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Ghrelin as a novel locally produced relaxing peptide of the iris sphincter and dilator muscles

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Abstract

Ghrelin is a recently described acylated peptide, which works as a somatosecretagogue and has described effects on the smooth, skeletal and cardiac muscle. We examined the production and effects of ghrelin on relaxation of the iris muscles. Contractile effects of 1–5 human ghrelin (frGhr, 10^{-9}–6×10^{-5} M) and 1–5 human des-octanoyl-ghrelin (d-frGhr; 10^{-9}–6×10^{-5} M) were tested on iris rabbit sphincter ($n = 11$ frGhr; $n = 7$ d-frGhr), dilator ($n = 6$ frGhr; $n = 6$ d-frGhr) and rat sphincter ($n = 6$ frGhr; $n = 8$ d-frGhr) precontracted muscles. On rabbit sphincter the effect of frGhr was also tested in presence of: i) L-NA (10^{-5} M; $n = 7$); ii) indomethacin (10^{-5} M; $n = 7$); iii) DLys^{3}GHRP6 (10^{-4} M; $n = 6$); and iv) apamin + carybdotoxin (10^{-6} M; $n = 6$). Furthermore, on rabbit dilator the effect of frGhr was tested in presence of DLys^{3}GHRP6 (10^{-4} M; $n = 7$). Finally, ghrelin mRNA production was assessed by "*in situ*" hybridization in Wistar rat eyes ($n = 8$). In all muscles, frGhr promoted a concentration-dependent relaxation, maximal at 6×10^{-5} M, 1.5–3 min after its addition, decreasing tension by $34.1 \pm 12.1\%$, $25.8 \pm 4.8\%$ and $52.1 \pm 10.3\%$ in the rabbit sphincter, dilator and rat sphincter, respectively. In the rabbit sphincter the relaxing effects of frGhr were: (i) enhanced in presence of DLys^{3}GHRP6 ($118.1 \pm 21.1\%$); (ii) blunted by indomethacin; and (iii) not altered by apamin + carybdotoxin ($36.4 \pm 14.4\%$) or L-NA ($52.4 \pm 11.4\%$). Relaxing effects of d-frGhr in rabbit ($43.3 \pm 5.2\%$) and rat ($77.1 \pm 15.3\%$) sphincter muscles were similar to those of frGhr. In rabbit dilator muscle, d-frGhr did not significantly alter active tension and the relaxing effect of frGhr was blunted by GHSR-1a blockage. Ghrelin mRNA was identified in iris posterior epithelium. In conclusion, ghrelin is a novel, locally produced, relaxing agent of iris dilator and sphincter muscles, an effect that is mediated by GHSR-1a in the former, but not in the latter. Furthermore, in the sphincter it seems to be mediated by prostaglandins, but not by NO or K_{Ca} channels.

Keywords: peptide hormones; iris; ciliary epithelium; muscle fibers

1. Introduction

Ghrelin is a recently described acylated peptide, initially isolated from rat stomach, which works as a somatosecretagogue (Kojima et al., 1999). Growth hormone secretagogues (GHS) promote GH release by direct action on the pituitary gland (Smith et al., 1997; Locatelli and Torsello, 1997; Bowers, 1998; Dieguez and Casanueva, 2000), stimulating a specific G Protein coupled receptor (GHS type 1a receptor,

Abbreviations: L-NA, N^{ω}-nitro-L-arginine; GHS, growth hormone somatosecretagogues; GHSR-1a, growth hormone somatosecretagogues receptor type 1a; COX, cycloxygenase; ET-1, endothelin 1; frGhr, 1–5 human ghrelin; d-frGhr, 1–5 human des-octanoyl-ghrelin; NO, nitric oxide; Pg, prostaglandins; EP_2, prostaglandin E receptor 2.

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GHSR-1a), which is different from GHRH receptor (Howard et al., 1996; Muccioli et al., 1998; Smith et al., 1999; Feighner et al., 1998). Binding sites to the somatosecretagogues have been described in several tissues such as (in order of decreasing binding activity) the myocardium, adrenal gland, gonads, arteries, lung, liver, skeletal muscle, kidney, pituitary, thyroid, adipose tissue, veins, uterus, skin and lymphnode (Papotti et al., 2000; Iglesias et al., 2004). Interestingly, in many of these tissues, the specific binding values were described as even higher than in the pituitary gland (Papotti et al., 2000; Bodart et al., 1999; Muccioli et al., 2000).

Several studies investigated the physiological role of ghrelin and GHSR-1a in cardiac, skeletal and smooth muscles. In cardiac muscle, ghrelin is synthesized and secreted by cardiomyocytes (Iglesias et al., 2004), and acts as a negative inotrope. This effect is modulated by cycloxygenase depends on endocardial endothelium and seems to be independent from GHSR-1a (Bedendi et al., 2003). In the skeletal muscle, ghrelin reduces membrane potential, apparently by increasing chloride's permeability. This action is directly dependent of GHSR-1a and is blocked by the specific inhibitor DLys^{3}GHRP6 (Pierno et al., 2003). In human vascular smooth muscle, ghrelin appears as the most potent endothelium-independent vasodilator, reversing effectively endothelin-1 (ET-1) mediated constrictions with potency similar to that of adrenomodullin (Wiley and Davenport, 2002). Ghrelin also has a potent hypotensive effect associated with reduced nitric oxide (NO) availability. This effect, in rats, is reversed by the blockage of the Ca^{2+} activated K^+ channels (Shinde et al., 2005).

In the iris sphincter and dilator muscles the relaxing effects of several substances have been investigated over the last decade. Geyer et al. (1998) showed that β_3 adrenergic stimulation elicits a significant relaxation of the bovine iris sphincter muscle, an effect that was later shown to be potentiated by muscarinic blockage (Barilan et al., 2003). In the same experimental preparation, it was demonstrated that the endogenous NO-guanylyl cyclase-cGMP cascade mediates the non-adrenergic-non-cholinergic relaxation (Pianka et al., 2000) and that adrenomodullin relaxes it by increasing intracellular cAMP (Yousufzai et al., 1999). Finally, both galanin and somatostantin inhibit the cholinergic response of the rabbit iris sphincter muscle, being galanin more potent than somatostantin in this effect (43% vs 16%) (Yamaji et al., 2003). On the other hand, pituitary adenylate cyclase activating peptide-27 promotes relaxation of rabbit iris dilator muscles submitted to electric field stimulation or pre-contracted by phenylephrine (Yoshitomi et al., 2002).

The effect of some peptides in the iris muscle tone can modify the aqueous humor drainage mechanisms and induce changes in the pupil diameter. With the purpose of studying the effects and physiological relevance of ghrelin in the modulation of iris smooth muscles relaxation we investigated its direct effects on the iris sphincter or dilator muscles of rabbit or rat eyes as well as the presence of its mRNA in the anterior segment of the rat's eye.

2. Methods

All animal procedures were performed in accordance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

2.1. Functional studies

2.1.1. Specimens preparation

The study was performed in isolated iris sphincter ($n = 44$) and iris dilator ($n = 19$) muscles from male New Zealand white rabbits (*Oryctolagus cuniculus*; 2.0–3.0 Kg) and in iris sphincter muscles ($n = 14$) from Wistar rats. Animals were euthanized after an injection of pentobarbital sodium salt (50 mg/kg) into the marginal ear vein (rabbits) or pentobarbital sodium salt (150 mg/kg) intraperitoneal. The eyes were immediately enucleated and placed in modified Krebs-Ringer (KR) solution at 35 °C, with the following composition in mM: NaCl 98; KCl 4.7; $MgSO_4.7H_2O$ 2.4; KH_2PO_4 1.2; glucose 4.5; $CaCl_2.2H_2O$ 1.8 (2.5 for the rat muscles); $NaHCO_3$ 17; $C_3H_3NaO_3$ 15 and CH_3COONa 5. After removal of the cornea, the iris sphincter or dilator muscles were quickly excised and immersed in the KR solution. After dissection, the ends of each piece were tied with silk thread for mounting in a 15 ml plexi glass organ bath containing the above-described solutions. One end of the specimen was connected to an electromagnetic length-tension transducer (University of Antwerp, Belgium), and the other end was secured to a clip at the bottom of the organ bath. In rabbits we tied all the entire strip of the sphincter muscle or a radial strip of the dilator with 2 mm of width; while in rats we tied the entire ring. All the surgical procedures were taken under microscope (Wild M 650, Leica Microsystems, Switzerland). Solutions were bubbled with 95% O_2 and 5% CO_2 and pH was maintained between 7.38–7.42.

Iris muscles were stabilized always at the same preload (1.1 mN for the rabbit sphincter, 0.5 mN for the rabbit dilator and 0.2 mN for the rat sphincter) and bathing solutions replaced every 20 min until muscle length stabilization. They were then switched to isometric conditions and the protocols initiated when muscle tension stabilized (usually after 20 min).

2.1.2. Experimental protocols

2.1.2.1. Rabbit iris sphincter muscles. After stabilization, the rabbit iris sphincter muscles were contracted, by adding Carbachol (10^{-7} M) to the organ bath. When a stable contraction was reached, increasing doses of 1–5 human ghrelin (frGhr; 10^{-9}–6×10^{-5} M) were added to the organ bath to generate concentration-response curves in the absence ($n = 7$) or presence of: i) D-Lys^{3}GHRP6 (10^{-4} M, $n = 6$), a GHS receptor 1a antagonist; ii) N^{ω}-nitro-L-Arginine (LNA: 10^{-5} M, $n = 7$), a NO-synthase inhibitor; iii) indomethacin (10^{-5} M, $n = 7$), a cycloxigenase inhibitor; and iv) apamin and carybdotoxin (10^{-6} M + 10^{-6} M, $n = 6$), inhibitor of the Ca^{2+} activated K^+ channels. In a subset of muscles ($n = 4$) a single dose of