Short communication

Anti-nociceptive effect of neuropeptide Y in periaqueductal grey in rats with inflammation

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Abstract

Experimental inflammation was induced by subcutaneous injection of carrageenan into the left hindpaw of rats. Intra-periaqueductal grey (PAG) injection of 0.02 or 0.1 nmol of neuropeptide Y (NPY), but not 0.004 nmol, induced significant increases in hindpaw withdrawal latency (HWL) to thermal and mechanical stimulation in rats with inflammation. Furthermore, the anti-nociceptive effect of NPY was blocked partly by following intra-PAG injection of the Y1 receptor antagonist NPY28-36. The results demonstrated that NPY plays an anti-nociceptive role in PAG in rats with inflammation, in which Y1 receptor is involved.

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Neuropeptide Y (NPY) is a 36-amino acid peptide belonging to the pancreatic polypeptide family that was first isolated in mammalian brain tissue in the early 1980s [12]. NPY is one of the most abundant peptides with several receptor subtypes in the central and peripheral nervous system [1–3]. It was found that NPY-like immunoreactivity and mRNA of NPY distributed in rats periaqueductal grey (PAG) [1,2,11]. Studies implicate that NPY plays an important role on the modulation of nociception in the central nervous system [4,7,9,15]. Recent study in our laboratory has demonstrated that intra-periaqueductal grey (PAG) administration of NPY resulted in an anti-nociception in normal rats [14].

Carrageenan-induced inflammation is a commonly used model for the study of pain [8,16,18]. The present study was performed to explore the effect of intra-PAG administration of NPY on nociceptive responses in rats with experimentally induced inflammation.

All experiments were performed on freely moving male Sprague–Dawley rats weighing between 200 and 300 g (Experimental Animal Center of Beijing Medical University, Beijing, China). The rats were housed in cages with free access to food and water, and maintained in a room temperature of 24±2°C with a 12-h light–dark cycle. All experiments were conducted according to the guidelines of the Animal Ethical Committee of Karolinska Institute and every effort was made to minimize animal suffering. The animals were anaesthetized by intraperitoneal pentobarbital (40 mg/kg) and were mounted on a stereotaxic instrument. A stainless steel guide cannula of 0.8 mm out-diameter was directed to PAG (AP 5.5, L 0.5, H 6.0 mm from the surface of the skull) according to Paxinos and Watson [10] and was fixed to the skull by dental acrylic. On the experimental day a stainless steel needle with a 0.4-mm diameter was directly inserted into the guide cannula, with 1 mm beyond the tip of the latter. One µl of solution was thereafter infused into PAG over 1 min.

Inflammation was induced by subcutaneous injection of 0.1 ml of 2% carrageenan (Sigma Chemical Company, St. Louis, MO, USA) into the plantar region of the rat left hindpaw [8,16,18]. Three hours later, NPY was injected into PAG and nociceptive tests were performed. The hindpaw volume was measured by a plethysmometer.
(UGO Basile, type 7150, Italy) before testing procedures started.

All rats were accustomed to the nociceptive test conditions for 5 days before the experiment was conducted. The latencies to hindpaw withdrawal during thermal and mechanical stimulation were measured. The thermal response was assessed by the hot-plate. The entire ventral surface of the rat’s hindpaw was placed manually on the hot-plate which was maintained at a temperature of 52°C (51.8–52.4°C) [16–18]. The time to hindpaw withdrawal was measured in seconds (s) to be referred to as the hindpaw withdrawal latency (HWL). The Randall Selitto Test (Ugo Basile, Type 7200, Italy) was used to assess the HWL to mechanical stimulation. A wedge-shaped pusher at a loading rate of 30 g/s was applied to the dorsal surface of the manually handled hindpaw and the latency required to initiate the struggle response was assessed and expressed in s [16–18]. Each rat was tested with both types of stimulation. The measurements before intra-P AG injection were regarded as the basal HWL. The HWLs recorded during subsequent experiments were expressed as percentage change of the basal level for each rat (% change of HWL). The HWLs were tested before and repeated at 5, 10, 15, 20, 30 and 60 min after intra-P AG injection. Solution for intra-PAG administration was prepared with sterilized 0.9% saline, each with a volume of 1 μl: (1) 0.004, 0.02 or 0.1 nmol of NPY (human NPY, Neosystem Laboratories, France); (2) 0.1 nmol of NPY28-36 ([Pro30, Tyr32, Leu34]NPY28-36, Neosystem Laboratories, France); (3) or 1 μl of 0.9% saline as a control. Data are presented as mean ± S.E.M. The difference between groups was determined by two-way analysis of variance (ANOVA) for repeated measures or Student’s t-test (two-tailed) where applicable.

Thirty-two rats with inflammation received intra-periaqueductal grey injection of: (1) 1 μl of 0.9% saline as a control (n=8); (2) 0.004 nmol of NPY (n=8); (3) 0.02 nmol of NPY (n=8); (4) 0.1 nmol of NPY (n=8). The results are shown in Fig. 1.

The HWL increased significantly after intra-PAG injection of 0.02 of (Hot plate test: \( F_{left/right} = 6.46, P<0.01; \) \( F_{left/right} = 15.08, P<0.001 \). Randall Selitto test: \( F_{left/right} =

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Fig. 1. Effects of intra-PAG injection of NPY on the HWL to thermal and mechanical stimulation in rats with inflammation induced by 0.1 ml of 2% carrageenan injected into the plantar region of left hindpaw. (A and B): hot-plate test; (C and D): Randall Selitto test. HWL: hindpaw withdrawal latency. NPY: neuropeptide Y. P AG: periaqueductal grey. Results are presented as mean ± S.E.M. The statistical difference between groups was evaluated by two-way ANOVA, **P<0.01 and ***P<0.001 compared with the control group.
2.06, *$P<0.001$; $F_{right/right}=14.5$, $P<0.001$) or 0.1 nmol of NPY (Hot plate test: $F_{left/left}=33.69$, $P<0.001$; $F_{right/right}=20.22$, $P<0.001$. Randall Selitto test: $F_{left/left}=62.80$, $P<0.001$; $F_{right/right}=38.08$, $P<0.001$) compared with the control group, but not 0.04 nmol of NPY (Hot plate test: $F_{left/left}=0.32$, $P=0.57$; $F_{right/right}=1.20$, $P=0.28$. Randall Selitto test: $F_{left/left}=17.35$, $P<0.001$; $F_{right/right}=7.94$, $P=0.06$).

Rats with inflammation received intra-PAG administration of 0.1 nmol of NPY, 5 min later by intra-PAG injection of either 0.1 nmol of the Y1 antagonist NPY28-36 ($n=8$) or 1 μl of 0.9% saline as a control ($n=8$). As shown in Fig. 2, the NPY-induced increases in HWLs to thermal and mechanical stimulation decreased significantly after intra-PAG injection of 0.1 nmol NPY28-36 (Thermal test: $F_{left/left}=26.89$, $P<0.001$; $F_{right/right}=36.54$, $P<0.001$. Mechanical test: $F_{left/left}=24.13$, $P<0.001$; $F_{right/right}=17.09$, $P<0.001$) compared with the control group.

It is well known that PAG is an important brain area involved in anti-nociception [6]. Studies demonstrated that moderate to high concentrations of NPY and NPY binding sites were found in PAG [1,2,5]. The present study demonstrated that intra-PAG injection of 0.02 or 0.1 nmol of NPY induced significant bilateral increases in HWLs to both thermal and mechanical stimulation in rats with inflammation. Furthermore, the increased HWLs induced by NPY were blocked by intra-PAG injection of the Y1 receptor antagonist NPY28-36. The results demonstrated that NPY plays an anti-nociceptive role in PAG in rats with experimentally induced inflammation, in which Y1 receptor is involved.

Pathological conditions can alter the nociceptive responses to NPY in animals [13,15]. It is more useful to assess the anti-nociceptive effect induced by intra-PAG administration of NPY in inflammatory rats than in intact rats. The peak anti-nociceptive effects induced by intra-PAG injection of NPY was higher in rats with inflammation (0.1 nmol of NPY increased about 45% of the HWL than the basal HWL) than in intact rats (0.2 nmol of NPY increased 50% of the HWL than the basal HWL) [14], indicating that there may be an up-regulation of NPY and/or NPY receptor system after experimentally induced inflammation.

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References


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Fig. 2. Effects of intra-PAG injection of 0.1 nmol of NPY28-36 on the NPY-induced increase in HWLs to thermal and mechanical stimulation in rats with carrageenan-induced inflammation. Control group: NPY 0.1 nmol + saline 1 μl; Experimental group: NPY 0.1 nmol + NPY28-36 0.1 nmol. (A): hot-plate test; (B): Randall Selitto test. HWL: hindpaw withdrawal latency. PAG: periaqueductal grey. NPY: neuropeptide Y. Results are presented as mean±S.E.M. The statistical difference between groups was evaluated by Student $t$-test (two-tails), *$P<0.05$, **$P<0.01$ and ***$P<0.001$ compared with the control group.


