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A synthetic kainoid, (2*S*,3*R*,4*R*)-3-carboxymethyl-4-(phenylthio) pyrrolidine-2-carboxylic acid (PSPA-1) serves as a novel anti-allodynic agent for neuropathic pain

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Abstract

In spite of prominent progress in basic pain research, neuropathic pain remains a significant medical problem, because it is often poorly relieved by conventional analgesics. Thus this situation encourages us to make more sophisticated efforts toward the discovery of new analgesics. We previously showed that i.t. administration of acromelic acid-A (ACRO-A), a Japanese mushroom poison, provoked prominent tactile pain (allodynia) at an extremely low dose of 1 fg/mouse. In the present study we synthesized ACRO-A analogues (2*S*,3*R*,4*R*)-3-carboxymethyl-4-phenoxypyrrolidine-2-carboxylic acid (POPA-2) and (2*S*,3*R*,4*R*)-3-carboxymethyl-4-(phenylthio)pyrrolidine-2-carboxylic acid (PSPA-1) chemically and examined their ability to induce allodynia in conscious mice. Whereas POPA-2 induced allodynia at extremely low doses from 1 to 100 fg/mouse, similar to ACRO-A, PSPA-1 did not induce allodynia; rather, it inhibited the ACRO-A-induced allodynia with an ID_{50} value (95% confidence limits) of 2.19 fg/mouse (0.04–31.8 fg/mouse). Furthermore, PSPA-1 relieved neuropathic pain produced by L5 spinal nerve transection on day 7 after the operation in a dose-dependent manner from 1 to 100 pg/mouse. In contrast, it did not affect thermal or mechanical nociception or inflammatory pain. PSPA-1 reduced the increase in neuronal nitric oxide synthase activity in the spinal cord of neuropathic pain mice assessed by NADPH-diaphorase histochemistry and blocked the allodynia induced by *N*-methyl-D-aspartate. These results demonstrate that PSPA-1 may represent a novel class of anti-allodynic agents for neuropathic pain acting by blocking the glutamate-nitric oxide pathway.

Keywords: Acromelic acid; Allodynia; Glutamate; Neuropathic pain; PSPA-1; Spinal cord

1. Introduction

The development of a variety of persistent pain models such as the inflammatory pain model induced by peripheral injection of chemical irritants and neuropathic pain models produced by peripheral nerve injury has promoted our understanding of the molecular and cellular mechanisms that contribute to enhanced nociceptive sensitivity under pathophysiological conditions (Ito et al., 2001; Julius and Basbaum, 2001). Although similar peripheral sensitization and spinal cord neuroplasticity are considered to contribute to persistent pain states, the efficacy of conventional analgesics such as opioids and non-steroidal anti-inflammatory drugs is clearly different between inflammatory pain and neuropathic pain. The latter remains a significant medical problem as it is often poorly relieved by such conventional analgesics (Backonja and Rowbotham, 2006). To link the progress in basic pain research and successful pain management in the clinic, we are asked to make a greater and more sophisticated effort to be directed toward the discovery of targets for new analgesics (Scholz and Woolf, 2002).

Glutamate is the main excitatory neurotransmitter in the central nervous system and mediates fast neurotransmission at

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the vast majority of excitatory synapses via *N*-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors (Nakanishi, 1992; Hollmann and Heinemann, 1994). The non-NMDA receptor group is further divided pharmacologically into kainate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtypes. The ingestion of *Clitocybe acromelalga*, a Japanese poisonous mushroom, causes marked erythromelalgia, *i.e.*, reddish edema and severe tactile pain (allodynia), in the tips of hands and feet, which sympoms continue for a month. By monitoring the lethal effect of *C. acromelalga* toxins in mice, 2 isomers of acromelic acid (ACRO), ACRO-A and ACRO-B, were isolated from this poisonous mushroom (Konno et al., 1983). ACRO belongs to a class of so-called kainoids bearing a pyrrolidine dicarboxylic acid represented by kainic acid. Continuous i.t. injection of ACRO-A induced long-lasting pure motor, rigid-spastic paraparesis in a dose-dependent manner, which was accompanied by selective degeneration of interneurons in laminae II–IV of the lower spinal cord, in marked contrast to the nonselective neuronal damage induced by kainic acid and AMPA (Kwak and Nakamura, 1995a,b). These behavioral and morphological changes were considerably ameliorated by concomitant infusion of a non-NMDA receptor antagonist, but not by an NMDA receptor antagonist (Kwak and Nakamura, 1995a). On the other hand, we recently showed that a single i.t. injection of ACRO-A induced prominent allodynia in a bell-shaped manner at doses from 1 ag to 10 fg/mouse, which doses were much lower than the lethal dose (10 ng/mouse) of ACRO-A or those (1 pg to 1 ng/mouse) for the induction of allodynia by ACRO-B (Minami et al., 2004). Furthermore, i.t. injection of kainic acid could not induce allodynia, and the allodynia induced by ACRO-A was blocked by NMDA receptor antagonists, but not by the AMPA/kainate receptor antagonists (Minami et al., 2004). Thus, ACRO as well as kainic acid causes strong depolarization of neurons, but the *in vivo* behavioral and pathological effects elicited by ACRO-A are different from those of kainic acid.

To clarify the structure–activity relationship of ACRO-A for the induction of allodynia, we designed and synthesized ACRO analogues with a simplified structure and examined their ability to induce allodynia in conscious mice. Here we present evidence that one of these ACRO analogues, (2*S*,3*R*,4*R*)-3-carboxymethyl-4-(phenylthio)pyrrolidine-2-carboxylic acid (PSPA-1), did not induce allodynia but conversely attenuated the ACRO-A-induced allodynia and neuropathic pain.

2. Materials and methods

2.1. Animals

Male ddY mice were purchased from Shizuoka Laboratory Center (Hamamatsu, Japan). The animals were housed under conditions of a 12-h light/12-h dark cycle, a constant temperature of 22±2 °C, and 60±10% humidity. They were allowed free access to food and water before testing. All animals conformed to the regulations of the Animal Care Committees of Osaka Medical College and Kansai Medical University, and received humane care in accordance with the guideline of Ethics Committee of the International Association for the Study of Pain (Zimmermann, 1983).

2.2. Mechanical allodynia induced by i.t. injection

Studies on mechanical allodynia induced by i.t. injection of ACRO-A or other agents were carried out as described previously (Minami et al., 2004). Briefly, a 27-gauge stainless-steel needle attached to a microsyringe was inserted between the L5 and L6 vertebrae and agents in saline (5 μl) or saline alone was injected slowly into the subarachnoid space of conscious mice. After i.t. injection, allodynia was assessed once every 5 min for 50 min, every day for the first week and every week afterward by light stroking of the flank of the mice with a paintbrush. The allodynic response was ranked as follows: 0, no response; 1, mild squeaking with attempts to move away from the stroking probe; 2, vigorous squeaking evoked by the stroking probe, biting at the probe or strong efforts to escape. The maximum possible scores for allodynia of 6 mice were $2\times6=12$ in any 5-min period and $2\times6\times10=120$ for 50 min, and they were taken as 100%. To evaluate the effect of PSPA-1 on agent-induced allodynia, we assessed the effects on the maximal possible score of allodynia obtained 15 and 10 min after i.t. injection of ACRO-A and NMDA, respectively. The animals were used only for a single experiment.

2.3. Nociceptive pain responses

Thermal sensitivity was assessed by measuring the latency to withdrawal from a radiant heat stimulus used in the plantar test (model 7370, UGO BASILE, Comerio, Italy). The intensity of the radiant heat source was adjusted to yield a mean baseline latency of approximately 10 s with the cut-off automatically set at 20 s to avoid possible tissue damage. The left hindpaw or right hindpaw was selected at random and tested once. The mechanical threshold was determined by using calibrated von Frey filaments (Stoelting, Wood Dale, IL, USA) applied in an ascending order 5 times at an interval of a few seconds to the plantar surface of the hindpaw from the mesh floor. The threshold was taken as the lowest force to elicit a withdrawal reflex of the paw.

2.4. Formalin test

To study inflammatory pain-like behaviors, we used the paw formalin test as described previously (Muratani et al., 2002). Twenty microliters of 2% formalin in 0.9% NaCl was injected subcutaneously into the left dorsal hindpaw of mice weighing 22±2 g by using a microsyringe fitted with a 26-gauge needle. After the formalin injection, each mouse was placed in the observation chamber. The amount of time spent licking and biting the injected paw was measured with a hand-held stopwatch for 5 min from 0 to 30 min. Two distinct periods of high licking activity could be identified, the first phase lasting the first 10 min and the second one lasting from 15 to 30 min after the injection of formalin.