British Journal of Pharmacology (2003) 139, 203–207
www.nature.com/bjp

SPECIAL REPORT

The neuromedin B receptor antagonist, BIM-23127, is a potent antagonist at human and rat urotensin-II receptors

*,[1]Christopher L. Herold, [1]David J. Behm, [2]Peter T. Buckley, [2]James J. Foley, [2]William E. Wixted, [2]Henry M. Sarau & [1]Stephen A. Douglas

[1]Cardiovascular and Urogenital Centre of Excellence for Drug Discovery, GlaxoSmithKline, King of Prussia, PA 19406-0939, U.S.A. and [2]Respiratory, Inflammation, & Respiratory Pathogens Centre of Excellence for Drug Discovery, GlaxoSmithKline, King of Prussia, PA 19406-0939, U.S.A.

The functional activity of the peptidic neuromedin B receptor antagonist BIM-23127 was investigated at recombinant and native urotensin-II receptors (UT receptors). Human urotensin-II (hU-II) promoted intracellular calcium mobilization in HEK293 cells expressing the human UT (hUT) or rat UT (rUT) receptors with pEC_{50} values of 9.80 ± 0.34 ($n=6$) and 9.06 ± 0.32 ($n=4$), respectively. While BIM-23127 alone had no effect on calcium responses in either cell line, it was a potent and competitive antagonist at both hUT ($pA_2 = 7.54 \pm 0.14$; $n=3$) and rUT ($pA_2 = 7.70 \pm 0.05$; $n=3$) receptors. Furthermore, BIM-23127 reversed hU-II-induced contractile tone in the rat-isolated aorta with a pIC_{50} of 6.66 ± 0.04 ($n=4$). In conclusion, BIM- 23127 is the first hUT receptor antagonist identified to date and should not be considered as a selective neuromedin B receptor antagonist.
British Journal of Pharmacology (2003) **139,** 203 – 207. doi:10.1038/sj.bjp.0705251

Keywords: Urotensin-II; UT receptor; BIM-23127; GPR14; neuromedin B

Abbreviations: $Ca^{2+}{}_i$, intracellular calcium; hU-II, human urotensin-II; hUT receptor, human UT receptor; rUT receptor, rat UT receptor; U-II, urotensin-II; UT receptor, urotensin-II receptor

Introduction

Urotensin-II (U-II) is a cyclic peptide first identified in the goby urophysis, an endocrine organ homologous in structure to the mammalian hypothalamoneurohypophysial axis (Pearson *et al.*, 1980). Following the cloning of carp U-II cDNAs (Ohsako *et al.*, 1986), U-II orthologues have since been cloned from additional vertebrates, including humans (Ames *et al.*, 1999; Coulouarn *et al.*, 1998). Human urotensin-II (hU-II) is a cyclic undecapeptide that has been identified as the endogenous ligand for the orphan G protein-coupled receptor, GPR14 (Ames *et al.*, 1999), subsequently redesignated the urotensin-II receptor (UT receptor) by the IUPHAR Committee on Receptor Nomenclature (Douglas & Ohlstein, 2000b).

Both hU-II and the human UT (hUT) receptor are expressed within several cardiovascular tissues (Ames *et al.*, 1999; Douglas & Ohlstein, 2000a), suggesting that hU-II may be an endogenous modulator of cardiovascular function. Indeed, hU-II has been shown to exert potent contractile effects in isolated vascular tissues from numerous species (Douglas *et al.*, 2000c; Paysant *et al.*, 2001), promote inotropic and arrhythmogenic actions in human cardiac trabeculae (Russell *et al.*, 2001), have synergistic effects with factors such as oxidized LDL on vascular smooth muscle cell proliferation (Watanabe *et al.*, 2001), and promote cardiomyocyte hypertrophy (Zou *et al.*, 2001). In addition, elevated hU-II levels were observed in patients with hypertension (Matsushita *et al.*, 2001) and congestive heart failure (Douglas *et al.*, 2002). Thus, because evidence exists suggesting a role for hU-II as a cardiovascular mediator, the UT receptor represents a potential target in the treatment of cardiovascular disorders. To this end, the development of selective UT receptor antagonists will facilitate a better understanding of the role of U-II in the mammalian cardiovascular system. Therefore, the present study has examined the functional activity of the neuromedin B receptor antagonist BIM-23127 (D-Nal-cyclo-[Cys-Tyr-D-Trp-Orn-Val-Cys]-2-Nal-NH_2; Nal = β-2-Napthyl-L-alanine) at recombinant and native UT receptors. BIM-23127 is a D-amino-acid substituted cyclo-somatostatin octapeptide analog (Orbuch *et al.*, 1993) that shares structural similarity with SB-710411, which functions as a competitive antagonist of the rat UT (rUT) receptor in the rat aorta (Behm *et al.*, 2002) and a full agonist at the recombinant hUT receptor (Herold *et al.*, 2001).

Methods

Cell culture

HEK293 cells stably expressing the hUT or rUT receptors were generated and propagated as described previously (Ames *et al.*, 1999).

Intracellular calcium ($Ca^{2+}{}_i$) mobilization assay

hUT-HEK293 cells or rUT-HEK293 cells were seeded in blackwalled, clear-bottomed 96-well Biocoat plates (Beckton

*Author for correspondence at: Vascular Biology, GlaxoSmithKline, UW2510, 709 Swedeland Road, Box 1539, King of Prussia, PA 19406-0939, U.S.A.; E-mail: heroldc1@yahoo.com

Dickinson, Bedford, MA, U.S.A.) at a density of 45,000 cells/well, grown in the incubator at 37°C for 18–24 h, and prepared for $Ca^{2+}{}_i$ measurements as described previously (Ames *et al.*, 1999). Plates were placed into the Fluorometric Imaging Plate Reader (Molecular Devices, Sunnyvale, CA, U.S.A.) where cells, loaded with Fluo 3 (Molecular Probes, Eugene, OR, U.S.A.), were exposed to excitation (488 nm) from a 6-W argon laser. Fluorescence was monitored at 566 nm emission for all 96 wells simultaneously, and data were read every 1 s for 1 min and then every 3 s thereafter. Agonist was added after 10 s and concentration–response curves were obtained by calculating the maximal fluorescent counts above background after addition of each concentration of agonist. For antagonist studies, BIM-23127 (Bachem, King of Prussia, PA, U.S.A.) was added 10 min prior to the addition of hU-II (California Peptide Research, Inc., Napa, CA, U.S.A.). Concentration-response curves were analyzed by nonlinear regression using GraphPad Prism 3.0 software (GraphPad, San Diego, CA, U.S.A.).

Preparation and utilization of rat isolated aortae

All procedures were performed in accredited facilities in accordance with institutional guidelines (Animal Care and Use Committee, GlaxoSmithKline) and the Guide for the Care and Use of Laboratory Animals (DHSS #NIH 85-23). Proximal descending thoracic aortae were isolated from male Sprague–Dawley rats (400 g) and prepared as described previously (Behm *et al.*, 2002). Following a 60 min equilibration period, vessels were exposed to standard concentrations of KCl (60 mM) and phenylephrine (1 μM). Paired thoracic aortae were pretreated with either vehicle (0.1% DMSO) or BIM-23127 (3 μM) for 30 min, following which cumulative concentration-response curves to hU-II (0.01–300 nM) were constructed.

For reversal of hU-II-induced tone experiments, separate vessels were contracted with 3 nM hU-II (predetermined EC_{80}) and once the response plateaued, contractile tone was reversed by adding increasing log unit concentrations of BIM-23127. For all experiments, each tissue was used to generate only one concentration–response curve and each response was allowed to plateau before the addition of subsequent agonist concentration. All values are expressed as mean ± s.e.m. and n represents the total number of animals from which the vessels were isolated. Where relevant, statistical comparisons were made using a paired, two-tailed t-test and differences were considered significant when $P \leqslant 0.05$.

Competition binding assay

[^{125}I]hU-II binding assays were performed using the scintillation proximity assay (SPA) method. Membranes from hUT and UT receptor-expressing HEK293 cells were prepared as described previously (Ames *et al.*, 1999) and precoupled to wheatgerm–agglutinin-coated SPA beads (Amersham, Arlington Heights, IL, U.S.A.). Binding conditions (200 μl final volume) consisted of 10–20 μg membrane protein, 0.4 mg SPA beads, and 0.3 nM [^{125}I]hU-II ([^{125}I]Tyr^{10}, 2000 Ci/mmol; Amersham) in the absence or presence of varying concentrations of unlabeled hU-II or BIM-23127 in assay buffer (20 mM Tris-HCl pH 7.4, 5 mM $MgCl_2$, and 0.05% BSA). Non-specific binding was determined with 1 μM unlabeled hU-II. Assays were performed in 96-well Optiplates (Packard Bioscience, Meriden, CT, U.S.A.). Assay plates were sealed, shaken gently for 1 h at room temperature, centrifuged at 2000 × g for 10 min, and counted in a Packard Top Count Scintillation Counter. Competition binding curves were analyzed by nonlinear regression using GraphPad Prism 3.0 software.

Results

hU-II caused a dose-dependent increase in $[Ca^{2+}]_i$; in hUT-HEK293 cells (Figure 1a) and rUT-HEK293 cells (Figure 1b) with pEC_{50} values of 9.80 ± 0.34 ($n = 6$) and 9.06 ± 0.32 ($n = 4$), respectively. In both cell lines, in the presence of increasing concentrations of BIM-23127 (33 nM–3.3 μM), the hU-II concentration–response curves were shifted progressively to the right in a parallel manner with no changes in the maximum response to hU-II, suggesting competitive antagonism. Schild plots derived from these data could be fit by straight lines with slopes near unity (1.04 ± 0.06; $n = 3$ for the hUT receptor and 1.03 ± 0.06; $n = 3$ for the rUT receptor) and yielded pA_2 values of 7.54 ± 0.14 ($n = 3$) and 7.70 ± 0.05 ($n = 3$) for the hUT

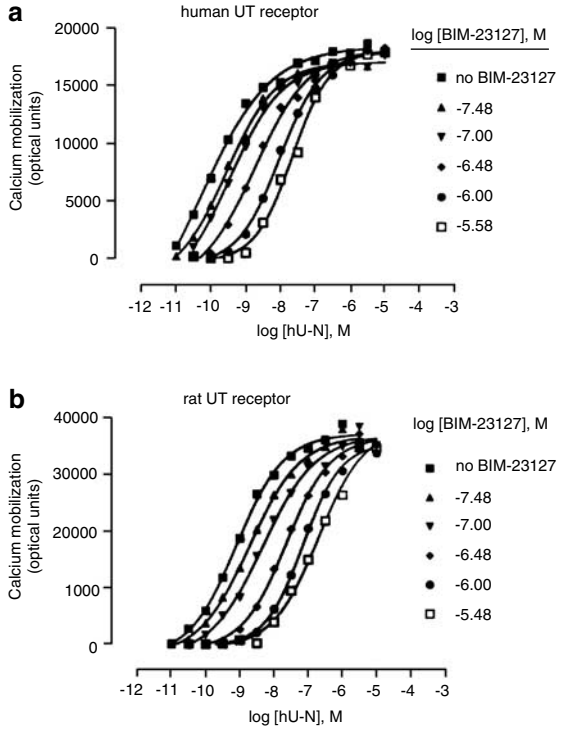

Figure 1 BIM-23127 is a competitive antagonist at hUT and rUT receptors. The effect of BIM-23127 on hU-II-induced $Ca^{2+}{}_i$ mobilization in HEK293 cells expressing either the hUT receptor (a) or the rUT receptor (b) was determined by generating hU-II concentration–response curves in the presence of vehicle increasing concentrations of BIM-23127. Data shown are the average $Ca^{2+}{}_i$ mobilization results (expressed as optical units) of a representative experiment performed in triplicate. pA_2 values reported in Results were derived from three separate experiments. Standard error bars were omitted for clarity but were typically <10% of the mean.

receptor and rUT receptor, respectively. BIM-23127 alone (10 μM) did not have agonist properties in either cell line, whereas somatostatin-14 was 5000-fold less potent than hU-II as an agonist (data not shown). In addition, the nonpeptide neuromedin B receptor antagonist PD 168368 (10 μM) had no effect on the hU-II concentration – response curves (data not shown).

BIM-23127 also displayed high affinity for UT receptors in competition binding experiments. Using membranes from transfected HEK293 cells, BIM-23127 bound with pK_is of 6.70 ± 0.05, $n=3$ (*vs* 8.45 ± 0.16, $n=3$ for hU-II) at the hUT receptor and 7.20 ± 0.14, $n=3$ (*vs* 8.42 ± 0.06, $n=3$ for hU-II) at the rUT receptor, whereas somatostatin-14 failed to compete for [^{125}I]hU-II binding at concentrations up to 3 μM (data not shown).

In the rat isolated aorta bioassay, hU-II evoked potent concentration–dependent contractions (Figure 2a) with a pEC_{50} of 8.98 ± 0.03 (E_{max} of 109 ± 14% response to 60 mM KCl; $n=4$). Exposure to 3 μM BIM-23127 produced a significant (~40%; $P<0.05$) suppression of the maximum contractile response to hU-II (E_{max} of 62 ± 7.0% response to 60 mM KCl; $n=4$) with a slight shift (~five fold) in the concentration–response curve ($pEC_{50} = 8.28 \pm 0.04$). While an affinity value for BIM-23127 could not be determined *in vitro* because of the insurmountable antagonism, BIM-23127 potently ($pIC_{50} = 6.66 \pm 0.04$; $n=4$) and efficaciously (100% suppression) reversed contractile tone established in the rat isolated aorta with an EC_{80} dose of hU-II (Figure 2b).

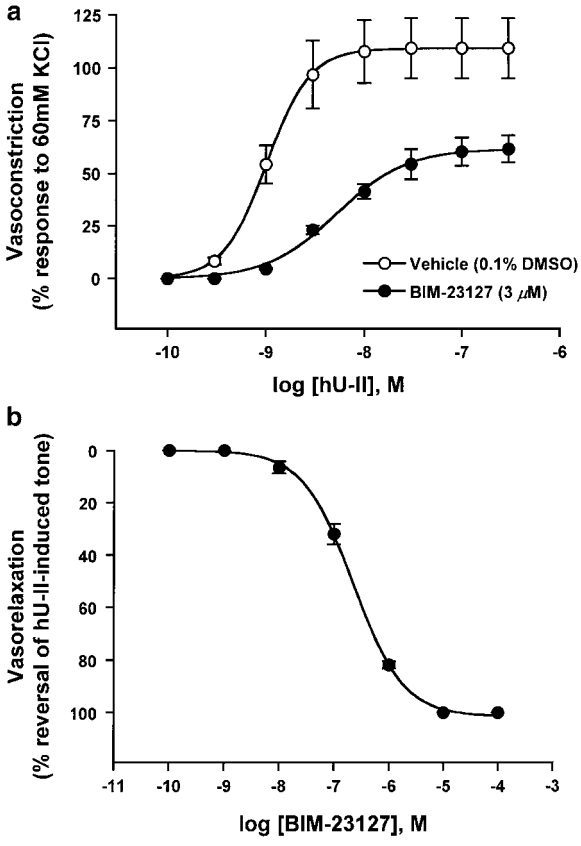

Figure 2 Antagonist effects of BIM-23127 in the rat isolated aorta. Concentration–response curves to hU-II were generated (a) in the absence (vehicle) or presence of 3 μM BIM- 23127. Concentration – dependent relaxation response curve to BIM-23127 (b) is expressed as percent reversal of the original tone established in endothelium-denuded aortae with 3 nM hU-II. Values shown in (a) and (b) are mean ± s.e.m ($n=4$) and curves were derived by fitting experimental data to a logistic equation (Behm *et al.*, 2002).

Discussion

hU-II has been shown to be a potent and efficacious spasmogen of mammalian isolated blood vessels and a mediator of additional cardiovascular functions (Douglas & Ohlstein, 2000a). Therefore, the development of novel UT receptor antagonists should be of utility in the management of cardiovascular pathologies such as hypertension, atherosclerosis, myocardial infarction, and congestive heart failure. To date, very few synthetic ligands have been reported for the UT receptor, thus hindering an understanding of the role of this ligand – receptor pair in cardiovascular homeostasis.

Owing to the striking sequence similarities between the neuromedin B receptor antagonist BIM-23127 and the UT receptor antagonist SB-710411, the ability of BIM-23127 to function as a UT receptor ligand was assessed. Interestingly, in $Ca^{2+}{}_i$ mobilization assays, BIM-23127 was a potent and competitive antagonist at both hUT and rUT receptors. While BIM-23127 competed with [^{125}I]hU-II for recombinant UT receptors with pK_i values several fold less potent than the pK_b values for the inhibition of hU-II-promoted $Ca^{2+}{}_i$ mobilization, such differences between affinities for functional assays and binding assays have been observed with other UT receptor ligands (Kinney *et al.*, 2002).

BIM-23127 also was an antagonist *in vitro* in the rat aorta bioassay where it dose dependently reversed hU-II-induced contractile tone with moderate potency. In addition, 3 μM BIM-23127 significantly suppressed the E_{max} for hU-II-promoted aortic ring contractions, suggesting non-competitive antagonism. While the mechanism responsible for this observation remains to be elucidated, its potency for reversing hU-II-induced contractile tone in the rat-isolated aorta is among the highest observed to date (Behm *et al.*, 2002; Rossowski *et al.*, 2002).

BIM-23127 did not display agonist activity at concentrations up to 10 μM in either the $Ca^{2+}{}_i$ mobilization assay or the rat aorta bioassay, suggesting that this ligand has applications as a pure antagonist at both recombinant UT receptors and natively expressed UT receptors. In contrast, the residual agonist activity observed in the rat-isolated aorta with the novel UT receptor ligand [Orn^8] hU-II described recently (Camarda *et al.*, 2002) potentially limits the utility of [Orn^8] hU-II as a UT receptor tool compound.

Given the potent effects of BIM-23127 at hUT and rUT receptors, these results indicate that BIM-23127 in fact is not a selective neuromedin B receptor antagonist as reported previously (Orbuch *et al.*, 1993). Moreover, BIM-23127 displayed affinity values for competition binding at recombinant UT receptors and inhibition of hU-II-promoted $Ca^{2+}{}_i$ mobilization in cells expressing hUT and rUT receptors that were roughly identical to those values determined for

competition binding at neuromedin B receptors and for its ability to inhibit neuromedin B-promoted DNA synthesis (Lach *et al.*, 1995). The nonpeptide neuromedin B receptor antagonist PD 168368 did not inhibit hU-II-induced $Ca^{2+}{}_i$ mobilization, suggesting that the observed antagonism was because of the U-II-like structure of the ligand (hexapeptide cyclic core sequence) and is not a general property of neuromedin B receptor antagonists. In accord with these findings, it has become increasingly evident that a class of D-amino-acid substituted cyclo-somatostatin octapeptide analogs, which are structurally related to the cyclic U-II core sequence, display submicromolar to micromolar potency at UT receptors. For example, SB-710411 competitively antagonized hU-II-induced contractions in the rat aorta with a K_b of $\sim$500 nM (Behm *et al.*, 2002) and was a full agonist at the hUT receptor expressed in HEK293 cells with an EC_{50} $\sim$100 nM (Herold *et al.*, 2001). Similarly, the putative UT receptor antagonists PRL-2882, PRL-2903, and PRL-2915 were capable of blocking hU-II-induced rat aorta ring contractions (although the nature of antagonism was not discussed) and competed for [^{125}I]U-II binding at recombinant hUT and rUT receptors (Rossowski *et al.*, 2002). The data presented herein indicate that BIM-23127 is the most potent UT receptor ligand from this class of substituted somatostatin analogs; in fact, BIM-23127 represents the first example of an hUT receptor antagonist described to date. Whilst the identification of a nonpeptide hUT receptor antagonist of moderate potency (IC_{50} = 400 nM as determined by $Ca^{2+}{}_i$ mobilization) was discussed recently (Flohr *et al.*, 2002), no data were reported for this lead compound. In addition, BIM-23127 is the most potent antagonist of the recombinant rUT receptor yet reported.

In summary, while data generated with BIM-23127 should be interpreted cautiously because of its roughly equal affinity with the neuromedin B receptor (Lach *et al.*, 1995), it nonetheless represents the most potent UT receptor antagonist characterized to date. Thus, BIM-23127 should find applications as an antagonist *in vitro* and *in vivo* for studying the actions of U-II and as a tool compound to examine recombinant UT receptor function from two species, human and rat. Furthermore, the D-amino-acid-substituted cyclo-somatostatin octapeptide analogs may serve as a template for the development of potent and selective UT receptor antagonists.

References

AMES, R.S., SARAU, H.M., CHAMBERS, J.K., WILLETTE, R.N., AIYAR, N.V., ROMANIC, A.M., LOUDEN, C.S., FOLEY, J.J., SAUERMELCH, C.F., COATNEY, R.W., AO, Z., DISA, J., HOLMES, S.D., STADEL, J.M., MARTIN, J.D., LIU, W.S., GLOVER, G.I., WILSON, S., McNULTY, D.E., ELLIS, C.E., ELSHOURBAGY, N.A., SHABON, U., TRILL, J.J., HAY, D.W.P., OHLSTEIN, E.H., BERGSMA, D.J. & DOUGLAS, S.A. (1999). Human urotensin-II is a potent vasoconstrictor and agonist for the orphan receptor GPR14. *Nature,* **401,** 282 – 286.

BEHM, D.J., HEROLD, C.L., OHLSTEIN, E.H., KNIGHT, S.D., DHANAK, D. & DOUGLAS, S.A. (2002). Pharmacological characterization of SB-710411 (Cpa-c[D-Cys-Pal-D-Trp- Lys- Val-Cys]-Cpa-amide), a novel peptidic urotensin-II receptor antagonist. *Br. J. Pharmacol.,* **137,** 449 – 458.

CAMARDA, V., GUERRINI, R., KOSTENIS, E., RIZZI, A., CALO, G., HATTENBERGER, A., ZUCCHINI, M., SALVADORI, S. & REGOLI, D. (2002). A new ligand for the urotensin II receptor. *Br. J. Pharmacol.,* **137,** 311 – 314.

COULOUARN, Y., LIHRMANN, I., JEGOU, S., ANOUAR, Y., TOSTIVINT, H., BEAUVILLAIN, J.C., CONLON, J.M., BERN, H.A. & VAUDRY, H. (1998). Cloning of the cDNA encoding the urotensin II precursor in frog and human reveals intense expression of the urotensin II gene in motoneurons of the spinal cord. *Proc. Natl. Acad. Sci. USA,* **95,** 15803 – 15808.

DOUGLAS, S.A. & OHLSTEIN, E.H. (2000a). Human urotensin-II, the most potent mammalian vasoconstrictor identified to date, as a therapeutic target for the management of cardiovascular disease. *Trends Cardiovasc Med.,* **10,** 229 – 237.

DOUGLAS, S.A. & OHLSTEIN, E.H. (2000b). Urotensin receptors. In: *The IUPHAR Compendium of Receptor Characterization and Classification*. ed. Girdlestone, D. pp. 365 – 372. London: IUPHAR Media.

DOUGLAS, S.A., SULPIZIO, A.C., PIERCY, V., SARAU, H.M., AMES, R.S., AIYAR, N.V., OHLSTEIN, E.H. & WILLETTE, R.N. (2000c). Differential vasoconstrictor activity of human urotensin-II in vascular tissue isolated from the rat, mouse, dog, pig, marmoset and cynomolgus monkey. *Br. J. Pharmacol.,* **131,** 1262 – 1274.

DOUGLAS, S.A., TAYARA, L., OHLSTEIN, E.H., HALAWA, N. & GIAID, A. (2002). Congestive heart failure and expression of myocardial urotensin II. *Lancet.,* **359,** 1990 – 1997.

FLOHR, S., KURZ, M., KOSTENIS, E., BRKOVICH, A., FOURNIER, A. & KLABUNDE, T. (2002). Identification of nonpeptidic urotensin II receptor antagonists by virtual screening based on a pharmacophore model derived from structure – activity relationships and nuclear magnetic resonance studies on urotensin II. *J. Med. Chem.,* **45,** 1799 – 1805.

HEROLD, C.L., BEHM, D.J., AIYAR, N.V., NASELSKY, D.P., KNIGHT, S.D., DHANAK, D. & DOUGLAS, S.A. (2001). The somatostatin derivative, Cpa-c-[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Cpa-amide, is an agonist at the human UT receptor but not the rat UT receptor in transfected HEK293 cells. *Pharmacologist,* **43,** 189.

KINNEY, W.A., ALMOND, H.R., QI, J., SMITH, C.E., SANTULLI, R.J., DE GARAVILLA, L., ANDRADE-GORDON, P., CHO, D.S., EVERSON, A.M., FEINSTEIN, M.A., LEUNG, P.A. & MARYANOFF, B.E. (2002). Structure – function analysis of urotensin II and its use in the construction of a ligand-receptor working model. *Angew. Chem. Int. Ed.,* **41,** 2940 – 2944.

LACH, E.B., BROAD, S. & ROZENGURT, E. (1995). Mitogenic signaling by transfected neuromedin B receptors in Rat-1 cells. *Cell Growth Differ.,* **6,** 1427 – 1435.

MATSUSHITA, M., SHICHIRI, M., IMAI, T., IWASHINA, M., TANAKA, H., TAKASU, N. & HIRATA, Y. (2001). Co-expression of urotensin II and its receptor (GPR14) in human cardiovascular and renal tissues. *J. Hypertens.,* **19,** 2185 – 2190.

OHSAKO, S., ISHIDA, I., ICHIKAWA, T. & DEGUCHI, T. (1986). Cloning and sequence analysis of cDNAs encoding precursors of urotensin II-alpha and -gamma. *J. Neurosci.,* **6,** 2730 – 2735.

ORBUCH, M., TAYLOR, J.E., COY, D.H., MROZINSKI, J.E., JR., MANTEY, S.A., BATTEY, J.F., MOREAU, J.P. & JENSEN, R.T. (1993). Discovery of a novel class of neuromedin B receptor antagonists, substituted somatostatin analogues. *Mol. Pharmacol.,* **44,** 841 – 850.

PAYSANT, J., RUPIN, A., SIMONET, S., FABIANI, J.N. & VERBEUREN, T.J. (2001). Comparison of the contractile responses of human coronary bypass grafts and monkey arteries to human urotensin-II. *Fundam. Clin. Pharmacol.,* **15,** 227 – 231.

PEARSON, D., SHIVELY, J.E., CLARK, B.R., GESCHWIND, II., BARKLEY, M., NISHIOKA, R.S. & BERN, H.A. (1980). Urotensin II: a somatostatin-like peptide in the caudal neurosecretory system of fishes. *Proc. Natl. Acad. Sci. U.S.A.,* **77,** 5021 – 5024.

ROSSOWSKI, W.J., CHENG, B.L., TAYLOR, J.E., DATTA, R. & COY, D.H. (2002). Human urotensin II-induced aorta ring contractions are mediated by protein kinase C, tyrosine kinases and Rho-kinase: inhibition by somatostatin receptor antagonists. *Eur. J. Pharmacol.,* **438,** 159 – 170.

RUSSELL, F.D., MOLENAAR, P. & O'BRIEN, D.M. (2001). Cardiostimulant effects of urotensin-II in human heart *in vitro*. *Br. J. Pharmacol.,* **132,** 5 – 9.

WATANABE, T., PAKALA, R., KATAGIRI, T. & BENEDICT, C.R. (2001). Synergistic effect of urotensin II with mildly oxidized LDL on DNA synthesis in vascular smooth muscle cells. *Circulation,* **104,** 16 – 18.

ZOU, Y., NAGAI, R. & YAMAZAKI, T. (2001). Urotensin II induces hypertrophic responses in cultured cardiomyocytes from neonatal rats. *FEBS Lett.,* **508,** 57 – 60.

(Received December 2, 2002
Revised February 10, 2003
Accepted February 21, 2003)