Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical Pharmacology 69 (2005) 1069–1079
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

# Cloning and pharmacological characterization of the cat urotensin-II receptor (UT)

Nambi Aiyar [a,1,*], Douglas G. Johns [a,1], Zhaohui Ao [a], Jyoti Disa [a], David J. Behm [a], James J. Foley [b], Peter T. Buckley [b], Henry M. Sarau [b], Harjeet K van-der-Keyl [c], Nabil A. Elshourbagy [c], Stephen A. Douglas [a]

[a] *Vascular Biology and Thrombosis Department, Cardiovascular and Urogenital Center for Excellence in Drug Discovery, GlaxoSmithKline, 709 Swedeland Rd, UW2510, King of Prussia, PA 19406, USA*
[b] *Biology Department, Respiratory and Inflammation Center for Excellence in Drug Discovery, GlaxoSmithKline, King of Prussia, PA, USA*
[c] *Gene Cloning, GlaxoSmithKline, King of Prussia, PA, USA*

Received 11 October 2004; accepted 23 December 2004

**Abstract**

Urotensin-II (U-II), acting through its G-protein-coupled receptor, UT, is a possible contributor to hypertension. Variable functional responses to U-II, both within and between species studied to date, complicate the characterization of UT antagonists. In the cat, however, U-II causes systemic hypertension and constricts arterial segments isolated from several vascular beds. The purpose of this study was to clone and pharmacologically characterize cat recombinant UT to determine whether this system represents a model for characterizing UT antagonists. Cloned cat UT displayed 74% identity to primate UT, and 77% identity to rodent UT. [$^{125}$I] hU-II bound in a saturable manner to a single site on recombinant cat UT with high affinity ($K_D$ 288 ± 13 pM) and high density ($B_{max}$ 747 ± 66 fmol/mg protein). U-II isopeptides displayed equipotent, high affinity binding to cat UT ($K_i$ 1.8–5.3 nM). Cat UT was coupled to intracellular [$Ca^{2+}$] release ($EC_{50}$ 0.6 ± 0.2 nM) and total inositol phosphate (IP) formation ($EC_{50}$ 0.4 ± 0.1 nM). Protein kinase C activation desensitized cat, but not human, UT-mediated IP formation. UT mRNA expression was detected in cat blood vessels, trachea, lung, and kidney, where the medulla ($K_D$ 815 ± 34) and cortex and ($K_D$ 316 ± 39 pM) displayed high affinity binding for human U-II (hU-II). The cat urotensin-II receptor represents a suitable in vitro model to examine the role of the U-II/UT system in the etiology of hypertension, assisting in the evaluation of the UT antagonists to help treat cardiovascular disease.
© 2005 Elsevier Inc. All rights reserved.

*Keywords:* Urotensin-II; UT antagonist; GPR-14; Feline; Radioligand binding; Signal transduction

## 1. Introduction

Urotensin-II (U-II) is the most potent mammalian vasoconstrictor identified to date [1]. Acting through its G-protein-coupled receptor (GPCR), UT, U-II has been implicated as a possible contributor to essential hypertension, heart failure, and diabetes [2–8]. A growing body of literature describes U-II as a direct vasoconstrictor in isolated blood vessels in a number of species, including rat, dog, rabbit, pig, monkey, and human with a potency 10–100-fold greater than endothelin-1 [9–12]. Specifically, in hypertension, the determination of the role of U-II is complicated by reports of variability in the contractile response to U-II, both between different species as well as between the anatomical location in the vascular tree within the same species [9]. Furthermore, reports of U-II-induced vasodilation in isolated rat and human blood vessels [13–15], and highly variable responses (pressor and depressor) to U-II in vivo [16–19], add to the challenge of elucidating a role for U-II in cardiovascular disease.

In order to evaluate the role of U-II in hypertension, a suitable pre-clinical model is required in which U-II exhibits consistent vascular reactivity through a wide range of blood vessel types (conduit and resistance vessels) and in which U-II displays a reproducible effect on total peripheral resistance and blood pressure. Recently, Behm et al. reported that U-II potently and efficaciously constricted aortic, renal, femoral, carotid, superior mesenteric,

* Corresponding author. Tel.: +1 610 270 5004; fax: +1 610 270 5681.
*E-mail address:* Nambi.Aiyar@gsk.com (N. Aiyar).
[1] They have given equal contribution.

0006-2952/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2004.12.016

and mesenteric resistance arterial segments isolated from the cat [20]. In addition, U-II increased mean arterial pressure and systemic vascular resistance when administered systemically in the cat, suggesting that this species could represent an appropriate model for the examination of the role of U-II in hypertension [20].

However, it is unknown whether UT antagonists have any affinity for cat UT, a property that would be required when considering the cat as a model for the study of UT antagonism as a potential therapeutic intervention. Indeed, rat and mouse UT share 92% identity, while the human and monkey share 95% identity, though the rodent and monkey isoforms are only 74% identical [1,8]. Whether the cat UT receptor displays ''rodent UT-like'' or ''primate UT-like'' characteristics has not been determined.

The purpose of the present study was to pharmacologically characterize cat UT by: (a) identifying and cloning full-length cat UT, (b) expressing and characterizing U-II-binding and signaling properties of cat recombinant UT, and effects of UT receptor antagonists on these phenomena, (c) determining the tissue distribution of cat UT mRNA, and (d) characterizing the binding properties of native cat UT. Such characterization of the cat U-II receptor will greatly add to the utility of the cat as a model to examine the role of the U-II/UT system in the etiology of hypertension, and will assist in the evaluation of the UT receptor as a potential site of therapeutic intervention in cardiovascular disease.

## 2. Methods

### 2.1. Cloning of cat UT

Degenerate primers were designed based on the conserved regions of the coding sequence of other known UT orthologs [1,21]. Since the UT receptor is an intronless gene in other species [22], cat genomic DNA was used as a substrate for polymerase chain reaction (PCR) amplification. The conditions for PCR were as follows: denaturing step of 35 cycles for 30 s each, followed by annealing and extension at 68 °C for 3 min using the Advantage GC-Polymerase enzyme system (Clontech, Palo Alto, CA). Several overlapping PCR fragments were identified to generate a 689 bp partial sequence characteristic of the UT receptor. 3′ and 5′ SMART libraries (Clontech) were prepared from cat heart RNA. Full-length sequence was determined by rapid amplification of cDNA ends (RACE) reactions using the SMART technology (Clontech). Full-length amplified DNA was cloned into pCDN3.1D (Invitrogen, Carlsbad, CA) vector. Sequence identity was determined using Lasergene software (DNAStar, Inc., Madison, WI). The optimal alignment of the deduced amino acid sequences of the mouse and monkey UT receptor were compared to the rat and human UT receptor using the Wisconsin program obtained from Devereux et al. [23]. The cat UT receptor was assigned the GenBank accession number AY787788.

### 2.2. Expression of cat UT in HEK-293 cells

HEK-293 cells were cultured in EMEM/Earle's salts, 2.2 g/l sodium bicarbonate, L-glutatmine, and non-essential amino acids with 10% fetal bovine serum (JRH Bioscience, Lenexa, KS). For transient transfection, HEK-293 cells were seeded in a six-well dish at a concentration of $3 \times 10^5$ cells/well in complete media. pCDN 3.1D cat UT was transfected into HEK-293 cells using lipofectamine reagent (Gibco/Life Technologies, Gaithersburg, MD) at a ratio of 1 μg plasmid DNA to 5 μl lipofectamine per well. After 48 h, cells were trypsinized and resuspended in complete media containing 0.4 mg/ml G-418. G-418 resistant clones were selected and tested for [$^{125}$I] hU-II binding. Fifteen HEK 293/cat UT receptor clonal cell lines were assessed under saturation conditions to determine radioligand binding density ($B_{max}$) and affinity ($K_D$).

### 2.3. HEK-293 cell membrane preparation

Cells were harvested by scraping in phosphate buffered saline (PBS), washing with PBS, and pelleting by centrifugation at 500 × *g* for 10 min. Cells were lysed by sonication in a buffer containing 10 mM Tris pH 7.5, 10 μM EDTA, 0.25 mg/ml bacitracin, and 0.2 mM phenylmethyl sulfonyl fluoride. The homogenates were centrifuged at 47,000 × *g* for 20 min at 4 °C, and the membrane pellets were washed twice by centrifugation in buffer containing 20 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 2 mM Na–EGTA, 0.1 mg/ml bacitracin (buffer B). The membranes were resuspended in the same buffer at (5 mg/ml) and stored at −70 °C. Protein concentrations were measured using the bicinchonic acid method (Pierce Biotechnology, Rockford, IL), with bovine serum albumin as the standard.

### 2.4. Radioligand binding studies (cultured cells)

[$^{125}$I] hU-II binding assays were performed on UT-transfected HEK-293 cell membrane preparations using a scintillation proximity assay (SPA). Binding assays were performed with [$^{125}$I] hU-II (20–600 pM for saturation binding and 300 pM for competition binding) in the presence or absence of unlabeled ligand in assay buffer (20 mM Tris–HCl [pH 7.4], 5 mM $MgCl_2$ and 0.05% BSA) and cell membranes pre-coupled to wheat germ agglutinin-SPA (WGA-SPA) beads (Amersham, Arlington Heights, IL) at a concentration of 5–10 μg membrane protein and 0.5 mg of WGA-SPA beads. The total assay volume was 150 μl in each well of a 96-well plate. For competition binding, 10 concentrations (1 pM to 1 μM) of unlabeled ligand (human full-length U-II and hU-II [4–11] fragment, goby-, rat-, and mouse-U-II, both forms of porcine U-II, and urotensin-related peptide [URP]) or

antagonists were used for displacement of labeled hU-II. Assay plates were sealed, shaken gently for 45 min at room temperature, and centrifuged at $2000 \times g$ for 10 min before counting in a Top Count Scintillation Counter (Perkin Elmer, Wellesley, MA). Binding data were analyzed by nonlinear regression (GraphPad Software, San Diego, CA) to calculate $B_{max}$, $K_D$, $K_i$ and Hill coefficient.

### 2.5. Inositol phosphate formation

Confluent HEK-293 cells expressing cat recombinant UT were pre-treated for 20 h with [$^3$H]myoinositol (0.25 μCi/well) in 24-well cluster dishes in inositol-free Dulbecco's modified Eagle's medium. Following pre-treatment, cells were washed twice with PBS, resuspended in PBS containing 10 mM LiCl, and incubated for 10 min at 37 °C. Cells were then incubated with different concentrations of hU-II for 20 min at 37 °C. The reaction was terminated by the addition of 0.1% tricholoroacetic acid (TCA). Total inositol phosphate was isolated by flowing lysates over an ion-exchange column (Dowex AG1-X8 [200–400 mesh, formate form] pre-washed with 20 mM ammonium formate). [$^3$H]inositol phosphates were eluted with 1 M ammonium formate/0.1 M formic acid solution and radioactivity was determined by liquid scintillation counting. In some experiments, cells were pre-treated with 10 μM phorbol dibutyrate (PDBu), a protein kinase C activator, or 10 μM α-phorbol didecanoate (αPDD), an inactive phorbol ester isomer, for 10 min prior to stimulation with hU-II.

### 2.6. hU-II-mediated calcium mobilization

HEK-293 cells stably expressing cat UT were seeded (50,000 cells/well) into poly-D-lysine coated 96-well black-walled, clear bottom microtitre plates (Biocoat plates from Becton Dickinson Labware, Bedford, MA) 24 h prior to assay. Cells were pre-treated for 1 h with 1 μM Fluo-4-AM fluorescent indicator dye (Molecular Probes, Eugene, OR) in Hanks Balanced Salts Solution (HBSS) containing 10 mM HEPES, 200 μM $Ca^{2+}$, 0.1% BSA, and 2.5 mM probenecid. Cells were washed three times with HBSS, then returned to the incubator for 10 min before measurement on a fluorometric imaging plate reader (FLIPR, Molecular Devices, Sunnyvale, CA). Maximum change in fluorescence over baseline was determined in response to U-II isopeptides.

### 2.7. Tissue distribution of cat UT mRNA

All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and were approved by the GlaxoSmithKline Animal Care and Use Committee. Tissues (lung, kidneys, spleen, brain, aorta, vena cava, heart, esophagus, stomach, small intestine, colon, liver, skeletal muscle, bladder, adipose) were harvested from healthy, adult male cats (4–5 kg; 12–24 months old; Liberty Research Inc., Waverly, NY) following euthanasia via sodium pentobarbital overdose. Tissues were flash frozen in liquid $N_2$ and stored at −80 °C prior to processing. Tissue was homogenized in liquid $N_2$ using a mortar and pestle and total RNA extracted using RNAzol, according to manufacturer's instructions (GIBCO BRL, Gaithersburg, MD).

RT-PCR was performed using DNase-I-treated cat total RNA. cDNA was generated using oligo ($dT_{16}$)-primed MultiScribe reverse transcriptase (TaqMan, Pekin-Elmer, Branchburg, NJ). The resultant samples were subjected to TaqMan quantitative real time PCR as described [24] using the following primer/probe sequences (5′ to 3′): forward CCG AGT TCT CTT CAG; reverse TGC TCA TGA CGG TCA; TaqMan probe TTC CTG ACC ATG CAC GCC (with FAM and TAMRA as the reporter and quencher dyes, respectively). Primers were validated using a plasmid containing a cDNA for cat UT, and via sequencing of the amplicon which showed 100% identity with its respective region in cat UT. β-Actin was used as a housekeeper control for normalization of UT copy number. PCR reactions for each tissue were performed in triplicate for $n = 4$ cats. The PCR data presented represent the average of a single triplicate PCR experiment for $n = 4$ cats. Experiments were performed three times and showed similar results.

### 2.8. Preparation of tissue membranes for binding

The portion of frozen tissues not used for RNA isolation were homogenized in 10 mM Tris–HCl containing 1 mM EDTA (pH 7.4) and centrifuged at $1000 \times g$ for 10 min. The supernatant was centrifuged at $47{,}000 \times g$ for 20 min and the resulting pellets were resuspended in 25 mM Tri-HCl containing 5 mM $MgCl_2$ (pH 7.4). The washing procedure was repeated twice, and the final pellets were resuspended and stored in small aliquots at −80 °C until use. The protein concentration was measured using the Pierce BCA method with bovine serum albumin as the standard.

### 2.9. Binding of [$^{125}$I] hU-II to cat tissue membrane preparations

Specific binding of radiolabeled hU-II was determined using 200 pM [$^{125}$I] hU-II (for all tissues studied). Membranes (50–70 μg protein) were incubated for 60 min at 25 °C in 200 μl buffer [25 mM Tri-HCl, pH7.4, 5 mM $MgCl_2$, 0.20 mg/ml bacitracin and 0.1% BSA] containing 200 pM [$^{125}$I] hU-II in the absence or presence of 1 μM unlabeled hU-II. The assay was carried out using 12 mm × 75 mm polystyrene tubes. The incubation was terminated by the addition of 2 ml of ice-cold wash buffer (0.9% NaCl) followed by rapid filtration over Whatman GF/C filters (Clifton, NJ) using a cell harvester (Brandel Research and Development Laboratories, Gaithersburg, MD). The filters were washed three times with 4 ml of

ice-cold wash buffer. Non-specific binding was determined in the presence of 1 μM unlabeled hU-II and filter papers were counted in a gamma counter (Packard Instrument Company, Meriden, CT). For saturation binding studies (renal cortex and medulla), increasing concentrations of [$^{125}$I] hU-II were added to membranes (30–50 μg of membrane protein per ml) in a total volume of 200 μl and incubated for 60 min at 25 °C. In competition binding studies (renal cortex and medulla), membranes (30–50μg of membrane protein per ml) were incubated with increasing concentrations (1 pM–1 μM) of competing ligand and 150–200 pM of [$^{125}$I] hU-II for 60 min at 25 °C.

### 2.10. Reagents

BIM-23127 and BIM-23042 [25,26] were purchased from Bachem Bioscience (King of Prussia, PA). hU-II, SB-710411 (Cpa-*cyclo*-[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Cpa-amide; 4-chlorophenylalanine (Cpa); 3-pyridylalanine (Pal)) [27] and GSK248451 (4-Cl-(*trans*)-cinnamoyl-c[D-Cys-4-Pal-D-Trp-Orn-Val-Cys]-His-amide; 4-pyridylalanine (4-Pal)) [28] were synthesized by California Peptide Research Inc. (Napa, CA). Goby, rat and mouse U-II isoforms were purchased from Phoenix Pharmaceuticals Inc. (Mountain View, CA). Porcine U-IIA and -B isoforms were synthesized at GlaxoSmithKline (King of Prussia, PA). Monoiodinated hU-II ([$^{125}$I]- Tyr$^{9}$, specific activity 2000 Ci/mmol) was custom synthesized by Amersham (Arlington Heights, IL). All other reagents were from Sigma Chemical Co. (St. Louis, MO).

## 3. Results

### 3.1. Sequence analysis of cat UT

Sequence analysis of the cat UT gene (GenBank accession number AY787788) revealed an open reading frame of 1125 nucleotides. Fig. 1 describes the amino acid sequence identity of cat UT compared to human, monkey, rat and mouse sequences. Full-length cat UT was determined to be 374 amino acids in length. In the cat UT sequence, a 10 amino acid deletion near the amino terminus, (between and including amino acids 13–22) distinguished it from the human, monkey, mouse, and rat orthologs, while the seven-transmembrane domains typical of GPCRs were conserved (Fig. 2A). Similar to the rodent receptor orthologs, in the C-terminal region cat UT contained a caveolin-binding domain [29] and a larger number of potential phosphorylation sites compared to the human and monkey isoforms (Fig. 2B). Cat UT shares 74% overall homology to the human and monkey orthologs, and 77% overall homology to the mouse and rat receptors.

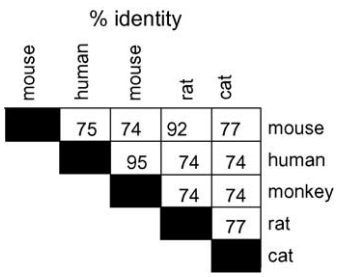


Fig. 1. Sequence identity among various species isoforms of UT. Sequence identity between cat UT and the human, monkey, rat, and mouse isoforms was determined as described in Section 2.1.

### 3.2. U-II binding properties to recombinant cat UT-receptor

Initial characterization of [$^{125}$I] hU-II binding to membranes from UT-transfected HEK-293 cells indicated that specific binding at 125 pM was >95% of total binding as defined using unlabeled hU-II (1 μM). [$^{125}$I] hU-II bound to membranes from HEK-293 cells containing cat UT receptor in a specific and saturable manner. Saturation binding analysis of [$^{125}$I] hU-II binding (Fig. 3) revealed a single set of sites with $K_D$ 288 ± 13 pM and $B_{max}$ 747 ± 66 fmol/mg protein. No such binding was detected on membranes prepared from HEK-293 cells transfected with empty vector DNA.

The specificity of U-II binding to the recombinant cat UT receptor was assessed by examining the ability of various U-II isoforms (human, hU-II [4–11] fragment, goby, rat, mouse, porcine A and B U-II isoforms, and urotensin-related peptide [URP]) to compete with [$^{125}$I] hU-II for membrane binding (Fig. 4). Competition binding revealed that all isoforms of U-II displaced the radioligand with comparable $K_i$s in the range of 0.9–7.4 nM (Fig. 4, Table 1). In all cases, the peptides displayed monophasic competition curves with Hill coefficients which were not significantly different from unity. The UT antagonists BIM-23127, BIM-23042 (neuromedin B analogs [25,26]), SB-710411 and GSK248451 (somatostatin analogs [27,28]) concentration-dependently inhibited [$^{125}$I] hU-II binding to HEK-293 cells transfected with cat UT with GSK248451 showing the greatest potency with a $K_i$ value of 1.0 ± 0.3 nM compared with BIM-23127, BIM-23042, and SB-710411, which had $K_i$ values ranging from 31 to 114 nM (Fig. 5, Table 1).

### 3.3. Phosphoinositide/calcium signaling and desensitization

The activation of UT-mediated signal transduction pathways was verified using HEK-293 cells expressing cat recombinant UT. U-II isopeptides stimulated phosphoinositide metabolism in a concentration-dependent manner with an $EC_{50}$ 0.4 ± 0.1 nM (Fig. 6A). No U-II-induced phosphoinositide hydrolysis was observed in cells transfected with the vector DNA alone. hU-II also increased intracellular $Ca^{2+}$ in a concentration-dependent manner

(A)

```
   UT receptor_cat  MALSPAPLSG FP-------- --EPSAAFNA SLNRSWASPT EPSSLEDLVA   40
 UT receptor_human  MALTPESPSS FPGLAATGSS VPEPPGGFNA TLNSSWASPT EPSSLEDLVA   50
UT receptor_monkey  MALTPESPSS FPGLAAIGSS VPEPPGSFNA TLNSSWASPT EPSSLEDLVA   50
 UT receptor_mouse  MALSLEST-S FPMLAVSRST ASELPGGFNV SHNSSWTGPT DPSSLQDLVA   49
   UT receptor_rat  MALSLESTTS FHMLTVSGST VTELPGDSNV SLNSSWSGPT DPSSLKDLVA   50

   UT receptor_cat  TGAIGAVLSA MGVVGVAGNA YTLVVMCRVL HTSASMSVYV VNLALADLLY   90
 UT receptor_human  TGTIGTLLSA MGVVGVVGNA YTLVVTCRSL RAVASMYVYV VNLALADLLY  100
UT receptor_monkey  TGAIGTLLSA MGVVGVVGNA YTLVVTCRSL RAVASMYIYV VNLALADLLY  100
 UT receptor_mouse  TGVIGAVLST MGVVGVVGNV YTLVVMCRFL RASASMYVYV VNLALADLLY   99
   UT receptor_rat  TGVIGAVLSA MGVVGMVGNV YTLVVMCRFL RASASMYVYV VNLALADLLY  100
                              1                                  2

   UT receptor_cat  LLSIPFIVAT YVTKEWHFGD VGCRVLFSLD FLTMHASIFT LTVMSSERYA  140
 UT receptor_human  LLSIPFIVAT YVTKEWHFGD VGCRVLFGLD FLTMHASIFT LTVMSSERYA  150
UT receptor_monkey  LLSIPFIVAT YITKEWHFGD VGCRVLFSLD FLTMHASIFT LTVMSSERYA  150
 UT receptor_mouse  LLSIPFIVAT YVTKDWHFGD VGCRVLFSLD FLTMHASIFT LTIMSSERYA  149
   UT receptor_rat  LLSIPFIIAT YVTKDWHFGD VGCRVLFSLD FLTMHASIFT LTIMSSERYA  150
                                          3

   UT receptor_cat  AVLRPLDTVQ RSKGYRKVLA LGTWLLALLL ALPMMLAIRL VHRGHKSLCL  190
 UT receptor_human  AVLRPLDTVQ RPKGYRKLLA LGTWLLALLL TLPVMLAMRL VRRGPKSLCL  200
UT receptor_monkey  AVLRPLDTVQ RPKGYRKLLA LGTWLLALLL TLPVMLAMRL VRRGPKSLCL  200
 UT receptor_mouse  AVLRPLDTVQ RSKGYRKLLA LGTWLLALLL TLPMMLAIRL VRRGSKSLCL  199
   UT receptor_rat  AVLRPLDTVQ RSKGYRKLLV LGTWLLALLL TLPMMLAIQL VRRGSKSLCL  200
                                          4

   UT receptor_cat  PVWGPRAHRA YLTLLFGTSI VGPGTVIGLL YVRLARAYWL SQRASFTQTR  240
 UT receptor_human  PAWGPRAHRA YLTLLFATSI AGPGLLIGLL YARLARAYRR SQRASFKRAR  250
UT receptor_monkey  PAWGPRAHRA YLTLLFATSI AGPGLLIGLL YARLARAYRR SQRASFKRAR  250
 UT receptor_mouse  PAWGPRAHRT YLTLLFGTSI VGPGLVIGLL YIRLARAYWL SQQASFKQTR  249
   UT receptor_rat  PAWGPRAHRT YLTLLFGTSI VGPGLVIGLL YVRLARAYWL SQQASFKQTR  250
                                          5

   UT receptor_cat  RLPNPKVLYL ILGIVLLFWA CFLPFWLWQL LAQYRGAQTL TPRTARIVNY  290
 UT receptor_human  R-PGARALRL VLGIVLLFWA CFLPFWLWQL LAQYHQA-PL APRTARIVNY  298
UT receptor_monkey  R-PGARALRL VLGIVLLFWA CFLPFWLWQL LAQYREA-PL APRTARIVNY  298
 UT receptor_mouse  RLPNPRVLYL ILGIVLLFWA CFLPFWLWQL LAQYHQAMPL TPETARIINY  299
   UT receptor_rat  RLPNPRVLYL ILGIVLLFWA CFLPFWLWQL LAQYHEAMPL TPETARIVNY  300
                                          6

   UT receptor_cat  LTTCLTYGNS CVNPFLYTLL TKNYREY--- RRRSLRARSA RGPAGARHSL  337
 UT receptor_human  LTTCLTYGNS CANPFLYTLL TRNYRDHLRG R---VRGPGS GGGRGPVPSL  345
UT receptor_monkey  LTTCLTYGNS CANPFLYTLL TRNYRDHLRG R---VRSPGS GGVRGPVPSL  345
 UT receptor_mouse  LTACLTYGNS CINPFLYTLL TKNYREYLRG RQRSL-GSSC RGPGSAGSFL  348
   UT receptor_rat  LTTCLTYGNS CINPFLYTLL TKNYREYLRG RQRSL-GSSC HSPGSPGSFL  349
                              7

   UT receptor_cat  PCRVRFQRGS GHSLCSSSQQ ATETITLSPA ASRAVCA               375
 UT receptor_human  QPRARFQRCS GRSLSSCSPQ PTDSLVLAPA APARPAPEGPRAPA        390
UT receptor_monkey  QPRARFQRGS GRSLSSCSPQ PTESLVLAAA APAGPALESPGDPA        390
 UT receptor_mouse  SSRVHLQQDS GRSLSSNSQQ ATETLVLSPV PPNGAFV               386
   UT receptor_rat  PSRVHLQQDS GRSLSSSSQQ ATETLMLSPV PRNGALL               387
```

(B)

```
Human   YTLL TRNYRDHLRG R - - - VRGPGS GGGRGPVPSL QPRARFQRCS
Cat     YTLL TKNYREY - - -  RRRSLRARSA RGPAGARHSL PCRVRFQRGS   347
        caveolin binding motif

Human   GRSLSSCSPQ PTDSLVLAPA APARPAPEGPRAPA-COOH
Cat     GHSLCSSSQQ ATETITLSPA  ASRAVCA-COOH
```

Fig. 2. Amino acid sequences alignments of cat, human, monkey, mouse, and rat UT. (A) Deduced amino acid residues are indicated beginning with the initiation methionine. The regions identifying the positive transmembrane as domains 1–7 are underlined and numbered sequentially. The potential *N*-glycosolation site (black boxes), and the characteristic G-protein-coupled receptor conserved cysteine, D/ERY, and $NPx_{2-3}Y$ motifs (red boxes) are indicated. (B) Comparison of C-terminal regions of cat and human UT, indicating the caveolin binding site (underlined) of cat UT, and putative phosphorylation sites (red). The optimal alignment of the deduced amino acid sequences UT receptor orthologs was determined as described in Section 2.1.

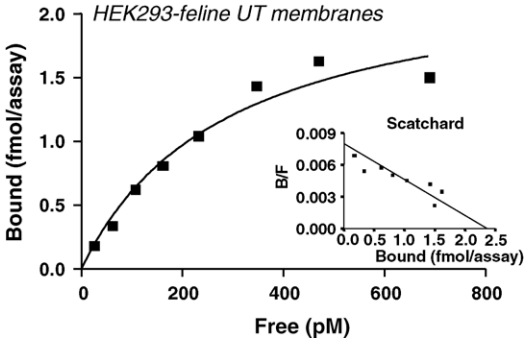


Fig. 3. Saturation binding of [$^{125}$I] hU-II to membranes of HEK-293 cells expressing cat UT. *Inset:* Scatchard plot of the specific binding. Points shown are from representative experiments performed in duplicate and repeated three times.

Table 1
$K_i$s of U-II isopeptides and antagonists for cat recombinant UT

| Competing ligand | $K_i$ (nM) |
|---|---|
| Human U-II | 0.9 ± 0.2 |
| Goby U-II | 1.3 ± 0.3 |
| Rat U-II | 2.9 ± 0.7 |
| Mouse U-II | 7.4 ± 3.1 |
| Porcine U-IIA | 5.9 ± 1.3 |
| Porcine U-IIB | 4.8 ± 1.4 |
| Human U-II [4–11] | 1.1 ± 0.3 |
| URP | 1.6 ± 0.1 |
| BIM-23042 | 224.1 ± 44.3 |
| BIM-23127 | 71.3 ± 20.5 |
| SB-710411 | 31.5 ± 5.5 |
| GSK248451 | 1.0 ± 0.3 |

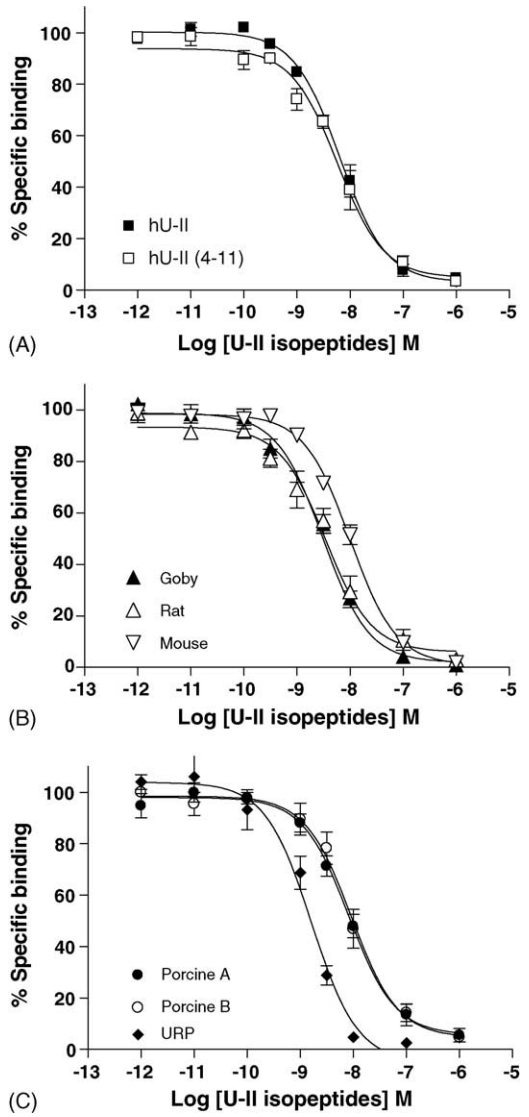


Fig. 4. Competition binding of U-II isoforms at recombinant cat UT. Determination of specific binding was performed as described in Section 2 in HEK-293 cells expressing cat UT for: (A) hU-II and [4–11] fragment, (B) goby, rat, and mouse U-II, and (C) porcine U-IIA, U-IIB, and urotensin-related peptide (URP). Data represent means ± S.E.M. of three independent experiments performed in duplicate.

(EC$_{50}$ of 0.6 ± 0.2 nM, Fig. 6B and C). SB-710411 (1 μM) and GSK248451 (100 nM) produced a parallel-rightward shift in the concentration-response curve for hU-II (p$K_b$ 79.4 ± 0.8 and 2.0 ± 0.5 nM, respectively, Fig. 6A). SB-710411 by itself displayed weak agonistic properties (30 ± 5% over basal at 10 μM), whereas GSK248451 alone had no effect.

Pretreatment of cat UT-expressing HEK-293 cells with 1 μM PDBu for 10 min inhibited U-II-mediated phosphoinositide accumulation (Fig. 7A). Up to 20 min pre-treatment with 10 μM αPDD, a phorbol ester that does not activate protein kinase C, had no effect on U-II-stimulated phosphoinositide accumulation. PDBu pre-treatment did not inhibit basal phosphoinositide levels. In contrast to the data obtained using cat UT, PDBu had no effect on U-II-induced IP accumulation in human UT-expressing HEK-293 cells (Fig. 7B).

[$^{125}$I] hU-II binding analysis of equilibrium competition by unlabeled hU-II revealed that 1 μM PDBu treatment of cat UT-expressing HEK-293 cells did not affect the affinity or number of U-II binding sites ($K_D$ 7.9 ± 1.2 and 5.9 ± 1.1 nM, and $B_{max}$ 871 ± 105 and 792 ± 95 fmol/mg protein in vehicle and PDBu-treated cells, respectively).

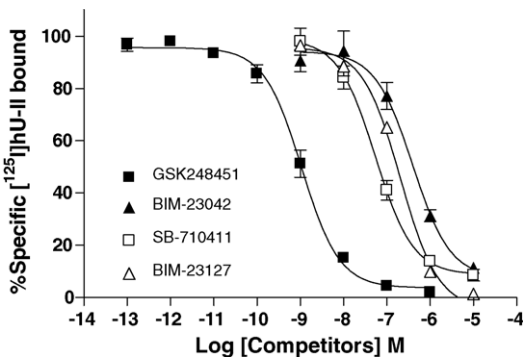


Fig. 5. Effect of UT receptor antagonists on hU-II binding to recombinant cat UT. Representative competition curves for BIM-23042, BIM-23127 and SB-710411 on membrane preparations of HEK-293 cells expressing cat UT with [$^{125}$I] hU-II as radioligand were generated as described in Section 2. Data represent means ± S.E.M. of three independent experiments performed in duplicate.

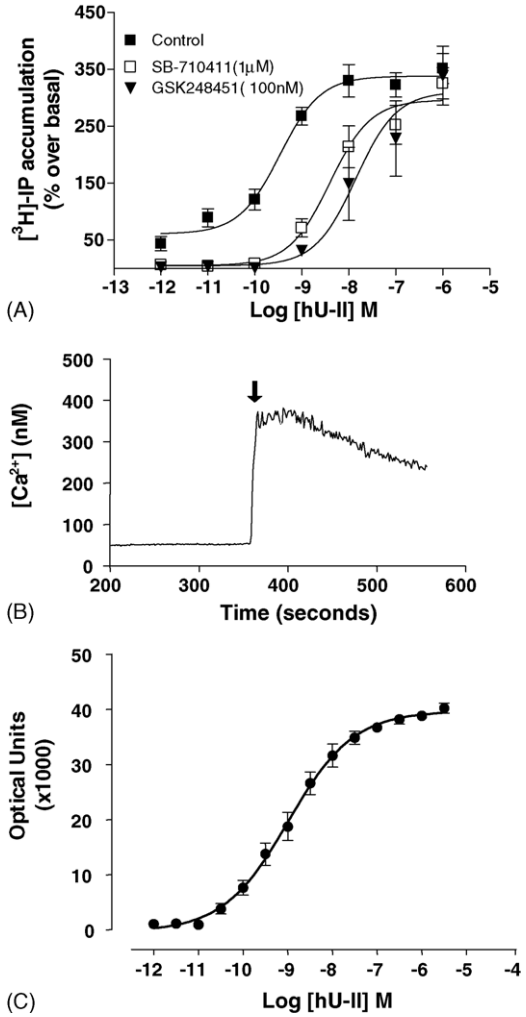


Fig. 6. Signal transduction properties of cat UT. (A) Inositol phosphate (IP) accumulation in response to hU-II was performed in HEK-293 cells expressing cat UT as described in Section 2. The basal accumulation of [$^3$H]IPs was 2582 ± 204 dpm (B) calcium tracing depicting intracellular calcium mobilization in response to 1 μM hU-II (arrow), (C) concentration response curve to hU-II showing that calcium response shows similar potency as IP accumulation. Data represent means ± S.E.M. of three independent experiments performed in duplicate.

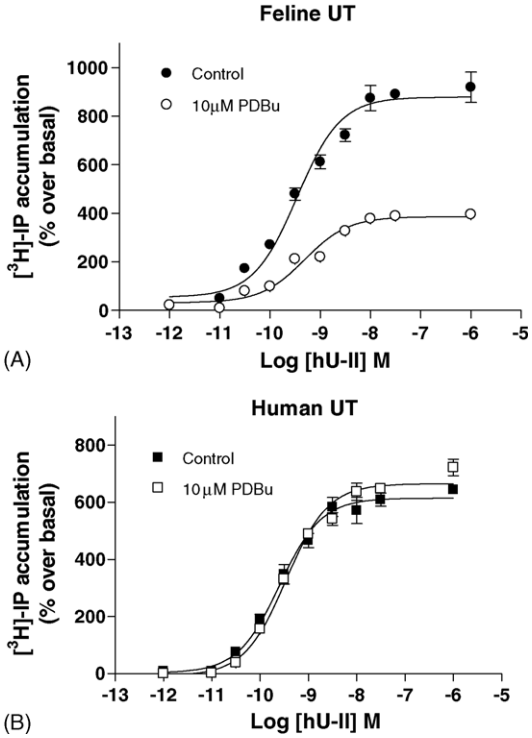


Fig. 7. Phorbol ester-induced desensitization of cat UT. Inositol phosphate accumulation in response to hU-II in the presence (open symbols) and absence (filled symbols) of 10 μM phorbol dibutyrate (PDBu), showing a lack of desensitization in (A) cat UT expressing cells, and a significant reduction in maximal IP response in (B) human UT expressing cells. Data represent means ± S.E.M. of three independent experiments performed in duplicate.

Similarly, no change in $K_D$ or $B_{max}$ was seen in PDBu-treated HEK-293 cells expressing human UT compared to vehicle treatment.

### *3.4. Tissue distribution and pharmacological characterization of native cat UT*

The tissue distribution of cat UT mRNA was determined using TaqMan/quantitative PCR. UT mRNA expression was detected in all tissues studied, with the highest copy levels seen in the aorta, lung, and spleen (Fig. 8).

To determine binding characteristics of native cat UT, tissues with higher mRNA expression levels (lung, spleen, aorta) were isolated and [$^{125}$I] hU-II specific binding was determined in the presence of 1 μM unlabeled U-II. Because rat kidney displays high specific binding of U-II (N. Aiyar, unpublished observation), the cat kidney was also isolated for determination of specific binding of U-II. Of the tissues examined, the intact kidney showed the highest level of specific binding (Fig. 9A). Therefore, kidney medulla and cortex were prepared for more complete pharmacological characterization of cat UT. Saturation studies using [$^{125}$I] hU-II revealed a single binding site in membranes from both medulla and cortex (Table 2, Fig. 9B and C). In kidney cortex and medulla, hU-II displaced [$^{125}$I] hU-II with similar $K_i$ values (Fig. 9D, Table 2).

## 4. Discussion

Identification of a suitable pre-clinical species for the study of U-II is critical to evaluating the potential of the U-II/UT system to be a novel site of therapeutic intervention. A recent study by Behm et al. [20] describes potent and efficacious constrictor responses to U-II in a broad spectrum of blood vessel types isolated from the cat, as well as marked pressor responses to U-II in vivo, suggesting that the cat may represent an ideal model for evaluating the U-II/UT system in cardiovascular diseases such as hypertension. Currently, it is not possible to fully appreciate the utility of available UT antagonists, such as

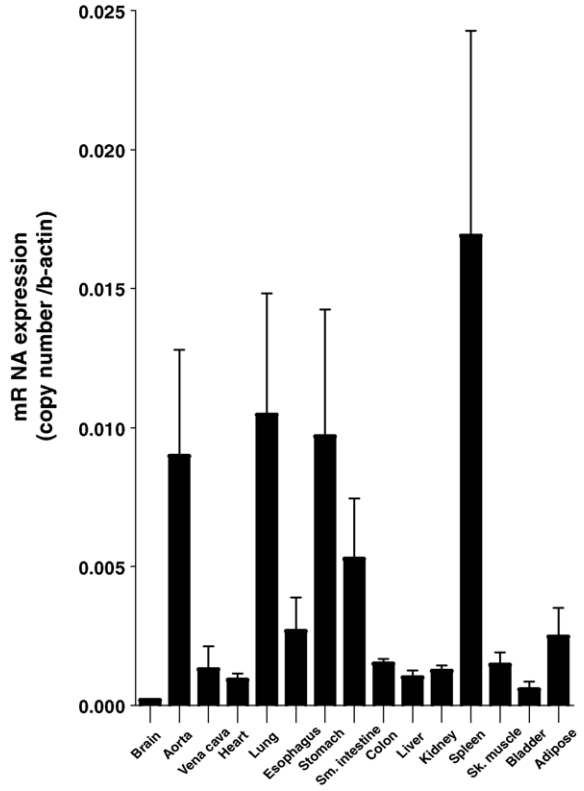


Fig. 8. Expression of UT mRNA in cat tissues. Quantitative RT-PCR was performed on RNA isolated from various cat tissues as described in Section 2. Abbreviations: Sm, small; Sk, skeletal.

SB-710411, given the fact that it functions as a rodent UT antagonist [27] and primate agonist [30]. As such, cloning and full pharmacological characterization of cat UT is required in order to determine whether this species can serve as a tool to evaluate UT antagonists.

The current study is the first report of the cloning of cat UT. As is the case with the human, monkey, rat, and mouse UT receptor [1,8], the cat UT receptor belongs to the G-protein-coupled receptor (GPCR) superfamily (type I or class A rhodopsin-like), due to the presence of seven hydrophobic domains whose sequence was similar to putative transmembrane (TM) domains, and because of the presence of a DERY motif at the interface of transmembrane domain 3 and intracellular loop 2 [1,8]. Studies that pharmacologically characterized human recombinant UT reported that [$^{125}$I] hU-II binds to a single class of high affinity sites in a saturable manner ($K_D$ 430 ± 10 pM and $B_{max}$ of 447 ± 58 fmol/mg protein), resulting in activation of phospholipase C, protein kinase C, and calcium signaling [1].

Further supporting this classification, cat UT was found to possess two potential N-linked glycosylation sites in the N-terminal extracellular domain, a palmitoylation site ($NPx_{2-3}Y$) in the seventh TM motif (TM-7), as well as conserved cysteine residues in the first and second extracellular loop (Fig. 2C). The alignment of the cat UT amino

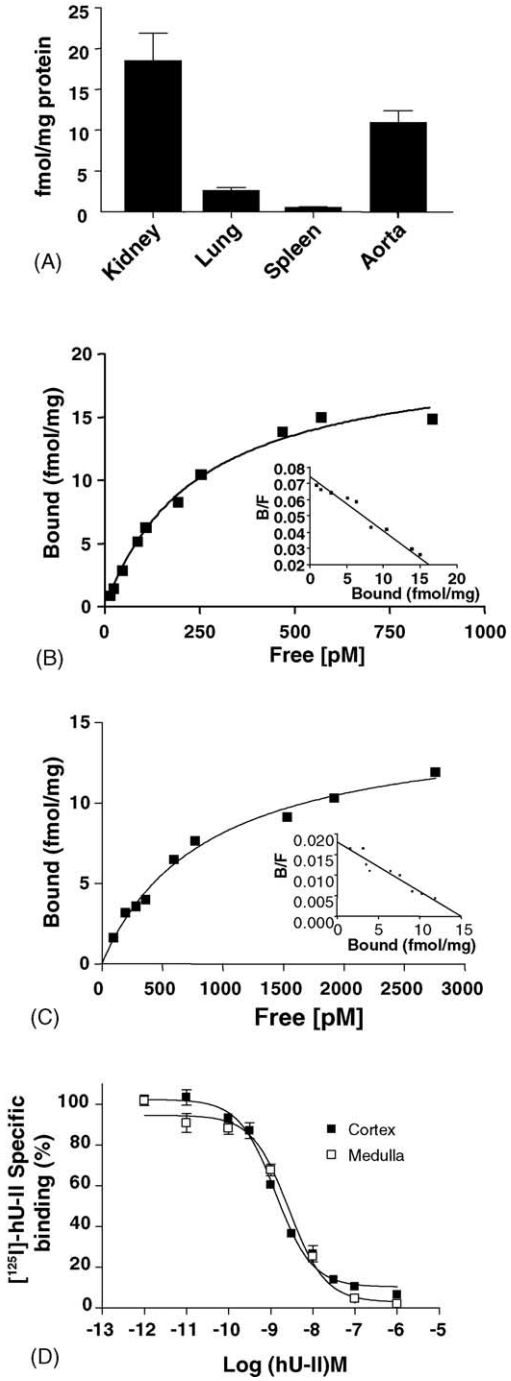


Fig. 9. Binding properties of native cat UT. (A) Specific binding in membrane preparations of cat kidney, lung, spleen, and aorta was performed as described in Section 2. Binding in fmol per mg of protein is the concentration of binding sites measured at a single ligand concentration (200 pM [$^{125}$I] hU-II). Saturation binding was performed in kidney (B) cortex, and (C) medulla. Data represent means ± S.E.M. of duplicate replicates of $n = 4$ cats. (D) Displacement of radiolabeled hU-II by unlabeled hU-II in kidney cortex and medulla.

Table 2
Binding properties of hU-II in cat kidney

| Kidney region | $K_D$ (pM) | $B_{max}$ (fmol/mg) | $K_i$ (nM) for hU-II |
|---|---|---|---|
| Cortex | 316 ± 39 | 21 ± 2 | 0.6 ± 0.1 |
| Medulla | 815 ± 34 | 13 ± 1 | 1.8 ± .2 |

acid sequence with those of other species indicated that cat UT is most homologous to rodent UT (76% identical to the rat sequence) and more divergent from that of monkey and human evidenced by the C-terminal end of cytoplasmic loop 3 (positions 248–258 of the human sequence) where a clear separation into the two groups (primate [human/monkey] versus cat/rodent) is seen. Another striking difference between cat and human UT is the number and position of C-terminal serine/threonine residues located at regions thought to harbor protein kinase A and protein kinase C consensus sequences [29]. This raises the possibility that phosphorylation-dependent regulation of cat/rodent UT receptor may be different than the human/monkey homolog (see below).

Saturation binding studies with recombinant cat UT using the radioligand [$^{125}$I] hU-II indicated that hU-II binds to a single, high affinity site, with a $K_D$ (288 pM) similar to that seen with the human receptor and other orthologs [1,8]. Similarly, the binding profiles of various U-II isoforms (human, human [4–11] fragment, rat, mouse, goby, porcine A and B) to recombinant cat UT are similar to those reported for recombinant human, monkey and mouse UT [1,8]. Furthermore, the $K_i$ values generated against cat UT for the peptidic UT antagonists (BIM-23127, BIM-23042, SB-710411, and GSK248451) are not dissimilar from those reported by others against the human receptor [26,31] The stimulation of phosphoinositide hydrolysis and $Ca^{2+}$ mobilization in cat UT-expressing cells by U-II verified expression of a functional receptor in HEK-293 cells. In addition, the potencies of the phosphoinositide and $Ca^{2+}$ responses (0.4 nM and 0.6 nM, respectively) are in close agreement to those reported for the human and primate receptor (~1 nM) [1,8]. Taken together, these data suggest that the sequence differences between cat and other species isoforms of UT do not result in differences in binding characteristics or the initiation of signal transduction, thereby making cat UT a prime candidate for evaluation of novel UT antagonists pre-clinically.

Because the somatostatin analogs SB-710411 and GSK248451 have been shown to block hU-II-induced contractions in rat thoracic aorta, these compounds were tested for their ability to inhibit inositol phosphate hydrolysis in cat UT expressing HEK-293 cells. Somatostatin analogs (SB-710411 and GSK248451) are relatively potent UT receptor ligands that do not harbor the hexapeptide cyclic core sequence, Cys-Phe-Trp-Lys-Tyr-Cys, which is retained in all U-II species isoforms identified to date and thought to be crucial for U-II bioactivity [28,31,32] The somatostatin analog, RC-160, which contains a hexapeptide cyclic sequence similar to that of SB-710411, has been reported to exhibit agonist activity at the rat UT receptor [33]. Thus, particular motifs exist that impart UT receptor agonist activity to certain somatostatin analogs and may form the basis for designing additional UT receptor ligands. Studies have shown that SB-710411 inhibits [$^{125}$I] hU-II binding to rat and monkey UT receptor with p$K_i$s of 7.5 ± 0.1 and 6.8 ± 0.1 [30]. However, its functional actions at these two UT orthologs differ radically. SB-710411 antagonized hU-II-induced inositol phosphate formation at rat UT (p$K_b$ 6.5 ± 0.1), whereas this ligand functioned as an agonist at monkey UT (p$EC_{50}$ 6.6 ± 0.4; $R_{max}$ 5.3 ± 0.7-fold over basal) [34]. In the present study using cat UT, this ligand displayed weak partial agonist activity, stimulating inositol phosphate formation only at high (>1 uM) concentrations, but antagonizing the effects of U-II stimulation. Similarly, GSK248451, purported to be the most potent peptidic UT antagonist [28], inhibited inositol phosphate formation with a p$K_b$ of 8.7 ± 0.3, but had no intrinsic agonist activity alone. The species-dependent differences in ligand activity at UT (agonist versus antagonist) imply that one cannot infer the activity of an antagonist characterized in one assay system to that characterized in a different system. For example, while the cat appears to be an appropriate model of U-II-dependent hypertension [20], in order to determine whether a ligand that is a UT antagonist will prevent U-II-induced hypertension in the cat, the antagonist must first be characterized in vitro. This highlights the need for in vitro pharmacological characterization of cat UT, as described in the current study.

The C-terminal region of cat UT shows a great amount of sequence divergence from the human receptor, including additional potential phosphorylation sites compared to the human ortholog, as described by Onan et al. [29]. Furthermore, a caveolin binding domain (ϕXϕXXXXϕXXϕ, where ϕ is a hydrophobic residue, and X is any amino acid [34], appears at the same position as in rat UT but is absent in the human ortholog. This region has been shown to play a key role in receptor trafficking and internalization of the $AT_1$ receptor [35]. It is possible, then, to speculate that the presence of this region in cat UT and absence in human UT might contribute to possible differences in regulation of internalization and desensitization of cat UT compared with human. While the presence of these sites implies that UT may undergo phosphorylation-dependent desensitization, similar to other $G_{\alpha q}$-coupled receptors including the $AT_1$ angiotensin-II receptor [32–33,36] it is unclear which, if any, are important in regulation of UT. Onan and Thomas [37] described poor phosphorylation-dependent internalization of UT, implying weak and atypical regulation of UT. Recently, however, Proulx et al. [38] demonstrated that substitution of a serine cluster (residues 365–368) with alanine residues in rat UT markedly reduced agonist-induced internalization by nearly 50% compared to the wild-type receptor, suggesting that this particular grouping is important in receptor internalization. This particular serine cluster also occurs in cat UT (residues 353–355), but not in human UT. In the current study, phorbol ester pre-treatment resulted in an attenuation of U-II-induced PI hydrolysis in cat UT-expressing cells, but not human UT-expressing cells, without a change

in basal PI levels or receptor binding for either isoform. This supports the notion that regulation of UT desensitization might be species-dependent, but this clearly warrants further investigation. The pseudo-irreversible nature of U-II binding to UT [1] makes determination of the precise mode of PKC desensitization (i.e. homologous versus heterologous) difficult. That is, because U-II is so difficult to wash out (due to an extremely slow off-rate, [39]), repeated stimulation of cells with U-II to measure deterioration of responses is virtually impossible.

Expression of native UT mRNA was fairly widespread throughout the cat, with highest levels found in vascular tissue, spleen, and lung. As was observed previously in human tissue [1], the relative expression of cat UT mRNA was greater in arterial than in venous vascular tissue which supports the observation by Behm et al. [20] that U-II constriction is limited to arterial tissue in cat. In contrast to the UT mRNA expression relative to other tissues, the specific binding was quite low in thoracic aorta. This was not terribly surprising, as it has been demonstrated that few U-II binding sites (∼20 fmol/mg protein) are required to elicit potent and efficacious contractile responses in isolated tissues, such as rat aorta [39,40]. Indeed, Behm et al. report U-II induced constriction in cat thoracic aorta with an $EC_{50}$ of 1.8 nM and maximum contractile response 127% that of 60 mM KCl [20].

Although mRNA for UT was lower in the kidney than in other cat tissues chosen for binding in the current study, a high degree of specific binding has been seen in rat kidney, despite relatively low mRNA levels (N. Aiyar, unpublished results). For this reason, the cat kidney was also examined for the presence of U-II binding, a more physiologically-relevant measure of receptor expression. Because of the robust specific binding in the kidney, this tissue was chosen to evaluate the binding characteristics of U-II with native cat UT. In both the renal cortex and medulla, the $K_D$ values for [$^{125}$I] hU-II were similar to that seen with the recombinant receptor. In addition, $K_i$ values for hU-II displacing radioligand were also quite similar to that seen with cat recombinant UT. These data suggest that the recombinant cat UT behaves identically to the native receptor, and represents a useful tool for evaluating receptor antagonists.

The relevance of high affinity binding sites in cat vascular tissue is clear from the Behm et al. report of U-II-induced vasoconstriction and acute in vivo pressor response in the cat. However, the role of U-II in kidney (patho)physiology has only recently been studied. Both U-II and UT expression have been described in the human kidney [8,33,39–41], and U-II has been shown to affect renal sodium and water handling in the rat [14]. Furthermore, increased plasma U-II levels have been reported in patients with renal failure [3], and marked renal UT upregulation has been reported in human diabetic nephropathy [42]. While the effects of U-II on renal function in cardiovascular disease are only now emerging, the presence of characteristic U-II binding sites in the cat kidney further support this species as a possible ideal system for studying such phenomena.

In summary, the pharmacological characterization of the cat urotensin-II receptor described in the current study will greatly add to the utility of the cat as a model to examine the role of the U-II/UT system in the etiology of hypertension, assisting in the evaluation of the UT receptor as a potential site of therapeutic intervention in cardiovascular disease.

## Acknowledgements

The authors would like to thank Christopher L. Herold, Ph.D., and Valeria Camarda, Ph.D., for their early contributions to this study.

## References

[1] Ames RS, Sarau HM, Chambers JK, Willette RN, Aiyar NV, Romanic AM, et al. Human urotensin-II is a potent vasoconstrictor and agonist for the orphan receptor GPR14. Nature 1999;401:282–6.
[2] Cheung BM, Leung R, Man YB, Wong LY. Plasma concentration of urotensin II is raised in hypertension. J Hypertens 2004;22:1341–4.
[3] Matsushita M, Shichiri M, Imai T, Iwashina M, Tanaka H, Takasu N, et al. Co-expression of urotensin II and its receptor (GPR14) in human cardiovascular and renal tissues. J Hypertens 2001;19:2185–90.
[4] Totsune K, Takahashi K, Arihara Z, Sone M, Satoh F, Ito S, et al. Role of uroetnsin II in patients on dialysis. Lancet 2001;358:810–1.
[5] Douglas SA, Tayara L, Ohlstein EH, Halawa N, Giaid A. Congestive heart failure and expression of myocardial urotensin II. Lancet 2002;359:1990–7.
[6] Richards AM, Nicholls MG, Lainchbury JG, Fisher S, Yandle TG. Plasma urotensin II in heart failure. Lancet 2002;360:545–6.
[7] Dschietzig T, Bartsch C, Pregla R, Zurbrugg HR, Armbruster FP, Richter C, et al. Plasma levels and cardiovascular gene expression of urotensin-II in human heart failure. Regul Pept 2002;110:33–8.
[8] Elshourbagy NA, Douglas SA, Shabon U, Harrison S, Duddy G, Sechler JL, et al. Molecular and pharmacological characterization of genes encoding urotensin-II peptides and their cognate G-protein-coupled receptors from the mouse and monkey. Br J Pharmacol 2002;136:9–22.
[9] Douglas SA, Sulpizio AC, Piercy V, Sarau HM, Ames RS, Aiyar NV, et al. Differential vasoconstrictor activity of human urotensin-II in vascular tissue isolated from the rat, mouse, dog, pig, marmoset and cynomolgus monkey. Br J Pharmacol 2000;131:1262–74.
[10] MacLean MR, Alexander D, Stirrat A, Gallagher M, Douglas SA, Ohlstein EH, et al. Contractile responses to human urotensin-II in rat and human pulmonary arteries: effect of endothelial factors and chronic hypoxia in the rat. Br J Pharmacol 2000;130:201–4.
[11] Saetrum Opgaard O, Nothacker H, Ehlert FJ, Krause DN. Human urotensin II mediates vasoconstriction via an increase in inositol phosphates. Eur J Pharmacol 2000;406:265–71.
[12] Camarda V, Rizzi A, Calo G, Gendron G, Perron SI, Kostenis E, et al. Effects of human urotensin II in isolated vessels of various species; comparison with other vasoactive agents. Naunyn-Schmiedeberg's Arch Pharmacol 2002;365:141–9.
[13] Bottrill FE, Douglas SA, Hiley CR, White R. Human urotensin-II is an endothelium-dependent vasodilator in rat small arteries. Br J Pharmacol 2000;130:1865–70.

[14] Zhang AY, Chen YF, Zhang DX, Yi FX, Qi J, Andrade-Gordon P, et al. Urotensin II is a nitric oxide-dependent vasodilator and natriuretic peptide in the rat kidney. Am J Physiol 2003;285:F792–8.
[15] Stirrat A, Gallagher M, Douglas SA, Ohlstein OH, Berry C, Kirk A, et al. Potent vasodilator responses to human urotensin-II in human pulmonary and abdominal resistance arteries. Am J Physiol 2001; 280:H925–8.
[16] Wilkinson IB, Affolter JT, de Haas SL, Lellegrini MP, Boyd J, Winter MJ, et al. High plasma concentrations of human urotensin II do not alter local or systemic haemodynamics in man. Cardiovasc Res 2002;53:341–7.
[17] Affolter JT, Newby DE, Wilkinson IB, Winter MJ, Balment RJ, Webb DJ. No effect on central or peripheral blood pressure of systemic urotensin II infusion in humans. Br J Clin Pharmacol 2002;54:617–21.
[18] Böhm F, Pernow J. Urotensin II evokes potent vasoconstriction in humans in vivo. Br J Pharmacol 2002;135:25–7.
[19] Leslie SJ, Denvir M, Webb DJ. Human urotensin-II causes vasoconstriction in the human skin microcirculation. Circulation 2000;102: II-113.
[20] Behm DJ, Doe CPA. Johns DG, Maniscalco KM, Stankus GP, Wibberley A, et al. Urotensin-II: a novel systemic hypertensive factor in the cat. Naunyn-Schmiedeberg's Arch Pharmacol 2004;369:274–80.
[21] Marchese A, Heiber M, Nguyen T, Heng HH, Saldivia VR, Cheng R, et al. Cloning and chromosomal mapping of three novel genes, GPR9, GPR10, and GPR14, encoding receptors related to interleukin 8, neuropeptide Y, and somatostatin receptors. Genomics 1995;29: 335–44.
[22] Protopopov A, Kashuba V, Podowski R, Gizatullin R, Sonnhammer E, Wahlestedt C, et al. Assignment of the GPR14 gene coding for the G-protein-coupled receptor 14 to human chromosome 17q25.3 by fluorescent in situ hybridization. Cytogenet Cell Genet 2000;88:312–3.
[23] Devereux J, Haeberli P, Smithies O. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res 1984;12: 387–95.
[24] Johns DG, Ao Z, Naselsky DA, Herold CL, Maniscalco M, Sarov-Blat L, et al. Urotensin II-dependent cardiomyocyte hypertrophy: role of inflammatory mediators and pharmacological antagonism. Naunyn-Schmeidemerg's Arch Pharmacol 2004;370:238–50.
[25] Herold CL, Behm DJ, Buckley PT, Foley JJ, Wixted WE, Sarau HM, et al. The neuromedin B receptor antagonist, BIM-23127, is a potent antagonist at human and rat urotensin-II receptors. Br J Pharmacol 2003;139:203–7.
[26] Herold CL, Behm DJ, Buckley PT, Foley JJ, Douglas SA. The peptidic somatostatin analogs lanreotide, BIM-23127, and BIM-23042 are urotensin-II receptor ligands. Pharmacologist 2002;44:170–1.
[27] Behm DJ, Herold CL, Ohlstein EH, Knight SD, Dhanak D, Douglas SA. 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 2002;137:449–58.
[28] Coy DH, Rossowski WJ, Cheng BL, Taylor JE. Highly potent heptapeptide antagonists of the vasoactive peptide urotensin II. In: Abstracts of papers, 225th ACS National meeting. Washington, DC: American Chemical Society, 2003.
[29] Onan D, Hannan RD, Thomas WG. Urotensin II: the old kid in town. Trends Endocrinol Metab 2004;15:175–82.
[30] Behm DJ, Herold CL, Camarda V, Aiyar NV, Douglas SA. Differential agonistic and antagonistic effects of the urotensin-II ligand SB-710411 at rodent and primate UT receptors. Eur J Pharmacol 2004;492:113–6.
[31] Coy DH, Rossowski WJ, Cheng BL, Hocard SJ, Taylor JE. Novel urotensin-II (UII) antagonists point to multiple receptor involvement in UII bioactivity. Regul Pept 2000;94:48.
[32] Mori M, Sugo T, Abe M, Shimomura Y, Kurihara M, Kitada C, et al. Urotensin II is the endogenous ligand of a G-protein-coupled orphan receptor, SENR (GPR14). Biochem Biophys Res Commun 1999;265: 123–9.
[33] Nothacker HP, Wang Z, McNeill AM, Saito Y, Merten S, O'Dowd B, et al. Identification of the natural ligand of an orphan G-protein-coupled receptor involved in the regulation of vasoconstriction. Nat Cell Biol 1999;6:383–5.
[34] Couet J, Li S, Okamoto T, Ikezo T, Lisanti MP. Identification of peptide and protein ligands for the caveolin-scaffolding domain. Implications for the interaction of caveolin with caveolae-associated proteins. J Biol Chem 1999;272:6525–33.
[35] Leclerc P, Auger-Messier M, Lanctot PM, Escher E, Leduc R, Guillemette G. A polyaromatic caveolin-binding-like motif in the cytoplasmic tail of the type 1 receptor for angiotensin II plays an important role in receptor trafficking and signaling. Endocrinology 2002;143:4702–10.
[36] Balmforth AJ, Shepherd FH, Warburton P, Ball SG. Evidence of an important and direct role for protein kinase C in agonist-induced phosphorylation leading to desensitization of the angiotensin AT1A receptor. Br J Pharmacol 1997;122:1469–77.
[37] Onan D, Thomas WG. Atypical regulation of the urotensin receptor. Eur J Biol 2003;PS01-0076:48 [Meeting Abstract (FEBS Special meeting on Signal Transduction)].
[38] Proulx CD, Simaan M, Escher E, Laporte SA, Guillemette G, Leduc R. Involvement of a cytoplasmic-tail serine cluster in urotensin II receptor internalization. Biochem J 2005;385:115–23.
[39] Maguire JJ, Kuc RE, Davenport AP. Orphan-receptor ligand human urotensin II: receptor localization in human tissues and comparison of vasoconstrictor responses with endothelin-1. Br J Pharmacol 2000; 13:441–6.
[40] Itoh H, McMaster D, Lederis K. Functional receptors for fish neuropeptide urotensin II in major rat arteries. Eur J Pharmacol 1988; 149:61–6.
[41] Shenouda A, Douglas SA, Ohlstein EH, Giaid A. Localization of urotensin-II immunoreactivity in normal human kidneys and renal carcinoma. J Histochem Cytochem 2002;50:885–9.
[42] Langham RG, Kelly DJ, Gow RM, Zhang Y, Dowling JK, Thomson NM, Gilbert RE. Increased expression of urotensin II and urotensin II receptor in human diabetic nephropathy. Am J Kidney Dis 2004;44: 826–31.