Short Communication

Ghrelin Suppression of Potassium Currents in Smooth Muscle Cells of Human Mesenteric Artery

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Abstract. Ghrelin is a 28-amino acid peptide hormone which modulates many physiological functions including cardiovascular homeostasis. Here we report some novel findings about the action of ghrelin on smooth muscle cells (SMC) freshly isolated from human mesenteric arteries. Ghrelin (10^{-7} mol/l) significantly suppressed the iberiotoxin-blockable component of potassium currents (I_K) and depolarized the cell membrane, while having no effect on Ca^{2+} currents. Inhibition of inositol-trisphosphate (IP_3)-activated Ca^{2+} release channels, depletion of sarcoplasmic reticulum (SR) Ca^{2+} stores, blockade of phospholipase D (PLD) or protein kinase C (PKC) each abolished the effect of ghrelin on I_K, while the inhibition of phospholipase C (PLC) did not. These data imply that in human mesenteric artery SMC ghrelin suppresses I_K *via* PLD, PKC and SR Ca^{2+}-dependent signaling pathway.

Key words: Ghrelin — Potassium currents — Human mesenteric artery — Phospholipase D — Patch-clamp

Ghrelin, a 28-amino acid peptide initially isolated from rat stomach mucosa, was identified as the first endogenous ligand of growth hormone secretagogue receptor in 1999 (Kojima et al. 1999). Since then numerous studies have demonstrated the physiological role of this hormone in growth hormone release, thermal regulation, fuel preference, appetite stimulation, regulation of cardiovascular and reproductive functions.

Ghrelin possesses a unique post-translational modification forming an *n*-octanoyl esther with Ser-2, that is essential for its hormonal activity. Desoctanoyl-ghrelin is the major ghrelin analogue in the circulation of humans, and has been shown to act *in vivo* as a full antagonist of acylghrelin-induced changes in energy balance and metabolism (see Kojima and Kanagawa 2005, for extensive review). Ghrelin is

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synthesized and released in a paracrine manner by many tissues, including cardiac muscle and cells of the diffuse endocrine system, most abundant in the gastrointestinal tract, where ghrelin receptors are expressed in vasculature, suggesting that the local concentrations of the hormone in these vessels may be much higher than in systemic circulation (Kleinz et al. 2005). This paper is the first to report the effects of ghrelin on ionic currents expressed by single smooth muscle cells (SMC) of human mesenteric arteries.

24 male (age = 66 ± 2.31 years) and 13 female (age = 56.8 ± 4.02 years) patients were included into the study in accordance with the ethical principles of the Helsinki Declaration. Half of the patients were operated for malignant growths (carcinoma sigma), and the rest – for nonmalignant conditions. In all cases, the mesenteric vessels taken for our study appeared without any abnormal structures. After gentle removal of the connective tissue, the vessels were dissected and placed in an enzyme-containing solution. Single SMC were then obtained using the procedure and solution compositions as those reported before (Hristov et al. 2004). The intracellular Ca^{2+} concentration was kept at 10^{-7} mol/l as verified with Ca^{2+}-sensitive electrode. Current signals were recorded and further analyzed as described elsewhere (Hristov et al. 2004). Outward currents were elicited from holding potential (V_h) of −50 mV, and inward currents – from V_h = −90 mV. All experiments were performed at room temperature. Data were assessed for statistical significance by the Student's *t*-test, at $p < 0.05$. Ghrelin, enzyme inhibitors, cyclopiazonic acid (CPA), ryanodine and iberiotoxin were produced by Tocris Cookson (UK). The rest of the drugs where purchased from Sigma Chemicals Co. (USA).

Using conventional patch-clamp technique, Smirnov and Aaronson (1992a,b) characterized the ionic currents and passive electrical properties of single SMC from human mesenteric artery. They described Ca^{2+}-activated and voltage-gated K^+ outward potassium currents (I_K) as well as inward currents through dihydropyridine-sensitive and dihydropyridine-insensitive Ca^{2+} channels, the latter being conductible only if cells were kept at a V_h more negative than −50 mV. In the presence of 2.10^{-5} mol/l nicardipine to block inward Ca^{2+} currents, and some metabolites from the Krebs' cycle (for details, see Hristov et al. 2004) into the cell dialyzing solution (Fig. 2A shows a typical record of K^+-currents expressed under these conditions), we found that the amplitudes of the whole-cell outward I_K were stable for more than 3 h, but decreased significantly 10 min following the bath application of 10^{-7} mol/l ghrelin ($n = 25$). The suppression was more pronounced at positive membrane potentials (Fig. 1A), and coincided with a depolarization of plasmalemma with 8 ± 2 mV ($n = 25$) as measured under current-clamp conditions. This effect of the hormone persisted in its presence, but was reversible upon 20 min washout. Even 10^{-5} mol/l ghrelin did not affect the amplitudes and kinetics of the whole-cell inward currents, carried by 10^{-2} mol/l barium (not shown).

15 min after the application of 10^{-7} mol/l ghrelin (Fig. 2B), the addition of 10^{-7} mol/l iberiotoxin to the bathing solution caused no statistically significant decrease in K^+ currents (Figs. 1A and 2C – representative original recording). *Vice versa*, iberiotoxin (10^{-7} mol/l) abolished about 90% of the total I_K (Fig. 1B), and

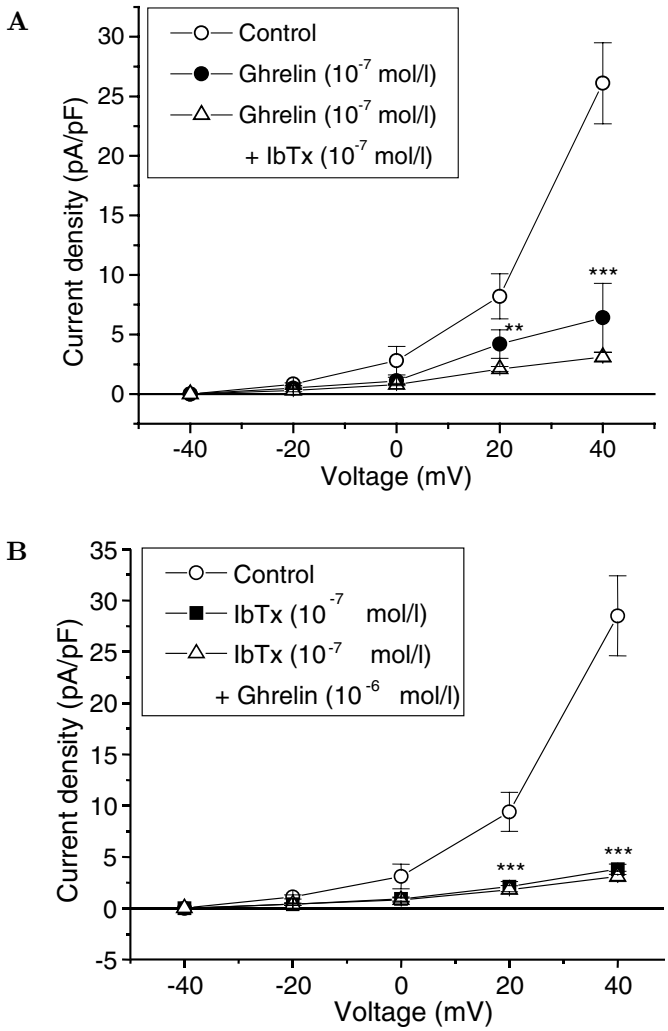

Figure 1. Effect of ghrelin on current-voltage relationships of outward potassium currents (I_K) expressed by single smooth muscle cells (SMC), isolated from human mesenteric artery. Depolarizing pulses of 500 ms duration were applied from −60 mV to +40 mV in 20 mV increments and 10 s inter-stimulus interval. I_K amplitudes were measured 150 ms after the start of each pulse (at the plateau of the current waveform) and the current densities were estimated. In both panels, control currents were measured before the addition of any drugs (open circles). **A.** Ghrelin (10^{-7} mol/l) caused a decrease in I_K, which stabilized in 10 to 15 min after its bath application (closed circles). The subsequent addition of 10^{-7} mol/l iberiotoxin (IbTx) had no significant effect on I_K densities (triangles). **B.** IbTx was applied first (closed circles) and after I_K had stabilized, ghrelin was added without any effect on I_K amplitudes (triangles). Data are means ± SEM of at least 7 independent measurements for each point. ** $p < 0.01$ and *** $p < 0.001$ show the difference between the control I_K and current measured in the presence of ghrelin.

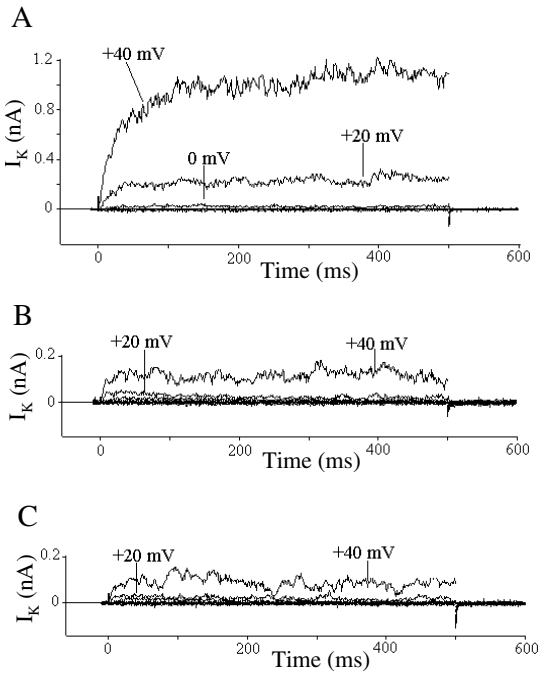

Figure 2. Representative original traces of K^+ outward current, expressed by human mesenteric artery smooth muscle cells in control conditions (A), 12 min after bath application of 10^{-7} mol/l ghrelin (B), and 5 min after the consecutive addition of 10^{-7} mol/l iberiotoxin (IbTx) to ghrelin-containing bath (C). Rectangular pulses in 20 mV increments from −60 mV to +40 mV were applied to the cell kept at a V_h of −50 mV with 15 s inter-stimulus interval. Cell with capacitance 42 pF.

subsequent addition of ghrelin had no further effect on I_K densities. These data suggest that ghrelin selectively inhibits the iberiotoxin-blockable component of I_K in a manner independent of the voltage-sensitive Ca^{2+} entry.

The participation of sarcoplasmic reticulum (SR) Ca^{2+} stores in ghrelin-induced I_K suppression was investigated by blocking the intracellular Ca^{2+} release with $2 \cdot 10^{-5}$ mol/l CPA and $2 \cdot 10^{-5}$ mol/l ryanodine added to the bathing solution – a drug combination known to result in Ca^{2+} depletion of SR (Gagov et al. 1993, 1994). 15 min after the application of this drug combination or CPA alone, I_K amplitudes were significantly higher than the control ones, most probably due to the vectorial release of Ca^{2+} from SR in the direction of the plasmalemma (Van Breemen and Saida 1989), but they spontaneously restored their initial values in the next 10 min. At that point, the application of 10^{-6} mol/l ghrelin caused no further changes in I_K amplitudes (Table 1) suggesting that the action of the hormone on I_K was somehow coupled to intracellular Ca^{2+} homeostasis. The I_K-decreasing activity of ghrelin was abolished also by the blockade of inositol-1,4,5-

Table 1. Effect of ghrelin of I_K densities in the presence of various drugs

	Control	Iberiotoxin (10^{-7} mol/l)	Heparin (10^{-7} mol/l)	CPA ($2{\cdot}10^{-5}$ mol/l)	CPA ($2{\cdot}10^{-5}$ mol/l) + ryanodine ($2{\cdot}10^{-6}$ mol/l)	U73122 ($5{\cdot}10^{-7}$ mol/l)	*n*-butanol (1% sol.)	GF-109203X (10^{-6} mol/l)
Control	100 ($n = 9$)	12.2 ± 3.1 ($n = 9$)	93 ± 7.2 ($n = 6$)	102 ± 3.0 ($n = 5$)	101 ± 6.2 ($n = 12$)	92 ± 9.8 ($n = 4$)	93 ± 7.5 ($n = 7$)	94.2 ± 5.6 ($n = 5$)
Ghrelin	13.3 ± 3.4 ($n = 10$)	11.5 ± 2.8 ($n = 8$)	95 ± 4.9 ($n = 6$)	96 ± 4.5 ($n = 6$)	98 ± 3.4 ($n = 12$)	16.0 ± 5.1 ($n = 5$)	95 ± 8.1 ($n = 7$)	97.3 ± 4.9 ($n = 5$)

Changes (in % of the control amplitudes taken as 100%) of I_K densities in the presence of various drugs or drug combinations, affecting the intracellular Ca^{2+} homeostasis or enzyme activities of PLC, PLD and PKC (first row). Ghrelin (10^{-7} mol/l) was applied to the bath 15 to 20 min following the application of each drug or drug combination. Data are means ± SEM of *n* number of experiments.

trisphosphate (IP_3)-induced Ca^{2+} release with 10^{-7} mol/l heparin, applied *via* the pipette solution (Table 1). Taken together, these data suggest that ghrelin reduces iberiotoxin-sensitive I_K *via* modulation of vectorial Ca^{2+} release from SR.

Surprisingly, the I_K-decreasing effect of ghrelin was virtually unaffected by the blockade of phospholipase C (PLC) with 5.10^{-7} mol/l [1-(6-[17-β-3-methoxyestra-1,3,5-(10)-triene-17-yl] amino/hexyl)-1H-pyrroledione] (U73122). On the other hand, pretreatment of cells with 1% solution of *n*-butanol, a competitive and selective inhibitor of phospholipase D (PLD) in such concentration (Chen et al. 1997) completely abolished the effect of ghrelin on I_K, indicating the participation of PLD in ghrelin-induced I_K suppression. The selective inhibitor of calcium-sensitive protein kinase C (PKC) isoenzymes GF-109203X (3-[1-[3-(dimethylamino)propyl]-1H-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyrolle-2,5-dione), (10^{-6} mol/l) also averted the action of ghrelin on I_K. These observations imply that in SMC of human mesenteric artery ghrelin reduces I_K *via* a signaling pathway involving PLD and PKC, possibly by modulating the IP_3-sensitive Ca^{2+} release from SR.

In conclusion, ghrelin was found to suppress significantly the iberiotoxin-blockable component of I_K and to depolarize the plasma membrane of human mesenteric arteries SMC. These effects of the hormone were prevented by inhibition of PLD and PKC activity, as well as by blocking Ca^{2+} release through IP_3-activated Ca^{2+} channels of SR, or by depletion of SR Ca^{2+} stores, but not by inhibition of PLC.

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References

Chen Y.-G., Siddhanta A., Austin C. D., Hammond S. M., Sung T.-C., Frohman M. A., Morris A. J., Shields D. (1997): Phospholipase D stimulates release of nascent secretory vesicles from the *trans*-Golgi network. J. Cell Biol. **138**, 495—504

Gagov H. S., Duridanova D. B., Boev K. K. (1993): Participation of calcium, released from the IP_3-sensitive Ca-store in activation of Ca-dependent potassium conductance in ileal smooth muscle cells. Gen. Physiol. Biophys. **12**, 199—211

Gagov H. S., Duridanova D. B., Boev K. K., Daniel E. E. (1994): L-type calcium channels may fill directly the IP_3-sensitive calcium store. Gen. Physiol. Biophys. **13**, 75—84

Hristov K., Altankova I., Gagov H., Bolton T., Boev K. K., Duridanova D. (2004): Calcium-dependent changes in potassium currents in guinea-pig coronary artery smooth muscle cells after acute cobalt loading in vivo. Pflügers Arch. **449**, 16—25

Kleinz M. J., Maguire J. J., Skepper J. N., Davenport A. P. (2005): Functional and immunocytochemical evidence for a role of ghrelin and des-octanoyl ghrelin in the regulation of vascular tone in man. Cardiovasc. Res. **69**, 227—235

Kojima M., Hosoda H., Date Y., Nakazato M., Matsuo H., Kangawa K. (1999): Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature **402**, 656—660

Kojima M., Kanagawa K. (2005): Ghrelin: structure and function. Physiol. Rev. **85**, 495—522

Smirnov S. V., Aaronson P. I. (1992a): Ca^{2+}-activated and voltage-gated K^+ currents in smooth muscle cells isolated from human mesenteric arteries. J. Physiol. (London) **457**, 431—454

Smirnov S. V., Aaronson P. I. (1992b): Ca^{2+} currents in single myocytes from human mesenteric arteries: evidence for a physiological role of L-type channels. J. Physiol. (London) **457**, 455—475

Van Breemen C., Saida K. (1989): Cellular mechanisms regulating $[Ca^{2+}]_i$ smooth muscle. Annu. Rev. Physiol. **51**, 315—329

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