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Research report

Anti-hyperalgesic effects of intrathecally administered neuropeptide W-23, and neuropeptide B, in tests of inflammatory pain in rats

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Abstract

Neuropeptide W-23 (NPW23) is an endogenous ligand of both GPR7 and GPR8, and neuropeptide B (NPB) is an endogenous ligand of GPR7. GPR7 mRNA has been detected in regions of the cortex, the hippocampus, the hypothalamus, and the spinal cord in the rat, but GPR8 has not been found in rodents. GPR7 and GPR8 receptors have structural features in common with both opioid and somatostatin receptors. The effects of intrathecal (i.t.) application of NPW23 and NPB were tested in two inflammatory pain models (plantar injection of formalin or carrageenan) and two thermal nociceptive tests (52.5 °C and 50.5 °C hot plates) and one mechanical nociceptive test in the rat. I.t. injection of either NPW23 or NPB decreased the number of agitation behaviors induced by paw formalin injection and attenuated the level of mechanical allodynia, but not the level of thermal hyperalgesia, induced by paw carrageenan injection in a dose-dependent manner at a dose between 0.1 and 10 μg, significantly. The effects of either 10 μg of NPW23 or 10 μg of NPB were not antagonized by 10 μg of naloxone. I.t. injection of either NPW23 or NPB had no effect in both the 52.5 °C hot plate test or in the 50.5 °C hot plate tests at a dose between 1 and 100 μg. I.t. injection of either 10 μg of NPW23 or 10 μg of NPB had no effect in the mechanical nociceptive test. I.t. injection of either 10 μg of NPW23 or 10 μg of NPB significantly suppressed the expression of Fos-like immunoreactivity of the L4–5 spinal dorsal horn induced by paw formalin injection. These data suggest that both spinally-applied NPW23 and NPB suppressed the input of nociceptive information to the spinal dorsal horn, produced an analgesic effect in the formalin test, and attenuated the level of mechanical allodynia in the carrageenan test, and that either spinally applied NPW23 or spinally applied NPB had no effect in the physiological condition.

Theme: Sensory system
Topic: Pain modulation: pharmacology

Keywords: GPR7; GPR8; Fos-like immunoreactivity; Spinal cord; Nociceptive transmission; Mechanical allodynia

1. Introduction

There are a large number of G protein-coupled receptors whose roles are still unknown. GPR7 and GPR8 are known as orphan G protein-coupled receptors. GPR7 and GPR8 share a high similarity to the opioid and somatostatin receptor families, but GPR7 receptor binds a non-selective opioid ligand, such as β-endorphin, only with low affinity [8]. In rodents, GPR8 was not found and only GPR7 was expressed [8]. Recently, two endogenous ligands for GPR7 and GPR8 were identified, neuropeptide W (NPW) and neuropeptide B (NPB) [3,9,10]. NPW has two forms of peptide ligand with lengths of 23 and 30 amino acid residues, NPW23 and NPW30 [9]. The amino acid sequence of NPW23 is completely identical to that of the N-terminal 23 residues of NPW30. NPW23 and NPW30 bind to and activate both GPR7 and GPR8 at similar effective doses and are thought to be the endogenous ligands for both GPR7 and GPR8 [9]. NPB is a peptide with 29 amino acid residues and

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the C-6 position of the indole moiety in the N-terminal Trp is brominated. NPB is thought to be an endogenous ligand for GPR7 [3].

Intracerebroventricular (i.c.v.) administration of NPW23 to rats increased food intake and stimulated prolactin release [9]. Moreover, i.c.v. injection of NPB produced an analgesic effect in the rat formalin test [10]. These data suggested that the activation of brain GPR7 modulates the central control of feeding, the neuroendocrine system, and central nociceptive transmission. In the rat, GPR7 mRNA was detected in regions of the cortex, the hippocampus, the hypothalamus, and the spinal cord [3,7], and the physiological role of spinal GPR7 is still not clear. In the present study, to explore the role of spinal GPR7, the effects of intrathecal (i.t.) administration of NPW23 or NPB were examined in two inflammatory pain models (plantar injection of formalin or carrageenan), the thermal nociceptive hot plate test and the mechanical nociceptive test in the rat.

Expression of Fos, which is the protein product of the immediate-early protooncogene c-*fos*, has been widely used to identify populations of neurons that are activated by noxious stimuli [6] and to concomitantly examine the ability of drugs to suppress the expression of Fos-like immunoreactivity (Fos-LI) in the spinal cord in the formalin test [4,15]. In the present study, the authors also examined the effect of i.t. administration of either NPW23 or NPB on the expression of Fos-LI induced by paw formalin injection.

2. Materials and methods

The following investigations were performed according to a protocol approved by the Institutional Animal Care Committee of Chiba University, Chiba, Japan. Male Sprague–Dawley rats weighing 250–300 g were fitted with long-term i.t. catheters and examined for the effects of the agents on the agitation behavior induced by plantar formalin injection (formalin test), the level of mechanical allodynia, and the level of thermal hyperalgesia induced by plantar carrageenan injection (carrageenan test), the 52.5 °C and 50.5 °C hot plate latency (hot plate test), and the frequency of paw withdrawal to repetitive application of a 46.5-g von Frey filament (mechanical nociceptive test). All animals were used once. After the experiment, the animals were killed with an overdose of barbiturate.

2.1. I.t. catheters

Chronic i.t. catheters were inserted, during halothane anesthesia, by passing a PE-10 catheter through an incision in the atlanto-occipital membrane to a position 8 cm caudal to the cisterna for drug administration at the level of lumbar enlargement [12]. For drug administration at the mid-thoracic level, a PE-10 catheter was passed through an incision in the atlanto-occipital membrane to a position 3 cm caudal to the cisterna. The catheter was externalized on the top of the skull and sealed with a steel wire, and the wound was closed with 3-0 silk sutures. The animals were allowed to recover for 1 week before being used experimentally. All animals displayed normal feeding and drinking behavior post-operatively. Rats showing neurological deficits were not studied.

2.2. Formalin test

Under halothane anesthesia, 50 μl of 5% formalin was injected subcutaneously, with a 27-gauge needle, into the plantar surface of the right hind paw. Animals regained their posture within 1 min and immediately displayed behavior typical of this model: the injected paw was held just off the floor and showed spontaneous flinching (rapid and brief withdrawal). This pain-related behavior was quantified by counting the number of flinches for 1-min periods at 1–2 and 5–6 min, and then for 1-min periods at 5-min intervals during the period from 10 to 60 min after the injection. Two phases of spontaneous flinching behavior were observed. An initial acute phase (phase 1, during the first 6 min after the formalin injection) was followed by a relative short quiescent period and then by a prolonged tonic response (phase 2, beginning about 10 min after the formalin injection).

2.3. Carrageenan test

Two milligrams of lambda carrageenan (Sigma, St. Louis, MO), suspended in 0.1 ml normal saline by sonication, was injected subcutaneously via a 24-G needle into the plantar surface of the right hind paw under halothane anesthesia.

Mechanical thresholds were measured using von Frey filaments with logarithmically incremental stiffnesses (0.41, 0.70, 1.20, 2.00, 3.63, 5.50, 8.50, and 15.1 g) (Stoelting, Wood Dale, IL) to calculate the 50% probability thresholds for mechanical paw withdrawal (von Frey threshold) [1]. Beginning with the 2.00 g probe, filaments were applied to the plantar surface of a hind paw for 6–8 s in a stepwise ascending or descending order following negative or positive withdrawal responses, respectively, until six consecutive responses were noted. Von Frey thresholds were calculated according to the method of Dixon [2]. In case where continuous negative or positive responses were observed to the limits of the stimulus set, values of 15.00 g and 0.25 g were assigned, respectively.

The thermal nociceptive threshold was measured with a device similar to that previously reported [5]. The rats were placed in a clear plastic cage (10 × 20 × 24 cm) placed on an elevated floor of clear glass (2 mm thick). A radiant heat source (eye projector halogen lamp JRC-12-V-100W, Iwasaki Electric Tokyo, Japan) with an aperture diameter of 5 mm was contained in a movable holder placed beneath the glass floor. The halogen lamp beneath the floor was then positioned so that it focused on the plantar surface of the carrageenan-injected paw that was in contact with the glass plate. The interval between the application of the light beam