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Research report

The role of neurotensin in positive reinforcement in the rat central nucleus of amygdala

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ABSTRACT

In the central nervous system neurotensin (NT) acts as a neurotransmitter and neuromodulator. It was shown that NT has positive reinforcing effects after its direct microinjection into the ventral tegmental area. The central nucleus of amygdala (CeA), part of the limbic system, plays an important role in learning, memory, regulation of feeding, anxiety and emotional behavior. By means of immunohistochemical and radioimmune methods it was shown that the amygdaloid body is relatively rich in NT immunoreactive elements and NT receptors.

The aim of our study was to examine the possible effects of NT on reinforcement and anxiety in the CeA. In conditioned place preference test male Wistar rats were microinjected bilaterally with 100 or 250 ng NT in volume of 0.4 μl or 35 ng neurotensin receptor 1 (NTS1) antagonist SR 48692 alone, or NTS1 antagonist 15 min before 100 ng NT treatment. Hundred or 250 ng NT significantly increased the time rats spent in the treatment quadrant. Prior treatment with the non-peptide NTS1 antagonist blocked the effects of NT. Antagonist itself did not influence the reinforcing effect. In elevated plus maze test we did not find differences among the groups as far as the anxiety index (time spent on the open arms) was concerned. Our results suggest that in the rat ACE NT has positive reinforcing effects. We clarified that NTS1s are involved in this action. It was also shown that NT does not influence anxiety behavior.

1. Introduction

NT is a tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH) that was first isolated from bovine hypothalamus during the purification of substance P [9]. NT interacting with specific G protein coupled receptors [28] was shown to operate as a hormone of the gastrointestinal tract. NT has been found in blood plasma, the gastrointestinal (GI) tract, anterior and intermediate lobes of the pituitary, thyroid gland, testis and it was detected in numerous other peripheral visceral organs such as the heart, kidney, liver, lung, pancreas, and spleen. [10,15,54]. It has been demonstrated that the elevated concentration of intraluminal lipid in the GI tract is accompanied by the facilitation of NT release into the plasma and the inhibition of intestinal motility [22]. When NT is administered intravenously to laboratory animals, it produces different cardiovascular and endocrine effects, including hypotension, hyperglycemia and vasodilation. NT and its receptors are widely distributed in the rat and human central nervous system (CNS) where NT acts as a neurotransmitter and/or neuromodulator [5,11,15,47,65]. Combined dysfunction of NT neurons and the mesocortical-mesolimbic dopamine (DA) system may play a role in different diseases including Parkinson's disease [21] and schizophrenia [38]. It has been revealed that NT has positive rewarding effects after its direct microinjection into the ventral tegmental area (VTA) [24]. NT is involved in stress and pain modulation, reward related processes and in the pathophysiology of drug addiction and depression [13,25,38,55].

NT mediates its effects through its neurotensin receptor 1, -2, -3 (NTS1, NTS2, NTS3), respectively [40,65]. The NTS1 was molecularly cloned from rat brain [60] and from human brain as well [67]. NT has high affinity to NTS1 and this receptor is distributed throughout the CNS. In the amygdaloid body (AMY) the NTS1 density is relatively high [26,47]. NTS1 is a high-affinity G-protein-coupled receptor, belonging to the seven transmembrane domain receptor family [60]. The SR 48692 [2-{1-(7-chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)-(1*H*-pyrazole-3-carbonyl)amino}-adamantane-2-carboxylic acid] is a potent non-peptide NTS1 antagonist [14]. SR 48692 has much higher affinity for NTS1 (IC_{50} = 5.6 nM) than for NTS2 (IC_{50} = 300 nM) [27], so it has been a good tool to differentiate the roles of NTS1 in certain physiological properties of NT [63].

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The AMY, part of the limbic system, is considered to be one of the key elements in the neural control of emotion [12], memory [46], and reinforcement [36,41]. It is well known that the AMY is an important structure for innate and conditioned fear behavior and results of lesion studies implicate the AMY in the acquisition and retention of aversively motivated tasks [12,36,41]. Extensive evidence indicates that memory storage is influenced by post-training intra-amygdaloid injections of drugs affecting several different neuromodulatory and neurotransmitter systems [31]. Conditioned place preference (CPP) studies reveal that the AMY may play an important role in reward processes and motivation [7,50]. The AMY is diffusely innervated by the mesolimbic DA system and the central and intercalated nuclei of the AMY (CeA) have more DA terminals than the other amygdaloid nuclei. The CeA connects with the nucleus accumbens and receives DA-ergic afferents from the VTA [37,68]. By means of immunohistochemical and radioimmune methods it was shown that the CeA is relatively rich in NT immunreactive elements and NT receptors [5,11,65].

In the AMY, however, possible positive reinforcing effects of NT or its modulatory role in anxiety have not been investigated yet. In the present experiments, therefore, we examined the effects of bilateral NT microinjections into the CeA on reinforcement in CPP paradigm and on anxiety in elevated plus maze (EPM) test. NT was injected in two different doses in order to demonstrate dose-dependent effects. We used specific receptor antagonist to study the involvement of NTS1 in the effects of NT. This was accomplished by examining the effect of prior treatment with the NTS1 antagonist SR 48692. This compound was selected, since it is a competitive antagonist at the NTS1 and several reports have shown that SR 48692 can inhibit behavioral effects of NT [27,51,59].

2. Materials and methods

2.1. Subjects

Hundred and nineteen adult male Wistar rats weighing 280–320 g at the beginning of the experiments were housed individually and cared for in accordance with institutional (Pécs University, Medical School) and international standards (National Institutes of Health Guidelines for Laboratory Animals). 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 behavioral 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 [66]. Cannulae were fixed to the skull with two 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 of 6 days postoperative recovery before experiments commenced, 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 in two different doses: 100 ng (54.6 pmol) or 250 ng (136.6 pmol) 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. NTS1 antagonist SR 48692 [gifted by Sanofi-Synthelabo Co., 35 ng (60 pmol)/0.4 μl] 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 in the experiment with NTS1 antagonist. In this experiment the following groups were used: the antagonist treated group (ANT) received SR 48692 and then 15 min later vehicle of NT (ANT + Veh1). The NT injected group pretreated with antagonist (ANT + NT) received SR 48692 15 min before being injected with 100 ng NT. The NT treated group (NT) received vehicle of antagonist and then 100 ng NT (Veh2 + NT). The Control group (Control) received two vehicle injections (Veh2 + Veh1). The antagonist or Veh2 were applied 15 min prior to NT or Veh1 injections, respectively. Solutions were kept in +4 °C before application. In this report all the doses mentioned are meant to be the 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 a syringe pump in volume of 0.4 μl (Cole Parmer, IITC, Life Sci. Instruments, California) over a 60 s interval. After injection cannulae were left in place for an additional 60 s to allow diffusion into the surrounding tissue. During the injections rats were gently hold in hand.

2.4. Conditioned place preference test (CPP)

The CPP paradigm has been used to measure hedonic properties of drugs of abuse as well as of natural reinforcers [5,64]. Our corral apparatus, consisted of a circular open field, with a diameter of 85 and 40 cm high wall. Black lines divided the floor into four quadrants of equal size. External visual cues in the surroundings helped the animals' spatial orientation inside the apparatus. The room was dimly lit by a 40 W bulb. The place preference procedure consisted of one Habituation (day 1), two Conditioning (days 2–3) and one Test (day 4) trials, each lasted of 900 s (15 min). The apparatus was cleaned and dried after each session. All trainings and testing were conducted in an isolated experimental room. In 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 that animals had spent in each of the four quadrants was measured. During Conditioning trials (days 2–3) animals received the drug injections (see in drugs and injection procedure) and subsequently rats were restricted to the treatment quadrant for 15 min by means of a plexiglass barrier. Treatment quadrant (TQ) was determined to be one of the four quadrants in which the animal had spent neither the longest nor the shortest time during habituation. On the fourth day (Test trial) animals had free access to all parts of the apparatus. The time that rats had spent in each of the four quadrants was measured again. Behavior 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 the four quadrants was also recorded during Habituation and Test trials, as a measure of gross locomotor activity. In order to gauge acute effects of NT on spontaneous behavior, frequency of rearing and grooming were also analyzed.

2.5. Elevated plus maze test

Anxiety was evaluated in an EPM test. The apparatus was constructed of grey coloured wooden planks. The equipment consisted of two opposite open arms (50 cm × 10 cm) and two opposite enclosed 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 administrations the animals were placed into the center of the maze (central platform), facing one of the enclosed arms. The trials lasted 5 min during which the number of entries into and time spent on the open and enclosed arms and the end of the open arms (end-arms) were measured. Each rat was tested only once. Data were stored and motion analysis was made by means of EthoVision Basic software.

2.6. Histology

At the end of experiments, 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 1 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 [66]. Only data from rats with correctly placed cannulae were analyzed.

2.7. Statistical analysis

Data are presented as mean ± standard error of the mean (S.E.M.). One-way and two-way ANOVAs followed by Tukey post hoc analysis were employed. Statistical significance was established at $p \leq 0.05$.

3. Results

3.1. Histology

Histological examination showed that in 107 cases of 119 animals the target place of the cannulae was precisely and symmetrically tipped to the target area (CeA). The tracks of cannulae and tip positions were determined on the basis of the existence of debris and moderate glial proliferation. Schematic illustration of cannula placements is shown in Fig. 1.

Considering 12 rats the reconstructed cannula placement was not correctly positioned in the target area, so these subjects were