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European Journal of Pharmacology 492 (2004) 177–182

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Effects of selective endothelin ET_A receptor antagonists on endothelin-1-induced potentiation of cancer pain

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Received 12 January 2004; received in revised form 31 March 2004; accepted 9 April 2004

Available online 10 May 2004

Abstract

In some diseases in which endothelin-1 production increases, e.g. prostate cancer, endothelin-1 is considered to be involved in the generation of pain. In the present study, we investigated the effects of a selective endothelin ET_A receptor antagonist, (E)-*N*-[6-methoxy-5-(2-methoxyphenoxy)[2,2′-bipyrimidin]-4-yl]-2-phenylethenesulfonamide monopotassium salt (YM598), on the nociception potentiated by endothelin-1 in a cancer inoculation-induced pain model in mice, induced by inoculation of the androgen-independent human prostate cancer cell line PPC-1 into the hind paws of severe combined immunodeficiency (SCID) mice. No pain responses were observed in the sham-operated mice, whereas monophasic pain responses were observed in the PPC-1-inoculated mice. Endothelin-1 (1 to 10 pmol/paw) but not sarafotoxin S6c potentiated the pain response in prostate cancer-inoculated mice. Both YM598 and atrasentan (0.3 to 3 mg/kg, p.o.) significantly inhibited the endothelin-1 (10 pmol/paw)-induced potentiation of nociception in a dose-dependent manner. These results suggest that selective endothelin ET_A receptor antagonists might relieve pain in patients with various diseases in which endothelin-1 production is increased, e.g. prostate cancer.

Keywords: YM598; Antagonist; Endothelin ET_A receptor; Nociception; Prostate cancer

1. Introduction

Pain is a frequent and disabling consequence of metastatic prostate cancer in humans. The cause of this pain is unknown, but may involve mediator-dependent signaling by tumor cells to spinal nerve roots. One candidate mediator is endothelin, which was originally identified in the supernatant of a porcine aortic endothelial cell culture (Yanagisawa et al., 1988; Hirata et al., 1989). Endothelin is a potent vasoconstrictor and mitogenic peptide consisting of 21 amino acid residues. Three isopeptides of endothelin, endothelin-1, endothelin-2, and endothelin-3, have been reported (Inoue et al., 1989). The physiological actions of endothelin are mediated by specific cell surface endothelin receptors, two subtypes of which have been cloned and stably expressed in mammals, endothelin ET_A and ET_B (Arai et al., 1990; Sakurai et al., 1990).

Endothelin-1 is secreted in high concentrations from metastatic prostate cancer cells. Elevated endothelin-1 is observed in the plasma of prostate cancer patients with bone metastasis (Nelson et al., 1995), and is known to induce pain-like behavior in animals and pain in humans (Dahlof et al., 1990; Piovezan et al., 1997, 1998; Davar et al., 1998; De-Melo et al., 1998; Jarvis et al., 2000; Gokin et al., 2001). Endothelin-1 is also found in high concentrations in dorsal root ganglion neurons (Giaid et al., 1989), and endothelin ET_A receptors are found on small- to medium-sized dorsal root ganglion neurons and their axons (Pomonis et al., 2001), which is further evidence of a potential role for endothelin-1 in pain signaling. In mice or rats, intraperitoneal or intraplantar administration of endothelin-1 produces pain behavior or an increase in the frequency of sensory nerve activity mediated by endothelin ET_A receptors (Raffa and Jacoby, 1991; Raffa et al., 1991, 1996;

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doi:10.1016/j.ejphar.2004.04.016

Gokin et al., 2001). Endothelin-1 is also reported to induce pain responses when topically applied to the sciatic nerve in rats through the activation of endothelin ET_A receptors (Davar et al., 1998). Similarly, intraplantar administration of endothelin-1 potentiates pain states in models of chemical- and inflammation-induced pain in mice or rats (Piovezan et al., 1997, 1998; De-Melo et al., 1998). In humans, muscular pain and edema are induced by endothelin-1 administered to the brachial artery (Dahlof et al., 1990). From these various findings, it is considered that endothelin-1 is both a pain-producing and a pain-potentiating substance, and that selective endothelin ET_A receptor antagonists may be effective in inhibiting pain responses in various nociceptive diseases, including metastatic prostate cancer.

Recently, we reported a novel, highly bioavailable and potent nonpeptidic selective endothelin ET_A receptor antagonist, (E)-*N*-[6-methoxy-5-(2-methoxyphenoxy)[2,2′-bipyrimidin]-4-yl]-2-phenylethenesulfonamide monopotassium salt (YM598; Yuyama et al., 2003). In the present study, to evaluate the clinical usefulness of selective endothelin ET_A receptor antagonists in the treatment of various nociceptive diseases, including metastatic prostate cancer, we investigated the effects of YM598 and the other selective endothelin ET_A receptor antagonist, atrasentan (Opgenorth et al., 1996), on the nociception potentiated by endothelin-1 in a cancer inoculation-induced pain model in mice.

2. Materials and methods

2.1. Materials

YM598 and atrasentan were synthesized at Yamanouchi Pharmaceutical (Ibaraki, Japan), and dissolved or suspended in a 0.5% methylcellulose aqueous solution. Doses of YM598 and atrasentan are represented in terms of the free form dose. Endothelin-1 and the selective ET_B receptor agonist sarafotoxin S6c (Williams et al., 1991) were purchased from Peptide Institute (Osaka, Japan), formalin from Wako (Osaka, Japan), Roswell Park Memorial Institute 1640 (RPMI1640) medium, fetal bovine serum and trypsin-EDTA from Gibco (Grand Island, NY, USA), and methylcellulose from Shin-Etsu Chemical (Tokyo, Japan). All other chemicals were of analytical grade.

2.2. Animals

Male severe combined immunodeficiency (SCID) mice (C.B17/Icr-scid mice, 5 weeks old) were obtained from CLEA Japan (Tokyo, Japan) to prepare a cancer inoculation-induced pain model. They were given solid food and tap water ad libitum during the breeding period before use in the experiments.

2.3. Cell and cell culture

The androgen-independent human prostate cancer cell line PPC-1 was kindly gifted by Dr. Brothman, AR (Cytogenetics Laboratory, University of Utah). PPC-1 cells were cultured at 37 °C in a humidified atmosphere with 5% CO_2 in RPMI1640 supplemented with 20% fetal bovine serum, 50 U/ml penicillin and 50 μg/ml streptomycin.

2.4. Experimental methods: cancer inoculation-induced pain model

The experiments on cancer inoculation-induced pain were conducted basically as described previously (Kuraishi, 2001) with minor modifications. PPC-1 cells in the growth stage were adjusted to a concentration of 1.6×10^7 cells/ml in RPMI1640 medium. Under mild restraint, the mice were inoculated with PPC-1 (4×10^5 cells/25 μl/paw) subcutaneously in the left paw using a microsyringe (Hamilton Company, NV, USA). Sham-operated mice were inoculated with RPMI1640 medium containing no tumor cells. Pain response was measured at 4 weeks after cell inoculation. Before measurement, the left hind limb volume was determined on the day before measurement using a volume meter (TK-105, Unicom, Tokyo, Japan) and the mice were divided into groups without bias. They were then placed for 5 min in observation cages for acclimation. After acclimation, 10 μl of vehicle alone or vehicle with endothelin-1 or sarafotoxin S6c was subcutaneously injected into the left hind paw. Immediately following injection, the animal was returned to the observation cage configured as for the formalin-induced pain study. In the cancer-induced pain study, the duration of the lifting responses which appeared immediately after injection was measured each 5 min for 40 min using a chronometer. After measurement, each animal was sacrificed by cervical dislocation and both hind paws were cut off at the ankle joint and weighed. The total response time (in seconds) was used as an index of pain, and the difference in weight (milligrams) between hind paws as an index of paw edema.

In the initial experiments, we confirmed whether endothelin-1 exerted an effect on the cancer inoculation-induced pain. Endothelin-1 (10 pmol/paw) or vehicle was injected subcutaneously in the left hind paw to the PPC-1-inoculated or sham-operated mice. In the second experiments, we performed dose–response examinations of endothelin on the cancer inoculation-induced pain. Endothelin-1 (1–10 pmol/paw), sarafotoxin S6c (1–10 pmol/paw), or vehicle was injected subcutaneously in the left hind paw to the PPC-1-inoculated mice. In the third experiments, we investigated the effects of YM598 and atrasentan on the potentiation by endothelin-1 of cancer inoculation-induced pain. Animals were orally administered either YM598 (0.3–3 mg/kg, p.o.), atrasentan (0.3–3 mg/kg, p.o.) or vehicle 1 h before intraplantar injection of endothelin-1 (10 pmol/paw).

2.5. Expression of results

Values are expressed as the mean ± S.E.M. Data were analyzed using the SAS software (SAS Institute, NC, USA). The difference between groups was analyzed by analysis of variance (ANOVA). When significant differences were identified by ANOVA, Dunnett's multiple range test was used. When differences between two groups were analyzed, Fisher's LSD test was used. A P value less than 0.05 was considered to be significant.

2.6. Ethical considerations

The protocol for this study was approved by the Animal Ethical Committee of Yamanouchi Pharmaceutical.

3. Results

3.1. Cancer inoculation-induced pain model

No pain responses were observed in the sham-operated animals, whereas monophasic pain responses were observed in the PPC-1 inoculated animals (Fig. 1).

Endothelin-1 (10 pmol/paw, s.c.) induced pain responses in the sham-operated animals and potentiated responses in the PPC-1 inoculated animals (Fig. 1). This potentiation of cancer pain by endothelin-1 (1 to 10 pmol/paw, s.c.) was dose-dependent (Fig. 2). In contrast, sarafotoxin S6c (1 to 10 pmol/paw, s.c.) did not potentiate cancer inoculation-induced pain at all (Fig. 2). No significant effects of endothelin-1 on the wet weight of the hind limbs of mice were observed (data not shown).

With regard to the effects of YM598 and atrasentan on the potentiation by endothelin-1 of cancer pain, prior administration of YM598 or atrasentan (0.3 to 3 mg/kg, p.o.) showed a dose-dependent inhibition of endothelin-1 (10 pmol/paw, s.c.)-induced potentiation in this model (Fig. 3). The inhibitory effects of the two compounds were almost equal (Fig. 3). No noticeable behavioral changes in mice were observed after oral administration of YM598 or atrasentan at these doses.

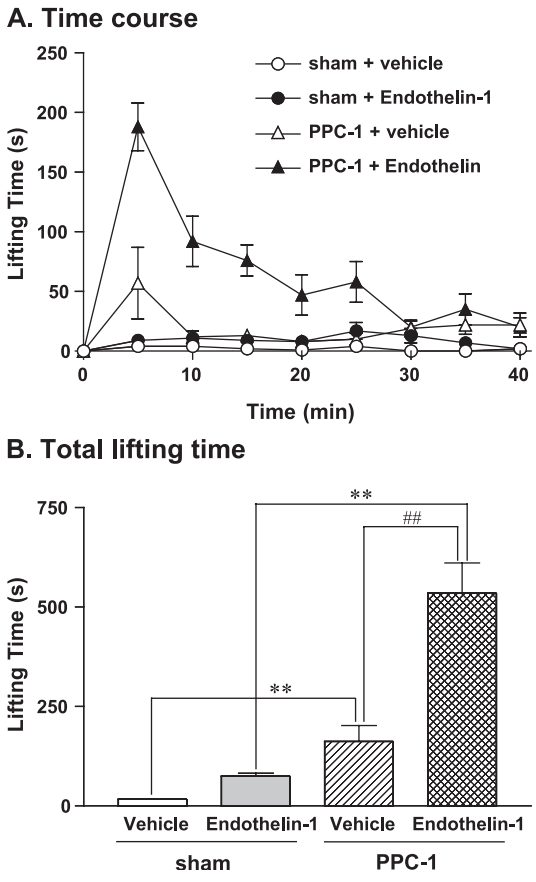

Fig. 1. Vehicle- or endothelin-1-induced pain responses in sham-operated and PPC-1-inoculated mice. Four weeks after inoculation of PPC-1 into the left hind paw of severe combined immunodeficiency mice, vehicle or endothelin-1 (10 pmol) was subcutaneously injected into the left hind paw of sham-operated or PPC-1-inoculated mice and the pain response was measured for 40 min using lifting time of the left hind paw as an index. (A) Time course and (B) sum of vehicle- or endothelin-1-induced lifting time (total lifting time). Each point or column with vertical line represents the mean ± S.E.M. of seven to eight independent determinations. Statistical analysis was performed by Fisher's LSD test. **: $P<0.01$ compared with the corresponding sham value, ##: $P<0.01$ compared with the vehicle value.

4. Discussion

In patients with prostate cancer, bone metastasis is frequent and is associated with pain in many cases (Kelly et al., 1993). This pain is considered almost unbearable and greatly reduces the patient's quality of life. At present, cancer pain is treated with narcotic analgesics such as morphine, but problems with insufficient efficacy, resistance and adverse drug reactions make the development of new drugs desirable. The mechanism of the onset of pain associated with bone metastasis in prostate cancer remains unclear, but zoledronic acid, a bisphosphonate, has recently shown a certain level of efficacy in these patients (for review, Keller, 2002), suggesting the involvement of osteolysis. Further, because malignant cancer cells secrete various factors that excite primary afferent nociceptors, and because bone cancer pain continued even after the complete inhibition of osteolysis in an osteolytic cancer pain model (Suzuki and Yamada, 1994; Safieh-Garabedian et al., 1995; Woolf et al., 1997; Honore et al., 2000), the involvement of tumor-secreted factors is also suspected.

One candidate mediator of pain induction is endothelin-1. Endothelin-1 is produced by various androgen-independent human prostate cancer cell lines and the elevation of endothelin-1 concentration in the plasma has been observed in prostate cancer patients with bone metastasis (Nelson et al., 1995), suggesting that endothelin-1 produced by prostate cancer cells is involved in the pain of prostate cancer associated with bone metastasis. In addition, endothelin-1

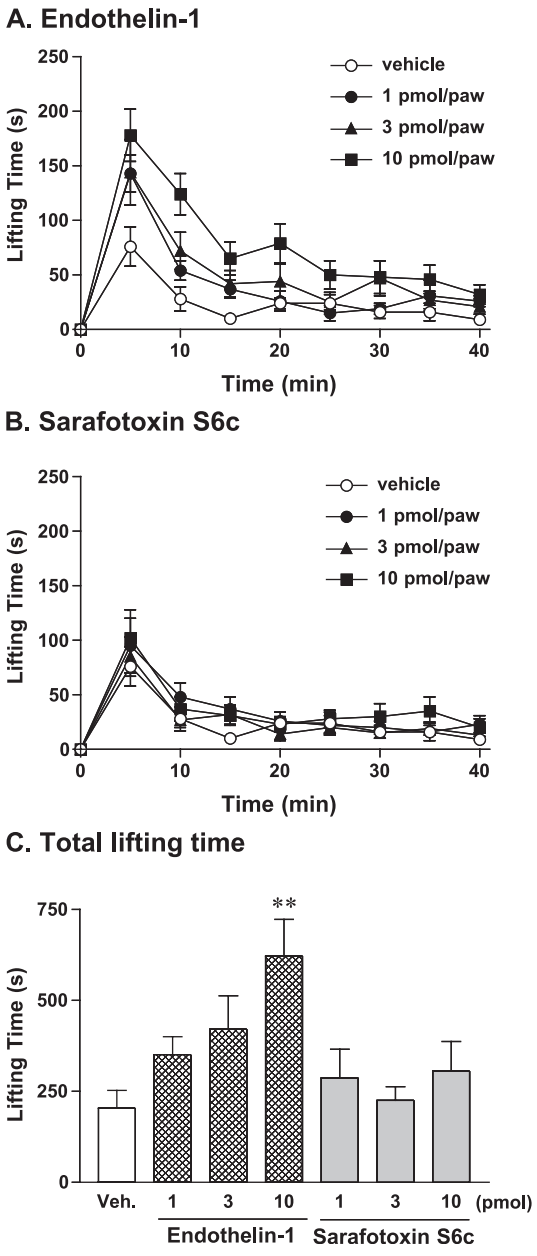

Fig. 2. Dose–response of endothelin-1- and sarafotoxin S6c-induced potentiation of pain responses in PPC-1-inoculated mice. Four weeks after inoculation of PPC-1 into the left hind paw of severe combined immunodeficiency mice, endothelin-1 or sarafotoxin S6c (S6c; 1, 3, 10 pmol) was subcutaneously injected into the left hind paw and the resulting pain responses were measured for 40 min. (A) Time course of endothelin-1-induced pain response, (B) time course of S6c-induced pain response, and (C) sum of lifting time (total lifting time). Each point or column with vertical line represents the mean ± S.E.M. of 9–10 independent determinations. Statistical analysis was performed by analysis of variance (ANOVA) followed by Dunnett's multiple range test. **: $P<0.01$ compared with the corresponding vehicle value.

potentiates pain states in various models of chemical- and inflammation-induced pain (Piovezan et al., 1997, 1998; De-Melo et al., 1998, Jarvis et al., 2000). On these bases, endothelin receptor antagonists may be effective against pain in various nociceptive diseases including prostate

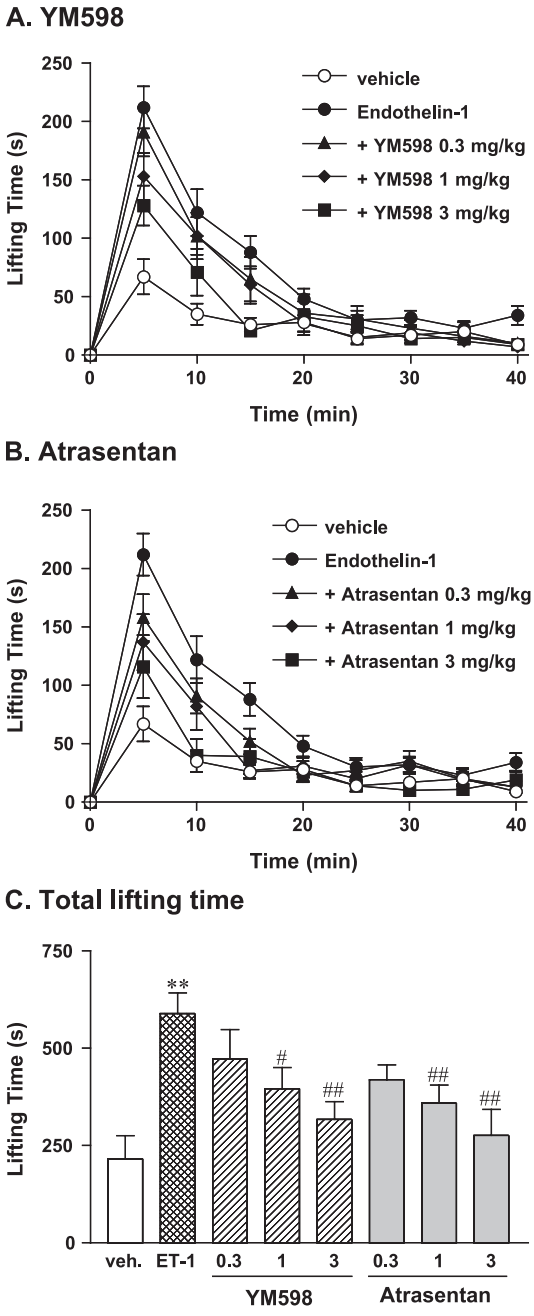

Fig. 3. Effects of YM598 and atrasentan on endothelin-1-induced potentiation of pain responses in PPC-1-inoculated mice. Four weeks after inoculation of PPC-1 into the left hind paw of severe combined immunodeficiency mice, YM598 (0.3, 1, 3 mg/kg) or atrasentan (0.3, 1, 3 mg/kg) was orally administered. One hour later, vehicle or endothelin-1 (ET-1; 10 pmol) was subcutaneously injected into the left hind paw and the resulting pain responses were measured for 40 min. (A) Time course of the effects of YM598, (B) time course of the effects of atrasentan, and (C) sum of lifting time (total lifting time). Each point or column with vertical line represents the mean ± S.E.M. of 10–12 independent determinations. Statistical analysis was performed by Fisher's LSD test or analysis of variance (ANOVA) followed by Dunnett's multiple range test. **: $P<0.05$ compared with vehicle value. #: $P<0.05$, ##: $P<0.01$ compared with the endothelin-1 value.

cancer, and thereby contribute to the improvement of patient quality of life. In the present study, we investigated the inhibitory effects of several endothelin ET_A receptor antagonists, including the novel, highly bioavailable and potent compound YM598, on endothelin-1-induced potentiation of cancer inoculation-induced pain model to confirm the usefulness of endothelin ET_A receptor antagonists on various nociceptive diseases including prostate cancer.

We have previously reported the in vivo endothelin receptor antagonistic activities of YM598 (Yuyama et al., 2003). In conscious rats, oral administration of YM598 inhibited big endothelin-1 (0.5 nmol/kg, i.v.)-induced pressor responses. The maximal inhibiting activity of YM598 was observed 30 min after administration, and the inhibiting activity at a dose of 1 mg/kg did not for change at least for 6.5 h after oral administration. On the other hand, YM598 at a dose of 10 mg/kg did not affect endothelin ET_B receptor-mediated responses in vivo. We selected the doses (0.3–3 mg/kg, p.o.) and pretreatment time (1 h before injection of endothelin-1) of YM598 for the present study on the basis of these findings to provide sufficient in vivo endothelin ET_A receptor inhibiting activity and selectivity.

For the investigation of cancer pain, we prepared a model in which the human prostate cancer cell line PPC-1 was inoculated into the hind paws of SCID mice. (Kuraishi et al. (2001) confirmed the establishment of cancer pain in a similar model using melanoma as the shortening of time until escape from a pain-inducing stimulus, namely withdrawal of a cancer-inoculated hind paw from heat radiation. We confirmed this shortening in the PPC-1-inoculated mice in the present study (data not shown). In addition, the pain responses observed on the administration of vehicle were higher in the PPC-1-inoculated animals than in the sham-operated controls. From these findings, we concluded that the establishment of a cancer pain model using human prostate cancer PPC-1 cells was possible. Endothelin-1-induced pain responses were markedly elevated in this model, confirming the potentiation of cancer pain by endothelin-1. In the formalin-induced pain models, biphasic pain response and edema were observed, and the potentiation of both pain responses and edema by endothelin-1 has been reported (Piovezan et al., 1997). These potentiations were considered to involve both direct nerve stimulation and indirect mechanisms, e.g. potentiation of inflammatory responses by endothelin-1. In the present cancer inoculation-induced pain model, the endothelin-1-induced pain responses were basically monophasic and, unlike the formalin-induced pain model, no edema was observed, suggesting that the pain resulted from direct nerve stimulation by endothelin-1. Further, the selective endothelin ET_B receptor agonist sarafotoxin S6c did not potentiate cancer pain, whereas the selective endothelin ET_A receptor antagonists YM598 and atrasentan dose-dependently inhibited the potentiation of cancer pain by endothelin-1, suggesting that endothelin-1 potentiates cancer pain via endothelin ET_A receptors. Wacnik et al. (2001) previously reported the exogenous and endogenous endothelin-1-induced potentiation of pain in a cancer pain model prepared using mouse fibrosarcoma and inhibition by the peptide selective endothelin ET_A receptor antagonist cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu-) (BQ-123). The results of the present study are consistent with these previous findings. Notably, furthermore, this is the first report to confirm the endothelin-1-induced potentiation of cancer pain using human prostate cancer cells and the potential of orally active selective endothelin ET_A receptor antagonists against endothelin-1-induced potentiation of pain associated with prostate cancer.

In conclusion, we have shown that endothelin-1 induces the potentiation of pain responses in a cancer inoculation-induced pain model through endothelin ET_A receptors. We have also confirmed the effectiveness of selective endothelin ET_A receptor antagonists against endothelin-1-induced potentiation of pain responses. These data suggest that selective endothelin ET_A receptor antagonists like YM598 may be effective against pain induced by various nociceptive diseases, including prostate cancer, and thereby help improve the quality of life of patients with these conditions.

References

Arai, H., Hori, S., Aramori, I., Ohkubo, H., Nakanishi, S., 1990. Cloning and expression of a cDNA encoding an endothelin receptor. Nature 348, 730–732.

Dahlof, B., Gustafsson, D., Hedner, T., Jern, S., Hansson, L., 1990. Regional haemodynamic effects of endothelin-1 in rat and man: unexpected adverse reaction. J. Hypertens. 8, 811–817.

Davar, G., Hans, G., Fareed, M.U., Sinnott, C., Strichartz, G., 1998. Behavioral signs of acute pain produced by application of endothelin-1 to rat sciatic nerve. NeuroReport 9, 2279–2283.

De-Melo, J.D., Tonussi, C.R., D'Orleans-Juste, P., Rae, G.A., 1998. Articular nociception induced by endothelin-1, carrageenan and LPS in naive and previously inflamed knee-joints in the rat: inhibition by endothelin receptor antagonists. Pain 77, 261–269.

Giaid, A., Gibson, S.J., Ibrahim, B.N., Legon, S., Bloom, S.R., Yanagisawa, M., Masaki, T., Varndell, I.M., Polak, J.M., 1989. Endothelin 1, an endothelium-derived peptide, is expressed in neurons of the human spinal cord and dorsal root ganglia. Proc. Natl. Acad. Sci. U. S. A. 86 (19), 7634–7638.

Gokin, A.P., Fareed, M.U., Pan, H.L., Hans, G., Strichartz, G.R., Davar, G., 2001. Local injection of endothelin-1 produces pain-like behavior and excitation of nociceptors in rats. J. Neurosci. 21, 5358–5366.

Hirata, Y., Takagi, Y., Fukuda, Y., Marumo, F., 1989. Endothelin is a potent mitogen for rat vascular smooth muscle cells. Atherosclerosis 78 (2–3), 225–228.

Honore, P., Luger, N.M., Sabino, M.A., Schwei, M.J., Rogers, S.D., Mach, D.B., O'keefe, P.F., Ramnaraine, M.L., Clohisy, D.R., Mantyh, P.W., 2000. Osteoprotegerin blocks bone cancer-induced skeletal destruction, skeletal pain and pain-related neurochemical reorganization of the spinal cord. Nat. Med. 6 (5), 521–528.

Inoue, A., Yanagisawa, M., Kimura, S., Kasuya, Y., Miyauchi, T., Goto, K., Masaki, T., 1989. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc. Natl. Acad. Sci. U. S. A. 86, 2863–2867.

Jarvis, M.F., Wessale, J.L., Zhu, C.Z., Lynch, J.J., Dayton, B.D., Calzadilla, S.V., Padley, R.J., Opgenorth, T.J., Kowaluk, E.A., 2000. ABT-627, an endothelin ET_A receptor-selective antagonist, attenuates tactile allody-

nia in a diabetic rat model of neuropathic pain. Eur. J. Pharmacol. 388 (1), 29–35.

Keller, E.T., 2002. The role of osteoclastic activity in prostate cancer skeletal metastases. Drugs Today 38 (2), 91–102.

Kelly, W.K., Scher, H.I., Mazumdar, M., Vlamis, V., Schwartz, M., Fossa, S.D., 1993. Prostate-specific antigen as a measure of disease outcome in metastatic hormone-refractory prostate cancer. J. Clin. Oncol. 11 (4), 607–615.

Kuraishi, Y., 2001. Effects of morphine on cancer pain and tumor growth and metastasis. Jpn. J. Clin. Med. 59 (9), 1669–1674.

Nelson, J.B., Hedican, S.P., George, D.J., Reddi, A.H., Piantadosi, S., Eisenberger, M.A., Simons, J.W., 1995. Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. Nat. Med. 1, 944–949.

Opgenorth, T.J., Adler, A.L., Calzadilla, S.V., Chiou, W.J., Dayton, B.D., Dixon, D.B., Gehrke, L.J., Hernandez, L., Magnuson, S.R., Marsh, K.C., Novosad, E.I., Von Geldern, T.W., Wessale, J.L., Winn, M., Wu-Wong, J.R., 1996. Pharmacological characterization of A-127722: an orally active and highly potent ET_A-selective receptor antagonist. J. Pharmacol. Exp. Ther. 276, 473–481.

Piovezan, A.P., D'Orleans-Juste, P., Tonussi, C.R., Rae, G.A., 1997. Endothelins potentiate formalin-induced nociception and paw edema in mice. Can. J. Physiol. Pharmacol. 75, 596–600.

Piovezan, A.P., D'Orleans-Juste, P., Tonussi, C.R., Rae, G.A., 1998. Effects of endothelin-1 on capsaicin-induced nociception in mice. Eur. J. Pharmacol. 351, 15–22.

Pomonis, J.D., Rogers, S.D., Peters, C.M., Ghilardi, J.R., Mantyh, P.W., 2001. Expression and localization of endothelin receptors: implications for the involvement of peripheral glia in nociception. J. Neurosci. 21 (3), 999–1006.

Raffa, R.B., Jacoby, H.I., 1991. Endothelin-1, -2 and -3 directly and big-endothelin-1 indirectly elicit an abdominal constriction response in mice. Life Sci. 48, PL85–PL90.

Raffa, R.B., Schupsky, J.J., Martinez, R.P., Jacoby, H.I., 1991. Endothelin-1-induced nociception. Life Sci. 49, PL61–PL65.

Raffa, R.B., Schupsky, J.J., Jacoby, H.I., 1996. Endothelin-induced nociception in mice: mediation by ET_A and ET_B receptors. J. Pharm. Exp. Ther. 276, 647–651.

Safieh-Garabedian, B., Poole, S., Allchorne, A., Winter, J., Woolf, C.J., 1995. Contribution of interleukin-1 beta to the inflammation-induced increase in nerve growth factor levels and inflammatory hyperalgesia. Br. J. Pharmacol. 115 (7), 1265–1275.

Sakurai, T., Yanagisawa, M., Takuwa, Y., Miyazaki, H., Kimura, S., Goto, K., Masaki, T., 1990. Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Nature 348, 732–735.

Suzuki, K., Yamada, S., 1994. Ascites sarcoma 180, a tumor associated with hypercalcemia, secretes potent bone-resorbing factors including transforming growth factor alpha, interleukin-1 alpha and interleukin-6. Bone. Mineral. 27 (3), 219–233.

Wacnik, P.W., Eikmeier, L.J., Ruggles, T.R., Ramnaraine, M.L., Walcheck, B.K., Beitz, A.J., Wilcox, G.L., 2001. Functional interactions between tumor and peripheral nerve: morphology, algogen identification, and behavioral characterization of a new murine model of cancer pain. J. Neurosci. 21 (23), 9355–9366.

Williams Jr., D.L., Jones, K.L., Pettibone, D.J., Lis, E.V., Clineschmidt, B.V., 1991. Sarafotoxin S6c: an agonist which distinguishes between endothelin receptor subtypes. Biochem. Biophys. Res. Commun. 175 (2), 556–561.

Woolf, C.J., Allchorne, A., Safieh-Garabedian, B., Poole, S., 1997. Cytokines, nerve growth factor and inflammatory hyperalgesia: the contribution of tumour necrosis factor alpha. Br. J. Pharmacol. 121 (3), 417–424.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., Masaki, T., 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332, 411–415.

Yuyama, H., Sanagi, M., Koakutsu, A., Mori, M., Fujimori, A., Hadrada, H., Sudoh, K., Miyata, K., 2003. Pharmacological Characterization of YM598, an orally active and highly potent selective ET_A receptor antagonist. Eur. J. Pharmacol. 478, 61–71.