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Research report

Positive reinforcing effect of neurotensin microinjection into the ventral pallidum in conditioned place preference test

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HIGHLIGHTS

- neurotensin was microinjected into the ventral pallidum (VP) of rats.
- 100 ng dose of neurotensin elicits conditioned place preference.
- NTR1 antagonist SR 48692 pretreatment prevents effects of neurotensin in the VP.
- results demonstrate positive reinforcing effects of ventral pallidal neurotensin.

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ABSTRACT

The ventral pallidum (VP) is innervated by the mesolimbic dopaminergic system and it has a key role in motivation, reward, and memory processes. Neurotensin (NT) is an important neuromodulator which has been shown to modulate reinforcement in the ventral tegmental area, in the ventral mesencephalic region and in the central nucleus of amygdala. Neurotensin receptor 1 (NTR1) has already been detected in the VP in abundance, but its role in rewarding and reinforcing processes is not fully understood yet.

In our present experiments, the effects of NT on positive reinforcement were investigated in the VP. In conditioned place preference (CPP) test, male Wistar rats were microinjected bilaterally with 100 ng or 250 ng NT in the volume of 0.4 μl. In other groups of animals, 35 ng NTR1 antagonist SR 48692 was applied by itself, or microinjected 15 min before 100 ng NT treatment.

One hundred ng dose of NT induced CPP, whereas animals injected with 250 ng NT did not exhibit significant differences from the vehicle group.

Antagonist pretreatment inhibited the effect of NT, while the antagonist applied by itself had no effect. Our results show that NT injected into the VP is involved in positive reinforcement. This effect is specific to NTR1 receptors because the development of CPP can be prevented by specific antagonist.

1. Introduction

The tridecapeptide neurotensin (NT) (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH) was originally isolated from bovine hypothalamus by Carraway and Leeman in 1973 [1]. It acts as a neurotransmitter and neuromodulator in the central nervous system and as a hormone in the gastrointestinal tract [2,3]. It was demonstrated to mediate its effects through three different types of receptors (NTR1, NTR2 and NTR3, respectively) [2,4].

NT has been shown to be involved in reward and memory processes, stress and pain modulation [3–7]. It has also important clinical relevance in drug addiction and depression [8–10]. Earlier investigations demonstrated that NT modulates dopaminergic [11,12], GABAergic [13], glutamatergic [14] and serotoninergic [15] neurotransmission in numerous brain structures. A broad literature also substantiates that NT has positive reinforcing effects after its direct microinjection into the *ventral tegmental area* (VTA) [5], *ventral mesencephalic area* [6], or into the *central nucleus of amygdala* [7].

The *ventral pallidum* (VP) was originally described by Heimer and Wilson [16]. The VP is known to be involved in the regulation

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of food intake and in processing of drug related motivational and reward signals and transformation of them into motor output, also known as limbic-motor integration [8,17]. Numerous neurotransmitters and neuromodulators are involved in the behavioral functions in the VP, including GABA, glutamate, dopamine, opioids, NT, substance P and vasopressin [8,18–22].

In the VP both NT neuron terminals and NT receptors have been detected: the presence of NT is one of the defining features of the ventromedial VP subregion (VPm), but it cannot be found in the dorsolateral part (VPl) [23,24]. The NTR1 receptors are present in the highest concentration [25] in the VPm, typically on the dendrites receiving striatopallidal boutons, and on neuronal perikarya [24,26]. NTR2 receptors can be identified in the VP only to a low or undetectable level [25,27], and there is no evidence of the existence of NTR3 in the VP. There are no data available in the literature of electrophysiological effects of direct ventral pallidal microinjection of NT and NT antagonist. However, it is known, that i. p. injections of NTR1 antagonists decrease the firing rate of neurons in the VPm, without any effects on the VPl [28].

The VPm receives most of its afferent fibers from the *nucleus accumbens* (almost exclusively from the shell region), the VTA, the *ventromedial caudate putamen*, and the *olfactory tubercle* [23,24,29,30]. The main source of NT in the VPm is the ventral striatopallidal pathway originating from the *nucleus accumbens*, however, the *extended amygdala* can be a probable additional source [23,24].

The VPm projects to the VTA and to the *mediodorsal nucleus of the thalamus* [31]. The VPm is in reciprocal connections with the VTA [31,32]. It is known, that the activation of the GABAergic accumbal afferents to the VP or direct inactivation of the VP by local infusion of GABA agonists increase the population activity of dopaminergic neurons in the VTA with an increase of the extracellular dopamine levels in the *nucleus accumbens* [33]. Increased dopamine level in the VTA and in the *nucleus accumbens* is associated with reward and positive reinforcement [34–36]. It is known, that NT(8–13) injection into the VP increases extracellular GABA level [22]. According to these, NT may also influence the activity of the dopaminergic neurons, and may play a role in regulation of these functions. There are contradictory data of the possible behavioral effects of NT in the VP. It is known, that injection of NT(8–13) in the VP inhibits cue primed reinstatement of drug seeking, however, it even potentiates cocaine primed reinstatement [22].

According to the above mentioned findings, the NTR1 receptors can play an important neuromodulatory role in the ventral striatopallidal projections that are intimately associated with reward and reinforcement functions [24,29]. Despite a relative abundance of these related data, the reinforcing effects of NTR1 receptor activation in the VP have not been investigated yet. Therefore, the conditioned place preference (CPP) paradigm has been used in the present experiments to evaluate the primary rewarding-reinforcing properties of NT and the efficacy of NTR1 receptor antagonist SR 48692 injected bilaterally into the VP.

2. Materials and methods

2.1. Animals and surgery

Ninety two 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 4:1 ratio 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 b. w. 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 [37]. Cannulae were fixed to the skull with self-polymerizing dental acrylic (Duracryl) anchored by 3 stainless steel screws. The guide tubes, when not being used for microinjection, were occluded with stainless steel obturators made of 27 gauge stainless steel wire.

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

2.2. Drugs and microinjection procedure

In the first experiment, bilateral microinjection of NT (Sigma–Aldrich Co., N 6383) was made in two different doses: 100 ng (54.6 pmol) or 250 ng (136.6 pmol) per side in 0.4 μl, respectively. NT 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). Control animals received this solution bilaterally as vehicle (veh1) in equal volume to that used for NT injections.

In the second experiment, the NTR1 antagonist SR 48692 (Tocris Co., Cat. No. 3721) was diluted in 0.15 M saline solution containing 2% dimethylsulfoxide and 0.01 M PBS, and its vehicle solution (veh2) was used for control injections. The NT treated group received veh2 and then 100 ng NT. The antagonist treated group received SR 48692 [35 ng (60.0 pmol)/0.4 μl per side] and then 15 min later veh1. The NT injected group pretreated with antagonist received SR 48692 15 min before being injected with 100 ng NT. Two subsequent vehicle microinjections (veh2 + veh1) were made in the control group. The antagonist or veh2 were applied 15 min prior to NT or veh1 injections, respectively. Solutions were kept at +4 °C before the applications.

In this paper, all the doses mentioned are meant to be the dose per side values. Drugs or vehicles were bilaterally microinjected 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 s interval. After accomplishing the microinjection, cannulae were left in place for an additional 60 s to allow diffusion into the surrounding tissue. Rats were gently held by hand during the injection procedure.

2.3. Conditioned place preference (CPP) test

The CPP apparatus consisted of a circular open field, with a diameter of 85 cm and 40 cm height. The apparatus (the walls and the floor) was made of plastic. The floor and the walls were gray-colored, black lines divided the floor into four quadrants of equal size. Visual cues in the surroundings assisted to distinguish the quadrants and helped the spatial orientation of animals [38]. The room was dimly lit by a 40 W bulb.

The place preference procedure consisted of one habituation (1st day), three conditioning (2nd–4th days) and one test (5th day)