Peripheral ghrelin administration increases bladder capacity without affecting the bladder contraction pressure or electroencephalogram in rats

Matsuta Y, Nagase K, Ishida H, Tanase K, Akino H and Yokoyama O
Department of Urology, Faculty of Medical Sciences, University of Fukui, Japan

Aim of study
Sleep disturbance is one of the causes of nocturia, and we have investigated the association between them. It has been known that ghrelin induces slow-wave sleep via the secretion of the growth hormone\(^1\). Recently, it has been suggested that ghrelin inhibits the contraction of bladder strips \textit{in vitro}\(^2,3\). Thus, we hypothesized that ghrelin alleviate both nocturia and sleep disturbance by increasing the bladder capacity (BC) and the amount of delta wave (i.e., slow-wave) on electroencephalogram (EEG). To evaluate the effects of ghrelin, the continuous cystometric and EEG recordings were performed in the present study.

Materials and methods
Female Sprague-Dawley rats (weighing 200-220 g) were used. During the recording, a rat was placed in the restraining cage. All measurements were performed after the recovery from anesthesia. A cystometric catheter was inserted into bladder dome, and BC and bladder contraction pressure (BCP) were measured. In study 1, ghrelin was administered into the left lateral ventricle to investigate its central effect. In study 2, ghrelin was administered into the right jugular vein during continuous EEG recording to investigate its peripheral effect. To record the EEG, we surgically placed silver ball electrodes in contact with the pia mater of the parietal cortex and occipital cortex (Figure 1). The recording and the analysis of cystometric and EEG data were performed by LabChart\(^5\) (ver. 7.1, ADInstruments). The frequency of delta wave and total power was defined as 0.5 – 3.5 Hz and 0.5 – 30.0 Hz, respectively. Since increasing sleep intensity was defined as an increase in delta wave, we focused on the absolute values of delta wave and the relative values of that to total power.

Ghrelin was dissolved in distilled water, and the concentration was adjusted by physiological saline. The injection volumes of intracerebroventricular (icv) and intravenous (iv) ghrelin administration were 5 μl and 0.1 ml, respectively.

The results are presented as means ± standard errors (SE). All data were analysed by ANOVA with Bonferroni test using IBM \(^\text{\textregistered}\) SPSS \(^\text{\textregistered}\) Statistics (version 19.0). Level of \(p\) < 0.05 was considered statistically significant.

Results

Study 1. \textit{Central effects of ghrelin on BC and BCP}

![Figure 2. Dose-response curves show mean ± SE effect of increasing ghrelin dose from 10μM to 100μM iv on percentage change in BC (A) and BCP (B). Intracerebroventricular ghrelin administration resulted in neither significant BC change nor BCP change.](Image)

Study 2-2. \textit{Peripheral effects of ghrelin on EEG parameters}

![Figure 4. Bars show mean ± SE effect of increasing ghrelin dose from 1μM to 100μM iv on EEG parameters: (A) absolute power values of delta wave, (B) absolute power values of total power, (C) absolute values of delta wave to total power ratio. Intravenous ghrelin administration did not show statistically significant changes on these EEG parameters.](Image)

Study 2-1. \textit{Peripheral effects of ghrelin on BC, BCP}

![Figure 3. Dose-response curves show mean ±SE effect of increasing ghrelin dose from 1μM to 100μM iv on percentage change in BC (A) and BCP (B). Vehicle group curve was obtained by repeated vehicle doses. Intravenous ghrelin administration resulted in significant BC change but did not change BCP. Asterisk indicates ghrelin vs. vehicle ANOVA with Bonferroni test \(p\) < 0.05.](Image)

Conclusions

The effects of ghrelin on smooth-muscle contraction have been controversial. Ghrelin stimulates the motility of the digestive tract; alternatively it has a relaxant effect on the smooth muscle of the vasculature\(^2\). In the present study, our results suggest that ghrelin increases the BC without affecting the BCP as a peripheral action. Ghrelin has been reported to induce sleep\(^1\), however, our results show no significant effect on EEG parameters by the intravenous ghrelin administration. To our knowledge, this is the first \textit{in vivo} study concerning the effect of ghrelin on bladder function. Further studies are required to elucidate the exact effect of ghrelin on the bladder function, and the refinement of experimental procedure might be needed.

References

![Figure 1. The position of EEG electrodes. The coordination of electrodes was as follows: parietal cortex (P) 1.0 mm posterior to bregma and 2.5 mm lateral to midline, occipital cortex (O) 1.0 mm anterior to lambda and 3.5 mm lateral to midline.](Image)