Endothelin ET_B Receptor Antagonist Reduces Mechanical Allodynia in Rats with Trigeminal Neuropathic Pain

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Trigeminal neuropathic pain, which is associated with marked orofacial mechanical allodynia, is frequently refractory to currently available drugs. Because endothelins (ETs) can contribute to nociceptive changes in animal models of inflammatory, cancer, and diabetic neuropathic pain, the present study evaluated the influence of ET_A and ET_B receptor antagonists on orofacial mechanical allodynia in a rat model of trigeminal neuropathic pain. Unilateral constriction (C) of the infraorbital nerve (ION) caused pronounced and sustained bilateral mechanical allodynia, evaluated by application of von Frey hairs to the vibrissal pad. Mechanical allodynia on postoperative days 12–15 after nerve injury was abolished for up to 90 mins by subcutaneous administration of 2.5 mg/kg morphine, but was fully refractory to intravenous (iv) administration of 10 mg/kg of the dual ET_A plus ET_B or selective ET_A receptor antagonists, bosentan and atrasentan, respectively. In sharp contrast, iv administration of 20 mg/kg of the selective ET_B receptor antagonist, A-192621, caused a net 61 ± 15% reduction of mechanical threshold, lasting 2 hrs. Co-injection of atrasentan plus A-192621 did not modify ION injury-induced mechanical allodynia. Injection of 10 pmol ET-1 into the upper lip of naive rats caused ipsilateral mechanical allodynia lasting up to 5 hrs. Thus, ET_B receptor–mediated mechanisms contribute to orofacial mechanical allodynia induced by CION injury, but, somehow, functional ET_A receptors are required for expression of the antiallodynic effect of ET_B receptor blockade. Exp Biol Med 231:1136–1140, 2006

The study was supported by the Brazilian National Research Council (CNPq), Coordenação de Aperfeicoamento de Pessoal de Nível Superior, Fundação de Amparo a Ciência e Tecnologia do Estado de Santa Catarina, and the Pronex program of the Brazilian Ministry of Science and Technology. J.G.C. is the recipient of a CNPq doctoral scholarship.

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Received September 8, 2005.
Accepted November 25, 2005.

1535-3702/06/2316-1136$15.00

Key words: trigeminal neuropathic pain; endothelin; rats; mechanical allodynia

Introduction

Trigeminal neuralgia (TN) is a form of neuropathic pain characterized by severe lancinating pain in orofacial regions innervated by the trigeminal nerve. Because currently available medical or surgical procedures for TN treatment fail to provide consistent and permanent pain relief in all patients, further studies characterizing its underlying mechanisms and exploring new effective treatment strategies are clearly warranted.

Chronic constriction (C) of the infraorbital nerve (ION), a branch of the trigeminal nerve, has been developed as an experimental model that reproduces important aspects of TN, including signs of abnormal spontaneous pain-related behavior, mechanical allodynia (1, 2), heat hyperalgesia (3), and inflammatory hypersensitivity (4). Enhanced responsiveness to mechanical stimulation is frequently observed in patients with TN and other chronic neuropathic pain forms (5). On the other hand, mechanical allodynia in the hind paw of rats with diabetic neuropathy is temporarily attenuated by atrasentan, a highly selective antagonist of endothelin $(ET)_A$ receptors (6). ET_A and ET_B receptors can be expressed in central and peripheral neurons (7, 8), and are specifically targeted by ETs, a family of peptides that includes ET-1, ET-2, and ET-3 (9, 10) and can be produced by many cell types in the central nervous system (11, 12), as well as in peripheral sensory ganglia, including cells of the trigeminal ganglion (13). ET-1 can trigger pain or overt nociception in humans and animals (14–17), as well as hyperalgesia to noxious chemical, mechanical, and thermal stimuli (17–20). Moreover, endogenous ETs contribute significantly to pain and/or hyperalgesia of inflammatory, immune, neuropathic, and neoplasic origins (6, 17, 21–23).

In light of these considerations, the present study aimed

to evaluate the influence of CION on nocifensive responses of rats to mechanical stimuli (using Von Frey hairs) applied to the snout, as well as the susceptibility of the resultant changes to reversal by treatment with morphine and ET_A and/or ET_B receptor antagonists.

Materials and Methods

Animals. Experiments were conducted on male Wistar rats weighing 180–200 g, housed five to a cage at 22 ± 1°C on a 12:12-hr light:dark cycle (lights on at 0700 hrs) with free access to laboratory chow and tap water. All experimental procedures were previously approved by Universidade Federal de Santa Catarina's Committee on the Ethical Use of Animals, where the study was conducted.

Surgical Procedure. The method for producing CION differed slightly from that originally proposed by Vos et al. (2). Briefly, rats were anesthetized with a mixture of 50 mg/kg ketamine and 10 mg/kg xylazine, and a small incision was made on the snout, under the right eye, approximately 3 mm caudal to the mystacial pads. The rostral end of the ION was exposed, at the point at which it emerged from the infraorbital fissure, and two silk 4–0 ligatures were tied loosely around it. The wound was closed with additional 4–0 silk sutures. Sham-operated rats were treated identically, but no ligatures were applied to the ION. After surgery, all rats were treated with intramuscular administration of 60 mg/kg oxytetracycline and maintained in a warm room until they recovered from anesthesia.

Mechanical Stimulation. Before each testing session, animals were placed in individual plastic cages and left to adapt to the environment for at least 1 hr. The mechanical threshold was measured using a graded series of 10 von Frey filaments ranging from 0.02 to 10 g (Semmes-Weinstein monofilaments, Stoelting, Wood Dale, IL) as described previously (24). Each filament was applied near the center of the vibrissal pad, to the point of bending, three times, at 30-sec intervals on the contralateral side and then on the nerve-injured side, for a total of six applications per rat. Each stimulation series began with the filament producing the lowest force, and proceded up to the filament that evoked one of the following nocifensive behaviors twice: brisk head withdrawal, escape or attack reactions, or short-lasting facial grooming. Only rats that did not react to application of the 10-g filament in the preoperative tests were included in this study, to avoid unspecific responses.

Drug Treatments. The influence of various drug treatments on CION-induced changes in nocifensive responsiveness was assessed on Days 12 or 15 after surgery, by determining the mechanical threshold for evoking responses before drug administration and then at 30-min intervals up to 8 hrs, thereafter. Drug treatments included: subcutaneous (sc) administration of 2.5 mg/kg morphine hydrochloride, intravenous (iv) administration of the non-peptidic ET receptor antagonists, bosentan (dual ET_A plus ET_B; 10 or 20 mg/kg), atrasentan (selective ET_A; 10 mg/kg), and A-192621 (selective ET_B; 20 mg/kg), prepared daily in heated (50°C) water alone (bosentan) or warm water containing 3% ethanol and 100 μl of 0.1 *N* NaOH. In some rats, 10 pmol ET-1 or its vehicle (phosphate-buffered saline [PBS]) was injected into the upper lip, and its effect on responses to mechanical stimulation was evaluated for up to 6 hrs. Control rats were always treated identically with the corresponding vehicle.

Drugs. The drugs used were: bosentan (kindly provided by Actelion A.G., Allschwil, Switzerland), atrasentan and A-192621.1 ([2R-{2a,3b,4a}]-4-[1,3-benzodioxol-5-yl]-1-[2-{2,6-diethylphenyl}amino]-2-oxoethyl-2-[4-propoxyphenyl]-3 pyrrolidinecarboxylic acid; both kindly provided by Abbott Laboratories, Abbott Park, IL), morphine hydrochloride (Merck AG, Darmstadt, Germany), ET-1 (Bachem, Natick, MA), and oxytetracycline (Terramicina; Pfizer, Guarulhos, Brazil).

Statistical Analysis. Results are presented as mean ± SEM at different time points after surgery for each group and were analyzed using the nonparametric Kruskal-Wallis ANOVA, followed by the Mann-Whitney *U* test for individual comparisons. Differences with *P* values less than 0.05 were considered significant.

Results

CION induced bilateral mechanical allodynia starting 9 days after surgery, which was maximal on both sides by Day 12 and remained at this level at least up to Day 120. Treatment of rats with 2.5 mg/kg morphine hydrochloride suppressed mechanical allodynia in the period between 30 and 90 mins after injection (Fig. 1). On the other hand, bilateral mechanical allodynia was unaffected by iv administration of either the dual ET_A plus ET_B receptor antagonist, bosentan (10 or 20 mg/kg; data not shown), or the selective ET_A receptor antagonist, atrasentan (10 mg/kg; Fig. 2). In sharp contrast, significant short-lasting and bilateral inhibitory effects were observed between 1 and 2 hrs after iv injection of the selective ET_B receptor antagonist A-192621 (20 mg/kg; Figs. 1 and 2 show only results regarding stimulation of the ipsilateral side). Combined iv treatment of rats with atrasentan plus A-192621, at the same doses used previously, failed to affect mechanical allodynia significantly (Fig. 2). Local sc injection of 50 μl of 10 pmol ET-1 caused mechanical allodynia that was restricted to the injected side and lasted up to 5 hrs (Fig. 3).

Discussion

The current study demonstrates that CION induces a state of persistent orofacial bilateral mechanical allodynia in rats that is amenable to temporary relief by opiates (morphine) or selective antagonists of ET_B receptors, yet is fully resistant to alleviation by selective ET_A receptor antagonists or to simultaneous blockade of ET_A and ET_B receptors, with either bosentan or a combination of atrasentan plus A-192621.

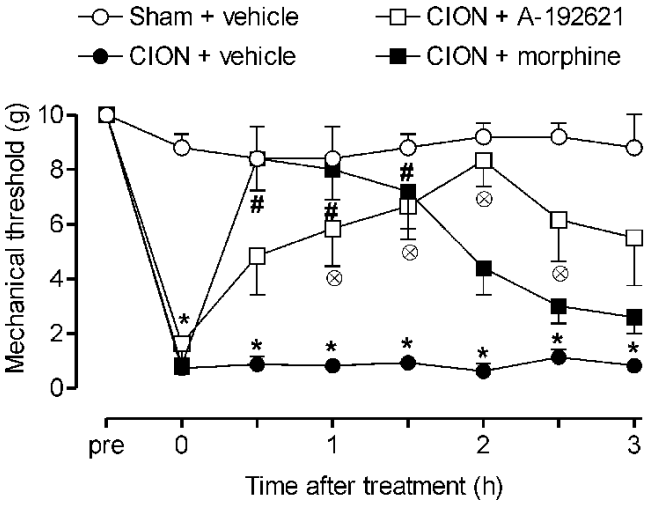

Figure 1. Effects of morphine hydrochloride or the selective ET_B receptor antagonist, A-192621, on CION-induced orofacial mechanical allodynia in rats. On Day 12 after surgery, rats received sc administration of 2.5 mg/kg morphine, iv administration of 20 mg/kg A-192621, or the respective vehicles. The mechanical threshold was assessed with von Frey filaments at different time intervals. Values represent the mean ± SEM of the minimal force required to elicit a nocifensive response when applied to the snout on the side ipsilateral to the nerve injury (n = 6–8). *$P < 0.05$ when compared with corresponding values of sham-operated rats; # and ⊗ indicate $P < 0.05$ when compared with corresponding value of CION rats treated with vehicle.

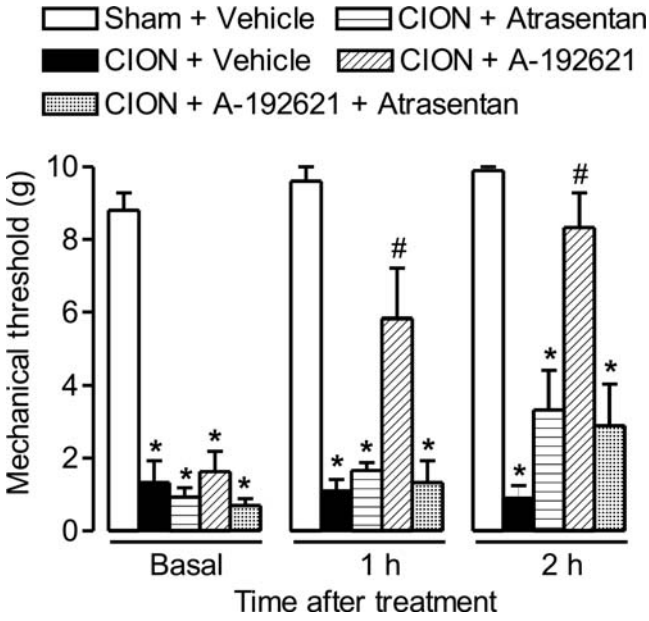

Figure 2. Effects of ET_A and/or ET_B receptor antagonists on CION-induced orofacial mechanical allodynia in rats. On Day 15 after surgery, rats received iv administration of 10 mg/kg atrasentan (ET_A receptor antagonist) or 20 mg/kg A-192621 (ET_B receptor antagonist) alone or in combination, or the respective vehicle. The mechanical threshold was assessed with von Frey filaments. Values represent the mean ± SEM of the minimal force required to elicit nocifensive response when applied to the snout on the side ipsilateral to the nerve injury before (basal) and 1 or 2 hrs after drug treatment (n = 6–8). Asterisks and fences denote $P < 0.05$ when compared with corresponding value of sham-operated or CION rats treated with vehicle, respectively.

Previous studies have disclosed differential roles for ET receptors in controlling nocifensive responsiveness in experimental pain models. In some models, ET-1–induced nociception occurs exclusively through ET_A receptor activation (17, 19, 21), which agrees well with their expression in small-sized peptidergic and nonpeptidergic sensory neurons in dorsal root ganglia (8). Likewise, ET_A receptor antagonists abolish hyperalgesic responses to thermal and chemical stimuli evoked by ET-1 in mice and attenuate the tactile allodynia in rats displaying diabetes-induced neuropathy (6, 17, 19, 20). However, the nociceptive effects of exogenous ETs in the writhing test and mechanical inflammatory hyperalgesia caused by carrageenan or complete Freund's adjuvant in the hind paw of mice involve both ET_A and ET_B receptors (15, 20), whereas phenylbenzoquinone-induced abdominal writhes in mice (22), articular nociception induced by lipopolysaccharide in a previously inflamed knee joint (21), and mechanical hyperalgesia induced by ET-1, interleukin (IL)-18, and IL-12 in rats seem to be mediated by ET_B (but not ET_A) receptors (25, 26).

Jarvis *et al.* (6) showed that mechanical allodynia detected in the hind paw of rats with diabetic neuropathy can be reduced temporarily by blockade of ET_A receptors with atrasentan, but not by blockade of ET_B receptors with A-192621. Corroborating such findings, the current study demonstrates that ETs can mediate mechanical allodynia in a distinct neuropathic model, that is, orofacial pain induced by CION. Nevertheless, it is important to emphasize that the receptors mediating the allodynic effects of the ET system in the diabetes and CION models are clearly opposite (ET_A and ET_B receptors, respectively). Despite this significant difference, the analgesic/antiallodynic effect produced by selec-

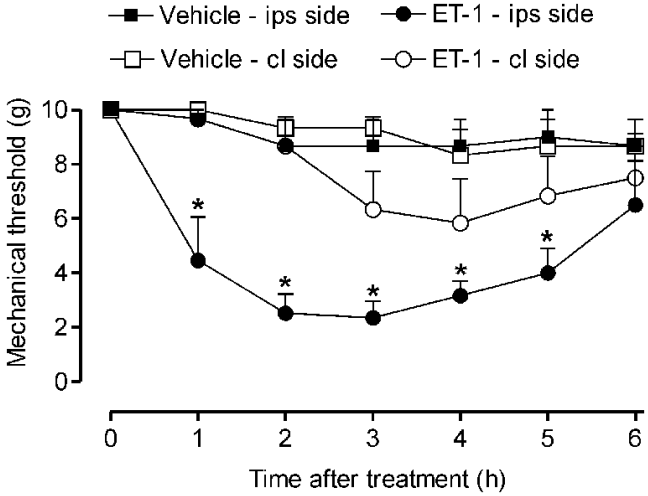

Figure 3. Effect of unilateral injection of 10 pmol ET-1 into the upper lip on the mechanical threshold of naive rats. Values represent the mean ± SEM of the minimal force required to elicit a nocifensive response from either ipsilateral (ips) or contralateral (cl) sides of the snout, relative to injection (n = 6). *$P < 0.05$ when compared with corresponding values of the control group.

tive antagonism of one receptor type in both the CION (present study) and diabetes neuropathy (6) models was attenuated by simultaneous blockade of the other receptor type. Specifically, suppression of CION-induced orofacial mechanical allodynia by ET_B receptor blockade the CION model was fully abrogated by concomitant antagonism of ET_A receptors, either by coadministration of A-192621 with atrasentan, or by treatment with bosentan. One could speculate that ET_A receptor blockade with atrasentan may have reduced the intensity of ET_B receptor blockade afforded by A-192621, by augmenting the concentration of ET-1 and, hence, its ability to compete with the latter antagonist. If this happens to be the case, the failure of ET_A receptor blockade alone to enhance mechanical allodynia might be caused by a floor effect of CION on noxious mechanical threshold (i.e., a maximal increase in sensitivity to mechanostimulation). A somewhat similar scenario could be envisaged to account for the lack of an antiallodynic effect of bosentan. However, the true mechanisms underlying this apparent interaction between ET_A and ET_B receptors on CION-induced orofacial mechanical allodynia clearly remain to be clarified.

On the other hand, because atrasentan does not cross the blood-brain barrier (27) and A-192621 displays a closely related chemical structure to atrasentan, it seems likely that peripheral, rather than central, ET_B receptor–dependent mechanisms are responsible for the mechanical allodynia induced by CION. This view is strengthened by a report that the trigeminal ganglion expresses a high density of ET_B receptors (28).

In summary, our results show that ET-dependent mechanisms mediated *via* ET_B receptors contribute to orofacial mechanical allodynia induced by CION in the rat. The results obtained with A-192621 suggest that ET_B receptor antagonists may represent promising candidates for the treatment of TN in humans.

The kind donations of bosentan by Dr. Martine Clozel (Actelion Pharmaceuticals, Allschwil, Switzerland), and atrasentan and A-192621 by Dr. Terry Opgenorth (Abbott Laboratories, Abbott Park, IL) are gratefully acknowledged.

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