Activation of Peripheral GABA<sub>a</sub> Receptors Inhibits Temporomandibular Joint–Evoked Jaw Muscle Activity

BRIAN E. CAIRNS, BARRY J. SESSLE, AND JAMES W. HU
Department of Oral Physiology, Faculty of Dentistry, University of Toronto, Toronto M5G 1G6 Canada


INTRODUCTION

Activation of either peripheral excitatory amino acid (EAA) or GABA<sub>a</sub> receptors located in cutaneous tissues of the tail of neonatal rats evokes a nociceptive reflex (Ault and Hildebrand 1993, 1994). Injection of glutamate into the temporomandibular joint (TMJ) region also reflexly induces a prolonged increase in the electromyographic (EMG) activity of both digastric and masseter muscles in adult rats (Cairns et al. 1998). This effect of glutamate is mediated through activation of peripheral EAA receptors located within the TMJ region because co-application of both N-methyl-D-aspartate (NMDA)– and non-NMDA–receptor antagonists significantly reduces the magnitude of glutamate-evoked jaw muscle EMG activity. However, it is not clear whether there are also peripheral GABA receptors located within the TMJ region and used to apply different chemicals. After surgery, the level of halothane was maintained at 0.8–1% for the duration of the experiment. All surgeries and procedures were approved by the University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada). At the end of each experiment, rats were euthanized with T61 (Hoechst).

METHODS

Surgical preparation

Forty-eight male Sprague-Dawley rats (250–450 g) were prepared for acute recording of jaw muscle EMG activity as previously described (Cairns et al. 1998). Briefly, under surgical anesthesia (O<sub>2</sub>: 0.3–0.4 l/min; N<sub>2</sub>O: 0.6–0.7 l/min; halothane: 1.5–2%) a tracheal cannula was inserted, and the left femoral vein was cannulated, bipolar electrodes were inserted bilaterally into the digastric and masseter muscles, and a catheter was carefully inserted into the TMJ region and used to apply different chemicals. After surgery, the level of halothane was maintained at 0.8–1% for the duration of the experiment. All surgeries and procedures were approved by the University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada). At the end of each experiment, rats were euthanized with T61 (Hoechst).

Drug solutions

The following chemicals were employed: glutamate, GABA, the GABA<sub>a</sub> receptor antagonist bicuculline and the GABA<sub>b</sub> receptor antagonist phaclofen (Research Biochemicals International; Natick, MA). All drugs were dissolved in isotonic saline, and the resulting solutions were adjusted to a pH of ≈7. Solutions (total volume per injection: 10 μL) were injected bilaterally into the TMJ capsule (Cairns et al. 1998). Intravenous administration of GABA (5 μmol in 0.1 ml normal saline) also was made through the femoral vein catheter.

Stimulation and recording

EMG activity was amplified (gain: 500×; bandwidth 30–1,000 Hz) and fed into a computer equipped with a CED 1401 Plus board and analysis software (Spike 2, Cambridge Electronics). EMG activity was recorded continuously for the duration of each experiment. Baseline EMG activity was observed for 10 min, and then glutamate (2.5 μmol) was injected into the TMJ region over 5 s. We previously demonstrated that application of this dose of glutamate to the TMJ region evokes EMG activity in both the ipsilateral digastric and masseter muscles by a reflex pathway through the trigeminal subnucleus caudalis (Cairns et al. 1998). Thirty minutes after the initial glutamate injection either glutamate (2.5 μmol) alone, GABA (0.005, 0.05, 0.5, or 5 μmol) alone, GABA in combination with glutamate, or GABA in combination with glutamate and either the selective GABA receptor antagonists bicuculline (0.05 μmol) or phaclofen (0.05 or 0.15 μmol) were injected into the TMJ region. All drugs were at room temperature immediately before injection. Osmolarity was adjusted by the addition of sodium chloride.

Data analysis

Recorded EMG data were rectified off-line, and EMG area bins (μV·min) were calculated. Mean baseline EMG activity was sub-

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Inhibition Mediated by Peripheral GABA<sub>A</sub> Receptors

Tract from each EMG area bin to yield residual EMG area bins. The area under the EMG response curve (AUC) was calculated by summing all residual EMG area bins greater than two times the SD of the mean baseline. The relative EMG response was then calculated by normalizing the AUC for the second application (AUC<sub>2</sub>) to the AUC evoked by glutamate (AUC<sub>1</sub>). For each dosing regimen, a mean relative EMG response was determined from the average of five experiments conducted in three rats.

To calculate ID<sub>50</sub>, nonlinear regression analysis was performed on the dose-response curve for GABA-mediated suppression of glutamate-evoked EMG activity according to the equation

\[
\text{Relative EMG response (AUC}_2/\text{AUC}_1) = \frac{1}{1 + \text{Dose}_{\text{GABA}}/\text{ID}_{50}}
\]

Comparisons of relative EMG responses were made with the use of analysis of variance (ANOVA) on ranks because the variances were always heterogeneous. Significant differences in the ID<sub>50</sub> were determined with a Student’s t-test.

**RESULTS**

Glutamate (2.5 μmol), GABA, or normal saline was injected into the TMJ region 30 min after an initial injection of glutamate (2.5 μmol) was applied. Repeated application of glutamate at an interval of 30 min evoked EMG responses in both ipsilateral jaw muscles (Fig. 1A) that were of comparable magnitude, in agreement with previous findings (Cairns et al. 1998). In contrast, the EMG activity evoked by GABA at all the doses applied (5, 0.5, 0.05, and 0.005 μmol) was significantly less than that evoked by glutamate and similar to that evoked by normal saline (P < 0.05 Kruskal-Wallis one-way ANOVA on ranks, Dunn’s method, Fig. 1, B and C).

The jaw muscle activity evoked by application of glutamate alone to the TMJ region was compared with that evoked by co-application of glutamate and GABA (5, 0.5, 0.05, or 0.005 μmol) or after systemic administration of GABA (5 μmol). Systemic administration of GABA (5 μmol) had no effect on glutamate-evoked EMG activity (Fig. 2A), but local application of GABA to the TMJ region suppressed glutamate-evoked EMG activity in a dose-dependent manner with an estimated mean (±SE) ID<sub>50</sub> of 0.17 ± 0.05 μmol and 0.031 ± 0.006 μmol for the digastric and masseter muscles, respectively (Figs. 2 and 3). The ID<sub>50</sub> for the digastric muscle was significantly larger than the ID<sub>50</sub> for the masseter muscle (P < 0.05, Student’s t-test).

Co-application of bicuculline (0.05 μmol) but not phaclofen (0.05 or 0.15 μmol) partially attenuated GABA-mediated suppression of glutamate-evoked EMG activity (Fig. 2C and D). Co-application of bicuculline also resulted in a rightward shift of the dose-response curves of GABA-mediated suppression for both the digastric and masseter muscles; the estimated ID<sub>50</sub> values increased to 7.5 ± 1.5 μmol and 1.5 ± 0.9 μmol for the digastric and masseter muscles, respectively (P < 0.05, Student’s t-test; Fig. 3).

**DISCUSSION**

Before this study it was not known whether peripheral GABA receptors exist within the TMJ region of adult rats or if activation of these receptors evokes jaw muscle activity analogous to that evoked by glutamate. In this study it was shown that when compared with glutamate local application of GABA to the TMJ region was not effective in evoking jaw muscle activity. However, TMJ application of GABA was found to inhibit glutamate-evoked jaw muscle EMG activity, and this GABA-mediated inhibition was reversed by co-application of the GABA<sub>A</sub> receptor antagonist bicuculline but not by the GABA<sub>B</sub> receptor antagonist phaclofen. These results suggest that GABA<sub>A</sub> receptors are located within the TMJ region and that their activation appears to attenuate the nociceptive reflex response that can be evoked from this region. In contrast, GABA application to the tail skin of the neonatal rat isolated spinal cord–tail preparation evokes a nociceptive reflex (Ault and Hildebrand 1994). This difference may reflect the use of an in vitro neonatal as opposed to an in vivo adult rat preparation and/or differences between cutaneous versus deep tissues.

Previous reports indicated that application of GABA to peripheral nerves has two effects. 1) It depolarizes certain nerve fibers, and 2) it concomitantly suppresses the conduction of sensory information along small-diameter (Aδ, C) primary afferent fibers that can convey nociceptive information from the periphery to the CNS (Brown and Marsh 1978; Bhisitkul et al. 1987; Morris et al. 1983). GABA-induced depolarization of...
peripheral nerve fibers is attenuated by GABA<sub>α</sub> receptor antagonists and mediated through the opening of chloride channels (Brown and Marsh 1978; Desarmenien et al. 1984; Deschenes et al. 1976; Gallagher et al. 1978; Morris et al. 1983). GABA-mediated activation of chloride channels may also act as a shunt, allowing current to flow out across the axon membrane instead along the nerve fiber and thus impede action potential conduction (Wall 1995).

The rat TMJ region is innervated almost exclusively by Aδ and C fibers (Kido et al. 1995), and there is recent evidence that suggests that GABA may block action potential conduction through small-diameter trigeminal primary afferents (Soja et al. 1998). We speculate that activation of peripheral GABA<sub>α</sub> channels located on the unmyelinated terminal segments of TMJ afferent fibers results in a current shunt that interferes with the conduction of action potentials (Brown and Marsh 1978; Wall 1995).

GABA is distributed widely in nonneural tissues and may also be synthesized and released by a subpopulation of trigeminal primary afferent fibers, providing evidence in favor of a modulatory role for GABA in the peripheral nervous system (Szabat et al. 1992; Tanaka 1985). Our findings also raise the possibility that activation of peripheral GABA<sub>α</sub> receptors within the TMJ region may result in a local analgesic effect, and so further study of these mechanisms may prove to have clinical significance in pain management.

The authors thank K. MacLeod for electronic services. This research was supported by National Institutes of Health Grant DE-11995. B. E. Cairns was supported by a Fellowship from the Canadian Arthritis Society and the Medical Research Council of Canada.

Address for reprint requests: J. W. Hu, Faculty of Dentistry, University of Toronto, 124 Edward St., Toronto, Ontario M5G 1G6 Canada.

Received 9 October 1998; accepted in final form 17 December 1998.

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![Fig. 2](http://jn.physiology.org/)

**Fig. 2.** Suppression of glutamate-evoked digastric (top) and masseter (bottom) muscle activity by co-application of GABA (5 μmol) to the TMJ region. For each of A–E the line plots to the left illustrate mean EMG activity (n = 5 experiments in 3 rats), whereas the bar graphs to the right summarize relative EMG response (AUC<sub>2</sub>/AUC<sub>1</sub>). In A, systemic administration of GABA (5 μmol) is indicated by a dotted line. Arrows indicate application of the following chemicals to the TMJ region: glutamate (open arrows), glutamate and GABA (5 μmol, closed arrow), glutamate, GABA, and bicuculline (0.05 μmol; vertically lined arrow), or glutamate, GABA, and phaclofen (D: 0.05 μmol; E: 0.15 μmol; horizontally lined arrow). Note that local but not systemic application of GABA suppressed glutamate-evoked EMG activity. GABA-mediated suppression was reversed by co-application of bicuculline but not phaclofen. *P < 0.05 Kruskal-Wallis one-way analysis of variance on ranks, Dunn's Method.

![Fig. 3](http://jn.physiology.org/)

**Fig. 3.** Dose-response relationship for GABA-mediated suppression. Each point in the graphs represents a mean value (n = 5 experiments in 3 rats). Bars indicate SE. Co-application of GABA with glutamate (●) decreased the glutamate-evoked EMG activity in a dose-dependent manner. Co-application of bicuculline (0.05 μmol) with glutamate and GABA (■) attenuated this GABA-mediated suppression. ◦ glutamate alone. □ bicuculline with glutamate.


