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Research paper

Effects of central administration of oxytocin-saporin cytotoxin on chronic inflammation and feeding/drinking behaviors in adjuvant arthritic rats

Takanori Matsuura[a,b], Makoto Kawasaki[b], Hirofumi Hashimoto[a], Mitsuhiro Yoshimura[a], Yasuhito Motojima[a,b], Reiko Saito[a], Hiromichi Ueno[a], Takashi Maruyama[a], Ken Sabanai[b], Toshiharu Mori[b], Hideo Ohnishi[b], Akinori Sakai[b], Yoichi Ueta[a,*]

[a] Department of Physiology, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan
[b] Department of Orthopaedics, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan

HIGHLIGHTS

- The arthritis index as a marker of chronic inflammation was enhanced in AA rats pretreated with OXT-SAP administered intrathecally.
- Suppression of food intake was attenuated transiently in AA rats pretreated with OXT-SAP administered fourth intracerebroventricularly.
- OXT-ergic pathways may be involved in anti-inflammation at the spinal level and suppression of feeding behavior at the forebrain-brainstem level in AA rats.

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ABSTRACT

An increase in the arthritis index as a marker of chronic inflammation and suppression of food intake are observed in adjuvant arthritic (AA) rats. Our previous study demonstrated that central oxytocin (OXT)-ergic pathways were activated potently in AA rats. In the present study, OXT-saporin (SAP) cytotoxin, which chemically disrupts OXT signaling was administered centrally to determine whether central OXT may be involved in the developments of chronic inflammation and alteration of feeding/drinking behavior in AA rats. The arthritis index was significantly enhanced in AA rats pretreated with OXT-SAP administered intrathecally (i.t.) but not intracerebroventricularly (i.c.v.). Suppression of food intake was significantly attenuated transiently in AA rats pretreated with OXT-SAP administered i.c.v. but not i.t. Suppression of drinking behavior was not affected by i.t. or i.c.v. administration of OXT-SAP in AA rats. In addition, intraperitoneal administration of an OXT receptor antagonist did not change the arthritis index or feeding/drinking behavior in AA rats. These results suggest that central OXT-ergic pathways may be involved in anti-inflammation at the spinal level and suppression of feeding behavior at the forebrain-brainstem level in AA rats.

1. Introduction

Adjuvant arthritis (AA) is a good experimental model of rheumatoid arthritis, which is a chronic inflammatory disease [19]. Arthritis can be induced in rats by subcutaneous (s.c.) injection at the base of their tails with heat-killed *Mycobacterium butyricum* (*M. butyricum*) [18]. Chronic inflammation is observed in AA rats, as indicated by an increase in the arthritis index, the suppression of food intake, and augmented sympathetic nerve activities [27].

Our recent study demonstrated that both oxytocin (OXT)-ergic hypothalamo-neurohypophysial/-spinal pathways were activated potently in AA rats [14]. However, little is known about the role

Abbreviations: AA, adjuvant arthritis; AVP, arginine vasopressin; CCK, cholecystokinin; CNS, central nervous system; i.c.v., intracerebroventricular; i.p., intraperitoneal; i.t., intrathecal; *M. butyricum*, *Mycobacterium butyricum*; NTS, nucleus of the solitary tract; OXT, oxytocin; OTR, oxytocin receptor; PVN, paraventricular nucleus; SEM, standard error of the mean; SAP, saporin; s.c., subcutaneous.

* Corresponding author at: Department of Physiology, School of Medicine, University of Occupational and Environmental Health, 1–1 Iseigaoka, Yahatanishi–ku, Kitakyushu 807–8555, Japan.
E-mail address: yoichi@med.uoeh-u.ac.jp (Y. Ueta).

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of activated OXT-ergic neurons in AA rats. OXT is a well-known neurohypophysial hormone that is synthesized in the paraventricular (PVN) and supraoptic nuclei of the hypothalamus, and endogenious OXT have two regulations. 1st regulation is humoral regulation, which OXT is delivered to target organs from posterior pituitary via the bloodstream. 2nd regulation is, nerve regulationis, which OXT from parvocellular neurosecretory neurons in the PVN directly project to the CNS. Many recent studies have shown that OXT is involved in various physiological functions including anti-nociception [28] and suppression of food intake [1]. Previous studies have demonstrated that s.c. administration of OXT decreases carrageenan-induced inflammation in rats [20] and that intracerebroventricular (i.c.v.) and s.c. administration of OXT results in reduced food intake in mice and rats [1,13]

In the present study, OXT-saporin (SAP) cytotoxin, which chemically disrupts OXT signaling was administered centrally and an OXT receptor (OTR) antagonist was administered peripherally to determine whether central and peripheral OXT is involved in chronic inflammation and feeding/drinking behavior in AA rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats were housed individually in plastic cages in an air-conditioned room (22–25 °C) under a 12: 12 h light/dark cycle (lights on at 07.00 h) with food and drinking water available ad libitum throughout the experiments. All of the experiments were performed in strict accordance with the guidelines on the use and care of laboratory animals, as determined by the Physiological Society of Japan and were approved by the Ethics Committee of Animal Care and Experimentation of the University of Occupational and Environmental Health, Japan

2.2. Adjuvant arthritis (AA) model

To induce AA, Wistar rats aged 7–9 weeks (weighing 250–420 g) were s.c. injected at the base of their tails with 1 mg of heat-killed *M. butyricum* (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) in paraffin liquid on day 0 [9]. The control rats were injected s.c. at the base of their tails with 100 μl of paraffin liquid in a similar manner on day 0. For experiments that involved injection of OXT-SAP or blank-SAP, the rats were injected with heat-killed *M. butyricum* 3 weeks after OXT-SAP or blank-SAP injection; the changes in food intake, body weight, and water intake were measured, and arthritis indices were scored every other day. The index was scored by assigning each paw a grade from 0 to 4 that was based on erythema, swelling and deformity of the joint (0 = no erythema or swelling; 1 = slight erythema or swelling of one of the toes; 2 = erythema and swelling of more than one toe; 3 = erythema and swelling of the ankle; 4 = complete erythema and swelling of the toes and ankle and the inability to bend the ankle). All four legs were scored as described previously [7], such that the highest score achievable was 16, and were evaluated by two independent investigators.

2.3. Fourth intracerebroventricular (i.c.v.) cannulation

For the fourth i.c.v. injection of solutions, animals were implanted with stainless steel cannulas in the fourth ventricle. Rats (5 weeks old) were anesthetized (sodium pentobarbital, 50 mg/kg body weight, intraperitoneal injection) and then placed in a stereotaxic frame. A stainless steel guide cannula (550-μl outer diameter, 10-mm length) was implanted stereotaxically at the following coordinates (from lambda: anteroposterior 3.2 mm, mediolateral 0 mm, and dorsoventral 7.2 mm from the skull surface) [2]. One stainless steel anchoring screw was fixed to the skull, and the cannula was secured in place by acrylic dental cement. The animals were then returned to their cages and allowed to recover for at least 5 days. During the recovery period, the animals were handled daily.

2.4. Chemical injections

OXT-SAP and blank-SAP were purchased from Funakoshi (Tokyo, Japan). OXT-SAP consists of OXT conjugated to SAP, which is a protein with *N*-glycosidase activity derived from the seeds of the *Saponaria officinalis* plant. Blank-SAP consists of saporin conjugated to a scrambled 11-amino acid peptide that is derived from alpha-melanocyte-stimulating hormone (α-MSH) and has no known cellular targets or biological activity. Rats were i.t. injected with OXT-SAP dissolved in saline (0.06 μg/μl) or blank-SAP dissolved in saline (0.06 μg/μl). These doses were based on the effective dose of targeted SAP toxins reported in a previous study [3].

The fourth i.c.v. injections of OXT-SAP or blank-SAP were conducted as described previously [6]. Three weeks before the animals were s.c. injected with heat-killed *M. butyricum*, a stainless steel injector (300-μm outer diameter) was introduced though the cannula at a depth 1.0 mm beyond the end of the guide. The total volume of the injected OXT-SAP or blank-SAP solution was 5 μl.

t. injections of OXT-SAP or blank-SAP were conducted as described previously [15]. Three weeks before the animals were s.c. injected with heat-killed *M. butyricum*, animals were anesthetized (under light sevoflurane), and a 25-gauge needle attached to a 25-ml Hamilton syringe was then inserted into the tissue between the dorsal aspects of the L5 and L6 spinal processes. The total volume of the injected OXT-SAP or blank SAP solution was 10 μl.

L-368,899 is an OTR antagonist and is a high-affinity, non-peptide molecule synthesized by Merck Research Laboratories. It was purchased from TOCRIS bioscience (Ellisville, MO). The dose used was based on the effective dose reported in a previous study [17]. The animals were i.p. injected with 1 mg/kg of L-368,899 or saline twice a day for 8 days (days 14–22) after they were s.c. injected heat-killed *M. butyricum*.

2.5. Verification of the effect of the fourth i.c.v. OXT-SAP

Previous studies suggested that the release of oxytocin in the nucleus tractus solitarius (NTS) of the hindbrain from descending projections that originate in the paraventricular nucleus can inhibit food intake by amplifying the satiety response to cholecystokinin (CCK)-8 [4]. We verified the effect of the fourth i.c.v. injected OXT-SAP indirectly using this response. CCK-8 was purchased from Sigma-Aldrich Japan Co. LLC. (Tokyo, Japan). Rats were i.p. injected with CCK-8 dissolved in saline (50 μg/kg, 2% body weight) 3 weeks after the rats were i.c.v. injected with OXT-SAP or blank-SAP; this dose was based on the effective dose of CCK-8 reported in a previous study [10]. The rats were fasted for 24 h, which ended immediately before the start of the dark cycle when they were given access to standard laboratory rat chow. CCK-8 was administered by i.p. injection immediately before the start of the dark cycle, when the animals normally begin eating and when CCK-8 has a potent effect on reducing food intake. OXT-SAP injected and blank-SAP-injected animals received CCK-8. Saline was also administered in separate experiments as vehicle using the same animals at 7-day intervals.

After the injections, food intake and water intake were measured after 1 h, 2 h, 3 h, 4 h and 6 h. The number of rats in each group was six.