1 agonists. The broken arrow illustrates the reduced transmission of the D_2-mediated dopamine signal and the two solid arrows illustrate the increased transmission of the D_1-mediated dopamine signal. Only the interactions demonstrated in the present study are indicated (see ref. 14).

tagonistic receptor–receptor interaction between NT and dopamine D_2 receptors at both the pre- and postsynaptic level. It may be speculated that the switch from dopamine D_2 toward D_1 transmission by NT is disturbed in schizophrenia and tardive dyskinesias, where dopamine D_2 transmission is thought to be inappropriately increased (43). This heteroregulation has the potential to substantially increase plasticity within the dopamine synapse.

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