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ABT-627, an endothelin ET_A receptor-selective antagonist, attenuates tactile allodynia in a diabetic rat model of neuropathic pain

Michael F. Jarvis *, Jerry L. Wessale, Chang Z. Zhu, James J. Lynch III, Brian D. Dayton, Samuel V. Calzadilla, Robert J. Padley, Terry J. Opgenorth, Elizabeth A. Kowaluk

Neurological and Urological Diseases Research and Metabolic Diseases Research, Pharmaceutical Products Division, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6123 USA

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Abstract

Tactile allodynia, the enhanced perception of pain in response to normally non-painful stimulation, represents a common complication of diabetic neuropathy. The activation of endothelin ET_A receptors has been implicated in diabetes-induced reductions in peripheral neurovascularization and concomitant endoneurial hypoxia. Endothelin receptor activation has also been shown to alter the peripheral and central processing of nociceptive information. The present study was conducted to evaluate the antinociceptive effects of the novel endothelin ET_A receptor-selective antagonist, 2*R*-(4-methoxyphenyl)-4*S*-(1,3-benzodioxol-5-yl)-1-(*N*,*N*-di(*n*-butyl)aminocarbonyl-methyl)-pyrrolidine-3*R*-carboxylic acid (ABT-627), in the streptozotocin-induced diabetic rat model of neuropathic pain. Rats were injected with 75 mg/kg streptozotocin (i.p.), and drug effects were assessed 8–12 weeks following streptozotocin treatment to allow for stabilization of blood glucose levels ($\geq$ 240 mg/dl) and tactile allodynia thresholds ($\leq$ 8.0 g). Systemic (i.p.) administration of ABT-627 (1 and 10 mg/kg) was found to produce a dose-dependent increase in tactile allodynia thresholds. A significant antinociceptive effect (40–50% increase in tactile allodynia thresholds, $P < 0.05$) was observed at the dose of 10 mg/kg, i.p., within 0.5–2-h post-dosing. The antinociceptive effects of ABT-627 (10 mg kg^{-1} day^{-1}, p.o.) were maintained following chronic administration of the antagonist in drinking water for 7 days. In comparison, morphine administered acutely at a dose of 8 mg/kg, i.p., produced a significant 90% increase in streptozotocin-induced tactile allodynia thresholds. The endothelin ET_B receptor-selective antagonist, 2*R*-(4-propoxyphenyl)-4*S*-(1,3-benzodioxol-5-yl)-1-(*N*-(2,6-diethylphenyl)aminocarbonyl-methyl)-pyrrolidine-3*R*-carboxylic acid (A-192621; 20 mg/kg, i.p.), did not significantly alter tactile allodynia thresholds in streptozotocin-treated rats. Although combined i.p. administration of ABT-627 and A-192621 produced a significant, acute increase in tactile allodynia thresholds, this effect was significantly less than that produced by ABT-627 alone. These results indicate that the selective blockade of endothelin ET_A receptors results in an attenuation of tactile allodynia in the streptozotocin-treated rat. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Allodynia; Endothelin; Streptozotocin; Diabetic neuropathy

1. Introduction

Neuropathic pain is generally considered to be one of the most common and troublesome complications afflicting diabetic patients (Vinik et al., 1992; Clark and Lee, 1995). Pain associated with diabetic neuropathy can occur either spontaneously, or as a result of exposure to only mildly painful stimuli (i.e., hyperalgesia) or to stimuli not normally perceived as painful (i.e., allodynia) (Brown and Asbury, 1984). Tactile allodynia (the perception of touch as painful) represents one of the most troublesome complaints, given the inevitability of contact with the physical environment.

Streptozotocin-induced destruction of pancreatic β-cells is a widely used model of Type I diabetes that exhibits a number of anomalies in pain perception (Hounsom and Tomlinson, 1997). For example, formalin-evoked flinching is exaggerated in streptozotocin-treated rats as compared to untreated, control animals (Calcutt et al., 1995, 1996).

* Corresponding author. Dept. 4PM, Bldg. AP9A-2, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6123. Tel.: +1-847-937-3919; fax: +1-847-937-9195.

E-mail address: michael.jarvis@abbott.com (M.F. Jarvis).

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Mechanical hyperalgesia and thermal allodynia have also been observed following streptozotocin treatment (Akunne and Soliman, 1987; Wuarin-Bierman et al., 1987; Ahlgren and Levine, 1993; Courteix et al., 1993). In addition, the development of tactile allodynia has recently been reported in this animal model of diabetes (Calcutt and Chaplan, 1997; Calcutt et al., 1996; Lynch et al., 1999).

While the specific etiology of diabetic neuropathy remains unknown, impairment of neurovascular blood flow leading to endoneurial hypoxia appears to contribute to diabetes-induced peripheral neuropathology (Tuck et al., 1984; Cameron and Cotter, 1997; Cameron et al., 1991, 1994; Zochodne, 1999). Such reductions in peripheral vascularization appear to be mediated by the reduction of endogenous vasodilatory mechanisms and a concomitant increase in the local actions of potent vasoconstrictors including endothelin-1 and angiotensin II (Cameron and Cotter, 1997; Zochodne and Cheng, 1999). In this regard, plasma immunoreactive endothelin-1 is significantly elevated in rats that were treated with streptozotocin 8 weeks earlier (Tada et al., 1994).

Previous studies have revealed that endothelin receptor antagonists can significantly improve peripheral neurovascular perfusion and nerve conduction velocities in the streptozotocin-treated rat (Stevens and Tomlinson, 1995; Cameron and Cotter, 1996; Cameron et al., 1994). The present experiments were conducted to characterize the ability of a novel endothelin ET_A receptor-selective antagonist, (2*R*-(4-methoxyphenyl)-4*S*-(1,3-benzodioxol-5-yl)-1-(*N*,*N*-di(*n*-butyl)aminocarbonyl-methyl)-pyrrolidine-3*R*-carboxylic acid (ABT-627) (Fig. 1), to reduce tactile allodynia thresholds in the streptozotocin model of neuropathic pain. ABT-627, also known as A-147627, is the active (+)-enantiomer of A-127722, a competitive antagonist that is highly selective for the endothelin ET_A receptor and is orally active in rats, dogs, and monkeys (Opgenorth et al., 1996; Winn et al., 1996). ABT-627 inhibits binding to cloned human endothelin ET_A and endothelin ET_B receptors with K_i values of 0.034 and 63 nM, respectively, and it potently blocks endothelin ET_A receptor-mediated vasoconstriction induced by exogenous endothelin-1 without affecting endothelin ET_B receptor-mediated vasodilation (Opgenorth et al., 1996).

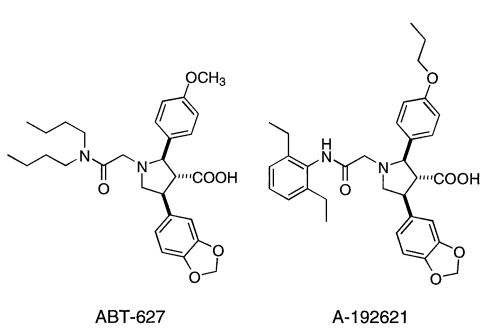

Fig. 1. Chemical structures of ABT-627 and A-192621.

Additional experiments were conducted to further define which of the two known endothelin receptor subtypes is involved in modulating nociception in this animal model by administering a recently described endothelin ET_B receptor-selective antagonist, 2*R*-(4-propoxyphenyl)-4*S*-(1,3-benzodioxol-5-yl)-1-(*N*-(2,6-diethylphenyl) aminocarbonyl-methyl)-pyrrolidine-3*R*-carboxylic acid (A-192621) (Fig. 1) (Von Geldern et al., 1999), alone and in combination with ABT-627. A-192621 has been shown to be ~700-fold more potent in inhibiting [^{125}I]ET-1 binding to human endothelin ET_B receptors ($K_i = 8.8$ nM) than to endothelin ET_A receptors ($K_i = 5.6$ μM) (Opgenorth et al., 1997; Von Geldern et al., 1999).

2. Methods

2.1. Streptozotocin treatment

Adult male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) were group-housed (four to five animals per cage) in standard plastic cages with wood chip bedding. Bedding was changed daily for all animals to maintain sanitary conditions. In individual experiments, rats (250–350 g) were injected in groups of approximately 100 animals with 75 mg/kg streptozotocin (Sigma, St. Louis, MO) dissolved in 0.9% saline, i.p. (Lynch et al., 1999). Another group of rats was injected with an equal volume of 0.9% saline and served as an age-matched, non-diabetic control group. Streptozotocin solutions were freshly prepared to limit instability of the compound. Blood glucose levels were determined in all animals using an Encore Glucometer (Bayer, Elkhart, IN) from blood samples obtained by tail vein bleeds 3–4 weeks following streptozotocin treatment (Courteix et al., 1993). Rats with blood glucose levels ≥ 240 mg/dl (≥ 13.3 mM) were considered diabetic and used for further studies. All animal handling and experimental protocols were approved by an institutional animal care and use committee (IACUC).

2.2. Assessment of tactile allodynia

Tactile allodynia thresholds were assessed, as described previously (Lynch et al., 1999). Briefly, animals were placed on a 1.27 × 1.27 cm elevated wire mesh screen to provide access to the ventral side of the rat's hind paws. An inverted, clear plastic cage (29 × 18 × 12 cm, $l \times w \times h$) was placed over each rat, and the animals were allowed to acclimate to the test environment for 20-min prior to baseline testing. Tactile allodynia thresholds were determined using von Frey hairs (Stoelting, Wood Dale, IL) as described by Chaplan et al. (1994), with each rat tested three times per session. Fifty percent threshold values were calculated according to the up-down method of Dixon (1980). In studies examining the effects of test compounds

on tactile allodynia in streptozotocin-treated animals, only rats with pre-dosing thresholds of ≤ 8.0 g were utilized. Tactile allodynia thresholds were also assessed in a control group consisting of age-matched, non-streptozotocin treated rats. A percent maximal protective effect value (% MPE) was calculated for each dose at each pre-treatment time according to the formula: ([post-drug threshold] − [baseline threshold])/([maximum threshold] − [baseline threshold]) × 100%, where maximum threshold was equal to 15 g (Lee and Yaksh, 1996). Data were analyzed (GB-STAT, Dynamics Microsystems, Silver Spring, MD) by analysis of variance with the repeated measure of time, and protected *t*-tests were then performed. Statistical significance was determined at $P < 0.05$. All data are expressed as mean ± SEM.

In acute drug studies, test compounds or appropriate vehicles were administered i.p. in a 1 ml/kg volume to individual groups of rats ($n = 6$ per drug dose). Tactile allodynia thresholds were assessed at 0.5, 1, and 2 h after drug administration. To evaluate the effects of chronic drug exposure, ABT-627 was administered p.o. in the drinking water at doses of 1 and 10 mg/kg ($n = 10$ per dose) for 7 days. These rats received continuous drug exposure until tactile allodynia thresholds were assessed at the beginning of the eighth day. As a vehicle control, a separate group of streptozotocin-treated rats ($n = 10$) received drinking water without the drug for 7 days. No significant differences in water intake were observed between the drug-treated and untreated (vehicle) groups of rats (data not shown). Doses and routes of administration of both ABT-627 and A-192621 used in the present studies were based on previous work demonstrating their in vivo activity to selectively block endothelin ET_A and endothelin ET_B receptor-mediated vasopressor responses, respectively (Opgenorth et al., 1996, 1997; Chen et al., 1997; Von Geldern et al., 1999).

2.3. Determination of drug plasma levels

In subgroups of diabetic rats, the concentration of the endothelin antagonist in plasma following p.o. or i.p. administration was determined to provide pharmacodynamic information on ABT-627 and A-192621 in this rodent model of neuropathic pain. Rats ($n = 4–5$ per dose) were administered ABT-627 at doses of 1 or 10 mg kg^{-1} day^{-1} in the drinking water for 7 days. In the morning (0900–1100 h) of day 8, a blood sample (1 ml) was obtained by bleeding of the periorbital sinus during a brief period of anesthesia induced by inhalation of 40% O_2/60% CO_2. In a second series, rats ($n = 6–7$ per group) were given a single i.p. injection of ABT-627 (1 or 10 mg/kg) or A-192621 (20 mg/kg). Two hours after dosing, the rats were anesthetized (methoxyflurane, Penthrane®, Abbott Laboratories, North Chicago, IL), and a 2-ml blood sample was obtained using the method of intracardiac puncture. Blood samples were placed in vials containing EDTA (5 mg/ml blood) and centrifuged; the plasma was removed by pipetting and stored at −20°C. Following liquid–liquid extraction of the plasma samples, parent drug concentrations were determined by high performance liquid chromatography using UV detection as previously described (Opgenorth et al., 1996).

2.4. Drugs

Morphine sulfate was obtained from Mallinckrodt (St. Louis, MO); ABT-627 and A-192621 were synthesized at Abbott Laboratories. Morphine was dissolved in a 0.9% saline solution. ABT-627 and A-192621 were dissolved in 0.5 N $NaHCO_3$ for i.p. administration and in distilled water for the chronic p.o. dosing study.

3. Results

The potential antinociceptive effects of ABT-627 were assessed during the first 2 h following systemic (i.p.) administration. ABT-627 was found to produce a dose-dependent increase in tactile allodynia thresholds (Fig. 2). At a dose of 10 mg/kg, i.p., ABT-627 treatment resulted in a statistically significant 40–50% increase in tactile allodynia thresholds 0.5- and 2-h post drug administration. ABT-627 (1 mg/kg, i.p.) produced a similar degree of antinociception 2-h post drug administration. No significant drug effect was observed 1-h post ABT-627 administration and may have been due to a relatively larger vehicle response at this time point as compared to the vehicle responses observed at 0.5- and 2-h post drug administration. As a

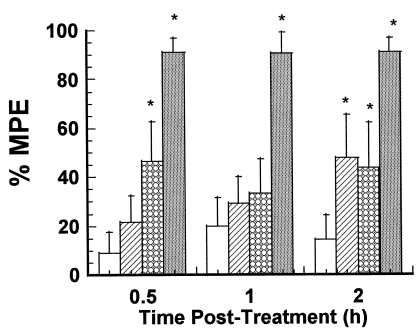

Fig. 2. ABT-627 (1 and 10 mg/kg, i.p.) significantly increased tactile allodynia thresholds in streptozotocin-treated rats ($F(3,20) = 10.01$, $P < 0.01$). Tactile allodynia was assessed 8 weeks following streptozotocin treatment. Drug vehicle (0.5 N $NaHCO_3$) responses in streptozotocin-treated rats are indicated by open bars. ABT-627 (1 mg/kg, i.p.; diagonal-lined bars) produced a 40% reversal of tactile allodynia 2-h post drug administration. A larger dose of ABT-627 (10 mg/kg, i.p.; patterned bars) produced a significant reversal of tactile allodynia at 0.5- and 2-h post drug administration. For comparison, morphine (8 mg/kg, i.p.; gray bars) produced a nearly complete reversal of tactile allodynia at all time points examined. $^*P < 0.05$ compared to streptozotocin-treated rats receiving drug vehicle. Values represent mean ± SEM., $n = 6$ per group.

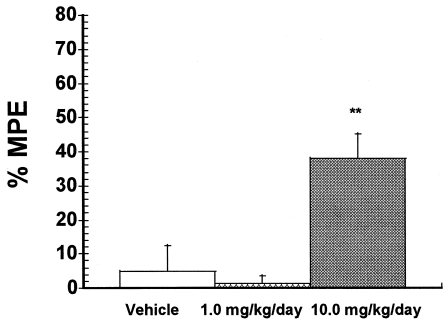

Fig. 3. Antinociceptive effect of ABT-627 in streptozotocin-treated rats following chronic exposure for 7 days. ABT-627 was administered p.o. in the drinking water at doses of 1 or 10 mg kg^{-1} day^{-1} (patterned and gray bars, respectively). A third group of streptozotocin-treated rats received no drug treatment (i.e., vehicle) and is indicated by the open bars. Tactile allodynia thresholds were assessed on day 8. Rats treated with ABT-627 (10 mg kg^{-1} day^{-1}, p.o.) for 7 days exhibited significantly less tactile allodynia ($F(2,27) = 14.8$, $P < 0.01$) as compared to untreated rats. $^{*}P < 0.05$ compared to rats receiving no drug treatment, $n = 10$ per group. Values represent mean ± SEM.

comparison, morphine at a dose of 8 mg/kg, i.p. produced a significant 90% increase in tactile allodynia thresholds across all time points (Fig. 2). Baseline tactile allodynia threshold for these streptozotocin-treated rats was 4.76 ± 0.12 g ($n = 24$) and were not significantly different ($P > 0.05$) between treatment groups.

To assess the effects of chronic administration of ABT-627, a separate group of rats was treated with streptozotocin and baseline tactile allodynia thresholds were determined 8 weeks later. The baseline tactile allodynia threshold for streptozotocin-treated ($n = 31$) rats at 8-weeks post-streptozotocin treatment was 2.98 ± 0.13 g. For comparison, a control group ($n = 8$) consisting of age-matched, non-streptozotocin treated rats exhibited tactile allodynia thresholds of 14.51 ± 0.27 g. Chronic administration of ABT-627 (10 mg kg^{-1} day^{-1} in the drinking water for 7 days) in streptozotocin-treated rats resulted in a significant 38% increase in tactile allodynia thresholds (Fig. 3). The magnitude of this effect was similar to that observed following the acute administration of ABT-627 (10 mg/kg, i.p.) (Fig. 2). No significant effect on tactile allodynia thresholds was observed following chronic administration of 1 mg kg^{-1} day^{-1}, p.o.

Following a 3-week washout period, a second study was conducted with these same rats to compare the acute effect of both a selective endothelin ET_A (ABT-627) and endothelin ET_B (A-192621) receptor antagonist alone, and in combination, on tactile allodynia. Baseline tactile allodynia thresholds were reassessed at week 12 for the streptozotocin-treated rats and were found not to be significantly different from the values determined at week 8 (range = 2.25 ± 0.32 to 2.83 ± 0.41 across four individual groups). The streptozotocin-treated rats were then randomly assigned to one of four treatment groups receiving the following: (1) ABT-627 (10 mg/kg i.p.), (2) A-192621 (20 mg/kg i.p.), (3) combination of ABT-627 (10 mg/kg) and A-192621 (20 mg/kg) by separate i.p. injections, and (4) vehicle only, i.p. Following the acute administration of ABT-627 (10 mg/kg, i.p.), a statistically significant 40% increase in tactile allodynia thresholds was observed at all time points examined (Fig. 4). In contrast, no significant elevation in tactile allodynia thresholds was observed following the acute administration of A-192621 (20 mg/kg, i.p.). The combined administration of ABT-627 and A-192621 produced a significantly smaller (approximately 25% MPE at 2 h) elevation in tactile allodynia thresholds compared to ABT-627 administered alone (Fig. 4).

Table 1 shows the results of additional experiments performed to assess the plasma concentrations of the endothelin ET receptor antagonists at the doses and routes used in streptozotocin-induced diabetic rats. With the exception of the 1 mg kg^{-1} day^{-1} p.o. dose, the plasma concentrations of ABT-627 achieved with the other doses or route of administration yielded antinociceptive effects in this animal model. These plasma concentrations were within the range of those shown previously to selectively antagonize the endothelin-1-induced vasopressor response in rats without discernible effect on the transient depressor activity of endothelin-1 (Opgenorth et al., 1996). Moreover, the plasma concentration of A-192621 achieved using a dose of 20 mg/kg, i.p., is consistent with that shown previously to provide significant, selective endothelin ET_B

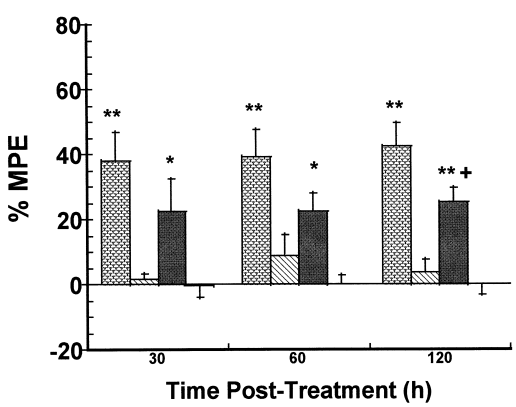

Fig 4. Antinociceptive effects of the selective endothelin ET_A receptor antagonist, ABT-627, and the selective endothelin ET_B receptor antagonist, A-192621, administered alone and in combination, to streptozotocin-treated rats. ABT-627 (10 mg/kg, i.p.; patterned bars) produced a significant, approximately 40% reduction in tactile allodynia thresholds ($F(3,20) = 8.5$, $P < 0.01$). A-192621 (20 mg/kg, i.p.; diagonal-lined bars) did not significantly ($P > 0.05$) alter tactile allodynia thresholds. When administered together (by separate injections), the combination (dark gray bars) of ABT-627 (10 mg/kg, i.p.) and A-192621 (20 mg/kg, i.p.) produced a statistically significant increase in tactile allodynia thresholds. However, the combined antinociceptive effect of both compounds was significantly ($P < 0.05$) less than that observed for ABT-627 alone 2-h post drug administration. Drug vehicle (0.5 N $NaHCO_3$) responses are indicated by open bars which ranged from 0.5–2% MPE. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to drug vehicle. + $P < 0.05$ compared to ABT-627 (10 mg/kg, i.p.) at 2 h, $n = 6$ per group. Values represent mean ± SEM.

Table 1
Plasma concentrations of ET antagonists in streptozotocin-induced diabetic rats.
Blood samples were drawn 2-h post drug (i.p.) administration, or during the morning (0900–1100 h) of day 8 during chronic (p.o.) exposure in the drinking water.

ET antagonist	Dose	Route of administration	*n*	Drug concentration in plasma (μg/ml)[a]
ABT-627	1 mg/kg	i.p.	7	0.09 ± 0.03
ABT-627	10 mg/kg	i.p.	7	0.42 ± 0.13
ABT-627	1 mg kg^{-1} day^{-1}	p.o.	4	0.02 ± 0.01
ABT-627	10 mg kg^{-1} day^{-1}	p.o.	5	0.14 ± 0.02
A-192621	20 mg/kg	i.p.	6	1.72 ± 0.32

[a] Mean ± SEM.

receptor blockade (Opgenorth et al., 1997; Von Geldern et al., 1999).

4. Discussion

The streptozotocin-treated diabetic rat develops a variety of anomalies in pain perception including thermal (Lee and McCarty, 1992) and mechanical (Ahlgren and Levine, 1993) hyperalgesia, chemically stimulated hyperalgesia (Courteix et al., 1994; Calcutt et al., 1995), as well as tactile allodynia (Calcutt, et al., 1996; Lynch et al., 1999). These patterns of increased nociception do not appear to be attributable to inherent streptozotocin-induced neurotoxicity (Calcutt and Chaplan, 1997), indicating that streptozotocin-induced hyperglycemia induces a variety of pathophysiological consequences that can lead to altered nociceptive responses (Hounsom and Tomlinson, 1997; Zochodne, 1999). The extent of streptozotocin-induced tactile allodynia and its sensitivity to morphine observed in the present study agree with previous data in this model (Lynch et al., 1999).

Diabetic neuropathy is associated with decreased neurovascular blood flow with concomitant endoneurial hypoxia leading to impaired nerve conduction velocities (Tuck et al., 1984; Cameron and Cotter, 1997; Cameron et al., 1991, 1994; Zochodne, 1999). These events may be mediated, in part, by the increased release of the potent vasoconstrictor, endothelin-1 (Takeda et al., 1991; Simonsen, 1993). In the streptozotocin-treated rat, hyperglycemia-induced deficits in neurovascular blood flow and nerve conduction velocities can be improved following systemic treatment with endothelin receptor antagonists (Stevens and Tomlinson, 1995; Cameron and Cotter, 1997). In this regard, selective blockade of the endothelin ET_A receptor using BQ-123 appears to be more effective than the non-selective endothelin receptor antagonist, bosentan, which can also block endothelin ET_B receptor mediated vasodilation (Cameron and Cotter, 1997; Cameron et al., 1994; Stevens and Tomlinson, 1995).

Endothelin-1, however, appears to play a complex role in the processing of nociceptive information. The intrathecal administration of endothelin receptor agonists attenuates chemically stimulated persistent pain as measured by the second phase of the formalin test (Yamamoto et al., 1994). In contrast, the systemic administration of endothelin or structurally related peptides produces peripheral nociceptive responses in mice that are behaviorally similar to those produced by the i.p. administration of acetylcholine or phenyl-*p*-quinone (Raffa et al., 1996b). Peripheral endothelin-induced nociceptive responses can be pharmacologically distinguished from those produced by other chemical agents, including acetylcholine or phenyl-*p*-quinone, since endothelin-induced nociception is not attenuated by systemically administered non-steriodal anti-inflammatory drugs such as indomethacin and ibuprofen (Raffa et al., 1996b). Additionally, the intraplantar administration of endothelin-1 into the rat hind paw has been demonstrated to enhance both the acute (Phase I) and persistent (Phase II) portions of the formalin test (Piovezan et al., 1997). Recent data have also shown that endothelin-1 applied directly to the sciatic nerve produces a formalin-like flinching response that can be blocked by morphine and the endothelin ET_A receptor antagonist BQ-123 but not by an endothelin ET_B receptor selective antagonist BQ-788 (Davar et al., 1998). In this latter study, the nociceptive effects of endothelin-1 were not mimicked by the direct application of epinephrine indicating that the vasoconstriction alone cannot account for the nociceptive actions of endothelin-1 on the sciatic nerve (Davar et al., 1998). The differential antinociceptive effects of selective endothelin receptor antagonists contrast with an earlier report indicating that both endothelin ET_A and endothelin ET_B receptor activation contributes to the nociceptive effects of endothelin (Raffa et al., 1996a), and suggest the possibility that specific endothelin receptor subtypes may differentially modulate nociceptive processes in acute versus chronic pain states (De-Melo et al., 1998; Piovezan et al., 1998). This hypothesis is further supported by recent data indicating that endothelin ET_B receptor knockout mice are insensitive to the nociceptive effects of i.p. phenyl-*p*-quinone, but retain sensitivity to acute noxious thermal stimuli as measured by the hot-plate test (Griswold et al., 1999).

The results of the present study provide support for a role of endothelin ET_A receptor activation in contributing to increased tactile allodynia in the streptozotocin-treated rat. Specifically, the systemic administration of ABT-627, a highly selective endothelin ET_A receptor antagonist produced a significant attenuation of streptozotocin-induced

tactile allodynia, and these effects were preserved during 7 days of chronic treatment. In contrast, no significant alteration of tactile allodynia thresholds was observed following the acute systemic administration of a highly selective endothelin ET_B receptor antagonist, A-192621. The results in this model also support the hypothesis that blockade of endothelin ET_B receptors may be undesirable for ameliorating some forms of endothelin-mediated nociceptive responses since the co-administration of both ABT-627 and A-192621 resulted in significantly less attenuation of tactile allodynia as compared to the antinociceptive effects of ABT-627 when administered alone. Endothelin ET_B receptors have been shown to mediate vasodilation via nitric oxide release (Warner et al., 1989; Ishikawa et al., 1994) and to clear endothelin-1 from the circulation (Fukuroda et al., 1994), two mechanisms which could serve to modulate the effects of endothelin ET_A receptor activation. Thus, there is developing evidence that activation of endothelin ET_A receptors contributes to nociception in some types of acute and chronic neurogenic pain (Davar et al., 1998, and the present data), whereas activation of endothelin ET_B receptors may contribute to the nociceptive effects of visceral and cutaneous inflammation (Griswold et al., 1999).

The potential antinociceptive effects of higher doses of ABT-627 were not examined in the present study. However, maximal antinociception may have been achieved since similar reductions in tactile allodynia were observed 2 h following the systemic (i.p.) administration of both 1 and 10 mg/kg ABT-627. Although ABT-627 is highly selective for the endothelin ET_A receptor, and the doses of ABT-627 examined in the present study have been shown to fully block the hemodynamic effects of endothelin ET_A receptor activation by endothelin-1 in vivo (Opgenorth et al., 1996), higher doses of ABT-627 may also block endothelin ET_B receptors. As the present data indicate, blockade of endothelin ET_B receptors does not contribute to the alleviation of tactile allodynia in streptozotocin-treated rats and may antagonize the actions of selective endothelin ET_A receptor blockade.

Taken together, these results suggest that, in the rat model of streptozotocin-induced neuropathic pain, the endothelin system is involved in the pathophysiology of tactile allodynia, and that the selective blockade of endothelin ET_A receptors can significantly increase tactile allodynia thresholds. The magnitude of the antinociceptive effects of ABT-627 were moderate compared to the antinociceptive effects of systemically administered morphine, but illustrates that a variety of pathophysiological mechanisms contribute to the expression of neuropathic pain in this model. This idea is further supported by recent demonstrations that a cholinergic channel modulator (Bannon et al., 1998) and an adenosine kinase inhibitor (Lynch et al., 1999) can effectively block nociception in the streptozotocin-treated rat. Importantly, the present data demonstrate that a potent and selective endothelin ET_A receptor antagonist produces antinociception in an animal model that is not dependent on the exogenous administration of endothelins. Furthermore, the present study has utilized a technique to quantitatively assess a nociceptive behavior in a conscious animal, in contrast to other studies which have used assessments of nerve conduction velocity or neurovascular perfusion in anesthetized animals to evaluate the neuroprotective effects of endothelin receptor antagonists (Stevens and Tomlinson, 1995). Further investigations of the behavioral effects of potent and selective antagonists for the different endothelin receptor subtypes will be useful in elucidating the respective roles of these receptors in contributing to nociceptive processes in different pain states.

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