Involvement of CGRP and CGRP1 receptor in nociception in the nucleus accumbens of rats

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Abstract

The present study was performed to investigate the role of calcitonin gene-related peptide (CGRP) and its antagonist CGRP8-37 on nociception in the nucleus accumbens of rats. Hindpaw withdrawal latencies (HWLs) to noxious stimulation induced by hot plate and Randall Selitto tests were measured. The HWL to both thermal and mechanical stimulation increased significantly after intra-nucleus accumbens administration of 0.5 or 1 nmol of CGRP, but not 0.1 nmol, indicating that CGRP plays an anti-nociceptive effect in the nucleus accumbens of rats. The anti-nociceptive effect induced by intra-nucleus accumbens administration of 1 nmol of CGRP was blocked significantly by following intra-nucleus accumbens administration of 1 nmol of CGRP8-37, a selective antagonist of CGRP1 receptor. Furthermore, the HWLs to both thermal and mechanical stimulation decreased significantly after intra-nucleus accumbens administration of 0.02, 0.1 and 0.5 nmol of CGRP8-37 alone. The hyperalgesic effect of intra-nucleus accumbens administration of CGRP8-37 lasted for more than 60 min after the injection, suggesting that CGRP1 receptor is involved in anti-nociception in the nucleus accumbens of rats. The results indicate that CGRP and CGRP1 receptor have important roles in nociceptive modulation in the nucleus accumbens of rats.

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1. Introduction

CGRP is a 37 amino acid neuropeptide with multiple physiological functions and exists widely in the nerve system [1,8,15]. It has been shown to play an important role in pain modulation [3,9,12,18,21]. CGRP potentiates the excitatory effect induced by substance P in the dorsal horn of the spinal cord [2]. Intrathecal injection of CGRP8-37, a selective antagonist of CGRP receptor, induced significant anti-nociceptive effect in intact rats, rats with inflammation and rats with mononeuropathy [21,23,24]. Furthermore, it was found that there was an interaction between CGRP and opioid peptides in the modulation of nociception in the spinal cord of rats [22]. Intracerebroventricular injection of CGRP produced an anti-nociceptive effect in rats and mouse [3,12]. It has been demonstrated that some brain structures or areas such as periaqueductal grey, nucleus raphe magnus and the nucleus accumbens play an important role in pain modulation [6,7,9,19,20]. Recent study in our laboratory demonstrated that CGRP produced significant anti-nociceptive effects in the nucleus raphe magnus of rats, and opioid receptors were involved in the CGRP-induced anti-nociception in nucleus raphe magnus [9]. Furthermore, our results showed that intra-periaqueductal grey administration of CGRP elicited dose-dependent increases in hindpaw withdrawal latencies to noxious thermal and mechanical stimulation in rats [18]. The above results of our laboratory demonstrated that CGRP plays an important role in anti-nociception in the periaqueductal grey and nucleus raphe magnus of rats. It is known that the nucleus accumbens plays an important role in pain modulation [5,7,19]. Morphological study has
shown that there is very high density of CGRP receptor binding sites in the nucleus accumbens, indicating that in the nucleus accumbens CGRP may be involved in pain modulation [15]. The present study was performed to investigate the role of CGRP in nociception in the nucleus accumbens of rats.

2. Materials and methods

2.1. Animals preparation

Experiments were performed on freely moving male Sprague–Dawley rats (weighing 200–250 g, Experimental Animal Center of Peking University, Beijing, China). The animals were individually housed in cages with free access to food and water, and in a room temperature of 24±2°C with a normal day/night cycle. All experiments were conducted according to the guidelines of the Animal Ethical Committee of Karolinska Institutet and every effort was made to minimize animal suffering.

2.2. Nociceptive tests

The hindpaw withdrawal latencies (HWLs) during noxious thermal and mechanical stimulation were measured. Briefly, the entire ventral surface of the rat hindpaw was placed manually on a hot plate which was maintained at a temperature of 52°C (51.8±52.4°C) [17,23,24]. The time to hindpaw withdrawal was measured in second (s) to be referred to as the HWL to thermal stimulation. 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. The latency required to initiate the withdrawal response was assessed and expressed in s. The average values obtained before intra-nucleus accumbens injection were regarded as the basal HWL in both tests. The HWLs recorded during subsequent experiments were expressed as percentage changes from the basal level for each rat. Each rat was tested with both types of stimulation. A cut-off limit of 15 s was set up to avoid tissue damage. All rats were accustomed to the testing conditions for 5 days before the starting of the experiment to minimize the stress induced by handling.

2.3. Intra-nucleus accumbens injection

The animals were anaesthetized by intraperitoneal pentobarbital (40 mg/kg) and were mounted on a stereotaxic frame. A stainless steel guide cannula of 0.8 mm outer diameter was directed to the nucleus accumbens (B 2.0, L 1.5, H 4.8 mm from the surface of the skull) [11], then fixed to the skull with dental acrylic. On the experimenta-

tion day, a stainless steel needle with 0.4 mm diameter was directly inserted into the guide cannula, so that it protruded 2 mm beyond the tip of the latter. One microliter of solution was thereafter infused into the nucleus accumbens over 1 min.

2.4. Data analysis

At the conclusion of the experiments, the location of the tip of the injection tube was verified and the results are showed in Fig. 1. Only the results from nociceptive tests where the tips of the injection tubes were within the nucleus accumbens were used for statistical analysis. Data from nociceptive tests were 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. P<0.05, P<0.01 and P<0.001 are considered as significant differences.

2.5. Chemicals

Solutions for intra-nucleus accumbens administration
were prepared with sterilized saline, each with a volume of 1 μl: (1) 0.1, 0.5 or 1 nmol of CGRP (rat-CGRP, Tocris Cookson Ltd., Bristol, BS11 8TA, UK); (2) 0.02, 0.1, 0.5 or 1 nmol of CGRP8-37 (rat-CGRP8-37, Tocris Cookson Ltd., Bristol, BS11 8TA, UK). Control group were given 1 μl of 0.9% saline.

3. Results

3.1. Anti-nociceptive effect induced by intra-nucleus accumbens injection of CGRP in rat

Four groups with eight rats in each received intra-nucleus accumbens injection of 0.1, 0.5 or 1 nmol of CGRP, or 1 μl of 0.9% saline as a control. The results are shown in Fig. 2.

Compared with the control group, there were significant increases in HWLs after intra-nucleus accumbens administration of 0.5 nmol (Thermal test: $F_{left/left}=17.77, P<0.001$; $F_{right/right}=22.95, P<0.001$). Mechanical test: $F_{left/left}=13.03, P<0.01$; $F_{right/right}=13.76, P<0.01$) or 1 nmol of CGRP (Thermal test: $F_{left/left}=37.90, P<0.001$; $F_{right/right}=67.54, P<0.001$). Mechanical test: $F_{left/left}=47.54, P<0.001$; $F_{right/right}=55.91, P<0.001$), but not 0.1 nmol of CGRP (Thermal test: $F_{left/left}=2.48, P=0.13$; $F_{right/right}=0.62, P=0.44$. Mechanical test: $F_{left/left}=2.25, P=0.15$; $F_{right/right}=1.94, P=0.18$).

3.2. Blockade effect of intra-nucleus accumbens administration of CGRP8-37 on CGRP-induced antinociception

Two groups of rats received intra-nucleus accumbens injection of 1 nmol of CGRP, followed 5 min later by intra-nucleus accumbens injection of 1 nmol of CGRP8-37 ($n=8$), or 1 μl of 0.9% saline as a control ($n=8$). Fig. 3 shows the data obtained at 15 min after the injection the CGRP8-37.

Compared with the control group the CGRP-induced increases in HWLs were attenuated significantly after intra-nucleus accumbens administration of CGRP8-37 (Thermal test: $t_{left/left}=3.77, P<0.01$; $t_{right/right}=4.01, P<0.01$; Mechanical test: $t_{left/left}=3.02, P<0.05$; $t_{right/right}=4.87, P<0.01$).

3.3. Hyperalgesia induced by intra-nucleus accumbens administration of CGRP8-37 in rat

Four groups with eight rats in each received intra-nucleus accumbens injection of 0.02, 0.1 or 0.5 nmol of
4. Discussion

The present study showed that intra-nucleus accumbens administration of CGRP induced significant increases in HWLs, indicating that CGRP produced an anti-nociceptive effect in that brain area of rats. The anti-nociceptive effect induced by CGRP was antagonized by following intra-nucleus accumbens injection of the CGRP antagonist CGRP8-37. Furthermore, the HWL decreased significantly after intra-nucleus accumbens injection of CGRP8-37 alone, and the hyperalgesic effect lasted for more than 60 min. The results indicate that in the nucleus accumbens the CGRP1 receptor plays a tonic role on pain modulation in rats.

Immunohistochemical studies demonstrated that CGRP-immunoreactive cell bodies and CGRP receptors are widely distributed in the brain [8,16]. Some reports have demonstrated the distribution of CGRP binding sites in the central nervous system. High levels of 125I-hCGRP binding sites were found in the nucleus accumbens, the central nucleus of the amygdala and periaqueductal grey [16]. It has been reported that injection of morphine into the nucleus accumbens induced an anti-nociceptive effect, indicating that the nucleus accumbens is one of the brain structures involved in endogenous pain modulation [5,7,19,20]. In the present study intra-nucleus accumbens injection of CGRP induced significant anti-nociceptive effect, which could be attenuated by a following administration of CGRP8-37, a CGRP antagonist [4], indicating that the anti-nociception of CGRP is induced by activating the CGRP receptor in the nucleus accumbens. CGRP8-37 is a selective antagonist of CGRP receptor, and mainly binds with CGRP1 subtype (pA2 value = 7–8) [1]. Because intra-nucleus accumbens injection of CGRP8-37 antagonized the anti-nociceptive effect induced by CGRP, it is likely that CGRP1 receptors were involved in the CGRP-induced anti-nociception in the nucleus accumbens of rats. Study has shown that nucleus accumbens sends nerve fibers to habenula, from which nerve fibers project directly or indirectly to the periaqueductal grey [13]. The existence of a neural pathway between the nucleus accumbens and the periaqueductal grey subserving modulation on nociception has been demonstrated [19,20]. It is possible that intra-nucleus accumbens administration of CGRP activates the descending pathway from nucleus accumbens to periaqueductal grey to producing anti-nociception in rats.

Biochemical and pharmacological studies have shown that the CGRP receptor belongs to the G-protein coupled receptor family and that CGRP binding affinity is sensitive to GTP or analogs in a variety of tissues [15]. CGRP also increases the transmembrane Ca2+ current [15]. Recent cloning studies have isolated receptors that confer specific responsiveness to CGRP and the related peptide adrenomedullin [15]. According to differences in sensitivity to receptor antagonists, CGRP receptors were divided into

CGRP8-37, or 1 μl of 0.9% saline as a control. The results are shown in Fig. 4.

Compared with the control group, the HWL decreased significantly after intra-nucleus accumbens injection of 0.02 (Thermal test: F_{left/left} = 42.74, P < 0.001; F_{right/right} = 41.38, P < 0.001; Mechanical test: F_{left/left} = 17.12, P < 0.001; F_{right/right} = 17.45, P < 0.001). 0.1 (Thermal test: F_{left/left} = 137.54, P < 0.001; F_{right/right} = 93.21, P < 0.001; Mechanical test: F_{left/left} = 49.43, P < 0.001; F_{right/right} = 50.62, P < 0.001) or 0.5 nmol of CGRP (Thermal test: F_{left/left} = 141.25, P < 0.001; F_{right/right} = 112.26, P < 0.001; Mechanical test: F_{left/left} = 110.48, P < 0.001; F_{right/right} = 101.22, P < 0.001). The effects of intra-nucleus accumbens administration of CGRP8-37 lasted for more than 60 min after the injection.
several subtypes and the CGRP1 receptor subtype is highly sensitive to CGRP8-37 [4,14]. Using in situ hybridization, Oliver et al. demonstrated the heterogeneous distribution of the mRNAs of two proposed CGRP1 receptors in the rat brain (RDC-1 and calcitonin receptor-like receptor, CRLR) [10]. In the present study intra-nucleus accumbens administration of 0.02, 0.1 or 0.5 nmol of the CGRP1 receptor antagonist CGRP8-37 alone produced significant hyperalgesic effects in rats and the effect of intra-nucleus accumbens administration of CGRP8-37 lasted for more than 60 min after the injection.

The results of the present study suggest that CGRP may play a tonic role on pain modulation in the nucleus accumbens of rats. It is possible that CGRP releases in nucleus accumbens and plays modulation on nociception in normal condition. It has been reported that there were high levels of $^{125}$I-hCGRP binding sites in the nucleus accumbens [16]. It is possible that intra-nucleus accumbens administration of CGRP may activate more CGRP receptors than the endogenous released CGRP so that produces anti-nociception. So it is possible that endogenous released CGRP binds to the available CGRP receptor in the nucleus accumbens during noxious stimulation subserving anti-nociception, intra-nucleus accumbens administration of CGRP8-37 could block the CGRP1 receptor causing hyperalgesia in rats. However, Yu et al. reported that intrathecal administration of CGRP8-37 induced significant anti-nociceptive effect in rats [21]. It is interesting to note that in the brain the roles of CGRP and CGRP8-37 have opposing effects to those in the spinal cord.

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