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Brain Research 1046 (2005) 68 – 76

BRAIN RESEARCH

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Research report

Peripheral NMDA receptor modulation of jaw muscle electromyographic activity induced by capsaicin injection into the temporomandibular joint of rats

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Accepted 15 March 2005
Available online 28 April 2005

Abstract

We have previously documented that peripheral *N*-methyl-D-aspartate (NMDA) receptor mechanisms are involved in nociceptive reflex increases in jaw muscle activity to injection of mustard oil or glutamate into the rat temporomandibular joint (TMJ). The aim of the present study was to determine whether peripheral NMDA receptor mechanisms are also involved in the nociceptive reflex responses in the jaw muscles evoked by injection of the inflammatory irritant and algesic chemical capsaicin into the TMJ. The effects of peripheral injection of NMDA receptor antagonists, MK-801 and APV, on the increases in electromyographic (EMG) activities of digastric and masseter muscles reflexly evoked by capsaicin injection into the TMJ were tested in halothane-anesthetized male rats. The capsaicin injection following pre-injection of vehicle evoked significant increases in EMG activity in both digastric and masseter muscles whereas pre-injection of MK-801 or APV into the TMJ resulted in a significant concentration-related reduction in the magnitude of capsaicin-evoked digastric and masseter EMG activity (ANOVA-on-ranks, $P < 0.05$). This finding indicates that capsaicin-evoked digastric and masseter EMG activity can be attenuated by pre-injection into the TMJ of NMDA receptor antagonists, and that the activation of peripheral NMDA receptors may be important in the mechanisms whereby capsaicin evokes nociceptive trigeminal responses.

Theme: Sensory system
Topic: Somatic and visceral afferent

Keywords: Temporomandibular joint; NMDA; Capsaicin; Electromyographic activity; Peripheral modulation

1. Introduction

The vanilloid type 1 receptor, TRPV1, is activated by noxious heat, protons or the inflammatory irritant, and small-fiber excitant capsaicin and is found on small-diameter afferent nerve fibers and dorsal root ganglion neurons [21,38,65]. TRPV1 receptors have also recently been described on trigeminal afferents and trigeminal ganglion neurons innervating the rat temporomandibular joint (TMJ) [37]. Furthermore, capsaicin injected into the rat TMJ reflexly evokes a dose-dependent increase in jaw muscle electromyographic (EMG) activity [63], produces an inflammatory response [26], and induces activation and sensitization in brainstem nociceptive neurons [42,44,45,64]. Intramuscular injection of capsaicin in humans also results in intense pain and hyperalgesia [70]. Both trigeminal afferent [46,47] and brainstem [44,64] nociceptive neuronal responses to capsaicin injected into the TMJ can be significantly increased following glutamate injection into the TMJ. These findings raise the possibility that peripheral NMDA receptors may contribute to the mechanisms whereby capsaicin evokes nociceptive responses.

We have previously shown that glutamate injection into the rat TMJ induces a concentration-related reflex increase in jaw muscle activity that can be significantly attenuated by

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doi:10.1016/j.brainres.2005.03.040

co-injection of NMDA receptor antagonists [16]. Similarly, glutamate injection into the human masseter muscle causes pain that can be attenuated by co-injection of an NMDA receptor antagonist [13,17,18,62]. Increases in jaw muscle reflex activity as a result of mustard oil application to the TMJ are also attenuated by TMJ pre-injection of an NMDA receptor antagonist [72]. In order to determine whether peripheral NMDA receptor mechanisms are involved in nociceptive reflex responses evoked by capsaicin application to the TMJ, the present study tested the possible effects of the peripheral (TMJ) application of NMDA receptor antagonists, MK-801 and APV, on the increases in jaw muscle EMG activity that could be reflexly evoked by capsaicin application to the TMJ.

A portion of this data has been previously presented in abstract form [36,43].

2. Methods

2.1. Animal preparation

A total of 45 male Sprague–Dawley rats (250–450 gm) were prepared for acute in vivo recording of jaw muscle EMG activity, as previously described [34,71–73]. Briefly, under surgical anesthesia (O_2: 1 L/min; halothane: 1.5–2.5%), a tracheal cannula was inserted and artificial ventilation initiated. The rat's head was then placed in a stereotaxic frame to facilitate placement of EMG recording electrodes. Bipolar electrodes were fashioned out of 40 gauge Teflon-coated single-strand stainless steel wire and inserted into the left digastric and masseter muscles.

After completion of all surgical procedures, the halothane level was titrated (1–1.3%) until noxious pressure applied to the hindpaw could not induce a flexion reflex of the hindlimb to ensure that an adequate level of anesthesia was maintained for the duration of the experiment. Heart rate and body core temperature were continuously monitored throughout the experiment and kept within the physiological range of 330–430/min and 37–37.5 °C, respectively. All methods and experimental approaches were approved by the University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada).

2.2. Drug solutions

For the TMJ administration of drug solutions, two 27-gauge cannulae were first cemented side-by-side and connected to two Hamilton syringes with polyethylene tubes. One cannula was filled with either the non-competitive NMDA receptor antagonist (+)-MK-801 (0.001, 0.01, or 0.1 M; 10 μL; n = 6 per group; Research Biochemicals International, Natick, MA), competitive NMDA receptor antagonist (±)-D-2-amino-5-phosphonovalerate, APV (0.005 or 0.05 M; 10 μL; n = 6 per group Research Biochemicals International, Natick, MA), or vehicle (sterile normal saline; 10 μL; n = 6), and the other with 1% capsaicin (10% capsaicin in ethanol:Tween-80: sterile normal saline in a 1:1:8 ratio by volume; 10 μL; Calbiochem, La Jolla, CA). Both NMDA receptor antagonists were dissolved in isotonic saline (pH 7.2–7.4). The concentrations of the NMDA receptor antagonists and capsaicin were chosen on the basis of our previous findings [16,26,63,72]. The two cannulae were then carefully inserted into the left TMJ, and capsaicin and NMDA receptor antagonists or vehicle were injected into the left TMJ via the needle and catheter. We have previously demonstrated that injection of capsaicin vehicle or isotonic saline into the TMJ region does not evoke any significant change in EMG activity in the digastric or masseter muscles [63,72,73].

2.3. Stimulation and recording techniques

Bipolar recordings were made of the EMG activities of the left digastric (jaw-opening) and left masseter (jaw-closing) muscles [34,73] before and after injections of MK-801, APV, or vehicle control, and capsaicin. EMG activity was amplified (gain, 500×; band width, 30–1000 Hz) and fed into a computer equipped with a CED 1401 Plus board and analysis software (Spike2; Cambridge Electronics; signal sampling rate was 2000 Hz). Recorded EMG activity was stored electronically and analyzed offline. The EMG activities with the jaw in resting position were first recorded for 20 min to establish a baseline, and either MK-801, APV, or saline vehicle was administrated into the left TMJ. In another series of experiments, MK-801 (0.1 M, n = 6) or APV (0.05 M, n = 3) was injected into the right TMJ to control for possible systemic effects. Five minutes following the administration, capsaicin was injected into the left TMJ and the resulting changes in EMG activity were continuously recorded for another 30 min. All drug solutions were slowly injected into the TMJ over ~5 s.

2.4. Data analysis and statistics

Recorded EMG data were rectified off-line, and EMG area bins (microvolts per minute) were calculated. Baseline EMG activity was calculated as a mean of EMG area bins recorded over the first 20 min before injection of agents into the TMJ. Relative EMG activity was calculated by normalizing EMG area bins to the baseline EMG activity and was used to illustrate the results of individual experiments. Agents applied to the TMJ were considered to have evoked jaw muscle activity if the value of the first EMG bin after TMJ application was 2 SD above the baseline [73]. The value of the baseline plus 2 SD was chosen as a signal-to-noise limit because it represents an approximation of the 95% confidence interval for the mean baseline activity. The relative area under the EMG response curve (AUC) was calculated by summing the value of the first and all subsequent EMG area bins greater than 2 SD above the mean baseline (20 min) EMG activity and defined as the