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Stimulatory effects of ghrelin on spontaneous contractions in the rat myometrium

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Abstract: Ghrelin, a recently isolated hormone, has been reported to have modulatory effects on smooth muscle contractility. In this study, we investigated the effects of ghrelin on spontaneous contractions in the rat uterus in vitro. Myometrium strips were removed from Wistar rats following decapitation and placed in a jacketed tissue bath. After initiation of spontaneous contractions, control contractions were recorded for 10 min and various concentrations of ghrelin were added to the tissue bath cumulatively. The Wilcoxon signed ranks test was used for statistical analysis. Application of ghrelin augmented the spontaneous contractions in myometrial strips. The mean peak amplitudes of contractions were 2.69 ± 0.32 g (n = 6), 2.71 ± 0.31 g (n = 6), 2.92 ± 0.36 g (n = 6), and 3.46 ± 0.24 g (n = 6) under control conditions and after application of 0.01 μM, 0.1 μM, and 1 μM ghrelin, respectively. The mean frequencies of contractions were 5.17 ± 0.75 (n = 6), 5.17 ± 0.75 (n = 6), 5.33 ± 0.48 (n = 6), and 8.0 ± 0.77 (n = 6) under control conditions and after application of 0.01 μM, 0.1 μM, and 1 μM ghrelin, respectively. The increase in amplitude and frequency of contractions was significant only after application of 1 μM ghrelin ($P < 0.05$). Data from this study demonstrated that ghrelin induces spontaneous contractions in a dose-dependent manner in the rat myometrium.

Key words: Ghrelin, spontaneous contractions, myometrium, rat

Ghrelin'in sıçan miyometriyumundaki spontan kasılmalara uyarıcı etkileri

Özet: Son yıllarda izole edilen ghrelinin düz kas kasılmaları üzerine düzenleyici etkiye sahip olduğu belirtilmektedir. Bu çalışmada, sıçan uterusundaki spontan kasılmalara ghrelin peptidinin etkileri in vitro olarak araştırıldı. Wistar sıçanların kesilmesini takiben miyometrial stripler izole organ banyosuna yerleştirildi. Spontan kasılmaların başlamasından sonra kontrol kasılmalar 10 dakikalık kayıtlar yapıldı ve izole organ banyosuna kümülatif olarak farklı konsantrasyonda ghrelin uygulamaları yapıldı. İstatistiksel analiz için Wilcoxon signed ranks testi kullanıldı. Kontrol ve 0,01 μM, 0,1 μM ve 1 μM ghrelin uygulanması ile oluşan kasılmaların amplitüdü sırasıyla 2,69 ± 0,32 g (n = 6), 2,71 ± 0,31 g (n = 6), 2,92 ± 0,36 g (n = 6) ve 3,46 ± 0,24 g (n = 6) olarak hesaplandı. Kasılmaların frekansı da sırasıyla 5,17 ± 0,75 (n = 6), 5,17 ± 0,75 (n = 6), 5,33 ± 0,48 (n = 6) ve 8,0 ± 0,77 (n = 6) olarak hesaplandı. Sadece kümülatif 1 μM ghrelin uygulama grubunda kasılmaların hem amplitüdü hem de frekansı bakımından istatistiksel olarak anlamlı artış gözlendi ($P < 0,05$). Bu çalışmadaki bilgiler, sıçan miyometriyumundaki spontan kasılmaları ghrelinin indüklediğini kanıtlamıştır.

Anahtar sözcükler: Ghrelin, spontan kasılmalar, miyometriyum, sıçan

Introduction

Ghrelin, an acylated 28-amino acid peptide known to exist in both acylated and des-acylated varieties, recently isolated from the mammalian stomach, has been identified as an endogenous ligand for growth hormone (GH) secretagogue receptors (1). This hormone exerts a strong stimulatory effect on GH secretion in humans (2) and rats (3). Ghrelin stimulates food intake after central and peripheral administration (4). Ghrelin levels in the blood are decreased by food intake in humans (5). Recent discoveries, however, suggest that ghrelin might be produced in other tissues, where it displays marked physiological activities (6-8). In addition to these effects, the reproductive system is another known target for this hormone. Ghrelin mRNA expression has been persistently detected in the rat ovary throughout pregnancy, with higher levels in early pregnancy and lower expression during the later part of gestation. Caminos et al. (9) provide novel evidence for the expression of ghrelin in the cyclic and pregnant rat ovary. Ghrelin is expressed in both human and rat placental tissues (10). Weak expression of ghrelin mRNA has been detected in the non-pregnant human endometrium, and it is dramatically increased in the decidualized endometrium (11). Administration of ghrelin causes a significant increase in milk yield in lactating rats (12). Papotti et al. (13) demonstrated the presence of ghrelin receptor in the uterus.

Its physiological and pharmacological actions on uterus contraction are unknown. Therefore, in the present study we examined the effects of ghrelin on myometrial contraction in rats in vitro.

Materials and methods

Virgin female Wistar rats (200-220 g) obtained from Fırat University Biomedical Unit were used in this study. The animals were kept in the laboratory on a 12-hour light/dark cycle at room temperature (20 ± 3 °C). Myometrial strips were obtained from virgin rats in estrous, determined by daily vaginal smear. These animals were not exposed to any chemical substances. Following decapitation, longitudinal strips (10 mm long, 2 mm wide, and 1 mm thick) were rapidly obtained from the anti-mesenteric edge of the uterine horn. Muscle strips were placed in a jacketed organ bath (5 mL volume) and tied at the bottom end to a fixed metal hook and at the top end to a force displacement transducer using a silk thread. The force displacement transducer was coupled to an amplifier and data acquisition system (Biopac Sys, Ankara, Turkey). The organ bath contained Krebs solution of the following composition: NaCl (154 mM); KCl (5.4 mM); $MgSO_4$ (1.2 mM); glucose (11.5 mM); $CaCl_2$ (2 mM); adjusted to pH 7.4 at 37 °C and continuously aired with 95% oxygen/5% carbon dioxide throughout the experimental period. In this system isometric contractions were recorded throughout the experiments. Following a 30-min equilibration period of the stabilization of myometrial strips with 0.5 g stretch tension and the development of spontaneous contractile activity, control contractions were recorded for 10 min and various concentrations of ghrelin were added to the tissue bath cumulatively. The amplitude (in g) and frequencies (number of contractions in 10 min) of contractions were evaluated at 10-min intervals before and after applications of each dose of ghrelin. All chemicals were purchased from Sigma, and the Wilcoxon signed ranks test was used for statistical analysis.

Results and discussion

After the manifestation of spontaneous contractions, the application of ghrelin augmented the contractile activity of the myometrial strips (Figure 1). The mean peak amplitude of contractions was 2.69 ± 0.32 g (n = 6) in control conditions. The mean peak amplitudes in periods of ghrelin administration were 2.71 ± 0.31 g (n = 6), 2.92 ± 0.36 g (n = 6), and 3.46 ± 0.24 g (n = 6) after applications of 0.01 μM, 0.1 μM, and 1 μM ghrelin, respectively. The ghrelin-induced augmentation in the peak amplitude values of contractions was significant compared to the control only for 1 μM concentration ($P < 0.05$, Figure 2).

The mean frequencies of contractions were 5.17 ± 0.75 (n = 6), 5.17 ± 0.75 (n = 6), 5.33 ± 0.48 (n = 6), and 8.0 ± 0.77 (n = 6) under control conditions and after application of 0.01 μM, 0.1 μM, and 1 μM ghrelin, respectively. The increase in frequency of contractions also was significant only after application of 1 μM ghrelin ($P < 0.05$, Figure 2).

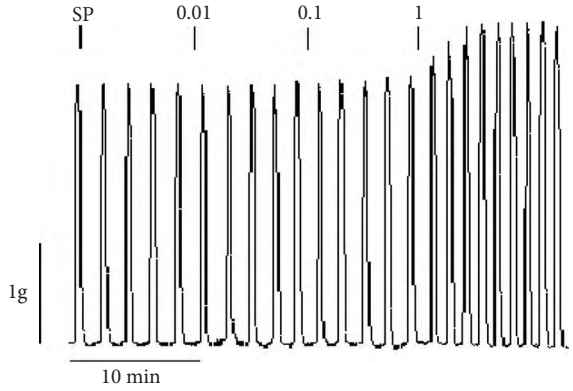

Figure 1. Original trace of a myometrial strip. SP: Spontaneous contractions, 0.01 μM, 0.1 μM, and 1 μM concentrations of ghrelin cumulatively.

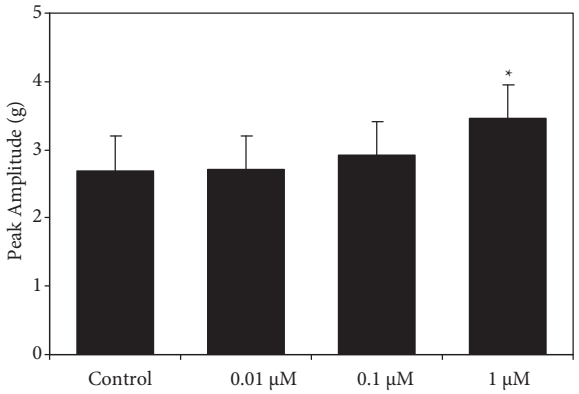

Figure 2. Peak amplitude (gram) levels of groups (n = 6): 0.01 μM, 0.1 μM, and 1 μM concentrations of ghrelin. * P < 0.05 compared to control group using Wilcoxon signed ranks test.

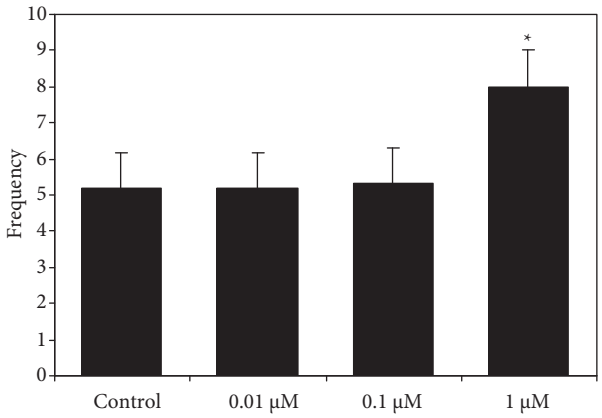

Figure 3. Peak frequencies (number of contractions in 10 min) groups (n = 6): 0.01 μM, 0.1 μM, and 1 μM concentrations of ghrelin. * P < 0.05 compared to control group using Wilcoxon signed ranks test.

The findings of the present study demonstrate that ghrelin has a stimulatory effect on spontaneous contractions in the rat myometrium. The effect was repeatable and long-lasting. To our knowledge this is the first study showing the stimulatory effect of the novel peptide hormone ghrelin on myometrial contractility. This is only a preliminary study and no attempt was made to clarify the possible mechanism of ghrelin's action on myometrial contractility. There are limited studies investigating the effects of ghrelin on smooth muscle contraction. Ghrelin has a relaxing effect on the isolated human internal mammary artery (14) and it has been shown to have in vivo vasodilator effects, possibly through nitric oxide-independent mechanisms (15). Furthermore, ghrelin stimulates gastric motility through activation of the vagal nerve in rats (16). Dimitrova et al. (17) show that, in single smooth muscle cells isolated from guinea-pig renal arteries, ghrelin by itself causes membrane hyperpolarization by activating Ca^{2+}-sensitive K^+ conductance. Our study was an in vitro study, and it is more likely that ghrelin is exerting a contractile effect directly on the myometrium.

The stimulatory effects of ghrelin are likely to be mediated by an increase in free calcium levels in the myometrium. This is plausible, since ghrelin directly interacts with NPY neurons in the arcuate nucleus to induce Ca^{2+} signalling via PKA and N-type calcium channel-dependent mechanisms in the rat (18). Ghrelin induces an increase in the intracellular calcium concentration in porcine somatotropes via an L-type calcium channel (19).

In conclusion, this study demonstrates that ghrelin induces spontaneous contractions in a dose dependent manner in the rat myometrium. There is a need for further studies including in vivo evaluations to determine the possible physiological role of ghrelin in the regulation of myometrial contractility. Its exact mechanism of action on myometrium remains to be clarified.

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