Stress decreases contraction of the colon, and the effects of stress are different among the regions of the colon.

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A running head: STRESS AFFECTS COLON MOTILITY
Stress affects a variety of organs. Diarrhea and constipation are closely related to stress, which involves the gastrointestinal motility of the colon. We compared the gastrointestinal motility of the proximal, mid, and distal colon in mice with stress. Stress was applied by water immersion restraint. Colon motility was measured using an isotonic transducer in the direction of the circular muscles. Electric field stimulation-induced contractions in stressed mice were reduced compared to control mice in the proximal and distal colon. On the other hand, in the mid colon, contraction in control mice and stressed mice were almost same. This interesting difference between the regions may provide a clue to the functional abnormalities in gastrointestinal motility associated with stress.

KEY WORDS: colon, contraction, smooth muscle, stress, transient receptor potential channel
Stress is the body's response to external stimuli [15]. The body naturally responds to stress and copes with difficult or dangerous situations. On the other hand, chronic and excessive stress can be harmful to the body [8]. Stress can cause a variety of diseases and affect a lot of organs in the body including the central nervous system [9, 16, 17], cardiovascular system [6], digestive system [1, 13], and immune system [4, 5]. Diarrhea and constipation are two of the most typical symptoms that appear with stress, and the colon is involved in these [10]. In a previous report, we reported that water immersion restraint stress affected small intestinal motility in mice [3], however we did not analyse colon motility. Based on this background, the purpose of this study is to measure the changes in colon motility due to stress. If the stress is too strong, it can affect various organs including the stomach. Therefore, in this study, we measured whether colon motility was affected under a weak stress.

Water immersion restraint stress was performed as described previously [3] with some modifications. Male C57BL/6 mice (8-10 weeks old) were purchased from CLEA Japan, Inc (Tokyo, Japan). Mice were restrained in a 50 mL conical centrifuge tube with multiple punctures and immersed vertically to the level of the xiphoid process into a 25°C water bath for 3 hr. As shown in Fig. 1, mice were immersed at regular times from 9 am to 12 am daily, and the experiment was carried out on day 7 after 6 days. All procedures used in this study complied with institutional policies of the Osaka Prefecture University Animal Care and Use Committee. (No.23-37 (2009-2014), No.27-56 (2015), No.28-62 (2016)).

Responses to electric field stimulation (EFS) were recorded using previously described methods [12]. Briefly, the muscle strips of the proximal, mid, and distal colons were prepared in the orientation of the layer of circular muscle. Specifically, the strips were exposed to EFS with trains of 100 pulses of 0.5 msec and 30 V for 60 sec. N-nitro-L-arginine (L-NNA; Wako Pure Chemical, Osaka, Japan) (30 μM) was treated 10 min prior to EFS. Contractions were
analyzed by measuring the extent of the maximal contraction in response to 60 mM KCl. Quantitative real-time PCR was performed using a previously described method [11]. The primers were used from a previously described method [3]. The results were expressed as the mean ± S.E. In comparison between the two groups, the statistical significance of the parametric data was evaluated using a two-tailed Student’s t-test. In comparison among 3 groups, statistical significance was determined using one-way ANOVA for non-repeated measures to detect differences among each group. The differences between groups were determined using the Tukey-Kramer test. A p value less than 0.05 was considered significant.

First, we report on mouse phenotype after water immersion restraint stress. There were no significant differences in the body weight of both control mouse and stressed mouse for each day, and in the amount of food intake, weight of stool, and quality of stool (soft or hard) (data not shown). Seven days later, there were no erosions or shallow ulcers in the stomach (data not shown). To measure the colon motility in a stress model, we investigated EFS-induced contractions in the circular smooth muscles obtained from the colon. We previously demonstrated that ACh plays roles as a contractile transmitter and NO plays roles as a relaxational transmitter in the mouse colon [2, 7]. Since we wanted to analyze the contractile response in this study, all experiments were performed in the presence of L-NNA, an inhibitor of NO synthase. Since the effects of stress may differ depending on the intensity of the stimulus, this study was performed under conditions of 1, 3, and 10 Hz. The contractile responses are normalized by the contraction induced by 60 mM KCl. We first confirmed that the magnitudes of the maximal contractions in response to 60 mM KCl were similar between control mouse and stressed mouse (Fig. 2). Figure 3A upper panel shows representative recording traces of contractions to EFS in the control proximal colon and stressed proximal colon. EFS-induced contraction was significantly reduced in the stressed proximal colon (Fig. 3A lower panel). In
Figure 3B, the same experiment was performed for the mid colon. Unlike the proximal colon, there was no change in EFS-induced contractions between control mid colon and stressed mid colon. In addition, the distal colon was analyzed. Figure 3C upper panel shows representative recording traces of contractions to EFS in the control distal colon and stressed distal colon. Like the proximal colon, EFS-induced contraction was significantly reduced in the stressed distal colon (Fig. 3C lower panel). We found that colon motility was reduced under the stress conditions in this study. Actually, the decreases seen for proximal and distal colon were not seen for mid colon. Thus, the reactivity in the three regions of the colon was different. When comparing the degree of decrease, the decrease in proximal colon (41.4-50.9%) was greater than the that in distal colon (49.7-58.8%).

The contractile transmitter of the colon is only acetylcholine, as it is completely eliminated by atropine pretreatment [2]. To investigate the direct response on the circular muscles, we examined the responses to Carbachol (CCh; Sigma-Aldrich, St. Louis, MO, USA). We used two different concentrations of CCh. The contractions induced by 0.3 and 3 μM CCh were significantly reduced in the stressed proximal and distal colon, but not mid colon (Fig. 4). The results also showed that stress affected the proximal and distal colon, but not the mid colon. This difference in the reactivity in the regions of the colon must be due to something. We focused on TRPC3, which we investigated in our previous report [3]. We examined the expression levels of TRPC3 in the colon of the control mouse and stressed mouse. In the control mouse, there was no significant difference in TRPC3 mRNA expression among three regions of the colon, although that in mid colon was lower than those in proximal or distal colon (Fig. 5). In the stressed mouse, TRPC3 expression were reduced in the proximal and distal colon, but not mid colon (Fig. 5). However, the difference was not significant. In a previous report, we found that TRPC3 expression was decreased in the ileum of stressed mice, resulting in a
reduced contractile mechanism [3]. TRP channels underlie non-selective cation currents [14].

TRP channels are downstream effectors of G protein-coupled receptors [14] including muscarinic receptors. It is possible that the decrease in TRPC3 expression due to stress reduced the function of the coupling muscarinic receptors, resulting in decreased contraction.

In summary, we revealed that stress caused a decreased contraction in the proximal and distal colon, but not mid colon. It is suggested that TRPC3 expression levels may be involved in the difference in reactivity among the regions on the colon.

CONFLICT OF INTEREST. We declare that we have no conflicts of interest to declare.

ACKNOWLEDGEMENTS. Declared none.
REFERENCES


Fig. 1. The protocol of water immersion restraint stress model.

Fig. 2. KCl-induced contraction in the colon. KCl-induced contractions in the proximal, mid, and distal colons in control mouse (control) (n=4) and stressed mouse (stress) (n=4). Quantitative data on KCl-induced contractions are expressed as mm.

Fig. 3. Electric field stimulation (EFS)-induced contractions in the colon. EFS-induced contractions in the proximal (A), mid (B), and distal (C) colons in control mouse (control) (n=4) and stressed mouse (stress) (n=4). (Upper) Representative recording traces of EFS-induced contractions are shown. Horizontal lines indicate the duration (60 sec) of EFS. Vertical dotted lines indicate the start and end of stimulation. (Lower) Quantitative data on EFS-induced contractions. Contractions were expressed as percentages of 60 mM KCl-induced contraction. **P<0.01 for vs. control.

Fig. 4. Carbachol-induced contractions in the colon. Carbachol-induced contractions in the proximal, mid, and distal colons in control mouse (control) (n=4) and stressed mouse (stress) (n=4). Carbachol-induced contractions were expressed as percentages of 60 mM KCl-induced contraction. *P<0.05, **P<0.01 for vs. control.

Fig. 5. Expressions of transient potential receptor (TRP) C3 in the colon. The mRNA expressions of TRPC3 from the proximal, mid, and distal colons in control mouse (n=4) were examined using quantitative real-time PCR.
Day 1

3 hr (9:00-12:00)
Water immersion restraint stress

Experiments

Figure 1
Figure 2

The figure shows a bar graph depicting KCl-induced contraction in proximal, mid, and distal regions. The graph compares control and stress conditions. The y-axis represents KCl-induced contraction in millimeters (mm), ranging from 0 to 120. The x-axis indicates the region: Proximal, Mid, and Distal. Each region has bars indicating control and stress conditions, with error bars showing variability.
A. Proximal colon

- EFS at 1 Hz, 3 Hz, and 10 Hz
- Control and stress conditions
- Graph showing contraction

B. Mid colon

- EFS at 1 Hz, 3 Hz, and 10 Hz
- Control and stress conditions
- Graph showing contraction

C. Distal colon

- EFS at 1 Hz, 3 Hz, and 10 Hz
- Control and stress conditions
- Graph showing contraction

Legend:
- Control
- Stress

Note: The graphs show the comparison of contraction responses between control and stress conditions across different frequencies for each colon region.
Figure 4
Figure 5

Bar graph showing the expression levels of TRPC3/HPRT in control and stress conditions across proximal, mid, and distal colon sections. The graph indicates a significant increase in expression in the distal colon under stress conditions compared to control, with a p-value of 0.0650.

**Proximal Colon**
- Control: ~1.0
- Stress: ~0.75

**Mid Colon**
- Control: ~1.0
- Stress: ~0.75

**Distal Colon**
- Control: ~1.25
- Stress: ~1.5

P-values:
- Proximal Colon: P=0.0896
- Distal Colon: P=0.0650