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Up-regulation of galanin and corticotropin-releasing hormone mRNAs in the key hypothalamic and amygdaloid nuclei in a mouse model of visceral pain

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Abstract

Cyclophosphamide (CP)-induced cystitis is often used as an animal model of visceral pain. Various neuropeptides in the hypothalamic and amygdaloid nuclei are implicated in pain-induced responses. However, little information is available regarding the regulation of the neuropeptides in response to visceral pain. In the present study, we examined the effects of CP-induced cystitis on the levels of mRNAs encoding galanin, corticotropin-releasing hormone (CRH), substance P, and enkephalins in the hypothalamic and limbic nuclei using *in situ* hybridization histochemistry in mouse. Galanin mRNA levels in CP-treated group increased significantly in the arcuate nucleus and the paraventricular nucleus (PVN) but not in the medial preoptic area after the intraperitoneal administration of CP (200 mg/kg body weight) in comparison to those in saline-treated group. CRH mRNA levels in CP-treated group also increased significantly in the central amygdala as well as the PVN after the CP administration. In contrast, CP-induced cystitis failed to upregulate the preprotachykinin-A and preproenkephalin genes which encode substance P and enkephalins, respectively in the hypothalamic and limbic nuclei at any of the time points examined. These results suggest that visceral nociception may upregulate both galanin and CRH gene expression in the hypothalamic and limbic nuclei.

Keywords: Galanin; Corticotropin-releasing hormone; Visceral pain; Chemical cystitis; Hypothalamus; *In situ* hybridization histochemistry

1. Introduction

Cyclophosphamide (CP)-induced cystitis is often used as a model for visceral pain [1–3]. Previous studies have demonstrated that CP-induced cystitis has some unique features compared to other visceral pain models [1–3]. Firstly, the stimulus is a pure visceral one confined to bladder. Secondly, its time course can be effectively monitored through behavioral and histological observations. Thirdly, it is reproducible and controllable without departing from ethical rules. This model is particularly useful for examining the mechanisms in the central nervous system (CNS) processing the visceral nociceptive information from the lower urinary tract, since no anesthesia is required [1–3].

Several studies using a CP-induced cystitis model have demonstrated that visceral pain activates the neural pathways in various brain regions [1–4]. Immunocytochemistry and *in situ* hybridization histochemistry have revealed that visceral nociception causes the induction of c-*fos* protein (Fos) as well as *c-fos* gene, a neural activational marker, in the CNS including the periaqueductal grey (PAG), the Edinger–Westphal nucleus, the lateral habenular nucleus, the bed nucleus of the stria terminalis (BNST), the paraventricular nucleus (PVN), and the central nucleus of amygdala (CeAMY) [2–5]. Moreover, visceral nociception has been reported to produce different patterns of *c-fos* gene and Fos expression in the brain in comparison to somatic nociception [1,3,5]. Activated neural pathway by nociception may lead to various biological responses including the regulation of transcription of neuropeptides.

Accumulating evidence revealed that nociceptive stimulation regulated hypothalamic neuropeptides, which may play a role in the regulation of pain and pain-induced stress responses [6–8]. We previously revealed that somatic pain, such as the subcutaneous injection of formalin into the bilateral hindpaws, caused neuroendocrine responses, such as the upregulation of corticotropin-releasing hormone (CRH) messenger (m)RNA, arginine vasopressin (AVP) heteronuclear (hn)RNA, neuronal nitric oxide synthase (nNOS) mRNA as well as the serum AVP levels

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[9]. Using a CP-induced cystitis mode, we recently demonstrated that visceral nociception caused different neuroendocrine responses in comparison to those caused by somatic nociception. Namely, the CRH mRNA level increased in the PVN, whereas the levels of AVP mRNA, AVP hnRNA and nNOS mRNA were unchanged in the PVN [10]. In addition to CRH in the PVN, several lines of studies demonstrate that various neuropeptides including galanin, substance P, and preproenkephalin (PPE) may also be implicated in the responses to visceral as well as somatic pain [7,8]. These neuropeptides were abundantly expressed in the hypothalamic and amygdaloid nuclei such as the PVN, the arcuate nucleus (ARC), the medial preoptic area (MPOA), the amygdala (AMY) which are key sites for emotional and physical responses to pain [7,11,12]. However, little information is available regarding the regulation of neuropeptides in response to visceral pain.

The present study was further conducted to elucidate the regulation of these neuropeptides expression in the mouse key hypothalamic and limbic nuclei in visceral nociceptive stimulation. In the present study, we examined the effects of CP-induced cystitis on the expression of genes encoding galanin, CRH, substance P, and enkephalins in the hypothalamic and limbic nuclei which includes the PVN, the ARC, the MPOA, the BNST, the CeAMY, the MeAMY, and the caudate putamen using an *in situ* hybridization histochemistry.

Although the exact cause of CP-induced cystitis is not fully understood, it has been proposed that urothelial damage occurs by acrolein, the hepatic aldehyde metabolite of CP [1–3,13]. A direct contact of the metabolite to the urothelium causes edema, infiltration of polymorphonuclear leukocyte, ulceration, and hemorrhages [3,13].

2. Methods

2.1. Mice

A total of 62 C57BL/6J male mice (10–12 weeks old, weighing 24–30 g) were used. They were group-housed (4–6 mice/cage) in plastic cages (30 × 20 × 12 cm) and maintained on a 12/12 h light/dark cycle (light off at 9:00 am, light on at 9:00 pm) at constant temperature (24 °C) throughout the studies. Food and water were available *ad libitum*.

A solution of 0.9% sterile pyrogen-free saline containing CP (200 mg/kg body weight) was injected intraperitoneally into the mice. The same volume of 0.9% sterile pyrogen-free saline was injected intraperitoneally into the control mice. Following the injection, the animals were returned to and kept in their home cages. The animals were euthanized at 1, 6, 12, 48, and 96 h after the CP or saline injection ($n = 5–7$ in each group). All mice were sacrificed at from 1:00–3:00 pm to minimize the diurnal variation. The brains and bladders were rapidly removed. The brains were frozen on dry ice and stored at −80 °C until they were used. The bladders were fixed in 4% formaldehyde and embedded in paraffin blocks. The bladder sections were stained with hematoxylin and eosin. Histological examination confirmed cystitis findings including edema, infiltration of polymorphonuclear leukocyte, ulceration, and hemorrhages as previously reported [10,14].

All experiments in the present study were done in accordance with guidelines on the use and care of laboratory animals as set out by the Physiological Society of Japan and approved by the animal care committee of our university.

2.2. In situ hybridization histochemistry

Frozen sections were cut in 12 μm thickness on a cryostat, mounted onto gelatin-coated slides (Matsunami Glass Ind., Lt, Osaka, Japan) and stored at −80 °C until they were used. The probes used were oligodeoxynucleotides complementary to mRNA coding for galanin (5′-GTG GTT GTC AAT GGC ATG TGG GCC CAG AAG GTA GCC AGC GGT GGT CAG-3′), CRH (5′-CAG TTT CCT GTT GCT GTG AGC TTG CTG AGC TAA CTG CTC TCG TGC CCT GGC-3′), preprotachykinin-A (PPT-A) (5′-GAA CTG CTG AGG CTT GGG TCT TCG GGC GAT TCT CTG AAG AAG ATG CTC-3′) which is

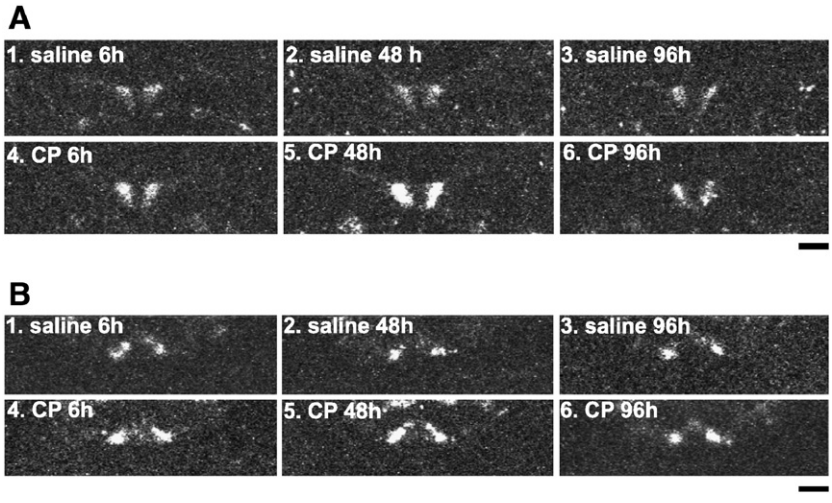

Fig. 1. Representative autoradiographs showing an increase of galanin mRNA in the paraventricular nucleus (PVN; A) and the arcuate nucleus (ARC; B) after an interaperitoneal administration of cyclophosphamide (CP; 200 mg/kg, body weight). The sections were hybridized to a ^{35}S-labelled oligodeoxynucleotide probe complementary to galanin gene. The scale bar represents 1 mm.