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BRAIN RESEARCH

Research Report

Sensitization of rat facial cutaneous mechanoreceptors by activation of peripheral N-methyl-D-aspartate receptors

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ABSTRACT

The effect of subcutaneous injection of glutamate on the mechanical sensitivity of rat facial cutaneous mechanoreceptors was examined. Individual facial mechanoreceptors were recorded in the trigeminal ganglion of anesthetized Sprague–Dawley rats. An electronic von Frey hair was used to measure the mechanical threshold (MT) of the afferent fibers at baseline and following subcutaneous injection of glutamate (0, 0.01, 0.1, 1 M; 10 μl) or glutamate (0, 0.1 M) plus the competitive N-methyl-D-aspartate (NMDA) receptor antagonist 2-amino-5-phosphonovalerate (APV; 0.01 M). Subcutaneous injections were randomized and the investigator was unaware of their content. Changes in MT were assessed with a repeated measure ANOVA with time, sex and treatment as factors. Immunohistochemistry was used to confirm NMDA receptor expression by cutaneous nerve fibers. A total of 100 (50 per sex) facial mechanoreceptors were recorded from 61 (32 females, 29 males) rats in two separate experiments. Subcutaneous injections of higher concentrations of glutamate (1, 0.1 M) induced a significant mechanical sensitization of skin afferent fibers (compared to 0 and 0.01 M). Females (EC_{50}=16.2 mM) were more sensitive to glutamate than males (EC_{50}=73.0 mM). Facial cutaneous nerve fibers in both sexes expressed NMDA receptors. APV blocked the mechanical sensitization of the afferent fibers treated by glutamate 0.1 M in both sexes with a lower effect in females at a 10–20 minute post-injection. Subcutaneous injection of glutamate mechanically sensitizes rat facial cutaneous mechanoreceptors through activation of peripheral NMDA receptors. Peripheral NMDA receptor antagonists may be considered for craniofacial pain.

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ANOVA, Analysis of variance; APV, 2-amino-5-phosphonovalerate; CGRP, Calcitonin gene-related peptide; CV, Conduction velocity; CY3, Cyanine; EC_{50}, Half maximal effective concentration; ELISA, Enzyme-linked immunosorbent assay; FITC, Fluorescein isothiocyanate; iGluRs, Ionotropic glutamate receptors; KA, Kainate; MT, Mechanical threshold; NGS, Normal goat serum; NMDA, N-methyl-D-aspartate; NR2B, N-methyl-D-aspartate receptor subunit 2B; PBS, Phosphate-buffered saline; PCR, Polymerase chain reaction; PGP 9.5, Protein gene product; rel MT, Relative MT; SP, Substance P

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1. Introduction

The excitatory amino acid glutamate may play an important role in both normal and pathophysiological nociception in the skin (Chizh, 2002). There is anatomical evidence in male rats for the expression of both ionotropic and metabotropic glutamate receptors on the terminal endings of sensory fibers, which innervate somatic cutaneous tissues (Carlton et al., 1995; Coggeshall and Carlton, 1998; Carlton and Coggeshall, 1999). The expression of these glutamate receptors in the periphery is elevated during the course of inflammation both in animals and humans (Carlton and Coggeshall, 1999; Tan et al., 2008). Endogenous peripheral levels of glutamate are also enhanced in peripheral tissues in response to nerve injury and inflammation (Lawand et al., 1997; Omote et al., 1998; deGroot et al., 2000; Lawand et al., 2000; McNearney et al., 2000; Jang et al., 2004; Rosendal et al., 2004). Endogenous sources for the glutamate include primary afferent fibers (Omote et al., 1998; deGroot et al., 2000), keratinocytes (Nordlind et al., 1993), macrophages (Piani et al., 1991), Schwann cells (Parpura et al., 1995) and serum (Erdo, 1991). Therefore, the activation of the glutamate receptors in the skin may contribute to pain, nociception and sensitization. Indeed, topical application of the glutamate receptor antagonist ketamine has been suggested to decrease both neuropathic (Lynch et al., 2005; Poyhia and Vainio, 2006) and inflammatory (Warncke et al., 1997) pain. Whether ketamine or other glutamate receptor antagonists could be used to treat craniofacial pain disorders, such as trigeminal neuralgia, atypical facial pain or burning mouth syndrome, requires further investigation.

Behavioral studies in male rats demonstrate that local subcutaneous administration of glutamate (Carlton et al., 1995; Jackson et al., 1995; Carlton, 2001; Beirith et al., 2002) or glutamate agonists (Zhou et al., 1996) evokes pain and thermal or mechanical hyperalgesia and allodynia, which is blocked by specific glutamate receptor antagonists (Zhou et al., 1996). However, it is not known whether glutamate or glutamate agonists have similar or different effects on nociceptive processing in female rats. In a model of trigeminal cutaneous pain wherein glutamate was injected under the skin of the forehead of human volunteers (Gazerani et al., 2006), it was also demonstrated that glutamate evokes pain, induces mechanical sensitization and increases vasomotor responses and that some of these effects were greater in women than in men. In an in vitro electrophysiological model of rat skin-nerve preparation elevated concentrations of glutamate induced thermal but not mechanical sensitization of individual cutaneous afferent fibers (Du et al., 2001). There is, however, no direct in vivo evidence for an effect of glutamate on nerves innervating the facial skin or the receptor mechanisms that might underlie such an effect.

Therefore, the aim of the present study was to examine whether subcutaneous injection of glutamate at concentrations that induced pain and mechanical sensitivity in humans could sensitize rat facial cutaneous mechanoreceptors to pressure stimulation and if so, whether this sensitization exhibited a sex-related difference and was mediated through activation of peripheral N-methyl-D-aspartate (NMDA) receptors.

2. Results

Single unit extracellular recordings were made from trigeminal ganglion neurons that had facial cutaneous mechanoreceptive fields (Fig. 1A). Antidromic collision was employed to confirm that all afferent fibers projected to the trigeminal subnucleus caudalis (Fig. 1A), a region of the trigeminal sensory nuclear complex that plays a critical role in the processing of nociceptive input from the face and oral cavity (Sessle, 2000). The same basic experimental paradigm shown in Fig. 1B was followed for all afferent fiber recording experiments.

2.1. *Experiment I: The effect of subcutaneous administration of glutamate on the excitability and mechanical sensitivity of facial cutaneous mechanoreceptors*

Although subcutaneous injection of glutamate (0.1 M) can induce facial pain and mechanical sensitization in healthy humans, it is unclear whether these effects are mediated through changes in the excitability of Aδ mechanoreceptive fibers (Gazerani et al., 2006).

2.2. *Glutamate-evoked responses of facial cutaneous afferent fibers*

Only a few fibers were excited by injection of glutamate. In females, 3 fibers responded with an increase in afferent discharge to glutamate injections (two fibers to glutamate 1 M and one to glutamate 0.01 M). One fiber responded to phosphate-buffered saline (PBS) injection. In males 3 fibers responded with an increase in afferent discharge to glutamate injections (two fibers to glutamate 1 M and one to glutamate 0.1 M). The median [interquartile range] delay between injection and the increase in activity was 21 [15–23] s. None of the other afferent fibers responded to subcutaneous injections of glutamate or PBS. Examples of afferent discharge following the administration of glutamate 1 M in both female and male rats are shown in Fig. 2. There was no difference between those fibers that fired and those that did not fire in terms of median CV (fired: 9.2 [4.8–9.8] m/s; did not fire: 9.09 [7.3–10.2] m/s), baseline MT (fired: 6.7 [4.8–10.4] g; did not fire: 5.7 [4.1–8.7] g) or location (random distribution) of the fibers.

2.3. *Glutamate-induced changes in afferent fiber MT*

A main effect of treatment ($F_{3,72}=20.830$, $P<0.001$) and time ($F_{3,216}=53.806$, $P<0.001$) on MT was found. Sex did not influence the change in MT ($F_{1,72}=0.188$, $P=0.666$). There was a significant interaction between treatment and time ($F_{9,216}=9.553$, $P<0.001$) but there was no interaction between sex and time ($F_{3,216}=0.194$, $P=0.900$) or between sex and time and treatments ($F_{9,216}=0.356$, $P=0.955$). *Post hoc* multiple comparisons revealed that at the first post-injection time period, glutamate 0.1 M and 1 M significantly decreased MT compared with glutamate 0.01 M and PBS ($P<0.01$). Fig. 3 illustrates the pattern of changes in MT following the injection of different concentrations of glutamate in all rats.

All fibers were sensitized following the administration of 1 M glutamate. No correlation was found between maximum