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Analysis of the ghrelin receptor-independent vascular actions of ulimorelin

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ABSTRACT

Ulimorelin (TZP101) is a ghrelin receptor agonist that stimulates intestinal motility, but also reduces blood pressure in rodents and humans and dilates blood vessels. It has been proposed as a treatment for intestinal motility disorders. Here we investigated the mechanisms through which ulimorelin affects vascular diameter. Actions of ulimorelin on wall tension of rodent arteries were investigated and compared with other ghrelin receptor agonists.

Saphenous, mesenteric and basilar arteries were obtained from Sprague-Dawley rats (male, 8 weeks) and saphenous arteries were obtained from wild type or ghrelin receptor null mice. These were mounted in myography chambers to record artery wall tension.

Ulimorelin (0.03–30 μM) inhibited phenylephrine-induced contractions of rat saphenous (IC_{50}=0.6 μM; I_{max}=66 ± 5%; n=3–6) and mesenteric arteries (IC_{50}=5 μM, I_{max}=113 ± 16%; n=3–4), but not those contracted by U46619, ET-1 or 60 mM [K^+]. Relaxation of phenylephrine-constricted arteries was not observed with ghrelin receptor agonists TZP102, capromorelin or AZP-531. In rat saphenous and basilar arteries, ulimorelin (10–100 μM) and TZP102 (10–100 μM) constricted arteries (EC_{50}=9.9 μM; E_{max}=50 ± 7% and EC_{50}=8 μM; E_{max}=99 ± 16% respectively), an effect not attenuated by the ghrelin receptor antagonist YIL 781 3 μM or mimicked by capromorelin or AZP-531. In mesenteric arteries, ulimorelin, 1–10 μM, caused a surmountable rightward shift in the response to phenylephrine (0.01–1000 μM; pA_2=5.7; n=3–4). Ulimorelin had similar actions in mouse saphenous artery from both wild type and ghrelin receptor null mice.

We conclude that ulimorelin causes vasorelaxation through competitive antagonist action at α_1-adrenoceptors and a constrictor action not mediated via the ghrelin receptor.

1. Introduction

The peptide hormone, ghrelin, has major roles in the control of appetite, growth hormone release and metabolic functions (Kojima and Kangawa, 2005, 2010; Delhanty and van der Lely, 2011). It reduces blood pressure in human and animals when administered intravenously (Nagaya et al., 2001; Okumura et al., 2002) and reduces sympathetic nerve activity when administered into the lower brain stem of rats and rabbits (Matsumura et al., 2002; Lin et al., 2004). Ghrelin has no direct vasodilator action on vessels from rat and the ghrelin receptor, growth hormone secretagogue receptor 1a (GHSR1a), is not expressed in rat vessels (Callaghan et al., 2012).

Ulimorelin, also known as TPZ101, is a macrocyclic molecule that is a potent agonist of the ghrelin receptor (Hoveyda et al., 2011). Due to its ability to stimulate gastrointestinal motility, ulimorelin has been evaluated as a possible treatment in gastroparesis (Ejskjaer et al., 2010), ileus (Fraser et al., 2009) and constipation (Pustovit et al., 2014).

We previously showed, in rats, that ulimorelin caused a biphasic reduction in blood pressure with an initial rapid decrease (resistant to ghrelin receptor antagonists), followed by a slower decrease (Callaghan et al., 2014). Ulimorelin also relaxed rat mesenteric arteries preconstricted with phenylephrine, an action not blocked by ghrelin receptor antagonists. Whether ulimorelin relaxes vessels constricted with other agents, or affects arteries in other vascular beds was not investigated, and mechanisms of action were also not investigated. In the current study, we investigated the mechanism(s) by which ulimorelin mediates its effects on vascular constriction, and investigated arteries supplying the viscera, limbs and central nervous system.

2. Materials and methods

Male Sprague-Dawley rats, mice with knockout of the gene for the ghrelin receptor, and wild-type C57BL6 mice were used.

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The knockout was created by inserting a transcriptional blocking cassette into intron 1 of GHSR1a in C57BL6 mice, creating a GHSR1a-null allele (Zigman et al., 2005). All animal procedures were approved by the University of Melbourne Animal Experimentation Ethics Committee. The procedures abided by the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

25 adult male Sprague-Dawley rats (300–400 g) and 8 adult mice (30–40 g) were killed by increasing atmospheric CO_2, followed by exsanguination. The saphenous artery was dissected from its origin at the femoral artery to a point just above the knee joint. Second/third order mesenteric arteries supplying the distal 15 cm of ileum were isolated from the mesentery. The whole of the basilar artery was isolated. After dissection, all vessels were maintained in physiological saline of the following composition (in mM): Na^+, 150.6; K^+, 4.7; Ca^{2+}, 2; Mg^{2+}, 1.2; Cl^-, 144.1; $H_2PO_4^{2-}$, 1.3; HCO_3^-, 16.3; glucose, 7.8. This solution was gassed with 95% O_2/5% CO_2.

The arteries were cut into 1.2–2 mm length sections, and mounted isometrically onto stainless steel wires. The rat saphenous arteries were mounted onto 50 μm diameter wires, whereas the rat mesenteric, basilar, and mouse saphenous arteries were mounted onto 40 μm diameter wires. The basal conditions were normalised by gradually stretching the vessel in small steps until the effective transmural pressure calculated using Laplace's equation [transmural pressure = wall tension/(internal circumference/2π), where wall tension = force/2 × vessel segment length] was 13.3 mN/mm^2 (100 mmHg; see Mulvany and Halpern, 1977). The rat and mouse saphenous arteries were suspended at an effective transmural pressure of 100 mmHg (Rummery et al., 2010). The rat mesenteric and basilar arteries were suspended at 90% of lumen circumference measured at an effective transmural pressure 100 mmHg (see Mulvany and Halpern, 1977). Under these conditions the vessels are mounted at close to the peak of their length–tension relationship. The baths were filled with 6 ml of physiological saline that was continuously bubbled with 95% O_2/5% CO_2 and heated to ~36.5 °C. Following mounting, the arteries were allowed to recover for 30 min.

At the start of all experiments, the tissues were stimulated with three applications of phenylephrine (3 or 10 μM) to confirm viability and when the contraction to the third application of phenylephrine had plateaued, carbachol (1 μM) was applied to determine if the endothelium was intact. Different tissues were used to assess the effects of each concentration of ghrelin receptor agonist on contractions induced by phenylephrine, methoxamine, U46619, ET-1 and 60 mM [K^+]. The 60 mM [K^+] experiments were performed in the presence of the α-adrenoceptor agonists prazosin 100 nM and idazoxan 1 μM to prevent the actions of noradrenaline released from the perivascular sympathetic nerve terminals. The test agonists (ghrelin, desacyl ghrelin, ulimorelin, capromorelin, or AZP-351) were applied and left in contact with the tissue for at least 5 min or until the relaxation produced had plateaued. When applied on their own, the concentration of ghrelin receptor agonists was increased cumulatively, with at least 8 min between each increase in concentration. For cumulative concentration curves to phenylephrine, at least 4 min was allowed to elapse between each addition of phenylephrine. When the effects of receptor antagonists, L-NAME or ion channel blockers were investigated, they were applied at least 30 min prior to application of ulimorelin.

Data were collected using a Powerlab and Chart 5 software (ADInstruments, Bella Vista, NSW, Australia) and were analysed using GraphPad Prism 5 (GraphPad Software, San Diego, CA). Responses to ghrelin receptor agonists in phenylephrine, methoxamine, endothelin-1, U46619 and 60 mM [K^+] contracted vessels were measured as the % change in active wall tension from that immediately prior to their addition. Contractions to ghrelin receptor agonists were measured as a % of the peak force of contraction to phenylephrine (3 μM for saphenous, 10 μM for mesenteric artery) or 60 mM [K^+] (in basilar artery). Both cumulative and non-cumulative curves were fitted using three parameter models. Data are reported as mean ± S.E.M or, for data generated by the three parameter models, the best-fit parameter determined by nonlinear regression and the standard error is presented; n is the number of animals. For the Schild plot a linear regression was performed using the EC_{50} values obtained from the phenylephrine concentration response curves. 1- or 2- way ANOVAs, followed by Dunnett's post-tests were used where indicated. $P < 0.05$ was considered significant.

The following compounds were used: ulimorelin and TZP102 from Tranzyme Pharma; capromorelin (CP424391) from Pfizer Pharmaceuticals, Sandwich, UK and synthesised by us; carbamylcholine chloride (carbachol), phenylephrine hydrochloride, methoxamine hydrochloride, propranolol hydrochloride, prazocin, idazoxan, atropine, glibenclamide, nifedipine, L-NAME, and ET-1 (from Sigma-Aldrich, Sydney, Australia); rat ghrelin (Auspep, Melbourne, Victoria, Australia); rat desacyl ghrelin (GL Biochem, China), AZP-531 (ChinaPeptides, Shanghai, China); YIL 781 and U46619 (Tocris, Bristol, UK). Stock solutions were prepared as follows; ghrelin, desacyl ghrelin, AZP-531 (all 1 mM pH 5.6 acetic acid buffer solution), U46619 (100 μM 70% ethanol), glibenclamide (10 mM in DMSO), nifedipine (1 mM 70% ethanol), YIL 781 (10 mM 10% DMSO). All other drugs used were prepared in dH_2O. The carrier solvents, acetic acid buffer, DMSO and ethanol, added alone in the same amounts as in experiments with drugs, had no effects on arterial wall tension in the vessels studied.

3. Results

3.1. Rat saphenous artery

In rat saphenous artery, ulimorelin (100 nM–30 μM) caused a concentration-dependent inhibition of vasoconstriction elicited by the α_1-adrenceptor agonist phenylephrine (3 μM; $pIC_{50} = 6.2 \pm 0.2$; $I_{max} = 66 \pm 5\%$; $n = 3$–6; Fig. 1). This effect was not mimicked by other ghrelin receptor agonists investigated, including ghrelin (1 nM–1 μM; $n = 3$–4), desacyl ghrelin (1 nM–1 μM; $n = 3$–4), TZP102 (10 nM–30 μM; $n = 3$–4), capromorelin (10 nM–10 μM), and AZP-531 (1 nM–1 μM; $n = 3$–4; Table 1) or the vehicles used (data not shown). Table 2 shows that the inhibition of constriction caused by ulimorelin (1–10 μM) was not reduced by the ghrelin receptor antagonist YIL 781 (3 μM, $n = 4$), the nitric oxide synthase inhibitor L-NAME (100 μM; $n = 4$), or the β-adrenoceptor antagonist propranolol (1 μM; $n = 4$; Table 2). The muscarinic acetylcholine receptor antagonist atropine (1 μM) caused a significant attenuation of the response to ulimorelin (10 μM; $n = 4$; $P < 0.01$; Table 2). The L-type calcium channel blocker nifedipine (1 μM; $n = 4$) and the K_{ATP} channel blocker glibenclamide (10 μM; $n = 4$) did not reduce the effects of ulimorelin (Table 2).

Ulimorelin (1–10 μM) also inhibited responses to the α_1-adrenoceptor agonist methoxamine (3 μM) by 42 ± 4% ($P > 0.05$) but did not inhibit contractions to the thromboxane A_2 agonist U46619 (100 nM, $n = 4$) or endothelin 1 (ET-1; 100 nM, $n = 3$–4, Table 3). The slow onset of the plateau phase of contraction to ET-1 is likely to be responsible for the apparent increase in artery tension following application of ulimorelin. In addition, ulimorelin did not reduce contractions to 60 mM [K^+] (Table 3). Ghrelin (1 μM) and desacyl ghrelin (1 μM) also had no effect on contractions to ET-1 or U46619 (both 100 nM; $n = 3$; Table 3).

At higher concentrations (10–100 μM), ulimorelin on its own caused an increase in artery tension ($pEC_{50} = 5.0 \pm 0.2$; $E_{max} = 50 \pm 7\%$ $n = 4$; Fig. 2), an effect that was attenuated by nifedipine (1 μM; $P < 0.001$ at 30 and 100 μM ulimorelin; $n = 3$). TZP102 (10–100 μM) also caused an increase in artery tension (estimated $pEC_{50} = 4.4$; $n = 4$). In contrast, ghrelin (1 nM–1 μM; $n = 3$), desacyl ghrelin (1 nM–1 μM; $n = 3$), capromorelin (10 nM–10 μM; $n = 3$), and