Novelty Stress Increases Fecal Pellet Output in Mongolian Gerbils: Effects of Several Drugs

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Abstract. Stress-induced colonic functional changes have been investigated mainly under conditions involving physical stress, like in the restraint stress model. In this study, we established a new stress-induced defecation model involving the placement of Mongolian gerbils in a novel environment (novelty stress) and determined the effects of several drugs on novelty stress-induced fecal pellet output. When animals kept in groups were placed individually in small cages, the fecal pellet output markedly increased, although the upper intestinal transit measured by charcoal method was not changed. The concentration of plasma adrenocorticotropic hormone was moderately but significantly increased by the novelty stress. Drugs reportedly effective for stress-induced defecation, like alosetron hydrochloride, atropine sulfate, and trimebutine maleate, inhibited both the novelty stress-induced increase in fecal pellet output and spontaneous defecation. In contrast, TAK-637, a tachykinin NK₁-receptor antagonist, and diazepam inhibited the novelty stress induced defecation but did not inhibit spontaneous defecation. The present study indicated that novelty stress increases fecal pellet output without affecting the upper intestinal transit; this model may be useful for evaluating the effects of drugs on stress-stimulated colonic motility.

Keywords: novelty stress, defecation, intestinal transit, fecal pellet, irritable bowel syndrome

Introduction

Physical or psychological stress affects various physiological factors, such as the secretion of hormones, the activity of autonomic neurons, and the regulation of the immune system. These factors are closely associated with the regulation of gastrointestinal functions. In humans and experimental animals, many reports have indicated that stress affects gastric emptying, gastric secretion, intestinal transit, and colonic motility (1, 2). In the colon, stress has been reported to stimulate motility via the activation of autonomic neurons, increasing fecal pellet output, or diarrhea (3, 4). To evaluate the relationship between stress and lower intestinal or colonic motility, several experimental models such as cage-restraint, wrap-restraint, and water-avoidance stress models have been used (5, 6). Among these models, the cage-restraint and wrap-restraint stress models are the most frequently used because these models do not induce organ injury and the procedure is simple (7, 8). Water-avoidance stress is a psychological stress model that does not involve physical restraint, but a large area is needed for the experiment. In humans, stress is mainly caused by psychological factors. Stress has been shown to increase colonic motility in both normal subjects and IBS patients (9, 10).

IBS is characterized by chronically abnormal bowel habits (either diarrhea or constipation or both), in most cases accompanied by abdominal pain. To develop new therapeutics for IBS, the effects of new drugs must be evaluated in experimental models under conditions similar to those causing the disease. Although the precise pathophysiological mechanisms of IBS have...
not yet been elucidated, the symptoms of patients reportedly worsened with mental stress and improved after the administration of anxiolytics in some serious cases, suggesting that psychological stress is deeply associated with the pathophysiology of IBS (11, 12).

The aim of this study was to establish a new non-physical stress model that would be useful for evaluating the effects of drugs on stress-induced colonic functions for the development of new therapeutics for IBS. Firstly we demonstrated that a novel environment stimulates fecal pellet output; secondly, we examined the effect of several drugs, reported to inhibit restraint stress-induced defecation, on novelty stress-induced defecation.

Materials and Methods

Experimental animals

Male, 15- to 18-week-old MGS/Sea Mongolian gerbils (Seac Yoshitomi, Fukuoka) weighing 60 – 120 g were used. Before the experiments, the animals were housed under standard controlled environmental conditions with a 12-h light/dark cycle and with food (CE-2; Clea Japan Inc., Tokyo) and water provided ad libitum. The animals were handled by the researcher every second day for two weeks before the experiment. For the experiment investigating intestinal transit during stress, the animals were not given any food overnight. The care and use of the animals and the experimental protocol of this study were approved by Takeda Pharmaceutical Company’s Experimental Animal Care and Use Committee.

Drugs and treatments

TAK-637 {(eR9R)}-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7H-[1,4]diazocino[2,1-g][1,7]naphthyridine-6,13-dione and alosetron hydrochloride were synthesized at Takeda Pharmaceutical Company, Ltd. (Osaka). Diazepam, atropine sulfate, and gum arabic were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Methylcellulose was purchased from Shinetsu Chemical Industries (Tokyo). All drugs were suspended in a 0.5% methylcellulose solution and administered orally in a volume of 4 mL/kg. Charcoal was suspended in a 5% gum arabic solution and administered in a volume of 0.5 mL/animal.

Novelty stress-induced fecal pellet output

The animals were housed in a standard home cage (size: 25.5 × 38 × 18.5 cm, 10 animals per cage) for at least 2 weeks to allow them to become accustomed to their environment. On the day of the experiment, each animal was put in an individual small cage (size: 13.5 × 27 × 12 cm) and left there for 2 h without any restraints. We named this procedure “novelty stress.” During the novelty stress, the animals were fed normal chow and supplied water ad libitum.

In the first series of experiments, we examined the time course changes in fecal pellet output during the novelty stress. The fecal pellets of each animal were collected every hour from 0 to 5 h after placement in the new environment. In the non-stress (normal) group, we collected all feces expelled by 10 animals in their home-cage every hour and obtained the average per animal. In the second series of experiments, we examined the effects of test drugs on novelty stress-induced fecal pellet output. The animals were given a test drug 0.5 h (alosetron) or 1 h (other drugs) before the 2-h novelty stress. Each animal in the control group was given the same volume of the vehicle alone. The animals in the normal group were left in the home cage for 2 h and the average excretion of fecal pellets by each animal was calculated.

Spontaneous defecation

The animals were placed individually in a small cage for over 20 h before the experiment to allow them to become acclimatized to their environment. The animals were fed normal chow and supplied water ad libitum. The fecal pellet output for 4 h after drug administration was determined.

Measurement of plasma ACTH concentration

The plasma ACTH concentration during the novelty stress was measured using the radioimmunoassay method. The animals were killed by detruncation at 0, 0.25, 0.5, 1, or 2 h after the start of the novelty stress. The ACTH value at 0 min was determined using the plasma concentration of the normal group in the home-cage. To prevent coagulation, 6 mM of ethylenediaminetetraacetic acid was added to 1/50th of the blood sample’s volume, and the blood samples were centrifuged for 15 min at 3,000 rpm. The plasma was collected and frozen at −80°C until assayed. The ACTH concentration was measured using a radioimmunoassay (ACTH IRMA Mitsubishi; Mitsubishi Chemical Industries, Tokyo).

Measurement of upper intestinal transit

Upper intestinal transit was evaluated using a charcoal method (13). The time required for upper intestinal transit through about half of the small intestine was determined to be 7 min in our preliminary study. Animals were deprived of food but given free access to
water for about 20 h before the experiment. Soon after
the oral administration of a suspended solution contain-
ing 10% charcoal and 5% gum arabic solution in dis-
tilled water, the animals were exposed to the novelty stress. Seven minutes later, the animals were killed by excess inhalation of carbon dioxide gas and the abdomen was opened. The stomach and intestine were dissected and laid out longitudinally. The intestine was not stretched to avoid displacing the intraluminal contents. The total length of the small intestine (A) and the length filled with the black charcoal (B) from the pyloric ring were measured. The upper intestinal transit index (%) was then calculated using the following formula:

Upper intestinal transit index (%) = (B / A) × 100

For comparison, the effect of cage-restraint stress on upper intestinal transit was also investigated. Animals housed individually in a small cage (size: 13.5 × 27 × 12 cm) for more than 2 h were placed individually in plastic cylinders (size: inner diameter = 3.6 cm, length = 14 cm), where they could not turn around. In the normal group, the animals were left in their individual cages without any restraints. The procedure for measuring upper intestinal transit was identical to that used in the novelty stress experiment.

Statistical analyses

All data were expressed as the mean ± S.E.M. The statistical significance of the differences among groups was determined using Dunnett’s test or Student’s t-test. In the experiment measuring the time course of the plasma ACTH concentration, the statistical evaluation was conducted using the log value. The inhibitory rate of each stress-stimulated group was calculated by defining the mean value of the normal group as 0% and that of the control group as 100%. The ID_{50} value of the inhibitory rate in each dosage group was calculated using a linear regression analysis.

Results

Novelty stress-induced fecal pellet output and plasma ACTH level

The animals that were moved from the home cage to individual cages displayed active exploratory behavior and excreted more fecal pellets than the non-stress group of animals. Figure 1 shows the time course of the changes in fecal pellet output during the novelty stress. Novelty stress markedly increased fecal pellet output during the first hour, after which defecation moderately decreased. Novelty stress significantly increased the plasma ACTH concentration (Fig. 2); the plasma ACTH concentration significantly increased 30 min after the start of the novelty stress, and the elevated plasma ACTH level was sustained for at least 1.5 h.

Upper intestinal transit during novelty or restraint stress

To evaluate whether novelty stress affects upper intestinal motility, we compared intestinal transit during novelty stress and during cage-restraint stress. Intestinal transit in the novelty stress group was almost the same as in the normal group; the upper intestinal transit index (%) in the non-stress group was 53 ± 5%, while that in the novelty stress group was 50 ± 4% (Fig. 3A). However, cage-restraint stress significantly inhibited intestinal transit; the upper intestinal transit indexes were 52 ± 2% in the non-stress group and 31 ± 3% (P<0.01) in the restraint stress group (Fig. 3B).
Effects of several drugs on novelty stress-induced defecation

The effects of several drugs on novelty stress-induced defecation were examined. All of the drugs examined have been reported to inhibit restraint stress-induced defecation: TAK-637 (a tachykinin NK1-receptor antagonist), alosetron hydrochloride (a 5-HT3-receptor antagonist), trimebutine maleate (a peripheral opioid receptor agonist), atropine sulfate (a muscarine-receptor agonist), and diazepam (a GABA_A-receptor agonist). TAK-637, alosetron hydrochloride, trimebutine maleate, and atropine sulfate exhibited dose-dependent and significant inhibitory effects on novelty stress-induced defecation, with ID_{50} values of 0.03, 0.38, 12, and 0.29 mg/kg, respectively (Fig. 4: A – D). Atropine sulfate caused the largest degree of inhibition: 86% at a dose of 3 mg/kg (Fig. 4D). The highest dose of diazepam significantly inhibited the increase in novelty stress-induced fecal pellet output (Fig. 4E).

Effect of various drugs on spontaneous defecation

To clarify whether the effect of the test drugs on stress-induced defecation may be ascribed to the inhibition of normal defecation, stress-stimulated defecation, or both, the effects of the drugs on the 4-h spontaneous fecal output of non-stressed animals was examined. TAK-637 did not inhibit normal defecation, as shown in Fig. 5A. The highest dose of diazepam slightly inhibited spontaneous defecation (Fig. 5E). However, alosetron hydrochloride, trimebutine maleate, and atropine sulfate significantly inhibited normal defecation, with ID_{50} values of 0.96, 22, and 0.56 mg/kg, respectively (Fig. 5: B, C, and D).

Discussion

In the present study, we demonstrated that gerbils placed in a new environment showed increased defecation and an elevated plasma ACTH level. In studies using rats in an open-field test, the challenge of a novel environment has been reported to produce a psychologically stressful condition, resulting in increased stool output or the stimulation of colonic motility accompanied by behavioral changes, like grooming, rearing, and sniffing (14 – 16). These behavioral changes have been associated with changes in dopamine and/or excitatory amino acid levels in the brain (17), and the involvement of GABA_A receptors and CRF2 receptors has been suggested (18, 19). In this study, the gerbils also displayed active mobility and exploratory behavior during the first hour of novelty stress, compared to the non-stressed group (data not shown). A significant increase in the plasma concentration of ACTH, a typical hormone secreted under stressful conditions, was detected in gerbils placed in a new cage, and this increase was sustained for at least 1.5 h. These findings indicated that the novel environment produced a stressful state in gerbils and stimulated colonic motility, resulting in the increase in fecal pellet output.

The plasma ACTH level of the gerbils exposed to a novelty stress in the present study was significantly increased to 1.5 times the value in the non-stress group. In an open-field-test study in rats, however, the plasma ACTH concentration was quadruple the value in the normal group (20). The smaller increase in the plasma ACTH level induced by the novelty stress in gerbils may be ascribed to the higher basal ACTH level in gerbils, compared to that in rats; the basal plasma ACTH levels were 258 ± 41 pg/mL in the gerbils (Fig. 2) and have been reported to be 64 – 87 pg/mL in rats (21). The hypothalamic-pituitary-adrenal axis is the endocrine system most central to the stress response, and ACTH is released from the pituitary in response to stress.
However, Williams et al. (2) reported that exogenously administered ACTH did not affect intestinal transit and that surgical ablation of the pituitary and adrenal glands did not affect the response of the small intestine to stress. These results suggested that stress-induced changes in gastrointestinal transit are not mediated by pituitary or adrenal gland-derived factors. CRH and TRH, secreted from the hypothalamus under stressful conditions (6, 22), are considered to stimulate the pituitary-adrenal axis and concurrently activate the paraventricular nucleus and dorsal vagal nucleus, resulting in the stimulation of the colonic motility (23). The contents of these two hormones in rats exposed to stress have been shown to be elevated in different areas of the CNS, and central or peripheral injections of these hormones reportedly stimulated colonic motility and fecal pellet output in rats (6). We previously reported that the i.c.v. administration of CRH increased the excretion of fecal pellets.

![Fig. 4. Effects of various drugs on the 2-h-novelty stress-induced increase in fecal pellet output in gerbils. Each column and vertical bar represents the mean ± S.E.M. for 12 animals. *P<0.05, **P<0.01, compared with the value for the control group (Dunnett’s test).](image)
pellets in gerbils (24). These findings suggest that CRH and/or TRH are involved in the novelty stress-induced increase in defecation; however, further investigations are needed to clarify the involvement of these factors.

Various stressors are known to affect gastrointestinal transit in experimental animals, and the affected site and the effect (increase/decrease) differ according to the kind of stress (25, 26). Upper gastrointestinal transit was strongly inhibited by restraint stress, as shown in this study in gerbils and in rats (2), but was not affected by force swimming in hot water in rats (27). Both acoustic stress and cold stress increase gastric emptying (28), and ether exposure decreases gastrointestinal motility (29). Colonic transit was accelerated by restraint.
or water-avoidance stress, but was not accelerated by electronic stress (6, 30). One of the interesting findings revealed in this study was that the novelty stress stimulated defecation without affecting upper intestinal transit. Although the release of CRH is associated with a variety of stresses, like restraint, ether exposure, and psychological stress, the central co-release of other neuropeptides, like TRH, catecholamine, and β-endorphin, might explain the differences in the gastrointestinal effects induced by different stressors.

Cholinergic neurons and 5-HT1 receptors have been reported to be involved in stress-induced defecation; Monnikes et al. (31) and Miyata et al. (5) reported that atropine and ramosetron inhibited restraint stress-induced defecation in rats. In our previous study, atropine and ondansetron reduced cage-restraint stress induced defecation in gerbils (24). Allosetron also significantly inhibited cage-restraint-induced defecation in our preliminary study. In the present study, atropine and alosetron inhibited novelty stress-induced defecation. These results coincided well with previous reports indicating the importance of cholinergic neurons and 5-HT1 receptors in the stress-induced stimulation of colonic motility in rats (5). TAK-637 inhibited restraint stress-induced and novelty stress-induced defecation in gerbils (24). In the enteric nervous system of the gastrointestinal tract, tachykinergic neurons, in addition to cholinergic neurons, play roles in the regulation of gastrointestinal motility and secretion (32, 33). The peripheral administration of NK1-receptor agonist stimulated defecation in gerbils, suggesting that peripheral NK1 receptors play a role in colonic motor functions (24). Trimebutine, a peripheral µ- and κ-opiate-receptor agonist, inhibited novelty stress-induced defecation in this study. Opiate-receptor agonists were reported to inhibit the release of ACh from enteric nerve endings (34), and this is considered to be the main inhibitory mechanism of trimebutine. In our previous study, trimebutine did not have a marked inhibitory effect on restraint stress-stimulated fecal pellet output in gerbils (24). Thus, the novelty stress model may be more sensitive than the restraint stress model for estimating the efficacy of drugs inhibiting stress-induced defe- cation.

Alosetron, atropine, and trimebutine inhibited spontaneous defecation at the same dosage used to inhibit stress-induced defecation, indicating that these drugs physiologically alter colonic transit. Alosetron has been approved for clinical use in diarrhea-predominant female IBS patients and has been reported to delay colonic transit after meals and to relieve colonic discomfort and pain produced by barostat bag distention (35). However, adverse effects like constipation and ischemic colitis have been observed in some patients (35). These clinical findings seem to be well associated with our finding that alosetron inhibited spontaneous defecation in gerbils. Another interesting finding in this study was that TAK-637 and low doses of diazepam did not inhibit spontaneous defecation but inhibited fecal pellet output stimulated by novelty stress or restraint stress. These results suggest that both NK1 receptors and GABA receptors play an important role in stress-stimulated colonic functions. Diazepam has been reported to inhibit the increase in plasma ACTH levels induced by stress, suggesting that diazepam acts centrally (36). In contrast, TAK-637 did not alter the plasma ACTH level stimulated by restraint stress but inhibited the defecation stimulated by CRH administered in the lateral ventricle (24). In our preliminary study, TAK-637 did not affect the novelty stress-induced increase in plasma ACTH levels. These findings indicate that TAK-637 inhibits defecation by acting at a more peripheral site, that is, the enteric nervous system of the gastrointestinal tract. Tachykinergic neurons in the enteric nervous system may be activated by stressful conditions.

In summary, novelty stress increased fecal pellet output in gerbils without affecting upper intestinal transit. Together with the effects of several drugs, which have been shown to inhibit restraint stress-stimulated fecal pellet output, on novelty stress-induced defecation, these findings suggest that this new model may be useful for evaluating drugs for the treatment of stress-induced lower bowel dysfunctions.

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References