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The role of intraamygdaloid neurotensin and dopamine interaction in conditioned place preference

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ABSTRACT

Tridecapeptide Neurotensin (NT) is widely distributed in the central nervous system where it acts as a neurotransmitter and neuromodulator. The central nucleus of amygdala (CeA), part of the limbic system, plays an important role in learning, memory, anxiety and reinforcing mechanisms. Our previous data showed that NT microinjected into the CeA has positive reinforcing properties. We supposed that these effects might be due to modulations of the mesolimbic dopamine system. The aim of our study was to examine in the CeA the possible effects of NT and dopamine interaction on reinforcement by conditioned place preference test.

Male Wistar rats were microinjected bilaterally with 100 ng NT or 2 μg D1 dopamine receptor antagonist alone, or D1 dopamine antagonist 15 min before 100 ng NT treatment or vehicle solution into the CeA. Other animals received 4 μg D2 dopamine receptor antagonist Sulpiride alone, or administration of D2 dopamine receptor antagonist 15 min before 100 ng NT treatment or vehicle solution into the CeA.

Rats that received 100 ng NT spent significantly more time in the treatment quadrant during the test session. Pre-treatment with the D1 dopamine antagonist, blocked the effects of NT. D2 dopamine receptor antagonist pretreatment could prevent the positive reinforcing effects of NT as well. Antagonists themselves did not influence the place preference.

Our results show that the rewarding effect of NT can be due to the modulation of DA system, since its effects could be blocked by either D1 dopamine or D2 dopamine antagonist preteatment.

1. Introduction

Tridecapeptide neurotensin (NT) was first isolated by Carraway and Leeman in 1973 [1]. NT is widely distributed in the mammalian central nervous system, where it acts as a neurotransmitter and neuromodulator [2,3]. High concentrations of NT were shown in the substantia nigra, nucleus accumbens (NAC), hypothalamus, periaqueductal gray matter and amygdala (AMY) [2,4–6]. Numerous NT-containing pathways have been identified to be originating from the ventral tegmental area (VTA), amygdala (AMY) and subiculum [7,8]. NT has been revealed to have rewarding effects in some mammalian brain structures [9–14]. Specifically, chemical self-stimulation can be induced by intravenous self-administration of NT or by direct self-application into the VTA [9,15]. NT has been shown to facilitate electrical intracranial self-stimulation in the VTA, mediocaudal NAC and subiculum as well [11,12,16]. NT has positive reinforcing effects in conditioned place preference in the VTA, ventral mesencephalon, ventral pallidum (VP) and CeA [10,12–14].

The common feature of the above mentioned structures is that they form the origin (i.e. the VTA) or they are the terminal fields of the mesolimbic dopaminergic system (MLDS). It is well known that the dopaminergic systems play crucial role in learning, motivation, and reward related behaviours [17,18]. Neurons of MLDS are originate from the VTA and their projections are sent to the NAC, hippocampus, septal area and amygdala (AMY). Numerous studies have indicated the interaction between neurotensinergic and dopaminergic systems. Co-localisation of NT and dopamine (DA) has been shown in the VTA, NAC, prefrontal cortex (PFC) and AMY [19,20]. NT has been revealed to modulate the DA release in various brain structures [20–25]. Our previous findings have shown that NT has positive reinforcing effects after its microinjection into the CeA or into the VP [13,14]. These brain structures are innervated by the MLDS. It is important to emphasize, that NT has been shown to modulate the dopaminergic neurotransmission in the MLDS [26]. More specifically, NT transmission is

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thought to be relevant in the regulation of dopaminergic neuronal pathways involved in reward related behaviour [12,27,28]. One of the key structures of MLDS is the VTA, that receives NTergic fibers from NAC, ventral pallidum (VP), AMY, olfactory tubercle and bed nucleus of stria terminalis [29,30]. Furthermore, NT receptors of VTA are localized mainly on dopaminergic neurons [31]. It has been shown that activation of NT receptors in the VTA leads to increased DA release in the NAC [32]. AMY, prefrontal cortex and NAC receive NTergic fibers originating from VTA [7,33]. NTergic neurons of CeA project to the bed nucleus of the stria terminalis, substantia nigra and lateral hypothalamus [33–35]. The above mentioned findings strongly indicate the role of NT in reward related behaviour and suggest the possible effect of NT and DA interaction on reinforcement and memory consolidation. Therefore, the aim of the present study was to examine in the rat CeA the effects of NT and D1 or D2 DA antagonist microinjection in conditioned place preference paradigm.

2. Materials and methods

2.1. Subjects

Seventy-two adult male Wistar rats weighing between 280–320 g at the beginning of the experiments were housed individually and cared 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). Rats were kept in a temperature and light controlled room (22 ± 2 °C; 12:12 h light–dark cycle with lights on at 6:00 a.m.). Standard laboratory food pellets (CRLT/N standard rodent food pellet, Charles River Kft, Budapest, Hungary) and tap water were available ad libitum. All behavioural tests were done during the rats' daylight period between 08:00 and 18:00 h.

2.2. Surgery

Rats were anesthetized i.p. by ketamine supplemented with diazepam (Calypsol and Seduxen, Richter Gedeon, Hungary, ketamine: 80 mg/kg body weight, diazepam: 20 mg/kg body weight). Animals were stereotaxically implanted bilaterally with 22 gauge stainless steel guide cannulae, directed toward and 1 mm above the dorsal border of the CeA (coordinates relative to bregma: AP: −2.3 mm, ML: ± 4.1 mm, DV: −6.5 mm) according to the rats' stereotaxic atlas [36]. Cannulae were fixed to the skull with three stainless steel screws and dental acrylic. When not being used for injection, the guide cannulae were occluded with 27 gauge stainless steel obturators. Animals were allowed a minimum 6 days of postoperative recovery period before starting the experiments, during which period they were handled daily.

2.3. Drugs and injection procedure

NT obtained from Sigma (Sigma–Aldrich Co., N 3010) was bilaterally microinjected with 100 ng dose (54.6 pmol) in 0.4 µl. 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 [37] in equal volume to that used for NT injections. D1 DA antagonist SCH 23390 [Sigma–Aldrich Co., R(+)-SCH-23390 hydrochloride 2 µg/0.4 µl] was diluted in 0.15 M saline solution containing 0.01 M Na-acetate and 0.01 M phosphate buffered saline (PBS, pH 7.4). Four groups of animals were involved in the first conditioned place preference (CPP) experiment: Control group (Veh + Veh) n = 8; 100 ng NT treated group (Veh + 100 ng NT) n = 8; 100 ng NT pretreated with D1 DA receptor antagonist group (D1 ANT + NT) n = 8; D1 dopamine receptor antagonist group (D1 ANT + Veh) n = 8. In the second CPP experiment, the effects of D2 DA receptor antagonist were investigated. D2 DA antagonist Sulpiride [Sigma–Aldrich Co., S7771, 4 µg/0.4 µl] was diluted in 0.15 M saline solution containing 0.01 M Na-acetate and 0.01 M phosphate buffered saline (PBS, pH 7.4). The following groups were examined: Control group (Veh + Veh) n = 9; 100 ng NT treated group (Veh + 100 ng NT) n = 9; 100 ng NT pretreated with D2 DA receptor antagonist group (D2 ANT + NT) n = 7; D2 DA receptor antagonist group (D2 ANT + Veh) n = 7. The antagonist or Veh treatment was applied 15 min prior to the NT or Veh injections, respectively. Solutions were kept in + 4 °C before application. In this article, all doses are reported as dose per side values. Drugs or vehicles were bilaterally microinjected through a 30 gauge stainless steel injection tube extending 1 mm below the tips of the implanted guide cannulae. The injection cannula was attached via polyethylene tubing (PE-10) to a 10 µl Hamilton microsyringe (Hamilton Co., Bonaduz, Switzerland). All injections were delivered by the syringe pump in a 0.4 µl volume (Cole Parmer, IITC, Life Sci. Instruments, California) over a 60 s interval. After an injection, cannulae were left in place for an additional 60 s to allow diffusion into the surrounding tissues. During the injections rats were gently held in hands.

2.4. Conditioned place preference test (CPP)

Positive reinforcing effects of drugs and memory processes can be measured by the conditioned place preference test [38,39]. Our corral apparatus consists of a circular open field with a diameter of 85 cm and 40 cm high wall. Black lines divided the floor into four quadrants of equal size. External visual cues placed on the surrounding wall guided the animals' spatial orientation inside the apparatus [40]. The room was dimly lit by a 40 W bulb. The place preference procedure consisted of one habituation (day 1), two conditioning (day 2–3) and one test (day 4) trials, each lasted for 900 s (15 min). The apparatus was cleaned and dried after each animal. All trainings and tests were conducted in an isolated experimental room. In the habituation trial (day 1) animals were placed into the apparatus and had free access to all parts of the apparatus for 900 s. The time the animals spent in each of the four quadrants was measured. During conditioning trials (day 2–3) animals received the drug injections (see in drugs and injection procedure) and subsequently rats were restricted in the treatment quadrant for 15 min by means of a plexiglass barrier. Treatment quadrant was determined to be one of the four quadrants in which the animal had spent neither the longest nor the shortest time during the habituation. In the test trial (day 4) animals had free access to all parts of the apparatus for 15 min. The time that rats had spent in each of the four quadrants was measured again. Behaviour of animals was recorded by a video camera. Data were stored and motion analysis was made by means of EthoVision Basic software (Noldus Information Technology B.V., Wageningen, The Netherlands). The number of entries into each of the four quadrants was also recorded during habituation and test trials, as a measure of gross locomotor activity. In order to measure its acute effects of the injected drugs on spontaneous behaviour, such as frequency of rearing and grooming were also analyzed.

2.5. Histology

At the end of the experiment, rats received an overdose of Calypsol and Seduxen mixed in the ratio of 4:1 and were transcardially perfused with isotonic saline followed by 10% formalin solution. After one week of postfixation, brains were frozen, cut into 40 µm serial sections and stained with Cresyl-violet. Injection sites were reconstructed according to the stereotaxic atlas of the rat brain [36]. Only data from rats with correctly placed cannulae were analyzed.

2.6. Statistical analysis

Data are presented as mean ± standard error of the mean (S.E.M.). Two-way ANOVAs followed by Tukey's post hoc analysis were employed. Statistical significance was established at $p < 0.05$.