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Short communication

Repeated activation of neurotensin receptors sensitizes to the stimulant effect of amphetamine

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Abstract

Effects of repeated intracerebroventricular microinjections of 18 nmol/10 μl of neurotensin, [D-Tyr^{11}]neurotensin, or saline were tested on motor activity in different groups of rats. One week after the fourth central injection, sensitivity to the behavioral stimulant effect of amphetamine (1 mg/kg, i.p.) was tested. As previously reported, neurotensin attenuated motor activity while [D-Tyr^{11}]neurotensin when compared to saline produced an initial suppression followed by an excitation. Despite such different behavioral effects, both peptides produced sensitization to the stimulant effect of amphetamine. These results show that repeated activation of neurotensin receptors produces long-lasting changes in responsiveness to a psychostimulant drug. © 1997 Elsevier Science B.V.

Keywords: Amphetamine; [D-Tyr^{11}]Neurotensin; Neurotensin; Sensitization

1. Introduction

Neurotensin is an endogenous tridecapeptide with behavioral effects similar to those produced by dopamine antagonists and indirect agonists. Intracerebroventricular microinjections of neurotensin, for instance, inhibit spontaneous locomotion as well as dopamine-induced locomotor activity (Kalivas et al., 1984). This behavioral inhibition contrasts with the psychomotor stimulant-like effect of neurotensin on behavior when it is injected directly into the ventral tegmental area (Kalivas and Duffy, 1990), and with its stimulatory effect on dopamine cell firing and release (Litwin and Goldstein, 1994; Blaha et al., 1990) when injected either into the ventricle or directly into the ventral tegmental area. Central injections of neurotensin also produce several other physiological effects, such as muscle relaxation, hypothermia and hypotension, that can, to some extent, attenuate spontaneous locomotor activity (see Nemeroff, 1986). Hence, I recently showed that ventricular microinjections of neurotensin potentiate brain stimulation reward, suggesting that it has a psychostimulant-like rather than a neuroleptic-like effect on this dopamine-dependent behavior (Rompré, 1995). In this study, the hypothesis that neurotensin can produce psychostimulant-like effects was tested by determining whether repeated activation of central neurotensin receptors produces sensitization to the locomotor-stimulant effect of amphetamine.

2. Materials and methods

2.1. Animals

Male Long-Evans rats (Charles River, St-Constant, Québec, Canada) weighing 300–350 g were used. They were housed individually 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).

2.2. Surgery

Animals were each injected with atropine methylnitrate (0.4 mg/kg, i.p.), anesthetized 20 min later with sodium pentobarbital (65 mg/kg, i.p.) and fixed in a stereotaxic instrument. The surface of the cranium was exposed and a guide cannula was inserted above the left lateral ventricle using the following stereotaxic coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral and 2.4 mm below the

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surface of the cranium (Paxinos and Watson, 1986). Four miniature screws were threaded into the bone and the guide cannula was fixed with acrylic dental cement.

2.3. Behavioral testing

Locomotor activity was measured with a bank of four Plexiglas boxes ($46 \times 35 \times 39$ cm) each equipped with seven photocells positioned 1 cm (5 photocells) and 6 cm (2 photocells) above a wire-mesh floor. The experiment consisted of two phases, a training phase and a sensitization test. During the training phase, motor activity was measured for 90 min, and on four occasions (every second day: Days 1, 3, 5 and 7), following an intracerebroventricular microinjection of 18 nmol/10 μl (30 μg) of neurotensin, [D-Tyr^{11}]neurotensin, or saline in different groups of rats; this dose of neurotensin was chosen because it produces significant attenuation of locomotion (Jolicoeur et al., 1981) while producing a psychostimulant-like effect on brain stimulation reward (Rompré, 1995). The cannula used for the microinjection extended at least 2 mm beyond the tip of the guide cannula and was connected with a polyethylene tubing to a 50 μl microsyringe. A 10 μl volume of peptide, or saline, was injected with a microinfusion pump over a period of 300 s in the freely moving rats; the injection cannula was left in place for an additional 60 s after the injection. Because it took approximately 27 min to inject the four animals tested in a single session, measures of motor activity were taken only between 30 and 120 min after the injection. Seven days after the training phase, animals were injected systemically with amphetamine sulphate (1.0 mg/kg, i.p.) and motor activity was measured immediately after for 120 min. Behavioral tests were always performed during the light phase of the daily cycle, in a room separate from the housing colony, and with the lights off.

2.4. Drugs

Neurotensin-(1–13) and [D-Tyr^{11}]neurotensin-(1–13) (Bachem, Sunnyvale, CA, USA) were dissolved in sterile 0.9% saline at a concentration of 1.8 nmol/μl and stored frozen at −20°C in 50 μl aliquots. Peptide solutions were thawed just before testing and used only once. Amphetamine sulphate was dissolved in saline and injected intraperitoneally in a volume of 1 ml/kg.

2.5. Histology

At the end of the experiment, animals were deeply anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and transcardially perfused with 0.9% saline followed by 10% formalin. Brains were removed, stored in 10% formalin and subsequently sliced in serial 40-μm sections that were stained with a formal-thionin solution. Location of the injection sites was determined under light microscopic examination. Only animals with a confirmed ventricular injection site were included in the study.

2.6. Data analysis

Total photocell counts were computed every 15 min and statistical significance was determined with a two-way analysis of variance for repeated measures on time factor. Comparisons among means were made with Duncan's multiple range test and the level of significance set at 0.05 (Statistica V5.0, StatSoft).

3. Results

Histological analysis revealed that for 30 of the 34 animals initially prepared, the injection site was within the left cerebral ventricle between 0.8 mm anterior and 1.0

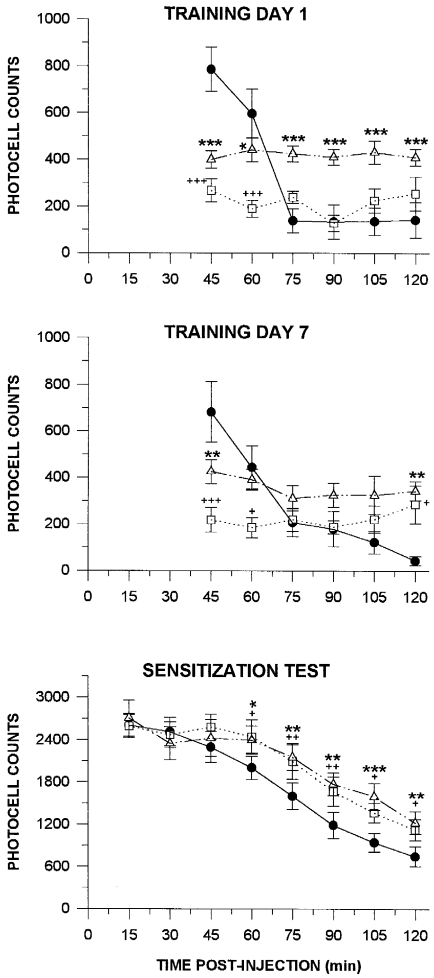

Fig. 1. Total motor activity scores recorded on day 1 (first injection, top panel) and on day 7 (fourth injection, middle panel) from each group of rats after the injection of neurotensin (squares, $n = 11$), [D-Tyr^{11}]neurotensin (triangles, $n = 7$) or saline (filled circles, $n = 10$). Each point represents the mean ± S.E.M. Total motor activity scores measured following amphetamine injection (sensitization test, day 14) are shown in the bottom panel; symbols represent same groups as above. In each panel, a statistically significant difference with the saline group is indicated by stars ([D-Tyr^{11}]neurotensin) and crosses (neurotensin) ($^{+,\star}$ $P < 0.05$; $^{++,\star\star}$ $P < 0.01$; $^{+++,\star\star\star}$ $P < 0.001$).

mm posterior to bregma; for the other four animals which were excluded from the study, the injection site was either in the cortex or in the striatum; results from two additional rats were lost during the sensitization test.

Total activity scores measured during the training phase are shown in the top and middle panels of Fig. 1. On the first day (top panel), neurotensin suppressed motor activity at 45 and 60 min post-injection, an effect that was still present after the fourth injection on day 7 (middle panel). During the second hour post-injection, the level of activity of the neurotensin-treated group was not different from the saline control group, except at day 7 in the last 15 min period where a small increase was observed. [D-Tyr^{11}]Neurotensin also suppressed motor activity during the first 30 min period on day 1, and on day 7, but to a smaller extent than neurotensin. During the second hour, however, this neurotensin analogue produced higher levels of activity compared to saline-treated rats, an effect that was less pronounced after the fourth injection (day 7). The bottom panel of Fig. 1 shows motor activity following administration of amphetamine (sensitization test), measured one week after the last central injection (day 14). Animals treated with neurotensin, and with [D-Tyr^{11}]neurotensin, showed higher activity scores than saline-treated animals from 60 to 120 min after the injection. No significant difference was found between neurotensin- and [D-Tyr^{11}]neurotensin-treated animals.

4. Discussion

The main finding of this study is that repeated central injection of neurotensin produces sensitization to the behavioral stimulant effect of amphetamine. That this effect is mediated by activation of neurotensin receptors is suggested by the similar effectiveness of [D-Tyr^{11}]neurotensin, a neurotensin analogue acting at the neurotensin receptors but with a greater potency due to its resistance to enzymatic degradation (Checler et al., 1983). This latter property can explain the comparatively greater stimulatory effect seen with this analogue (days 1 and 7), a finding consistent with previous reports (Castel et al., 1989; Jolicoeur et al., 1981). The finding that the sensitization to amphetamine produced by the two peptides was of comparable magnitude suggests that the neurotensin-induced sensitization is independent of its ability to stimulate locomotor activity. Indeed, previous data show that sensitization to amphetamine can be produced by ventral midbrain microinjection of amphetamine, a treatment that in fact suppresses locomotor activity (Kalivas and Weber, 1988). Although the mechanism involved in the development of amphetamine sensitization is not clear, several results suggest that it involves midbrain dopamine neurons (Kalivas and Stewart, 1991). Since neurotensin stimulates dopamine cell firing and release (Litwin and Goldstein, 1994; Blaha et al., 1990), it is tempting to hypothesize that the sensitization effect observed in this study is due to activation of ventral tegmental neurotensin receptors. This is unlikely, however, as repeated midbrain microinjections of neurotensin, at doses that stimulate locomotion, produce sensitization to neurotensin, but not to systemic amphetamine (Elliott and Nemeroff, 1986). An alternative site of action is the medial prefrontal cortex, a brain region that sends efferent projections to the ventral midbrain (Sesack and Pickel, 1992). Hence, ibotenic acid lesion of the medial prefrontal cortex was reported to attenuate amphetamine sensitization (Wolf et al., 1995) and activation of neurotensin receptors in this region was found to stimulate midbrain dopamine cell firing (Rompré and Boye, 1994). Furthermore, there is evidence that medial prefrontal cortex efferents to the ventral midbrain contain glutamate and/or aspartate (Christie et al., 1985), neurotransmitters that can modulate subcortical dopamine neurotransmission (see Kalivas and Duffy, 1995). Since the development of amphetamine sensitization is prevented by co-treatment with dizocilpine, a NMDA receptor antagonist (Karler et al., 1989), it may be the case that neurotensin-induced sensitization to amphetamine occurs via activation, or disinhibition, of an excitatory cortical input to midbrain dopamine neurons. The present results together with those on brain stimulation reward (Rompré, 1995) provide evidence that a centrally acting neurotensin agonist is expected to produce psychostimulant rather than neuroleptic-like effects on dopamine-dependent behaviors.

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