Development of an Experimental System for Evaluation of the Effect of Stress on Intestinal Motility Using a Radionuclide, ^{51}Cr , in the Rat

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We attempted to develop an experimental system for evaluation of the influence of stress on small intestinal motility using a radionuclide, ^{51}Cr , and an acute restraint stress model. Each rat was immobilized in an adjustable restraint device for 1 to 5 h. The rat was given ^{51}Cr immediately after the end of stress loading through a catheter inserted into the duodenum, and sacrificed 20 min after administration. The small intestine was removed and the ^{51}Cr radioactivity was continuously monitored with an NaI-scintillation survey meter. The small intestinal motility was estimated by the distance of the radioactive peak from the top of the duodenum. The small intestinal motility was significantly inhibited by 3 h-restraint stress. This experimental system is easy, rapid, and simple for the evaluation of the effect of stress on small intestinal motility.

Key words ^{51}Cr ; restraint stress; small intestine; motility

Today, a variety of types of stress from various disappointments and anxieties caused by aspects of every-day life impair our health. Much clinical evidence has indicated that stress is associated with gastrointestinal dysfunction, including abdominal pain and diarrhea.¹⁻⁴⁾ Experimental studies have also demonstrated that stress alters the colonic motility in not only patients with irritable bowel syndrome but also 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 produced by stress.⁹⁻¹⁴⁾ However, there are very few reports about the effect of stress on small intestinal motility.¹³⁻¹⁵⁾ Therefore, the etiology of small intestinal dysfunction caused by stress is not completely understood. The main reason for the absence of such investigations may be the lack of an appropriate experimental system for evaluating the effect of stress on small intestinal motility. In this study, we attempted to develop a suitable experimental system to estimate the effect of stress on small intestinal motility using the radionuclide, ^{51}Cr .

Kaneka, Tokyo, Japan) was inserted into the duodenum toward the jejunum and stuck to the lumen of the duodenum with surgical adhesive agent. The cannula was brought subcutaneously to the posterior head region and fixed to the scalp with surgical adhesive agent (Fig. 1). Rats were housed in groups of four in wire-mesh cages and allowed to recover from surgery for 2 d. All the experiments were performed under non-fasted condition.

Stress Loading and Measurement of Small Intestinal Transit Each rat was immobilized in an adjustable restraint device (Fig. 2) for 1, 3 and 5 h. Sodium chromate (^{51}Cr) solution was diluted to 1 MBq/ml with sterile physiological saline and 0.2 ml of the diluted solution (200 kBq) was directly infused into the duodenum via the implanted cannula, immediately after the end of stress loading. Twenty min after the administration of radioactive marker, the rat was killed by cervical dislocation and the small intestine (from the duodenum to the ileum) was removed without spillage of its contents. The radioactivity of ^{51}Cr in the small intestine was continuously monitored every 2 cm segment for ten seconds with an

MATERIALS AND METHODS

Animals Male Wistar rats (6-week old, 150–200 g) were used in all the experiments. The animals were housed in a wire-mesh cage in a temperature ($23 \pm 1^\circ\text{C}$) and humidity ($55 \pm 5\%$) controlled room under a 12 h light–dark cycle (light 8:30 am to 8:30 pm) and allowed free access to tap water and Rat Chow.

Reagents Sodium chromate, ^{51}Cr (37 MBq/ml) was purchased from Amersham Pharmacia Biotech (Piscataway, NJ, U.S.A.), sterile physiological saline from Fuso Pharmaceutical 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 Operative procedure was a modified method of Tadano *et al.*¹⁶⁾ Rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), the abdomen was opened by midline incision (approximately 2–3 cm length), and a chronic indwelling silicon cannula (0.5 mm internal diameter and 0.9 mm outer diameter, medical tube SH No. 00;

Fig. 1. Cannula Implantation

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Fig. 2. Apparatus for Restraint Stress

Fig. 3. Radioactivity Monitoring System for Small Intestine

NaI scintillation survey meter (Fig. 3). The small intestinal motility was estimated by the distance of the radioactive peak from the top of the duodenum.

Statistics Statistical differences among groups were analyzed by one-way analysis of variance followed by Scheffe's *F* test.

RESULTS AND DISCUSION

Stress is associated with gastrointestinal dysfunction, including abdominal pain and diarrhea.¹⁻⁴ Some studies have demonstrated stress-related alterations in colonic motility in both healthy subjects and patients with irritable bowel syndrome.⁵⁻⁷ However, the etiology of intestinal dysfunction produced by stress is completely unknown, and the lack of an appropriate animal model has been an obstacle to studies to find the cause of the effect of stress on small intestinal motility.

In contrast to many reports describing stress effects on colonic motility, only a few investigators have reported alterations in small intestinal motility produced by stress.¹³⁻¹⁵ In the present study, we attempted to develop an experimental system and stress model to examine small intestinal motility.

Table 1 shows the effect of stress on small intestinal motility. The mean distance of small intestinal transit of normal

Table 1. Effect of Stress on Small Intestinal Motility

Stress condition	Transit of ⁵¹ Cr in small intestine (mean ± S.E.) (cm)
Normal	40.0 ± 1.1
1 h-restraint	42.0 ± 1.1
3 h-restraint	32.0 ± 1.0 ^{a)}
5 h-restraint	31.3 ± 2.1 ^{b)}

Significantly different from normal value by one-way ANOVA. a) *p* < 0.05. b) *p* < 0.01. Number of animals in each group was 4 to 6.

Fig. 4. Typical Patterns of Small Intestinal Motility in Normal and Restrainted Rats

—●— normal, —■— 1 h-restraint, —▲— 3 h-restraint, —◆— 5 h-restraint.

rats was 40 cm. On the other hand, the mean distance of small intestinal transit of 1 h-, 3 h-, and 5 h-restraint stressed rats was 42, 32 and 31 cm, respectively. Inhibition of small intestinal motility was observed after stress produced by 3 h- and 5 h-restraint. Figure 4 shows a typical pattern of small intestinal motility in normal and restraint-stressed rats. In contrast to the stimulation of colonic motility by stress,⁹⁻¹⁴ small intestinal motility was significantly inhibited by stress produced by 3 h- and 5 h-restraint. This inhibitory effect of stress is in good agreement with the report of Williams *et al.*^{13,14} Since almost the same inhibitory potencies were observed for stress produced by 3 h- and 5 h-restraint, the shorter 3 h-restraint seems appropriate for the estimation of the effect of stress on small intestinal motility.

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