

MODULATORY EFFECTS OF PEPTIDE GHRELIN ON URINARY BLADDER AND ITS ROLE IN DIABETES

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Abstract

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Plasma ghrelin levels manifest “biphasic changes” in diabetes mellitus. In order to investigate ghrelin effects and mechanisms of action in diabetes, firstly we should know how this peptide modulates urinary bladder muscles under normal conditions. We found no statistically significant changes in contractile activity after application of ghrelin alone as compared to the spontaneous activity. The effects of ghrelin were displayed when it was applied in combination with other peptides. For example, 30 min after ghrelin application, the administration of Ang II did not lead to the typical tonic contractions occurring when only Ang II was administrated. The amplitude of the Ang II stimulated contractions was reduced from 1.90 ± 0.20 g to 0.78 ± 0.09 g in the presence of ghrelin ($n = 21$, $P < 0.05$). Based on these results we can assume that the urinary bladder possesses receptors for ghrelin, which are different from those in the digestive tract, with respect to the kind of intracellular signalling mechanism to which they are coupled.

Key words: ghrelin, urinary bladder, contractile activity, smooth muscle, diabetes

Abbreviations: Ang II – angiotensin II; GOAT – O-n-octanoylation at serine 3 through the enzyme ghrelin O-acyltransferase; AVP – vasopressin; AUC – area under the curve

Introduction

Urinary bladder problems can have a profound effect on quality of life. Diabetes damages the nerves that control detrusor muscle and bladder function. As a result men and women with diabetes commonly have bladder symptoms that include: i) feeling of urinary urgency; ii) getting up at night to urinate often; iii) leakage of urine (incontinence). The effects of the peptide ghrelin on various organs and systems are not well established however it is known that this peptide affects the performance of smooth muscles. Plasma ghrelin levels manifest “biphasic changes” in diabetes mellitus (Chen et al., 2009). In the early stage of diabetes ghre-

lin levels are significantly higher in correlation with diabetic hyperphagic feeding and accelerated gastrointestinal motility. In the late stage of diabetes plasma ghrelin levels are lower, which might be linked with anorexia/muscle wasting and delayed gastrointestinal transit. In order to investigate ghrelin effects and mechanisms of action in diabetes, firstly we should know how this peptide modulates urinary bladder muscles under normal conditions. The aim of the present study was to investigate the influence of ghrelin alone as in combination with angiotensin II (Ang II) on the contractile activity of urinary bladder smooth muscle strips from rat detrusor.

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Materials and Methods

Wistar rats weighting 200–250 g were used. The animals were anesthetized with Nembutal 50 mg/kg intraperitoneally and exsanguinated. The experiments have been performed according to the national regulations and European Communities Council Directive (86/609/EEC) also “Principles of laboratory animal care” (NIH publication No. 85-23) concerning the protection of animals used for scientific and experimental purposes.

Abdominal cavity was opened and the urinary bladder was dissected out and immediately placed in cold Krebs solution (3°C), containing the following composition (in mmol): NaCl 118.0, KCl 4.74, NaHCO₃ 25.0, MgSO₄ 1.2, CaCl₂ 2.0, KH₂PO₄ 1.2 and glucose 11.0. Strips (approximately 2 mm wide, 0.5 mm thick and 8 mm long) were dissected from the detrusor following the direction of the muscle bundles. The two ends of each strip were tied with silk ligatures. The distal end was connected to the organ holder; the proximal end was stretched and attached to a mechano-electrical transducer FSG-01 (Experimetria, Ltd., Hungary) via a hook. The preparations were mounted in organ baths TSZ-04/01, containing Krebs solution, pH 7.4, continuously bubbled with carbogen (95% O₂, 5% CO₂). The organ baths were mounted in parallel above an enclosed water bath, maintaining the solution temperature at 37°C. Strips were placed under an initial tension (preload) of 1 g and allowed to equilibrate for at least 75 min (three periods: 15 min, 45 min and 15 min and two washes with Krebs solution between them). All preparations displayed rhythmic spontaneous contractions. Mechanical activity was digitized and recorded using ISOSYS 1.0 computer program. The conversion of the data for later analysis was performed with KORELIA-IzoSys program. Differences among experimental groups were evaluated either by Student-Fisher *t*-test or by one-way analysis of variance (ANOVA) followed by Newman-Keuls post-hoc test.

Results

The effect of ghrelin on urinary bladder smooth muscle strips is summarized in Figure 1. We found no statistically significant changes in contractile activity after application of ghrelin alone as compared to the spontaneous activity (Figure 1). However, the effects of ghrelin were displayed when it was applied in combination with other peptides. For example, 30 min after ghrelin application and the administration of Ang II did not lead to the typical tonic contractions occurring when only Ang II was administrated (Figure 2). The amplitude of the Ang II stimulated contractions was reduced from 1.90 ± 0.20 g to 0.78 ± 0.09 g in the presence of ghrelin ($n = 21$, $P < 0.001$).

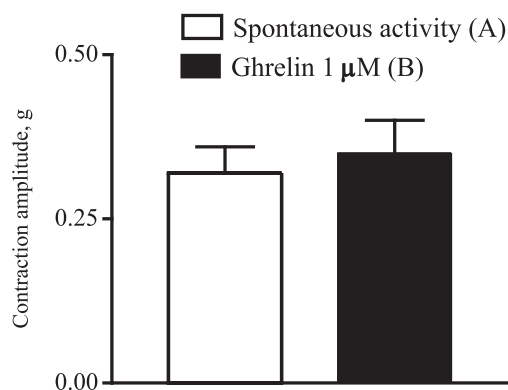


Fig. 1. Amplitude of spontaneous contractile activity of rat urinary bladder smooth muscle strips before (A) and after Ghrelin (B). Means \pm standard error of the mean of 16 experiments are presented. Differences among experimental groups were evaluated by Student-Fisher *t*-test

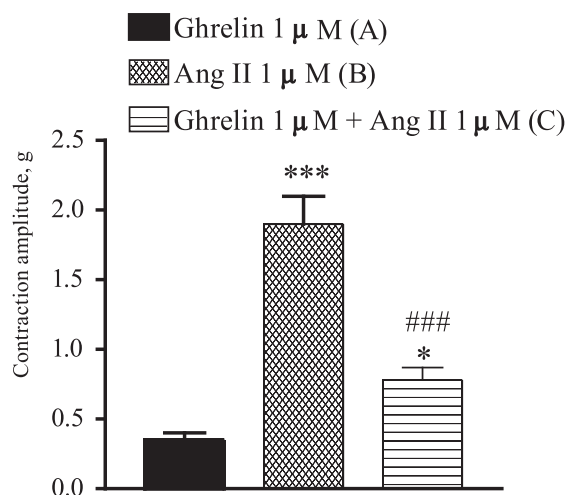


Fig. 2. Effects of Ghrelin (A), Angiotensin II alone (B) and in the presence of Ghrelin (C) on spontaneous contractile activity of rat urinary bladder smooth muscle strips. Means \pm standard error of the mean of 21 experiments are presented. Differences among experimental groups were evaluated by ANOVA ($F_{2,55} = 32.42$) followed by Newman-Keuls post-hoc test. Significant differences are indicated: *** $P < 0.001$ (B vs. A); * $P < 0.05$ (C vs. A); ### $P < 0.001$ (C vs. B)

Discussion

Ang II receptors have been discovered in the detrusor of many species including humans, and there is a wide variety in the responses to this peptide (Andersson and Arner, 2004). On the other hand, Ang II stimulates the activity of L/T-type voltage dependent calcium channels in vascular smooth-muscle cells (Lu et al., 1996). We can assume that in the smooth-muscle cells of the rat bladder a similar effect is realized.

Ghrelin peptide is the only peripheral signal to enhance food intake – ghrelin enhances appetite while leptin is a satiety signal (Ukkola, 2004). Ghrelin peptide is unique for its post-translational modification of O-n-octanoylation at serine 3 through the enzyme ghrelin O-acyltransferase (GOAT), which enables ghrelin to activate the ghrelin receptor (Chen et al., 2009). The unique ghrelin system may be the most important player compared to the other hindgut hormones participating in the “entero-insular axis”. Something more, the modulation of GOAT or ghrelin signaling may be a clinically relevant strategy to treat non-insulin-dependent Type 2 diabetes.

The receptors for ghrelin described in the literature are associated with activation of phospholipase C and increase in intracellular calcium (Davenport et al., 2005). Therefore, the application of ghrelin on muscle strips of urinary bladder would lead to the occurrence of tonic contractions. During the experiments we found no statistically significant changes in contractile activity after application of ghrelin as compared to the spontaneous activity. The effects of ghrelin are displayed when it is applied in combination with other peptides. In combination with Ang II, ghrelin reduces its

effect on the bladder (Ilieva et al., 2008). The combination of ghrelin with AVP leads to a similar yet significantly less manifested decrease, especially in the AUC. Based on these results we can assume that the urinary bladder possesses receptors for ghrelin, which are different from those in the digestive tract, with respect to the kind of intracellular signalling mechanism to which they are coupled.

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