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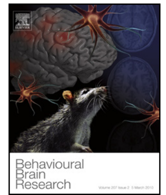

Research report

Anxiolytic effect of neurotensin microinjection into the ventral pallidum

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HIGHLIGHTS

- Neurotensin was microinjected into the ventral pallidum (VP) of rats.
- 100 ng dose of neurotensin exhibited anxiolytic effect in the VP.
- The anxiolytic effect could be prevented by NTR1 antagonist SR 48,692 pretreatment.
- Neither neurotensin nor SR 48,692 influenced locomotor activity.

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ABSTRACT

Neurotensin (NT) acts as a neurotransmitter and neuromodulator in the central nervous system. NT is involved in reward and memory processes, drug addiction and also in the regulation of anxiety. The ventral pallidum (VP) receives neurotensinergic innervation from the ventral striatopallidal pathway originating from the nucleus accumbens. Positive reinforcing effects of NT in the VP had been shown recently, however the possible effects of NT on anxiety have not been examined yet.

In our present experiments, the effects of NT on anxiety were investigated in the VP. In male Wistar rats bilateral microinjections of 100 ng or 250 ng NT were delivered in the volume of 0.4 μl into the VP, and elevated plus maze (EPM) test was performed. In another groups of animals, 35 ng NT-receptor 1 (NTR1) antagonist SR 48,692 was applied by itself, or microinjected 15 min before 100 ng NT treatment. Open field test (OPF) was also conducted.

The 100 ng dose of NT had anxiolytic effect, but the 250 ng NT did not influence anxiety. The antagonist pretreatment inhibited the effect of NT, while the antagonist itself had no effect. In the OPF test there was no difference among the groups. Our present results show that microinjection of NT into the VP induces anxiolytic effect, which is specific to the NTR1 receptors because it can be eliminated by a specific NTR1 antagonist. It is also substantiated that neither the NT, nor the NTR1 antagonist in the VP influences locomotor activity.

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1. Introduction

Anxiety and fear are physiological responses to environmental stimuli. Anxiety is an important risk factor of chronic organic disorders [1]. Anxiety is regulated by different limbic structures, such as the orbitofrontal cortex [2], the amygdala [3], the nucleus accumbens (NAC) [4], and the ventral pallidum (VP) [3,5]. Additionally, numerous neurotransmitters and neuromodulators are involved in anxiety, such as GABA [6], glutamate [7], and various monoamines [4,8–11]. In the latter group, one of the important neurotransmitters is dopamine, whose effects are partly associated to the mesolimbic dopaminergic system [4]. Neuropeptides, such as substance P [12–14] or neurotensin (NT) [15,16] had also been proven to be involved in the regulation of anxiety.

NT is a tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH), which acts as a neurotransmitter and neuromodulator in the central nervous system [17,18]. NT mediates its effects through three different types of receptors: NTR1, NTR2 and NTR3, respectively [17,19]. It has also been

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demonstrated that NT plays a role in reward and memory processes [20,21] and in drug addiction as well [22]. In addition to the above, the NT has also been shown to take part in the regulation of anxiety. Systemic administration of selective NTR1 agonist PD 149163 inhibits fear-potentiated startle [15] and the footshock-induced ultrasonic vocalization [16]. NT and NT receptors had been identified throughout the central nervous system: in the cerebral cortex, in the amygdala, in the NAC, and also in the VP [23–26].

The VP is an important constituent of the basal forebrain circuitry [27]: it is known to be involved in the regulation of motivational and reward signals, and in the transformation of such signals into motor output, the process known as limbic-motor integration [28,29]. The VP also plays a role in the regulation of anxiety: elevated activity of vasopressin 1a receptor in the VP increases anxiety [30], on the other hand, substance P had anxiolytic effects in the VP [5].

The VP can be divided into the following subregions: dorsolateral (VPl), ventromedial (VPm), ventrolateral (VPvl), and rostral (VPr) areas [31]. NTergic immunoreactivity can be detected only in the VPm, but it cannot be found in the VPl or the other subregions [24,31,32]. In the VPm, the NTR1 receptors are detected in the highest density [25,33], the NTR2 receptors can be identified in the VP only to a low or undetectable level [26,33]. There is no evidence of the existence of NTR3 in the VP. The VPm receives most of its afferent fibers from the NAC (almost exclusively from the shell region), the ventral tegmental area (VTA), the ventromedial caudate putamen, and the olfactory tubercle [24,32,34,35]. The NTergic fibers arise mostly from the ventral striatopallidal pathway originating from the NAC, and additionally from the extended amygdala [24,32]. The VPm projects to the VTA (which has reciprocal connections with the VPm) and to the mediodorsal nucleus of the thalamus [36,37]. Evidence has been obtained for that the VP influences the population activity of dopaminergic neurons in the VTA [3,38–40], and the VTA, even in such way, has been shown to be involved in the regulation of anxiety [41,42].

Although the electrophysiological effects of direct ventral pallidal microinjection of NT and NT antagonist had not been investigated yet, there is evidence for that i. p. injection of NTR1 antagonists decreases the firing rate of VPm neurons, without any effects on the VPl [43]. It is well known, that NT in the VP potentiates cocaine primed reinstatement, but it inhibits cue primed reinstatement of drug seeking [44]. Positive reinforcing and rewarding effect of NT in the VP has also been shown recently [21]. Nevertheless, details of the possible effects of NT in the VP on anxiety are not known yet. The aim of the present experiments was to investigate the possible effect of NT and the role of NT-receptor 1 (NTR1) in the VP on anxiety in elevated plus maze test (EPM) and on locomotor activity in open field test (OPF).

2. Materials and methods

2.1. Animals and surgery

One-hundred and five male Wistar rats weighing 280–320 g at the beginning of the experiments were housed individually and cared for in accordance with institutional (BA02/2000-8/2012), national (Hungarian Government Decree, 40/2013. (II. 14.)) and international standards (European Community Council Directive, 86/609/EEC, 1986, 2010). Animals were kept in a temperature- and light-controlled room (21 ± 2 °C, 12:12 h light–dark cycle with lights on at 7:00 a.m.) for one week before surgery. Standard laboratory food pellets (CRLT/N Charles River Kft, Budapest, Hungary) and tap water were available *ad libitum.*

Operations were carried out under general anesthesia by intraperitoneal injection of a mixture of ketamine (Calypsol, Richter Gedeon, Hungary, 80 mg/kg body weight) and diazepam (Seduxen, Richter Gedeon, Hungary, 20 mg/kg body weight, 2 ml/kg bw. (body weight) of the mixture).

By means of the stereotaxic technique, 22 gauge stainless steel guide tubes were bilaterally implanted 0.5 mm above the target area (coordinates referring to the bregma: AP: −0.26 mm, ML: 2.2 mm, DV: 7.1 mm from the surface of the dura) according to the stereotaxic rat brain atlas of Paxinos and Watson [45]. Cannulae were fixed to the skull with self-polymerizing dental acrylic (Duracryl) anchored by 3 stainless steel screws. The guide tubes, except when being used for microinjection, were occluded with stainless steel obturators made of 27 gauge stainless steel wire.

Before starting the behavioral experiments, animals were allowed a minimum of 6 days for postoperative recovery and during this period they were frequently handled. Behavioral tests were performed during the daylight period between 08:00 and 18:00 h.

2.2. Drugs and microinjection procedure

NT (Sigma–Aldrich Co., N 6383) was dissolved in 0.15 M sterile saline solution containing 0.01 M Na-acetate and 0.01 M phosphate buffered saline (PBS, pH 7.4), and it was microinjected in two different doses: 100 ng (54.6 pmol) or 250 ng (136.6 pmol). The NTR1 antagonist SR 48692 (Tocris Co., Cat. No. 3721) was diluted in 0.15 M saline solution containing 2% dimethyl sulfoxide and 0.01 M PBS. SR 48692 was microinjected in a dose of 35 ng (60.0 pmol).

In this paper, all the doses mentioned are meant to be the dose per side values. All substances were microinjected bilaterally through 27 gauge stainless steel microinjection tubes extending 0.5 mm below the tips of the implanted guide cannulae. The delivery cannula was attached to a 10 μl Hamilton microsyringe (Hamilton Co., Bonaduz, Switzerland) via polyethylene tubing (PE-10). All injections were delivered by a syringe pump in the volume of 0.4 μl (Cole Parmer, IITC, Life Sci. Instruments, California) over a 60 sec period. After accomplishing the microinjection, cannulae were left in place for an additional 60 sec to allow diffusion into the surrounding tissue and to prevent backflow of the solution. Rats were gently held by hand during the microinjection procedure.

In the first EPM experiment animals received 100 ng ($n = 8$) or 250 ng NT ($n = 8$). Control animals ($n = 9$) received vehicle of NT (veh1) also bilaterally.

In the second EPM experiment, the NT treated group ($n = 8$) received vehicle of SR 48692 (veh2) and then 100 ng NT. The antagonist treated group ($n = 9$) received SR 48692 and then 15 min later veh1. The NT injected group pretreated with antagonist ($n = 8$) received SR 48692 15 min prior being injected with the 100 ng NT. Two subsequent vehicle microinjections (veh2 + veh1) were made in the control group ($n = 9$). The antagonist or veh2 were applied 15 min prior to the NT or veh1 injections, respectively.

In the OPF experiment, NT was microinjected in 100 ng ($n = 8$) or 250 ng doses ($n = 6$), similar to the first EPM experiment. The antagonist treated group ($n = 6$) received SR 48692 and then 15 min later veh1, the NT injected group pretreated with antagonist ($n = 6$) received SR 48692 15 min before being injected with 100 ng NT (similar to the second EPM experiment). Veh2 and then 15 min later veh1 were microinjected in the control group ($n = 8$).

2.3. Elevated plus maze test (EPM)

Anxiety was evaluated in the EPM test. The apparatus was constructed of grey colored wooden planks. The equipment consisted of two opposite open arms (50 cm × 10 cm) and two opposite closed arms (50 cm × 10 cm × 40 cm) with an open roof. The maze was elevated to a height of 100 cm above the floor. After drug or vehicle administrations, animals were placed into the center of the maze (central platform), facing one of the closed arms. Trials lasted for

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