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Inhibitory effect of crotoxin on the pain-evoked discharge of neurons in thalamic parafascicular nucleus in rats ☆

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Abstract

Crotoxin (Cro), the principal neurotoxic component of *Crotalus durissus terrificus*, has been previously reported to have a behavioral analgesic effect in rats and mice. The present study investigated electrophysiologically the effect of Cro on pain-evoked unit discharge of neurons in thalamic parafascicular nucleus (Pf) and underlying mechanisms of its effect. The electrical discharge of Pf neurons was recorded with the microelectrode technique in rats. Intracerebroventricular (icv) injection of Cro at 0.25, 0.45 and 0.65 μg/kg resulted in a dose-dependent inhibitory effect on the pain-evoked discharge of Pf neurons. The discharge frequency and the discharge duration significantly ($P<0.05$) decreased after Cro administration. This inhibitory effect was significantly ($P<0.05$) attenuated after pretreatment with *para*-chlorophenylalanine (pCPA), or electrolytic lesion of dorsal raphe (DR) nucleus. In contrast, icv injection of atropine (muscarinic receptor antagonist, 5 μg) or naloxone (opioid receptor antagonist, 4 μg) had no effect on Cro-induced inhibition of discharge of Pf neurons. The results suggested that Cro has an analgesic effect, which is mediated, at least partially, by the central serotonergic system.

Keywords: Crotoxin; Analgesia; Thalamic parafascicular nucleus; Unit discharge; Serotonin; pCPA; Dorsal raphe nucleus; Atropine; Naloxone

☆ Ethical statement: On behalf of, and having obtained permission from all the authors, Xing-Hong Jiang declares that: (a) the material has not been published in whole or in part elsewhere; (b) the paper is not currently being considered for publication elsewhere; (c) all authors have been personally and actively involved in substantive work leading to the report, and will hold themselves jointly and individually responsible for its content; and (d) all experimental procedures in this study were reviewed and approved by the Animal Care and Use Committee of Soochow University. Xing-Hong Jiang testifies to the accuracy of the above on behalf of all the authors.

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1. Introduction

Among the various analgesics used in the clinic, opioids are the most effective. Although opioids are the most potent pain relievers, their use in the clinic is limited to addressing acute pain due to their potential for addiction and severe adverse effects such as; respiratory depression and digestive inhibition. There is also the risk of developing tolerance to their analgesic effects with chronic use. As a result, substantial effort has been made to identify alternatives to opioids use in the management of pain (Nestler et al., 2001).

Pharmacological studies have demonstrated that cobrotoxin, a neurotoxin from *Naja naja atra* and other cobra neurotoxins, produces strong analgesic effects in animal models (Chen and Robinson, 1990; Pu et al., 1995; Chen et al., 2006), suggesting that snake neurotoxins could provide new tools for combating pain.

Early observations noted that the venom of *Crotalus durissus terrificus* and peptides isolated had analgesic activity (Giorgi et al., 1993; Picolo et al., 1998; Brigatte et al., 2001). Furthermore, one component isolated from the venom of *C. durissus terrificus*, crotamine, has been proven to have analgesic effects (Mancin et al., 1998). Crotoxin (Cro), another neurotoxic component of this venom is a heterodimer composed of a basic weakly toxic secretory phospholipase A_2 subunit and of an acidic non-toxic and non-enzymatic subunit (Aird et al., 1985, 1986). Data from the literature have indicated that crotoxin (Cro) and its subunits inhibit inflammatory responses, as well as the growth of tumors (Landucci et al., 1995; Donato et al., 1996; Sampaio et al., 2005). Recently, it has been demonstrated in our laboratory that Cro also has analgesic activity in rodent models of pain, including the hotplate test, acetic acid-writhing test and tail-flick assay (Zhang et al., 2006). In all these tests the analgesic activity was determined by behavioral responses, which could vary, to certain degrees, with the state of the animal as well as experimenter bias. Therefore, such behavioral evidence alone is not sufficient proof and usually requires verification using more objective research techniques. Furthermore, the mechanisms of Cro's analgesic actions remain largely unclear and research should be extended to provide greater insight into the underlying mechanisms.

The thalamic parafascicular nucleus (Pf) is one of the important relays in the ascending nociceptive pathways. The electrical activity of neurons in this nucleus varies with the intensity of pain stimulation and can be used as an objective criterion of pain (Zhang, 1973; Weigel and Krauss, 2004). Therefore, the effects of Cro on the pain-evoked discharge of neurons in thalamic Pf were examined and the mechanisms of these effects were analyzed in the present study.

2. Materials and methods

2.1. Animals

Male Wistar adult rats, weighing 250–300 g, were purchased from the Experimental Animal Center, Soochow University School of Medicine (Grade II, Certificate No. SYXK2002-0037). All experimental procedures in this study were reviewed and approved by the Animal Care and Use Committee of Soochow University.

2.2. Materials

Purified Cro was supplied by Celtic Biotech Ltd. (Dublin, Ireland). Morphine, naloxone hydrochloride, atropine sulfate and *para*-chlorophenylalanine (pCPA) were purchased from Sigma (St. Louis, MO, USA).

2.3. Unit discharge recording

The pain-evoked unit discharge of Pf neurons was recorded extracellularly by the conventional microelectrode technique as described previously (Chen et al., 1986). Briefly, the animals were anesthetized with chloral hydrate (400 mg/kg, intraperitoneally (ip)) and fixed in the stereotaxic apparatus (SN-3, Narishige, Japan). The right sciatic nerve was exposed for noxious stimulation, which was carried out with twin rectangular pulses of 0.5 ms duration, 10 ms interval and 25 V intensity, and the trachea was intubated for artificial respiration. According to the coordinates of the brain atlas (Paxinos and Watson, 1982), a stainless-steel guide cannula was implanted into the lateral ventricle (0.8 mm posterior to the bregma, 1.5 mm lateral to midline, 4.0 mm below the skull surface) for drug administration and a hole was drilled in the skull (4.2–4.3 mm posterior to the bregma, 1.2 mm lateral to midline) for inserting the microelectrode to record the unit discharge of Pf neurons. During the experiment rats were paralyzed with gallamine triethiodide (60 mg/kg, ip) and artificially ventilated. A glass