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Differential agonistic and antagonistic effects of the urotensin-II ligand SB-710411 at rodent and primate UT receptors

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Abstract

SB-710411 (Cpa-c[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Cpa-amide) inhibited [^{125}I]urotensin-II rat and monkey UT receptor binding (pK_is 7.50 ± 0.07 and 6.82 ± 0.06). However, whereas SB-710411 antagonized urotensin-II-induced inositol phosphate formation at the rat UT receptor (pK_b 6.54 ± 0.05), this ligand functioned as an agonist at the monkey UT receptor (pEC_{50} 6.56 ± 0.35, E_{max} 5.27 ± 0.65-fold over basal). Indeed, in contrast to the rat UT receptor (and rat isolated arteries), SB-710411 exhibited intrinsic activity in monkey arteries acting as an efficacious vasoconstrictor (pEC_{50}s 5.03 ± 0.18 to 5.71 ± 0.21, E_{max}s 101 ± 4 to 218 ± 58% KCl). These data demonstrate that caution must be taken when extrapolating the pharmacology of a specific ligand(s) between the rodent and primate UT receptors.

Keywords: Urotensin-II; UT receptor; GPR14; SB-710411; Somatostatin

1. Introduction

Urotensin-II and its G-protein-coupled receptor, UT, are believed to play a role in the (patho)physiological regulation of mammalian cardiovascular function (Douglas, 2003). The search for a definitive role for urotensin-II and its receptor in the control of cardiovascular homeostasis would be greatly assisted by the development of selective UT receptor antagonists. To this end, an increasing number of UT receptor antagonists have been described recently (see Douglas, 2003). One such putative antagonist is SB-710411 (Cpa-c[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Cpa-amide), a cyclic somatostatin analogue (Coy et al., 2000). SB-710411 inhibits urotensin-II-induced contraction in the rat isolated aorta (K_b ~ 500 nM; Behm et al., 2002). However, as with most putative peptidic antagonists described to date, little is known about the pharmacology of this ligand in other species. Such a consideration is important, potentially, since rodent and primate UT receptor orthologues exhibit significant sequence differences at the amino acid level (Elshourbagy et al., 2002).

Whereas mouse and rat UT receptors are ~ 93% identical, the homology between rodent and primate UT receptors is considerably lower at ~ 76% identity (monkey and human UT receptors are 97% identical; Elshourbagy et al., 2002). These sequence differences raise the distinct possibility that the pharmacological effects of any given UT receptor modulator might differ between primate and non-primate species. Support for this contention comes from a recent observation that the UT receptor ligand BIM-23042 (D-2-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-2-Nal-amide) exhibits differential agonist/antagonist activity across several UT receptor orthologues (Herold et al., 2002). In order to address this issue, the present study examined the pharmacological effects of SB-710411 at rat and monkey recombinant UT receptors. Further, the intrinsic activity of this ligand was also evaluated in monkey isolated tissues.

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2. Materials and methods

2.1. Radioligand binding studies in recombinant cells

[^{125}I]Urotensin-II competition binding studies were performed with membranes prepared from human embryonic kidney (HEK-293) cells stably transfected with rat or monkey UT receptors using a scintillation proximity assay as described previously (Ames et al., 1999; Elshourbagy et al., 2002). Non-specific binding was defined using 1 μM unlabeled urotensin-II. Competition binding curves were analyzed by nonlinear regression: $K_i=(IC_{50}/([S]/K_D))+1$, where [S] is the concentration of [^{125}I]urotensin-II and K_D is the dissociation constant (GraphPad Prism 3.0).

2.2. Inositol phosphate formation in recombinant cells

Cells were seeded in Biocoat 96-well plates (25,000 cells/well; Becton Dickinson, Bedford, MA) for evaluation 3 days later. Cellular inositol lipids were radiolabeled following incubation overnight in 0.1 ml serum-free, inositol-free Dulbecco's modified Eagle's medium (DMEM) containing 1.0 g/l glucose and 0.75 μCi myo-[2-^{3}H]inositol (21.0 Ci/mmol, NEN, Boston, MA). Cells, preincubated for 10 min with 20 mM LiCl in PBS containing Ca^{2+} and Mg^{2+} (37 °C), were exposed to UT receptor ligands (in 50 μl PBS containing 20 mM LiCl) for 20 min. The assay was terminated with 20 μl ice-cold 100% trichloro-acetic acid. 200 μl supernatant were neutralized with 40 μl 1 M Tris (pH 9.8) and samples were transferred to MultiScreen 96-well filtration plates (Millipore, Bedford, MA) preloaded with Dowex AG 1-X8 resin, formate form (Bio-Rad Laboratories, Hercules, CA). Samples were washed (5 ×) with 200 μl 20 mM ammonium formate and total [^{3}H]inositol phosphates eluted with 200 μl 1 M ammonium formate (containing 0.1 M formic acid) and quantified by liquid scintillation counting.

2.3. Isolated vascular tissue

The thoracic aorta and renal, mesenteric and carotid arteries were isolated from adult male cynomolgus monkeys (4–7 kg; Primate Products, Miami, FL; Covance, Alice, TX; Charles River, Andover, MA; Mannheimer, Homestead, FL) following pentobarbital overdose (Douglas et al., 2000). After a 60 min equilibration period, SB-710411-induced contractile responses were assessed (0.01–30 μM, cumulative addition) in endothelium-denuded vessels (responses were normalized to 60 mM KCl). Concentration–response curves were fitted to a logistic equation as previously described (Behm et al., 2002). Animal procedures were reviewed and approved by the GSK Animal Care and Use Committee, and were performed in accredited facilities in accordance with institutional guidelines and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources [1996], National Research Council).

2.4. Statistics

All values are expressed as mean ± S.E.M. and n represents either the number of independent experiments done in triplicate or the number of total animals from which vessels were isolated. Statistical comparisons were made using a paired, two-tailed t-test and differences were considered significant where $P \leq 0.05$.

2.5. Drugs and reagents

Human urotensin-II and SB-710411 (Cpa-c[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Cpa-amide; Cpa, 4-chlorophenylalanine and Pal, 3-pyridylalanine; Coy et al., 2000; Behm et al., 2002) were synthesized by California Peptide Research (Napa, CA). DMEM-H, G-418, penicillin, streptomycin, amphotericin B and HBSS were from Gibco (Rockville, MD). All other reagents used were of analytical grade.

3. Results

3.1. Radioligand binding studies in recombinant cells

Urotensin-II and SB-710411 both competed for [^{125}I]urotensin-II binding at the recombinant rat UT receptor (pK_is 9.26 ± 0.14 and 7.50 ± 0.07, respectively; $n=3$, Fig. 1A). As with the rat UT receptor, urotensin-II and SB-710411 were both able to inhibit [^{125}I]urotensin-II binding at the recombinant monkey UT receptor with pK_is of 8.78 ± 0.12 and 6.82 ± 0.06, respectively ($n=3$, Fig. 1B).

3.2. Inositol phosphate formation in recombinant cells

SB-710411 failed to stimulate inositol phosphate formation in rat recombinant UT-HEK293 cells ($n=4$, Fig. 1C). Indeed, as predicted, SB-710411 (10 μM) was a functional antagonist in this inositol phosphate formation assay, inhibiting the agonist actions of urotensin-II with a pK_b of 6.54 ± 0.05 ($n=3$, Fig. 1D). In contrast to the recombinant rat UT receptor, however, SB-710411 exhibited significant intrinsic activity in the monkey UT receptor inositol phosphate assay. Indeed, SB-710411 was as efficacious as urotensin-II (i.e. a full agonist) in this system (pEC_{50}s of 6.56 ± 0.20 and 8.58 ± 0.20; E_{max}s of 5.27 ± 0.38- and 5.79 ± 0.56-fold over basal, respectively; $n=4$; Fig. 1E).

3.3. SB-710411 is an agonist (spasmogen) in monkey isolated arteries

As might be anticipated from the observations made in the monkey inositol phosphate assay, SB-710411 produced efficacious contractions in all monkey isolated arteries studied (EC_{50}s approximately 2–10 μM and E_{max}s from 101 ± 4% to 218 ± 58% 60 mM KCl; $n=4$–5; Fig. 1F, Table 1). As with urotensin-II, the contractile responses to

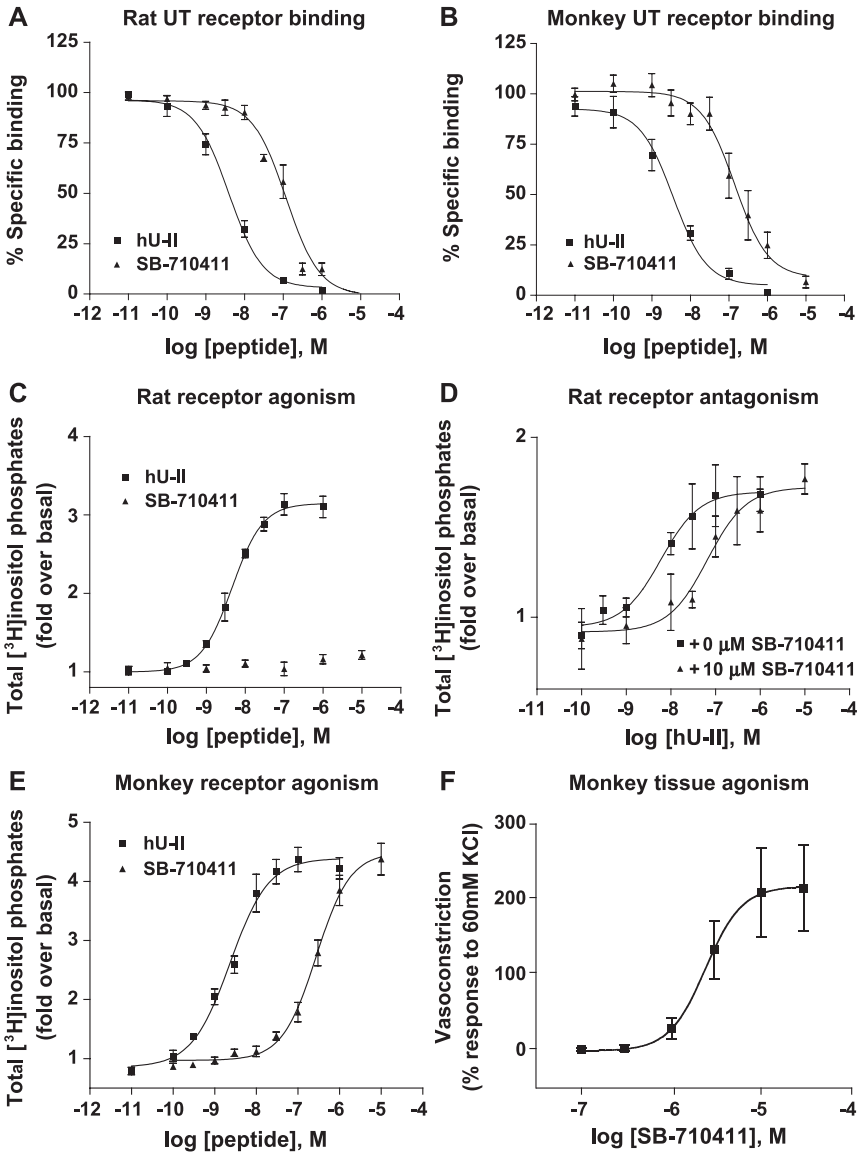

Fig. 1. (A) SB-710411 and urotensin-II compete with [^{125}I]urotensin-II for binding at recombinant rat UT receptors. SB-710411 is an antagonist at the recombinant rat UT receptor since this peptidic moiety (B) possesses no intrinsic activity and (C) 10 μM SB-710411 inhibits urotensin-II-induced IP formation. (D) SB-710411 and urotensin-II compete with [^{125}I]urotensin-II for binding at recombinant monkey UT receptors. In contrast to the rat UT receptor, however, (E) SB-710411 is a full agonist at the recombinant monkey UT receptor and (F) contracts the monkey isolated common carotid artery.

SB-710411 were slow in onset and characteristically sustained (the times taken for contractile responses to reach a maximum following addition of 10 μM SB-710411 to the isolated thoracic aorta and renal, mesenteric and carotid arteries were 140.0 ± 30.8, 45.0 ± 6.5, 65.0 ± 9.6 and 90.0 ± 12.9 min, respectively).

4. Discussion

SB-710411 acts as a ligand for both the recombinant rat and monkey UT receptors (30–150 nM affinities, 56- and 87-fold less potent than urotensin-II, respectively). However, the present study reveals that the functional behaviour of SB-710411 at these two UT receptor orthologues differs radically.

As was predicted, based on the previous observation that SB-710411 is an antagonist of urotensin-II-induced contraction in the rat isolated aorta (Behm et al., 2002), SB-710411 inhibited urotensin-II induced inositol phosphate mobilization in recombinant rat UT-HEK293 cells. SB-710411 itself did not promote inositol phosphate formation (i.e. at concentrations ≤ 10 μM, no intrinsic activity was evident) but competitively inhibited the agonistic actions of urotensin-II with similar potency to that recorded in the rat aorta (K_b 290 versus 525 nM, respectively; Behm et al., 2002).

In contrast to the rat, SB-710411 behaved as a full agonist at the recombinant monkey UT receptor (SB-

Table 1
Contractile effects of urotensin-II and SB-710411 in isolated arteries

Isolated artery	pEC_{50}		E_{max} (% KCl)	
	Urotensin-II	SB-710411	Urotensin-II	SB-710411
Rat				
Thoracic aorta	8.71 ± 0.15	–	66 ± 9	–
Monkey				
Thoracic aorta	8.96 ± 0.15	5.03 ± 0.18	224 ± 11	109 ± 11
Common carotid	9.22 ± 0.21	5.62 ± 0.04	242 ± 37	218 ± 58
Renal	9.59 ± 0.25	5.71 ± 0.21	144 ± 44	101 ± 4
Superior mesenteric	9.35 ± 0.26	5.39 ± 0.12	161 ± 41	102 ± 17

Data for urotensin-II and SB-710411 in rat aortae (where SB-710411 antagonized urotensin-II-induced contraction; Behm et al., 2002) and urotensin-II in monkey arteries (Douglas et al., 2000) are included for ease of reference.

710411 itself promoted inositol phosphate formation with an EC_{50} of 275 nM, approximately 100-fold less potent than urotensin-II). Although SB-710411 was less potent than urotensin-II, the maximum response to either ligand was similar, i.e. SB-710411 is a full agonist at the recombinant monkey UT receptor. Based on these observations, made using the monkey recombinant UT receptor, and previous observations in UT receptor knockout mice, where urotensin-II-induced vasoconstriction was directly linked with the UT receptor (Behm et al., 2003), it was predicted that SB-710411 should evoke a contractile response in monkey isolated blood vessels. Indeed, SB-710411 contracted all monkey isolated arteries studied, an observation that contrasted the antagonistic properties described previously for this peptide by Behm et al. (2002) in rat isolated aortae. Compared to the contractile responses to urotensin-II recorded previously in monkey arteries (E_{max}s ranged from 140% to 240% KCl; Douglas et al., 2000), SB-710411 was also an efficacious spasmogen in these vessels (E_{max}s ranged from 100% to 220% KCl). As such, although no direct comparison was made in the present study between urotensin-II and SB-710411, SB-710411 appears to function as a full agonist in monkey isolated arteries (perhaps with the exception of the aorta).

The present study suggests that the functional response of UT receptor modulators (such as SB-710411) at the rodent UT receptor do not necessarily predict those observed at non-rodent UT receptors (and vice versa). This is, perhaps, not too surprising given the relatively low sequence homology between rodent and primate UT receptors (~ 76%; Elshourbagy et al., 2002). Although there are many possible explanations, the apparent disparity (rodent antagonist, primate agonist) might result from alterations in receptor number and/or coupling efficiency (as has been proposed for an alternate UT receptor ligand, Orn^8[hU-II]; Camarda et al., 2002). It is of note, however, that this phenomenon might be of clinical relevance since it also appears to apply to octreotide and lanreotide, somatostatin-derivatives used therapeutically to manage several clinical conditions such as acromegaly. Octreotide and lanreotide appear to behave as either human UT receptor agonists or rat UT receptor antagonists (Herold et al., 2002; Heller et al., 2003).

In summary, the present study demonstrates that, in contrast to the antagonistic effects of SB-710411 observed at the rat UT receptor, SB-710411 functions as a full agonist at the monkey UT receptor. As such, the present findings indicate that the functional response of UT receptor modulators at the rodent UT receptor do not necessarily predict the functional response at non-rodent UT receptors (and vice versa). Thus, in terms of pharmacological actions, caution must be taken when extrapolating between rodent and primate UT receptors.

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