Mechanism of Inhibition of Small Intestinal Motility by Restraint Stress Differs from That with Norepinephrine Treatment in Rats

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We have previously reported that restraint stress inhibits small intestinal motility in rats, and that the adrenergic β₁-antagonist SR59230A administration recovered the inhibition. In the present study, we compared the effects of restraint stress and norepinephrine on small intestinal motility using α₁- and β₁-adrenergic antagonists. SR59230A did not recover the norepinephrine-induced inhibition of small intestinal motility. The norepinephrine-induced inhibition of small intestinal motility was recovered after administration of the α₁-antagonist yohimbine, but not by α₁-, β₁-, and β₂-antagonists. Considering these results, it is reasonable to assume that the mechanisms of inhibition of small intestinal motility due to restraint stress and norepinephrine treatment are different.

Key words restraint stress; norepinephrine; small intestine; motility; adrenoceptor antagonist

The sensitivity of the gastrointestinal tract to stress has been demonstrated in the clinical setting for over half a century.1,2 Psychological and physical stress stimuli are associated with gastrointestinal dysfunction, including abdominal pain and diarrhea.3–5 One clinical study demonstrated stress-induced alteration of colonic motility in both healthy subjects and patients with irritable bowel syndrome.6 The effects of stress on gastrointestinal motility are conflicting: an acceleration as well as a delay of the motility by stress have been reported in small intestine,6–9 and in colon.10,11–13

We observed that small intestinal motility was inhibited by restraint stress,14,15 and stimulation of β₁-adrenergic receptors is associated with this inhibition while α₁- and α₂-β₁- and β₂-adrenoceptors are not.16 It is well known that the sympathetic nervous system is strongly stimulated by restraint stress.17,18 To ascertain whether the inhibition mechanism of restraint stress is similar to that of norepinephrine, we examined the effect of norepinephrine on small intestinal motility and the influence of the adrenoceptor antagonists prazosin (α₁-antagonist), yohimbine (α₂-antagonist), atenolol (β₁-antagonist), ICI-118,551 (β₂-antagonist), and SR59230A (α₁-antagonist) on the action of norepinephrine.

MATERIALS AND METHODS

Animals Male Wistar rats (6 weeks old, 150–200 g) were purchased from Japan Shizuoka Laboratory Animal Center (Hamamatsu, Japan) and used in all the experiments according to the Guidelines for Animal Experimentation of Tohoku Pharmaceutical University. The animals were housed in a wire-mesh cage [26 (W)×38 (D)×19 (H) cm] placed in a chamber with a constant temperature (23±1 °C), humidity (55±5%), and a 12-h light–dark cycle (light 08:30 to 20:30). The animals were given free access to tap water and rat chow.

Reagents Sodium chromate (51Cr) was purchased from Amersham Pharmacia Biotech (Piscataway, NJ, U.S.A.), heparin sodium from Novo Nordisk ( Bagsvaerd, Denmark), pentobarbital sodium (Nembutal, 50 mg/ml) from Dainippon Pharmaceutical (Osaka, Japan), l-noradrenaline bitartate from Tokyo Kasei Co., Ltd., prazosin HCl from Sigma Aldrich Japan (Tokyo), yohimbine HCl from Nacalai Tesque (Kyoto, Japan), atenolol from Wako Pure Chemicals (Tokyo), and ICI-118,551 Na salt (1-[(2,3-dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride) from Funakoshi (Tokyo). SR59230A (3-(2-ethylphenoxy)-1-[(1S)-1,2,3,4-tetrahydro-naphtha-1-ylamino]-(2S)-2-propanol oxalate) was a kind gift from Mitsubishi-Tokyo Pharmaceutical (Tokyo). All other chemicals were of reagent grade.

Cannula Implantation Cannula implantation was performed according to the method of Tsukada et al.19 The rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), the abdomen opened by a midline incision (approximately 2–3 cm in length), and a chronic indwelling silicon cannula (0.5 mm internal diameter and 0.9 mm outer diameter, medical tube SH No. 00; Kaneka, Tokyo) was inserted into the duodenum toward the jejunum and fixed to the lumen of the duodenum with a surgical adhesive agent. The cannula was passed subcutaneously to the posterior head region and fixed to the scalp with a surgical adhesive agent. The rats were housed in groups of four in wire-mesh cages and allowed to recover from the surgery for 2 d. All experiments were performed under nonfasting conditions.

Stress Loading and Drug Administration Stress loading was performed according to a previously reported method.14 Each rat was immobilized in an adjustable restraint device for 3 h. Norepinephrine (0.4 mg/kg as free base) was intraperitoneally injected into each normal rat. Adrenergic α₁- and β-intagonists (1 mg/kg as free base) were intraperitoneally injected 10 min before the stress loading or administration of norepinephrine.

Measurement of Small Intestinal Motility 51Cr solution was diluted to 250 kBq/ml with sterile physiological saline. A 0.2-ml aliquot of the diluted solution (50 kBq) was directly infused into the duodenum via the implanted cannula immediately after the end of the stress loading or 30 min after the administration of norepinephrine. Ten minutes after the administration of 51Cr, the rats were killed by cervical dislocation and the small intestine (from duodenum to ileum) removed without spillage of its contents. The small intestine was divided into 20 equal segments. Each segment was placed into a glass tube and the 51Cr radioactivity counted in...
a well-type NaI scintillation γ-counter (ARC-300, Aloka, Japan). The geometric center (GC) of motility was calculated as a quantitative evaluation, according to the following equation:

\[ \text{GC} = \sum \frac{\text{counts per segment} \times \text{segment number}}{\text{total count}} \]

The GC values ranged from 1 to 20, with a GC value of 1 indicating no motility and a value of 20 indicating maximal motility.

**Statistical Analysis** All values are represented as the mean±S.E.M. 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**

Figure 1 shows the changes in the distribution pattern of \(^{51}\text{Cr}\) in the rat small intestine after restraint stress or administration of norepinephrine, and the effects of the adrenergic \( \beta_2 \)-antagonist SR59230A (1 mg/kg) on the changes. Both restraint stress and administration of norepinephrine (0.4 mg/kg) inhibited small intestinal motility. However, the inhibition patterns were different from each other. The peak of \(^{51}\text{Cr}\) after restraint stress shifted to 45—55% of the total small intestinal length. On the other hand, after norepinephrine administration, the peak appeared in 55% and appreciable radioactivity existed in 0—5% of the total small intestinal length. Pretreatment with SR59230A overcame the restraint stress-induced inhibition, but did not influence the norepinephrine-induced inhibition.

Figure 2 shows the effects of adrenergic \( \alpha_1 \), \( \alpha_2 \), \( \beta_1 \), and \( \beta_2 \)-antagonists on the changes in the distribution pattern of \(^{51}\text{Cr}\) in the small intestine after administration of norepinephrine. The peak of \(^{51}\text{Cr}\) after treatment with the \( \alpha_1 \)-antagonist prazosin shifted to 45% from 55% (norepinephrine only) of the total small intestinal length, but the radioactivity in 0—5% of the length decreased. On the other hand, the peak after treatment with the \( \alpha_2 \)-antagonist yohimbine shifted to 75% of the total length, and the radioactivity in 55% (norepinephrine only) decreased. Both \( \beta_1 \)-antagonist atenolol and \( \beta_2 \)-antagonist ICI-118,551 had no effect on the norepinephrine-induced inhibition.

![Fig. 1. Changes in Distribution Pattern of \(^{51}\text{Cr}\) by Restraint Stress or Administration of Norepinephrine (0.4 mg/kg), and the Effect of the \( \beta_2 \)-Adrenoceptor Antagonist SR59230A (1 mg/kg) on the Changes](image1)

A) Normal, B) restraint stress and SR59230A plus restraint stress, C) norepinephrine and SR59230A plus norepinephrine. Each value represents the mean±S.E.M. of 5—9 rats.

![Fig. 2. Changes in Distribution Pattern of \(^{51}\text{Cr}\) after Administration of Norepinephrine (0.4 mg/kg) and the Effects of \( \alpha_1 \), \( \alpha_2 \), \( \beta_1 \), and \( \beta_2 \)-Adrenoceptor Antagonists (1 mg/kg) on the Changes](image2)

Each value represents the mean±S.E.M. of 5—9 rats.
adrenoceptors may include a presynaptic one, since norepinephrine-induced inhibition of small intestinal motility was observed that small intestinal motility was inhibited by the stimulation of 51Cr with yohimbine pretreatment after norepinephrine.16) The plasma level of norepinephrine has been not result in recovery of the norepinephrine-induced inhibition after norepinephrine. The decrease in the GC value after norepinephrine was significantly recovered by pretreatment with yohimbine, but not by that with prazosin, atenolol, and ICI-118,551. 

**DISCUSSION**

We have previously demonstrated that small intestinal motility is inhibited by restraint stress,14,15) and that this inhibition is recovered by the β2-adrenoceptor antagonist SR59230A, but not by α1-, α2-, β2-, and β2-adrenoceptor antagonists.16) The plasma level of norepinephrine has been known to increase with restraint stress.17,18) Therefore we compared the effects of restraint stress and norepinephrine on small intestinal motility using α and β-adrenergic antagonists. From the results of GC value calculation, restraint stress and norepinephrine administration was not similar. Moreover, the adrenergic β2-antagonist SR59230A did not result in recovery of the norepinephrine-induced inhibition of small intestinal motility, although the restraint stress-induced inhibition was recovered.16) In terms of GC value, the norepinephrine-induced inhibition of small intestinal motility was not recovered by α1-, β1-, and β2-antagonists, but was recovered by the α2-antagonist yohimbine. On the other hand, we have reported that yohimbine did not recover the restraint stress-induced inhibition.16) In addition, we also observed that small intestinal motility was inhibited by the α2-agonist clonidine (data not shown). These findings suggest that norepinephrine inhibits!small intestinal motility via stimulation of α2-adrenoceptors. This stimulation of α2-adrenoceptors may include a presynaptic one, since norepinephrine inhibits the release of acetylcholine via activation of presynaptic α2-adrenoceptors.20,21) Moreover, the distribution pattern of 51Cr with yohimbine pretreatment after norepinephrine is different from that of SR59230A-induced recovery of the small intestinal motility inhibition due to restraint stress. Considering these results, it is reasonable to assume that the mechanisms of inhibition of small intestinal motility by restraint stress and administration of norepinephrine are different. It is suggested that the restraint stress-induced inhibition of small intestinal motility does not occur only by stimulation of the sympathetic nervous system, since inhibition of the opioidergic pathway may be also involved.22) With restraint stress, it is possible that α2-adrenergic action is inhibited, or alternatively that β2-adrenergic action predominates as a result of neural interactions between nervous systems activated by the stress.

**REFERENCES**