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Contractile effect of ghrelin on isolated guinea-pig renal arteries

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Abstract

Ghrelin, a 28-amino acid peptide, known to exist in both acylated and des-acylated varieties, was identified as the first endogenous ligand of growth hormone secretagogue receptor in 1999. Various arteries are known to express ghrelin receptors, but the direct action of ghrelin on blood vessels has been unclear. In the present study we show that ghrelin concentration-dependently potentiates endothelin-1 (ET-1) induced tension development of guinea-pig renal artery, as measured using a wire-type isometric myography of vascular segments. In vascular smooth muscle cells (SMC) ghrelin caused activation of potassium outward currents via phospholipase C (PLC)→inositol-1,4,5-trisphosphate (IP_3) and PLC→protein kinase C (PKC) signalling cascade, resulting in hyperpolarizaton of the cell membrane. On a tissue level ghrelin by itself had no effect on isometric tone, but augmented ET-1 induced contraction by a mechanism, involving PLC, Rho-kinase and intracellular IP_3-sensitive Ca^{2+} release, and not nucleotide-sensitive protein kinases or PKC. Together with our previous findings the data in this study suggest that ghrelin exerts its contractile activity on guinea-pig renal artery by facilitation of ET-1 triggered intracellular signalling in SMC, and/or by stimulating the release of a yet unknown contractile mediator from endothelium.

Keywords: Ghrelin; Renal artery; Guinea-pig; Endothelin-1; Potassium currents

1. Introduction

Ghrelin, an acylated peptide, was identified as the first endogenous ligand of growth hormone secretagogue receptor by Kojima and collaborators in 1999 (Kojima et al., 1999). The major active form of ghrelin is a 28-amino acid peptide containing an *n*-octanoyl modification at Ser(3) that is essential for its hormonal activity. Studies have ascertained multiple physiological functions for ghrelin, including growth hormone release, appetite stimulation, modulation of cardiovascular functions and metabolic fuel preference (Kojima and Kanagawa, 2005; Korbonits et al., 2004). In humans, ghrelin is inactivated via desoctanoylation by various serum esterases, while its peptidic moiety is stable in the blood (De Vriese et al., 2004).

Until very recently it was believed that the main source of ghrelin is the stomach mucosa and other sources of this hormone in mammals including hypothalamus, placenta and the cells of so-called diffuse endocrine system (Rindi et al., 2004, for review). Latest discoveries however suggest that ghrelin could be produced in other tissues, where it displays marked physiological activities (Caminos et al., 2005; Iglesias et al., 2004; Xu et al., 2005). Therefore, it is reasonable to suggest that ghrelin synthesis might occur in multiple systems and multiple organs where the hormone affects tissue-specific functions.

In vivo desacyl-ghrelin antagonizes the effects of ghrelin on energy balance and metabolism (Asakawa et al., 2005; Gauna et al., 2005). However, centrally mediated effects of ghrelin and desacyl-ghrelin on cardiovascular system have been shown to be equally and exclusively suppressive: hypotensive and bradycardic (Tsubota et al., 2005) as well as cardioprotective (Chang et al., 2004; Iglesias et al., 2004). These cardiovascular

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effects of ghrelin and desacyl-ghrelin are assumingly ascribed to their binding to a receptor different from growth hormone secretagogue one (for review see Nagaya et al., 2006).

Expression of ghrelin receptors on various vasculatures has been known (Katugampola et al., 2001), but astonishingly, the direct action of ghrelin on blood vessels has been largely unknown and most of the studies have been performed on systemic circulation. Vascular actions of ghrelin were thought to be mostly relaxant. The first report about direct relaxatory effect of ghrelin on vessels suggested no physiological relation to endothelium-derived NO (Okumura et al., 2002), while the studies of Wiley and Davenport (2002) proposed a functional antagonism between ghrelin and endothelin-1 (ET-1) on human mammary artery. On the other hand, vasopressor activity of ghrelin has also been documented in rat coronary (Pemberton et al., 2004) and human mesentery (Dimitrova, 2006) arteries. As for the effect of ghrelin on smooth muscle cell ionic currents, to the best of our knowledge only one study has been published to date (Mladenov et al., 2006). Thus, the mechanisms of both vasodilative and vasopressive activities of ghrelin have not been explained as yet.

Our preliminary studies have shown a contractile effect of ghrelin on ET-1 precontracted guinea-pig renal artery, where this hormone supposedly affected simultaneously the function of vascular smooth muscle, endothelium and neuronal projections (Mihov and Duridanova, 2005). Many studies have shown that various bioactive substances could modify the arterial tone by modulating both smooth muscle membrane ionic currents and the release of vasoactive mediators from adjacent vascular tissues (De Oliveira et al., 2006; Hall et al., 2006; Silva et al., 2007). In order to test whether ghrelin acts independently on smooth muscle membrane excitability and vascular contractility we investigated its effect on ionic currents, expressed by single renal artery myocytes, and its action on vascular tone, induced by endothelin-1. Changing the conditions of the contractile experiments by deendothelizing the vessels, by blocking the neurotransmitter release or both, we tried to discriminate the effects of the hormone on each arterial tissue. Thus, in the present paper we report our findings concerning the mechanisms by which ghrelin exercises a vasopressor activity with the emphasis on its action on renal artery smooth muscle.

2. Materials and methods

2.1. Contraction studies

Male guinea pigs, 500–600 g body weight, were killed by stun on the head. Kidneys were removed and placed in ice-cold, low Ca^{2+} physiological salt solution (PSS), containing (in mM): 118 NaCl, 5 KCl, 1.2 $MgCl_2$, 0.16 $CaCl_2$, 10 glucose, 1.2 Na_2HPO_4, 24 HEPES. Kidneys were dissected into two halves. Connective tissue was removed and renal arteries (diameter 300–400 μM) of 2 mm long were isolated and mounted on an automated wire-type myograph (model 410A, DMT — Danish Mio Technology, Denmark). Isometric force was recorded by program Miodaq (DMT, Denmark). After the vessels were mounted, 2.5 mM $CaCl_2$ was added to the solution and the bath was heated up to 37±0.5 °C. The vessels were radially stretched to their optimal lumen diameter d_o corresponding to 90% of the passive diameter of the vessel at 100 mm Hg, and allowed to stabilize for 20 min. Reactivity of the preparations was assessed by two consecutive applications of 10^{-6} M noradrenaline (NA) — a procedure, which did not modify tissue response to ET-1 or ghrelin, as has been shown in control experiments. The maximal contraction produced by NA was 5.4±0.8 N/m (n=25).

The vessels were further pre-constricted with 10^{-8} M ET-1, the most potent endogenous contractile agonist in renal circulation (Berthold et al., 1999). This was the experimentally found concentration of ET-1 that elicited a constriction response approximately 30% of maximal tension achieved after 10^{-6} M NA (1.35±0.15 N/m, n=18). In some experiments the preconstriction was induced by high potassium (42 mM)-containing PSS, which was prepared by equimolar replacement of NaCl, or by 3×10^{-6} M prostaglandin F_{2alpha}. Other contractile agonists have also been tested: angiotensin II, histamine, serotonin. All these mediators produced only unstable transitory contractions with comparatively rapid decline. Moreover, repetitive applications led to desensitisation and almost complete loss of contractile response. Thus, we were forced to work with ET-1 — the only contractile agent producing relatively stable tone in isometrically stretched preparations of renal artery.

After application of agonist in standardized time conditions for incubation (35–45 min), the maximal ET-1 induced contraction reached was taken as 100% contractile force. After the tone has been stabilized (steady-state was achieved 30–35 min after ET-1 application), ghrelin was added in increasing concentrations. The amplitudes of the tension achieved after each concentration of the hormone were measured at the plateau, and represented as a percentage of ET-1 maximal force (100%). The control preparations were treated the same way, but instead of ghrelin-containing solution the same volume of distilled water was added at corresponding time intervals. Such a procedure was proven appropriate by preliminary experiments showing that application of each single concentration alone caused the same contractile response as if administered cumulatively, i.e. no desensitisation to ghrelin was observed. Experiments were performed in the presence or absence of functional endothelium. The endothelium was removed using rat whisker, a procedure that abolished the acetylcholine-induced relaxation. Some experiments were conducted in the presence of 3×10^{-7} M tetrodotoxin (TTX) to block the neurotransmission. In the experiments in which inhibitors of intracellular signalling pathways 2-APB, NCDC, Y-27632 and GF-109203X were used, the substances were applied on the constriction plateau of ET-1 elicited tension and incubated with renal arterial rings for 30 min before application of ghrelin. All drugs were applied directly to the experimental chamber.

2.2. Patch-clamp studies

The renal artery was cut into 3 mm long segments and placed in physiological salt solution (PSS), containing (in mM): 126 NaCl, 6.1 KCl, 20 HEPES, 10 taurine, 20 glucose, 1.2 $MgCl_2$, 0.8 $CaCl_2$, 5 Na-pyruvate (pH adjusted to 7.4 with NaOH). To this