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Short communication

Effects of neurotensin receptor activation on brain stimulation reward in Fischer 344 and Lewis rats

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Abstract

The effects of intracerebroventricular injections of 18 nmol/10 μl of neurotensin, [D-Tyr^{11}]neurotensin, or saline on operant responding for brain stimulation reward were investigated in Fischer 344 (F344) and Lewis (LEW) rats using the curve–shift paradigm. [D-Tyr^{11}]neurotensin, but not neurotensin, decreased reward threshold in F344 rats while it increased thresholds in LEW rats. Both peptides suppressed maximal rates of responding; this effect was of greater magnitude and longer lasting in LEW than in F344 rats. These findings show that F344 and LEW rat strains are differentially sensitive to activation of central neurotensin receptors that modulate reward-relevant circuitry.

Keywords: Dopamine; Neurotensin; [D-Tyr^{11}]Neurotensin; Performance; Reward

1. Introduction

Fischer 344 (F344) and Lewis (LEW) are two strains of inbred rats that show marked differential appetitive and consummatory responses to several drugs of abuse. LEW rats, for instance, more readily self-administer opioids (Martin et al., 1999) and cocaine (Kosten et al., 1997) than F344 rats. Compared to F344, LEW rats more readily establish conditioned place-preferences to environments associated with opioids and with cocaine (Guitart et al., 1992; Kosten et al., 1994). Based on the large body of evidence implicating midbrain dopamine neurons in the rewarding effects of drugs of abuse, a significant amount of research has been dedicated to determining differences in the functional architecture of midbrain dopamine neurons to account for the differential sensitivity of F344 and LEW rats to these drugs. These two strains exhibit significant differences in dopamine synthesis, transport (Beitner-Johnson and Nestler, 1991; Guitart et al., 1992), and release (Strecker et al., 1995) as well as in second messenger and immediate early gene expression linked to dopamine receptor activation (Guitart et al., 1993). Such neurochemical differences in the midbrain dopamine system have been proposed to represent the neurobiological substrate of drug abuse-liability (Nestler et al., 1993). Given that neurotensin interacts closely with dopamine to modulate its function at both pre- and postsynaptic levels in several reward-relevant limbic regions (Nemeroff et al., 1983; Litwin and Goldstein, 1994), we hypothesize that this neuropeptide may play a significant role in the differential sensitivity of F344 and LEW rats to drug reward. This hypothesis is further supported by studies showing that central injections of neurotensin potentiate brain stimulation reward (Rompré et al., 1992; Rompré, 1995) and sensitize Long–Evans rats to the locomotor-stimulant effect of amphetamine (Rompré, 1997). The present experiment was thus aimed at determining whether F344 and LEW rats are differentially sensitive to the reward-potentiating effects of centrally injected neurotensin, and its long-lasting analog, [D-Tyr^{11}]neurotensin.

2. Materials and methods

2.1. Animals

Adult F344 and LEW male rats (Charles River, St-Constant, Québec, Canada) weighing 250–325 g at the time of surgery were used. They were housed individually in polyethylene cages with wood chip bedding and with free access to food and water in a climate-controlled room. Lighting was maintained on a 12-h light/dark cycle; lights

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on at 06:30 h. This experiment was carried out in accordance with the Canadian Council on Animal Care Guide to the Care and Use of Experimental Animals and the European Community guidelines for the use of experimental animals. An internal animal care committee approved the experiment and all efforts were made to minimize animal suffering.

2.2. Surgery

Each animal was injected with atropine methylnitrate (0.4 mg/kg, i.p.), anesthetized 20 min later with sodium pentobarbital (65 mg/kg, i.p.) and mounted onto a stereotaxic apparatus. The surface of the skull was exposed and a guide cannula (Plastic One, VA, USA, model C315G) was implanted above the left lateral ventricle using the following flat-skull coordinates: 0.8 mm posterior to bregma, 1.2 mm lateral to the midline and 2.8 mm below the skull surface (Paxinos and Watson, 1998). A moveable stimulation electrode (Kinetrods, Ottawa, Ontario, model SME-01) was then implanted in the ventral central gray at the following coordinates: 7.6 mm posterior to bregma, 0.0 mm lateral and 6.8 mm below the skull surface. A bare wire wrapped around four stainless steel screws and threaded into the skull served as the indifferent electrode. The cannula/electrode assembly was anchored to the skull with dental cement.

2.3. Behavioral testing

One week after surgery, animals were trained to lever-press for electrical stimulation of the ventral central gray region using procedures and stimulation parameters previously described (Rompré, 1995; also see Bauco and Wise, 1997). The animals were then trained to lever-press during discrete 40-s trials, each trial being followed by a 10-s interval during which the lever was disconnected. The beginning of each trial was signalled by 10 400-ms trains of non-contingent priming stimulation delivered at a rate of two trains/s. For each animal, pulse frequency on the first trial of each series was set at 67.5 Hz (27 pulses/train) and lowered on each subsequent trial by approximately 12% (0.05 log units) until it reached 12.5 Hz (5 pulses/train). Stimulation current (0.1 ms cathodal, rectangular pulses) was adjusted to initiate responding near 20–25 Hz. Reward threshold was derived from the function relating the rate of lever-presses to the pulse frequency and was defined as the pulse frequency sustaining half-maximal rate of responding (M50). Drug testing began when mean daily reward threshold varied by less than 10% across 3 consecutive days. Drug and vehicle tests were performed on separate test sessions, each of which consisted of two test periods. Three baseline frequency thresholds were first determined; the first threshold determination was considered as a warm-up and therefore not included in any of the analyses. Following this baseline, each animal received an intracerebroventricular (i.c.v.) microinjection of 18 nmol/10 μl of neurotensin or an equal volume of saline (see Rompré, 1995 for injection procedure). Beginning immediately after the injection, 10 reward threshold determinations were obtained during a second test period that lasted approximately 2.5 h. At least 1 week separated each drug or vehicle test and the order was counterbalanced. In some of these animals, the effect of 18 nmol/10 μl of [D-Tyr^{11}]neurotensin was also tested using the same procedures. The concentration of peptides tested was chosen on the basis of a previous report showing that 18 nmol/10 μl neurotensin significantly alters brain stimulation reward when administered i.c.v. (Rompré, 1995).

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 pre-coated with silicone (Sigmacote, Sigma, St. Louis, MO, USA). Peptide solutions were thawed just prior to testing.

2.5. Histology

At the end of the experiment, animals were deeply anesthetized with urethane, and the stimulation site was marked with a 1.5-mA anodal current of 15 s duration. They were then perfused with physiological saline followed by a 10% formalin solution containing 10% formalin, 3% potassium ferrocyanide, 3% potassium ferricyanide, and 0.5% trichloroacetic acid (see Bauco and Wise, 1997 for elaboration) . Brains were subsequently sliced in 40-μm serial sections and stained with a formal–thionin solution. Only animals with a confirmed ventricular injection site were included in the study.

2.6. Data analysis

Reward threshold and maximum rates of responding were calculated prior to drug injection and values used as a baseline; measures obtained after drug injection were expressed as percentage of the baseline. Group means were analyzed with a three-way analysis of variance (strain, drug and time) for repeated measures on time; differences among means were determined with Duncan's multiple range post hoc test and level of significance set at 0.05 (Statistica V5.0, StatSoft, USA).

3. Results

Rate–frequency functions obtained from one F344 (left panels) and one LEW (right panels) rat before and at 30-min intervals following saline, neurotensin or [D-Tyr^{11}]neurotensin injections are shown in Fig. 1. [D-

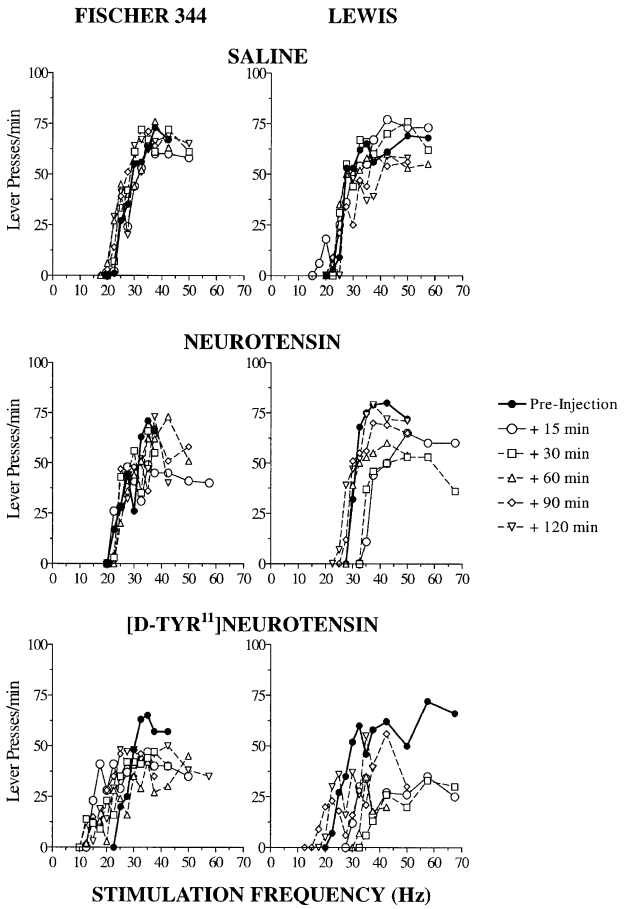

Fig. 1. Rate–frequency curves from one Fischer 344 (left column) and one Lewis rat (right column) obtained prior to (filled circles), and at 15 (unfilled circles), 30 (unfilled squares), 60 (unfilled triangles), 90 (unfilled diamonds) and 120 min (unfilled inverse triangles) after injection of saline (top panels), neurotensin (middle panels), and [D-Tyr^{11}]neurotensin (bottom panels).

Tyr^{11}]neurotensin suppressed maximal rates in both strains, but shifted the curves to the left in F344 and to the right in LEW rats, reflecting, respectively, a potentiation and an attenuation of reward. Neurotensin suppressed rates in both strains, but like saline, it produced no lateral shift of the rate–frequency functions.

Analysis of variance performed on percent change in reward threshold yielded a significant effect of strain ([$F(1,43) = 18.7$; $P < 0.0001$), time ($F(9,387) = 12.54$; $P < 0.01$), and drug by time interaction ($F(18,387) = 2.97$; $P < 0.0001$). Post hoc tests revealed that in F344 rats, reward thresholds were significantly lower after [D-Tyr^{11}]neurotensin injection compared to saline for the entire test session (Fig. 2, top left panel); such an effect was not seen after neurotensin. [D-Tyr^{11}]neurotensin produced an opposite effect in LEW rats, elevating reward threshold compared to saline between 30 and 60 min, and at 90 min after the injection (Fig. 2, top right panel). Changes in reward threshold produced [D-Tyr^{11}]neurotensin were statistically different at all time periods between F344 and LEW rats. No difference was found between reward thresholds in F344 and LEW following saline or neurotensin.

Analysis of variance performed on percent change in maximal rates yielded a significant effect of strain ($F(1,43) = 16.2$; $P < 0.001$), drug ($F(2,43) = 23.2$; $P < 0.0001$), and time ($F(9,387) = 5.6$; $P < 0.0001$). Post hoc tests revealed that in F344 rats neurotensin and [D-Tyr^{11}]neurotensin respectively suppressed maximal rates at 15 min, and between 15 and 105 min after the injection (Fig. 2, bottom left panel). Such suppression was also observed in LEW rats, but with a larger magnitude and a longer duration than in F344 rats (Fig. 2, bottom right panel). Post hoc tests confirmed that the suppression effect of neurotensin (30–60 min after injection) and of [D-Tyr^{11}]neurotensin (15–150 min after injection) were statistically different between F344 and LEW rats; no strain differences were found following injection of saline.

Analysis of variance confirmed that baseline reward thresholds measured prior to saline, and prior to each of the peptide injections, were not statistically different between tests within each strain. There were also no signifi-

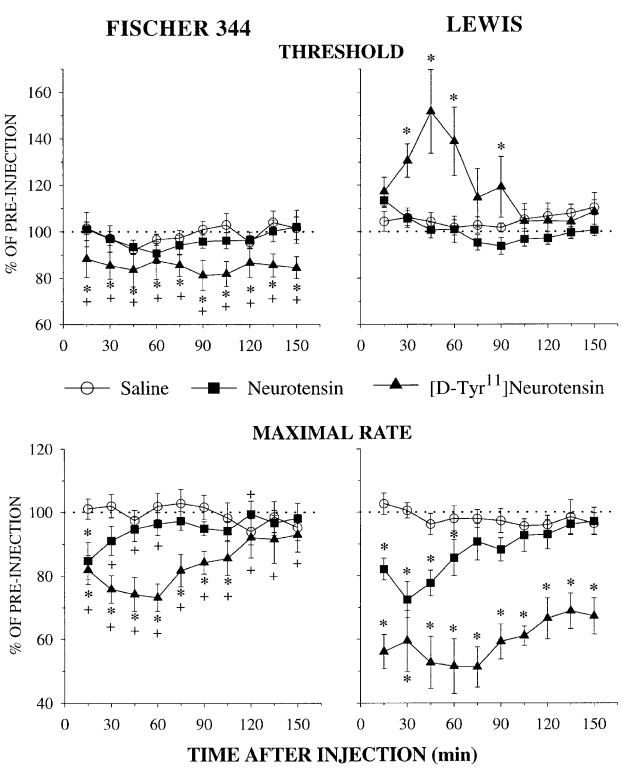

Fig. 2. Changes in reward threshold (top panels) and maximal rates of responding (bottom panels) as a function of time after injection of saline (unfilled circles; F344, $n = 9$; LEW, $n = 10$), neurotensin (filled squares; F344, $n = 9$; LEW, $n = 10$) and [D-Tyr^{11}]neurotensin (filled triangles; F344, $n = 6$; LEW, $n = 5$) for each rat strain. Data are expressed as a percentage of pre-injection values and each point represents the group mean (±S.E.M.). Asterisks and crosses indicate statistical difference ($p < 0.05$) with saline, and between strains for the corresponding drug treatment, respectively.

cant differences in baseline reward thresholds between the two strains (data not shown).

4. Discussion

A previous study performed with Long–Evans rats has shown that at the dose tested in the present study, i.c.v. neurotensin produced a decrease in reward threshold as reflected by a leftward shift of rate–frequency functions (Rompré, 1995), an effect not observed here neither with F344 nor with LEW rats. An equimolar concentration of the analog, [D-Tyr^{11}]neurotensin, did, however, decrease reward threshold only in F344 rats; surprisingly, an opposite effect was observed in LEW rats. [D-Tyr^{11}]neurotensin displays a lower affinity at the neurotensin receptor type 1 and type 2 than neurotensin (Kitabgi et al., 1980; Mazella et al., 1996) but in vivo, it is more potent than neurotensin at inducing physiological changes, an effect attributed to its lower sensitivity to enzymatic degradation (Kitabgi et al., 1980; Checler et al., 1983). Its effectiveness over neurotensin in F344 suggests that this strain is less sensitive than Long–Evans rats to activation of the neurotensin receptor that mediates reward potentiation. Previous studies have shown that activation of neurotensin receptors in the ventral tegmentum and in the nucleus accumbens, respectively, potentiates and suppresses brain stimulation reward (Nemeroff et al., 1982; Rompré et al., 1992). Rompré (1995) has suggested that the reward potentiation measured after i.c.v. neurotensin in Long–Evans rats is a result of its net effect at both sites. According to this hypothesis, the reward potentiation seen in F344 would reflect a stronger action of [D-Tyr^{11}]neurotensin in the ventral tegmentum than in the nucleus accumbens. These data suggest the opposite hypothesis for LEW rats as the analog was found to inhibit rather than potentiate reward. Conversely, it cannot be excluded that in F344 rats, the relevant neurotensin receptors in the ventral tegmentum are functionally more sensitive than in LEW rats. The present findings demonstrate that the reward-relevant pathway is differentially modulated by neurotensin receptor activation in F344 and LEW rats. Further studies need to be performed to determine which neurotensin relevant physiologic function(s) is/are likely involved in this differential modulation.

Both peptides were effective at suppressing maximal rates of responding in F344 and LEW rats but to a different degree and with a different time course. The suppression observed in F344 rats following neurotensin was weaker, and shorter lasting than that seen in LEW rats; interestingly, the suppression observed in LEW rats was very similar to that previously reported in Long–Evans (Rompré, 1995). Such a decrease in maximal rates reflects a decrease in the ability of the animal to produce the operant response (Miliaressis et al., 1986). When administered i.c.v., neurotensin produces muscle relaxation (Nemeroff, 1980; Jolicoeur et al., 1981), an effect that is likely to interfere with the ability of the animal to produce an operant response, thus accounting for the decrease in rates. It is noteworthy that the magnitude of the suppression of rates produced by [D-Tyr^{11}]neurotensin in F344 and in LEW rats is not correlated with the changes in reward threshold, therefore further confirming the effectiveness of the curve–shift method at dissociating both effects. This also shows that the substrate that mediates neurotensin receptor activation-induced reward changes is different than that mediating neurotensin receptor activation-induced suppression of performance, and that the latter effect is differentially sensitive in F344 and LEW rats.

The present results show that F344 and LEW rats respond differentially to neurotensin receptor activation-induced reward changes, a characteristic that may have some relevance to the differential sensitivity of the two strains to drugs of abuse.

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