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Short Communication

Activation of neurotensin receptors in the prefrontal cortex stimulates midbrain dopamine cell firing

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Abstract

The effects of medial prefrontal cortex microinjections of 3 nmol/0.5 μl of neurotensin-(1–13), the inactive fragment neurotensin-(1–8), or vehicle on the firing rate of midbrain dopamine neurons were studied in anesthetized rats. Twelve of 19 cells tested with neurotensin-(1–13) showed an average 20–25% increase in firing rate between 10 and 20 min after the injection. This effect was not mimicked by neurotensin-(1–8) (9 cells), nor by a control injection (10 cells) suggesting that it is mediated by high-affinity neurotensin receptors. These results suggest that activation of neurotensin receptors in the medial prefrontal cortex can modulate neural activity of a subpopulation of midbrain dopamine neurons. © 1998 Elsevier Science B.V.

Keywords: Dopamine; Medial prefrontal cortex; Neurotensin; Ventral tegmental area

1. Introduction

Recent evidence suggests that efferents from the medial prefrontal cortex can modulate subcortical dopamine neurotransmission. Hence, Sesack and Pickel (1992) demonstrated that some efferents from the prefrontal cortex establish synaptic contacts with midbrain dopamine neurons and Gariano and Groves (1988) reported that electrical stimulation of the prefrontal cortex increases midbrain dopamine cell firing. Prefrontal cortex electrical stimulation was also found to increase dopamine release in the nucleus accumbens, an effect that is blocked by application of NMDA receptor antagonists into the ventral tegmental area (Taber et al., 1995). Other studies suggest that dopamine itself may play an important role in the modulation of cortical efferents to the ventral midbrain. Substances that block or potentiate dopamine neurotransmission in the prefrontal cortex, for instance, were reported to potentiate and inhibit respectively subcortical dopamine function (Vezina et al., 1991; Doherty and Gratton, 1996). This modulation of subcortical dopamine neurotransmission may also be under the control of neurotensin, a neuropeptide that is co-localized and co-released with dopamine in the prefrontal cortex (Bean and Roth, 1991). An interaction between dopamine and neurotensin in the prefrontal cortex is suggested by the presence of high-affinity neurotensin receptors in the deep layers of the cortex innervated by dopamine terminals and by electrophysiological results showing that neurotensin attenuates the inhibitory effect of dopamine on prefrontal cortex neurons (Beauregard et al., 1992). In further support are recent results showing that microinjections of neurotensin in the prefrontal cortex attenuate the inhibition of locomotor activity produced by local injection of GBR 12909, an indirect dopamine agonist (Radcliffe and Erwin, 1996). The present study was aimed at testing the effects of activating neurotensin receptors in the prefrontal cortex on the firing activity of midbrain dopamine neurons.

2. Materials and methods

2.1. Animals

Male Long–Evans rats (Charles River, St. Constant, Québec) weighing 300–350 g were used. They were housed two per cage with free access to food and water in a temperature- and humidity-controlled room with a 12 h light/dark cycle (lights on at 06:30 h).

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2.2. Surgery and recording

Animals were anesthetized with urethane (1.2 g/kg, i.p.) and mounted on a stereotaxic instrument. The surface of the cranium was exposed and a guide cannula was implanted in the medial prefrontal cortex at the following stereotaxic coordinates: 3.2–3.6 mm anterior to bregma, 0.6 mm lateral and 3 mm below the cranium (Paxinos and Watson, 1986). The bone and dura above the ipsilateral midbrain were removed and action potentials from dopamine neurons were recorded with a glass micropipette that was lowered with a microdrive. The micropipette was pulled to a tip diameter of 1–2 mm and filled with 2 M NaCl solution that contained 0.1% Fast green solution; impedance ranged from 2–6 MΩ at 1000 Hz. The recorded signal was fed into a high input impedance amplifier and displayed on a digital oscillosope. Putative dopamine action potentials were identified according to the classical criteria: (i) bi- or triphasic action potentials with a duration larger than 2.5 ms with an initial positive-going segment followed by a wide negative segment; (ii) baseline rate of firing between 1 and 8 Hz; and (iii) location in the ventral tegmental area or the medial substantia nigra (see Bunney and Grace, 1978).

Once a dopamine action potential was identified, its baseline firing rate was recorded for at least 5 min and a 0.5 μl solution of 3 nmol of neurotensin-(1–13), neurotensin-(1–8), or saline, was injected into the prefrontal

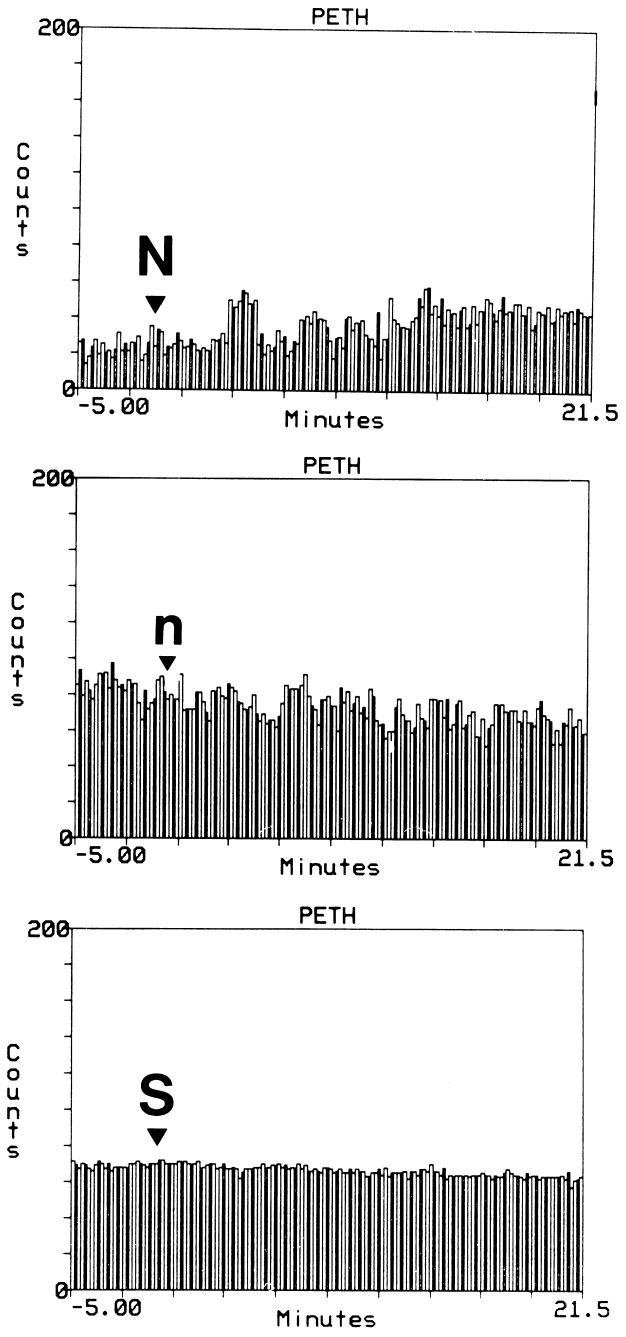

Fig. 1. Peri-event time histograms illustrating the rate of firing measured before and after an injection of neurotensin-(1–13)[N, top panel], neurotensin-(1–8)[n, middle panel] and saline (S, bottom panel); time of injection is indicated by the arrow. Each bar represents the number of action potentials per 10 s period (counts).

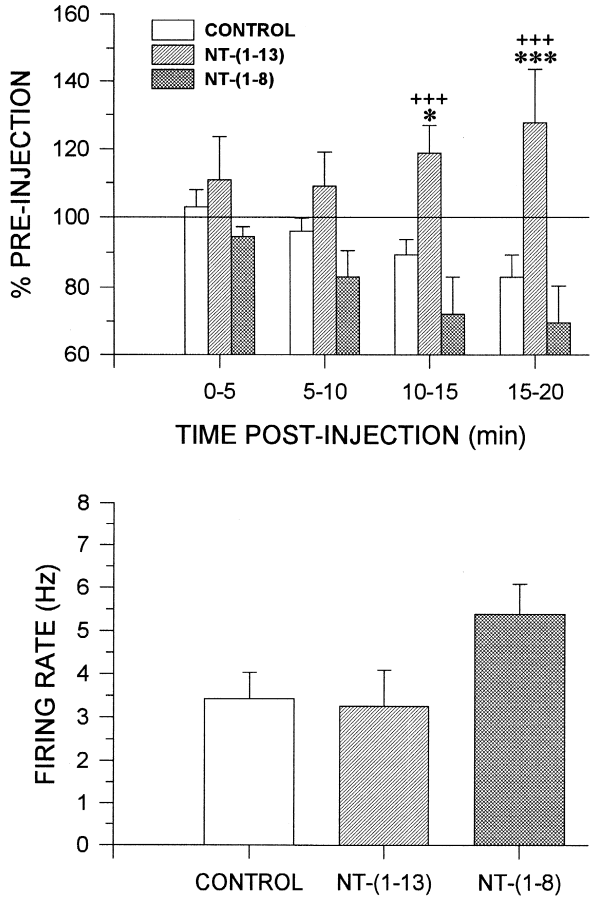

Fig. 2. Percent of baseline firing rates (mean ± S.E.M.) measured at each of the four 5 min periods that followed the central injection for each group (top panel). Mean baseline firing rates of the cells included in each group are shown in the bottom panel (mean ± S.E.M.). Asterisks and the crosses (top panel) indicate a statistically significant difference from the control and the neurotensin-(1–8) group, respectively.

cortex over a period of 60–90 s. The injection cannula was connected to a 2 μl Hamilton syringe and was at least 1 mm longer than the guide. The injection cannula was inserted into the brain at the beginning of the recording session and left in place for the entire recording period. The rate of firing was recorded for at least 20 min after the injection, and in some animals, the sensitivity of the cell to quinpirole (100 μg/kg, s.c.) was tested. For some cells, rate of firing was recorded for a period of about 30 min (no injection) and served as a control (see below). Only one cell was recorded per animal.

2.3. Drugs

Neurotensin-(1–13) and neurotensin-(1–8) (Bachem, CA) were dissolved in sterile 0.9% saline at a concentration of 6 nmol/μl and stored frozen at −20°C in 50 μl aliquots. Peptide solutions were thawed just before testing and used only once. Quinpirole (RBI, USA) was dissolved in saline and injected s.c. in a volume of 1 ml/kg.

2.4. Histology

At the end of the experiment, the recording site was marked with Fast Green by passing a 25 μA direct cathodal current for 30 min through the recording pipette. Deeply anesthetized animals were then transcardially perfused with 0.9% saline followed by 10% formalin. Brains were removed, stored in 10% formalin and subsequently sliced in serial 20 μm sections that were stained with a formal-thionin solution. Location of the recording sites as marked by Fast Green dye deposit, and of the injection site were determined under light microscopic examination.

2.5. Data analysis

The recorded signal was digitized and stored on a CPU disk for off-line analysis. Dopamine action potentials were discriminated from noise using the Spike Sort Module of Discovery Software (DataWave Technologies, CO, USA) and peri-event time histograms were generated using 10 s bin periods. For each cell, mean firing rates were calculated for the 5 min period that preceded the injection and for every 5 min period that followed. Values were then expressed as percent of baseline firing rate, averaged and grouped according to drug treatment. A cell was considered as being activated when its mean rate of firing increased by more than 10% when compared to pre-injection. Control group data consisted of results obtained from four cells in which no injection was made and from 6 additional cells tested with saline. Statistical significance was determined with a two-way analysis of variance for repeated measures on time factor. Differences among means were determined with the LSD post-hoc test with the level of significance set to 0.05 (Statistica V5.0, Statsoft, USA).

3. Results

A total of 55 dopamine cells were recorded and complete sets of data were obtained from 38 of them. For the other 17 cells [4 tested with neurotensin-(1–13), 8 with neurotensin-(1–8) and 5 with saline], the rate of firing became erratic soon after the injection and action potentials disappeared. Nineteen cells were tested with neurotensin-(1–13) and of these, 12 showed an increase in firing; the firing rate of the other 7 cells did not change. Nine cells were tested with neurotensin-(1–8) and all of them showed a small decrease in firing during the 20 min period that followed the injection. Examples of the results obtained after each treatment is shown in Fig. 1. Neurotensin-(1–13) (top panel) produced a clear increase in firing occurring, in this case, approximately 10 min after the injection, an effect not seen with neurotensin-(1–8) (middle panel) nor with saline (bottom panel). Mean percentage change in firing rates measured after the injection for each treatment group are shown in Fig. 2 (top panel); only the 12 cells that showed an increase in firing are included in the neurotensin-(1–13) group. The analysis of variance yielded a significant effect of treatment ($F(2,28) = 13.3$, $P < 0.001$), and the post-hoc test revealed that neurotensin-(1–13) produced a significant increase in firing compared to control between 10 and 20 min after the injection ($P < 0.05$ and 0.001); the effects of neurotensin-(1–13) were also significantly different from the effects of neurotensin-(1–8) at the same time periods ($P < 0.001$). Although neurotensin-(1–8) produced a decrease in firing, a similar effect was observed in the control group so that there was no difference between these conditions. The mean baseline firing rates of those cells included in the control and in the neurotensin-(1–13) group were very similar, while the one for cells included in the neurotensin-(1–8) group was slightly higher; the analysis of variance, however, yielded no significant main effect ($F(2,28) = 2.3$, $P > 0.11$). The mean baseline firing rates of the seven cells that did not respond to neurotensin-(1–13) was 4.1 (±0.96) Hz, a value similar to that of DA cells that showed an increase in firing (Fig. 2). Eleven of the 38 cells were tested with quinpirole (data not shown), and all of them responded with a decreased firing, an effect that generally occurred between 6 and 10 min after the injection.

Histological analysis showed that the recorded cells were located in the ventral tegmental area, or near the medial substantia nigra, between 5.8 and 4.8 mm posterior to bregma. Injection sites were all located in the medial prefrontal cortex between 2.7 and 3.7 mm anterior to bregma, within the infralimbic or the ventral cingulate cortex. The locations of those cells that did not respond to

neurotensin-(1–13) were not clearly different from those that increased firing, nor were the injection sites different.

4. Discussion

The major finding of this study is that activation of neurotensin receptors in the medial prefrontal cortex stimulates firing activity of a significant number of midbrain dopamine neurons. Several characteristics of the recorded signal suggest that they were from dopamine cells. The shape and duration of the action potentials and the baseline firing rate were similar to those reported for identified dopamine neurons (Bunney and Grace, 1978). Moreover, all cells were located within ventral midbrain, areas known to contain dopamine cell bodies, and as expected, all cells tested with quinpirole showed a decrease in firing. The hypothesis that the increase in dopamine cell firing observed with neurotensin-(1–13) is due to activation of neurotensin receptors is supported by the fact that its effect was not mimicked by an equimolar concentration of neurotensin-(1–8), a neurotensin fragment that displays no physiological activity at the high affinity neurotensin receptor and by the presence of neurotensin receptors in the regions of injection (Hervé et al., 1986). Although the exact mechanism by which neurotensin increases midbrain dopamine cell firing remains to be determined, it can be hypothesized that it acts by stimulating, or disinhibiting, medial prefrontal cortex glutamatergic efferents to midbrain dopamine neurons. A previous anatomical study has revealed that prefrontal cortex efferents to the midbrain make synaptic contacts with presumed dopamine neurons (Sesack and Pickel, 1992). Electrophysiological and neurochemical studies have shown that prefrontal cortex electrical stimulation increases glutamate neurotransmission (Taber et al., 1995) and dopamine cell firing (Gariano and Groves, 1988). Moreover, in vitro studies have shown that pyramidal neurons in the prefrontal cortex are excited by neurotensin (Audinat et al., 1989). It cannot be excluded, however, that neurotensin acts indirectly by stimulating or inhibiting interneurons either in the prefrontal cortex or in the ventral midbrain or by increasing dopamine release in the prefrontal cortex. However, in the light of previous studies suggesting that activation of dopamine receptors in this region attenuates subcortical dopamine neurotransmission (see Doherty and Gratton, 1996), this hypothesis is unlikely. Moreover, neurotensin in the prefrontal cortex seems to attenuate the effect of dopamine (Beauregard et al., 1992).

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