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Sex differences in ileal somatostatin-response after stress conditioning in rats

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Abstract: We investigated the effects of somatostatin on the ileal movements after gravity stress and compared these effects between male and female rats. Using an *in vitro* preparation, measurements of ileal movements evoked by somatostatin application were done at 1, 3, 15 and 30 days after 3G gravity loading-conditioning. Mixed phasic and tonic patterns were observed in the ileal muscle activities. Gravity-stress decreased or antagonized somatostatin inhibitory effects on tonic ileal contraction at an early stage (at day 3) in females, but not in males, indicating that sex differences exist in the way that changes of somatostatin sensitivity is modulated by stress conditioning.

Keywords: Somatostatin, Gravity Stress, Ileum, Sex Difference, Rat

1. Introduction

Stress induces various physiological and hormonal disturbances; in particular, concerning feeding. Previous studies have reported that there is a close relationship between stress and feeding behavior [1-3]. Usually evidenced in both human and animal studies, stress has been shown to affect food intake feeding behavior in a bidirectional fashion, inducing either increases or decreases in food intake [4].

In rats, the severity of the stressor may critically influence the pattern of feeding behavior. For example, chronic and strong stress induces a decrease of in food intake and loss of body weight [5]. By contrast, mild stress increases food intake, especially of highly palatable foods [6,7]. These changes may be in part explained by functional adaptations within the endocrine system [8-10]. For instance, it is generally accepted that corticotropin-releasing hormone (CRH) plays a critical role in the adaptation of the organism to stress. It serves as the main regulating hormone of the hypothalamic-pituitary-adrenal axis, which is activated after exposure to acute stress [11-13]. On the other hand, one of the important gastric hormones to control digestive movements is somatostatin; it inhibits peristalsis in the small intestine of rats

and mice. In the small intestine of rats and mice, somatostatin inhibits peristalsis in the small intestine [14], and in the ileum of guinea pigs, it can have both excitatory and inhibitory effects [15-17]. In addition, there are increasing data concerning a functional relationship between stress and somatostatin. It has been found that in the male rat, cold-restraint stress decreases somatostatin sensitivity in the stomach [18], and that ether-stress increases somatostatin release into the pituitary gland [19]. Therefore, it is thought that the effects of stress on digestive function, and by extension on feeding behavior, could take place through a somatostatin-induced functional modulation of peristalsis. Nevertheless, the relationship between stress and somatostatin is still controversial. Further, sex differences are usually observed in stress responses, including changes in digestive function and feeding behavior [20-22].

Here, we investigated the effects of somatostatin on the ileal movements modulated by gravity stress-conditioning and compared these effects between male and female rats.

2. Methods

The methods described here follow the ethical guidelines and received approval by the Animal Welfare Committee of

Japan Women's University.

One hundred and twenty eight rats of both sexes (Wistar, SPF) were divided into Control (1G) and 3G groups. Measurements of ileal movements were done at 1, 3, 15 and 30 days of gravity-stress conditioning. Each group consisted of 8 rats. Initial body weight (at day 1) was 59.3 g (mean) in males and 60.0 g in females and final body weight (at day 30) was 267.2g in males and 179.2 g in females, respectively.

2.1. Ileal Movement Analysis

For the *in vitro* preparation, a 1 cm-long portion of the ileum was isolated under barbiturate anesthesia (Nembutal, 20 mg/kg, i.p.). The inner contents of the isolated ileum were washed away with Tyrode solution. After that, the isolated ileum was fixed into a Magnus-type chamber filled with Tyrode solution (temperature: 37° C) as reported elsewhere [21]. The proximal end of the preparation was set upward and connected to a strain gauge through a cotton thread (Daruma #30, Yokoi, Osaka, Japan). The distal end of the preparation was fixed to the bottom of the chamber. Ileal movements were amplified by a strain-gauge amplifier (x100) and recorded continuously on a pen-recorder (SS259F2, SEKONIK, Tokyo, Japan).

Ileal movements (spontaneous peristalsis) were observed every 20 s during 60 s following the application of somatostatin. Somatostatin solution (0.3 ml total volume, at the concentration of 10-9 mg/ml) was applied topically onto the ileum preparation using an injection syringe. The recovery time after wash-out was too long to do further experiments, thus somatostatin was not tested in other concentrations. Two phases of ileal movements were estimated, that is, the magnitudes of the somatostatin-evoked peak-to-peak amplitudes of the phasic contraction and the tonic amplitudes between baseline and wave peak of the phasic contraction (see Fig.1). Baseline was defined as the bottom line of the phasic deflection just before somatostatin application. The magnitude of the phasic contraction was estimated as the peak-to-peak amplitude of ileal contraction. The magnitude of the tonic contraction was estimated as the baseline-to-peak phasic amplitudes at 20, 40 and 60s after somatostatin application.

2.2. Stress Conditioning

3G stress was loaded by a centrifugal apparatus (H26-F, Kokusan, Tokyo, Japan) every day at 10:00 AM for 10 min during 30 days, as described elsewhere [20]. During gravity-loading, rats were placed in the mesh case of the centrifuge with a head-central orientation. In the control group, the rats were placed in the centrifuge for 10 min but without rotation.

2.3. Statistics

A one-way ANOVA with repeated measures was used to determine the differences in tonic ileal contraction between stress and control groups. Where significant differences were found, pairwise comparisons at each time point were done by the post-hoc Bonferroni test. P values lower than 0.05 were considered statistically significant. All data are expressed as means ± SEM.

3. Results

Mixed phasic and tonic patterns were observed in the spontaneous activity of ileal smooth muscles. The former were peristalsis-like movements and the latter presented as a slow negative deflection from the baseline (tonus level) (Fig.1 dotted lines).

Somatostatin induced some irregular rhythms but no change in the average peak-to-peak amplitude of the phasic ileal movements. There were no changes in the phasic responses induced by somatostatin application in relation to stress conditioning.

By contrast, tonic contractions were clearly suppressed by somatostatin application (Fig. 1A). Stress conditioning reversed somatostatin suppression of ileal tonic contraction as compared to control (Fig. 1B). Amplitudes of negative deflection induced by somatostatin application were smaller in female, but not in male rats, after 3 days of stress conditioning. (ANOVA with repeated measures, $F_{(2,32)} = 6.038$, $P = 0.006$).
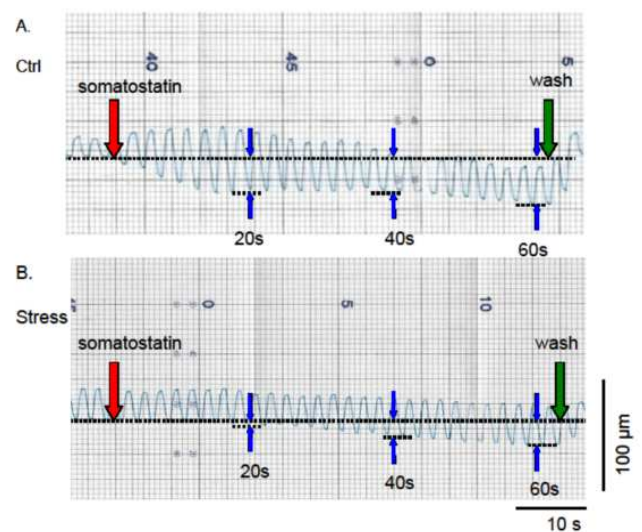

Figure 1. *Typical examples of ileum ileal movements and effects of somatostatin application in the female rat. A: Upper trace:Ileal movements in additional gravity (1G) control rat. B: Lower trace: Ileal movements in 3G stress-conditioned rat. Slow downward movement from the below baseline (dotted line) shows a decrease in tonus. Note that inhibitory effects of somatostatin on the ileal tonus are decreased in 3G gravity stress-conditioned rat as compared to control.*

Significant changes were observed from 20 to 60 s after somatostatin application at day 3 in females (Fig. 2, ANOVA followed by Bonferroni post-hoc test, $P < 0.05$). At day 1, 15 and 30, there were no significant differences in the somatostatin effects between control and stress groups both in males and females.
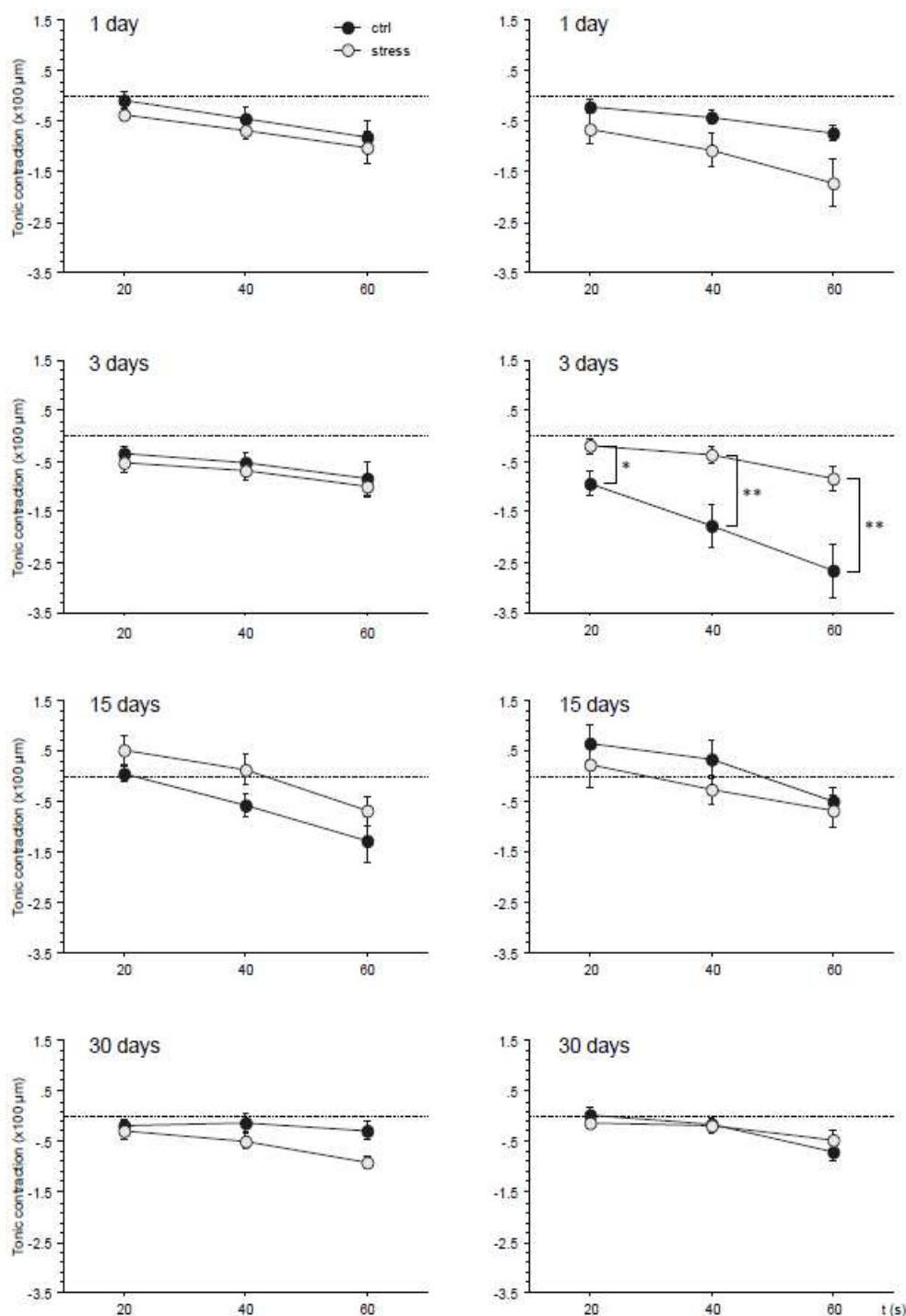
Figure 2. *Somatostatin effects on the ileum tonus in males (left-side graphs) and females (right-side graphs) at day 3. Note that at 3 days of stress conditioning, in female rats, the effects of somatostatin were significant antagonized 40-60 s after its application. *P < 0.05, **P < 0.01, in ANOVA followed by Bonferroni post-hoc test.*

4. Discussion

Several reports indicate that somatostatin inhibits peristalsis in the rat and mouse jejunum [14,16]. Similarly, in the rat ileum, our present data showed that somatostatin inhibited smooth ileum muscle activities, especially its tonic contraction. In addition, we showed that stress-conditioning decreased or antagonized the somatostatin inhibitory effect on tonic ileal contraction transiently in females at an early stage (at day 3 of conditioning), but not in males. This showed clearly that sex differences exist in the way somatostatin sensitivity can be modulated by gravity-stress. These results suggest that, at least in females, alarm mechanisms against stress were activated either by increasing

the ileum tonus level or by decreasing the somatostatin sensitivity. In more detail, the fact that stress antagonized the somatostatin-induced inhibition (i.e., disinhibition) on of the tonic ileal contraction suggests that gravity-stress may decrease the somatostatin sensitivity in the female ileum. Because somatostatin release is reported to increase about 2-fold under stress [19], it is possible that stress conditioning could cause desensitization followed by down-regulation of somatostatin receptors in the ileum.

There are several reports on the sex differences for stress response in the digestive system. In this study, it is not clearly shown why the stress-induced antagonization of the somatostatin effects on the ileal movements was different between males and females. One possible explanation is in the way the endocrine system responds to stress in males and females, which is thought to be critical in determining various physiological functions including digestion [10,23]. The present results in relation to somatostatin responsivity expand on previous data that showed sex differences in stress-induced ileal movements [21,22].

In summary, the present study in rats showed that stress reversed the inhibitory effects of somatostatin on the ileal tonus in female, but not in male, suggesting that sex differences exist in the stress-somatostatin interaction in the digestive system. Changes in the sensitivity to somatostatin may partly explain the differences between males and females in the way the digestive system responds to stress.

Acknowledgement

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