Evaluation of the Effects of Restraint and Footshock Stress on Small Intestinal Motility by an Improved Method Using a Radionuclide, $^{51}$Cr, in the Rat

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The effect of two different stress stimuli, restraint stress and footshock stress, on small intestinal motility was evaluated by a more reliable method with improvement of the previous method using a radionuclide, $^{51}$Cr. The small intestinal transit was significantly inhibited by restraint stress, but not by footshock stress, although plasma corticosterone levels were significantly elevated to the same extent by restraint stress and footshock stress. These results suggest that restraint stress and footshock stress stimuli influence small intestinal motility via different mechanisms, but the reason for the difference is unclear. This experimental system using $^{51}$Cr seems to be useful for the elucidation of mechanisms for restraint stress-induced dysfunction of small intestinal motility because of its excellent quantitative evaluation of small intestinal transit.

Key words restraint stress; footshock stress; small intestine; motility

Today, stress from various disappointments and anxieties caused by the environment of everyday life impairs our health. Clinical evidence has indicated that stress can be associated with gastrointestinal dysfunction, including abdominal pain and diarrhea.1—4 Some clinical studies have also demonstrated that stress alters colonic motility not only in patients with irritable bowel syndrome but also in healthy subjects.5—7 In addition, it has been shown that a relapse of inflammatory bowel disease is frequently preceded by periods of stress.8 Many investigators have reported alterations in colonic motility caused by stress.9—14 However, there are very few reports concerning the influence of stress on small intestinal motility.13—15 Therefore, the etiology of small intestinal dysfunction caused by stress is not completely understood.

We previously developed an available experimental system to evaluate the stress effect on small intestinal motility using a radionuclide, $^{51}$Cr.16 In the present study, we improved the previous method to establish a more reliable experimental system, and compared the effects of restraint stress and footshock stress on small intestinal motility.

MATERIALS AND METHODS

Animals Male Wistar rats (6-weeks old, 150—200 g) were purchased from Japan Shizuoka Laboratory Animal Center (Hamamatsu, Japan), and these were used in all the experiments. The animals were housed in a wire-mesh cage (Osaka, Japan). Other chemicals were of reagent grade.

Reagents Sodium chromate, $^{51}$Cr (37 MBq/ml) was purchased from Amersharm Pharmacia Biotech (Piscataway, NJ, U.S.A.), sterile physiological saline from Fusco Pharmaceuti
cal Industries (Osaka, Japan), heparin sodium from Novo Nordisk (Bagsvaerd, Denmark), and pentobarbital sodium (Nembutal, 50 mg/ml) from Dainippon Pharmaceutical (Osaka, Japan). Other chemicals were of reagent grade.

Cannula Implantation A silicon cannula was implanted by the method of Tsukada et al.16 as follows: the abdomen of anesthetized rats was opened, and a chronic indwelling sili
con cannula (0.5 mm internal diameter and 0.9 mm outer di
diameter, medical tube SH No. 00; Kaneka, Tokyo, Japan) was inserted into the duodenum toward the jejunum and stuck to the lumen of the duodenum with surgical adhesive agent. Rats were housed in groups of four in wire-mesh cages [26 (W)×38 (D)×19 (H) cm] and were allowed to recover from surgery for 2 d. All the experiments were performed in non-fasted rats.

Stress Loading The restraint stress and footshock stress stimuli were used in the present experiment. For the restraint stress, each rat was immobilized in an adjustable restraint de
tive placed on a home cage for 3 h individually, as described previously.16 Footshock stress was performed by applying a constant current (0.6 mA) for 2 s every 30 s for 0.5 h using a 3-channel shock generator (Muromachi Co., Ltd.).17

Measurement of Small Intestinal Transit Sodium chromate ($^{51}$Cr) solution was diluted to 250 kBq/ml with ster
e physiological saline. A 0.2 ml portion of the diluted solu
tion (50 kBq) was directly infused into the duodenum via the implanted cannula immediately after the end of stress-loading. Twenty minutes after the administration of the radioac
tive marker, the rat was killed by cervical dislocation and the small intestine (from duodenum to ileum) was removed with
tout spillage of contents. The small intestine was divided into 20 eq segments. Each segment was placed into a glass tube and the radioactivity of $^{51}$Cr was counted in a well-type γ-counter (ARC-300, ALOKA, Tokyo). The geometric center (GC) of transit was calculated as a quantitative evaluation,18 according to the following equation:

\[ \text{GC} = \frac{1}{n} \sum (\text{counts per segment} \times \text{segment number/total count}) \]

The GC values ranged from 1 to 20, such that the GC value of 1 indicated no transit, and the GC value of 20 indicated the maximal transit.

Measurement of Plasma Corticosterone Blood was drawn from the abdominal aorta after cervical dislocation and centrifuged at 1900×g for 10 min at 4 °C. Plasma cortico
costerone was determined by a commercially available im-
munoassay (double antibody \([^{125}\text{I}]\)RIA kit, ICN Biomedicals, Inc., Costa Mesa, CA, U.S.A.).

**Statistics** The differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by Fisher’s protected least significant difference test (PLSD) with a significance level of \(p<0.05\).

**RESULTS**

The changes in plasma corticosterone levels after stress loading are shown in Fig. 1. The plasma corticosterone concentration was 15.3±3.3 ng/ml (mean±S.E.M.) in normal rats. Plasma corticosterone concentrations of 3 h-restraint stress and 0.5 h-footshock stress were significantly elevated, to 473.1±30.7 and 476.7±32.2 ng/ml, respectively.

The distribution patterns of \(^{51}\text{Cr}\) in normal and stress loading rats are shown in Fig. 2. The peak radioactivity of \(^{51}\text{Cr}\) existed at 60—65% of the total small intestinal length in normal rats. In restraint stress loading rats, the peak radioactivity of \(^{51}\text{Cr}\) shifted to 45—55% of the total small intestinal length. However, in 0.5 h-footshock stress-loading rats, a broad peak of \(^{51}\text{Cr}\)-distribution, 55—70% of the total small intestinal length, was observed.

The changes in GC values after stress loading are shown in Fig. 3. The GC value of normal rats was 5.33±0.09 (mean±S.E.M.). The GC value was significantly inhibited to 4.21±0.29 by restraint stress, but was not influenced by 0.5 h-footshock stress.

**DISCUSSION**

Gastrointestinal dysfunction is produced by stress.1—4) Experimental studies have reported that stress induces an alteration in the colonic motility of both healthy subjects and patients with irritable bowel syndrome.5—7) However, the actual cause of intestinal dysfunction by stress is completely unknown, and the lack of an appropriate animal model has been an obstacle to studies of causality of the influence of stress on small intestinal motility.

We have already reported an experimental system for the evaluation of the effect of stress on small intestinal motility using a radionuclide, \(^{51}\text{Cr}\).16) However, only an approximate value could be obtained by the previous method, since a NaI-scintillation survey meter was used for continuous monitoring of radioactivity. In the present study, we compared the effects of restraint stress and footshock stress on small intestinal motility using an improved method of the previous one, with some modifications, as follows: a) lower radioactivity of \(^{51}\text{Cr}\), 50 kBq, was injected; b) the small intestine was divided into 20 eq segments, and the radioactivity of each individual segment was measured by a well-type \(\gamma\)-counter; c) estimation of small intestinal motility was performed by the calculation of GC. There was no difference in \(^{51}\text{Cr}\)-distribution patterns of the small intestine when compared with that of a previous method.16)

Sufficient stress stimuli seem to be given in both the restraint stress and footshock stress models, because of a similar increase in plasma corticosterone levels. The distribution patterns of \(^{51}\text{Cr}\), however, were different between both the stress models. Small intestinal motility was inhibited by restraint stress, but not by footshock stress, as shown in Fig. 2.
The GC method has been shown to be a more reliable measure of intestinal transit than the most distal segment and slope methods. The GC value of small intestinal transit was significantly decreased by restraint stress, but not by footshock stress as shown in Fig. 3. The GC value of the small intestinal transit of footshock-stressed rats was not different from that of normal rats. It has been well known that both footshock stress and restraint stress elevate the plasma concentration of catecholamines.24) Generally, opioid peptides have been well known to inhibit small intestinal propulsive motility in normal species.25) Taniyama and coworkers have shown that the activation of enteric μ-opioid receptors leads to the inhibition of cholinergic and adrenergic neurons, resulting in dual effects on the motility of guinea pig intestine in vivo.26) Considering these findings, the ineffectiveness of footshock stress on small intestinal transit could be due to the significant increase in plasma β-endorphin22,23) whereas Williams et al. have reported that the restraint stress only slightly elevates plasma β-endorphin concentration.13) We also observed that the increase in plasma β-endorphin after footshock stress was higher than that after restraint stress (data not shown). Galligan et al. have reported that footshock stress hardly affects gastrointestinal motility.24) Generally, opioid peptides have been well known to inhibit small intestinal propulsive motility in normal species.25) Taniyama and coworkers have shown that the activation of enteric μ-opioid receptors leads to the inhibition of cholinergic and adrenergic neurons, resulting in dual effects on the motility of guinea pig intestine in vivo.26) Considering these findings, the ineffectiveness of footshock stress on small intestinal transit could be due to the significant increase in plasma β-endorphin.

Thus, we plan to investigate the mechanism of restraint stress-induced inhibition of small intestinal motility using the present experimental system. Furthermore, this experimental system promises to be useful for developing anti-stress drugs against gastrointestinal dysfunction.

REFERENCES