Effect of Some Amino Acids on the Rectal Irritation Caused by Sodium Caprate in Conscious Rats

Yoshihito Kinouchi* and Noboru Yata

Pharmaceutical Research Lab., Taiho Pharmaceutical Co., Kawauchi-cho, Tokushima 771-01, Japan and Institute of Pharmaceutical Sciences, Hiroshima University, Minami-ku, Hiroshima 734, Japan.
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The effects of some amino acids such as L-glutamine (Gln), L-arginine (Arg) and L-methionine (Met) on rectal irritation caused by sodium caprate were studied in rats. Rectal irritation was assessed by the balloon method in fasting conscious rats. This method is based on measuring rectal contractions due to possible irritation caused by the presence of drugs and/or adjuvants in the rectum. Strong contractions were observed after rectal administration of an aqueous solution of 100 mM sodium caprate. However, the presence of Gln, Arg or Met (100 mM) in sodium caprate (100 mM) solution resulted in a significant decrease in the intensity of the rectal contraction caused by sodium caprate. The rectal absorption-promoting effect of sodium caprate on 6-carboxyfluorescein (6-CF) was examined following administration with amino acids in rats. The absorption of 6-CF was not influenced by the concurrent administration of amino acids. In addition, the rectal tissue interaction of sodium caprate, with or without Gln, was examined. The concentration of sodium caprate in rectal tissue was reduced by the presence of Gln.

Key words rectal irritation; sodium caprate; amino acid; rectal contraction; balloon method; rectal absorption

Some essential amino acids can significantly prevent mucosal damage induced by irritant drugs. However, there have been no experimental studies on the relief of defecating sensation caused by irritant drugs in preclinical studies. Previously, we demonstrated that rectal contraction detected by the balloon method after rectal administration of a drug solution in conscious rats could be a useful index for predicting the defecating sensation caused by suppositories in humans. Here, we have examined the effects of several amino acids on the inhibitory activity against rectal contraction induced by sodium caprate in rats. The effects of some amino acids on the rectal absorption-promoting effect and tissue interaction of sodium caprate were also examined.

MATERIALS AND METHODS

Materials Sodium caprate was purchased from Tokyo Kasei Kogyo Co., Japan. Sodium cholate, L-glutamine (Gln), L-arginine (Arg) and L-methionine (Met) were obtained from Wako Pure Chemical Industries Co., Japan. All other chemicals and solvents were of reagent grade and used without further purification.

Assessment of Rectal Irritation Male Wistar rats weighing from 250 to 300 g were used. They were fasted for 24 h prior to the experiments, but were allowed free access to water. Conscious rats were individually housed in a modified metabolic cage during the experiment. The body temperature was maintained at 37±1°C by heating with a 50 W incandescent lamp housed in a reflector suspended about 25 cm over the animal during the experiment. Rectal contractations were measured by using the balloon method as reported previously.

Processing of Rectal Contraction Signals In this study, a strong contraction was defined as an increase in pressure of >50 mmHg lasting for at least 10 s. The intensity of the contraction was assessed by the sum of the peak areas of all strong contractions for 20 min after administration of a test solution.

Preparation of a Test Solution The test solution was prepared by dissolving sodium caprate (100 mM) in 50 mM Tris–HCl buffer (pH 8.0), with or without amino acids (100 mM) such as Gln, Arg and Met. The osmotic pressure of the test solution was adjusted to 580 mOsm/kg H2O by addition of NaCl, measured using a Fiske Osmometer-3400 (Fiske Associates, Massachusetts, U.S.A.), and the pH of the solution was adjusted to pH 8.0 by the addition of HCl or NaOH (pH Meter F-16, Horiba, Tokyo, Japan).

Absorption Experiment Male Wistar rats (270—300 g) were fasted for 24 h prior to the experiment but water was given freely. They were anaesthetized by intraperitoneal injection of sodium pentobarbital (Nembutal sodium solution, Abbott Laboratories) at a dose of 30 mg/kg and held supine on the surface kept at 37±1°C to maintain their body temperature above 36°C. A buffered solution (0.1 ml) of 0.1% (w/v) 6-carboxyfluorescein (6-CF) containing sodium caprate (100 mM), with or without amino acids (100 mM) such as Gln, Arg or Met, was administered into the rectum through a catheter. Blood samples (0.1 ml) were taken from a jugular vein by heparinized syringe at appropriate intervals for 120 min. The whole blood concentration of 6-CF was assayed by HPLC.

A mixture of blood (0.1 ml) and 0.1 N HCl (1 ml) was applied to a Bond-Elut® column (No. 1210-2025, Analytichem International, CA, U.S.A.) preconditioned with methanol (2 ml) and purified water (2 ml). The column was then washed with 10% (v/v) methanol (1.5 X 3 ml). Then, absorbed drug was eluted with 1 ml 50 mM phosphate buffer (pH 8.2)-acetonitrile (90:10, v/v). The procedure was repeated twice. Ten µl of the eluted solutions were injected onto the HPLC column (TSK gel Octadecl-4PW, 150 mm X 4.6 mm i.d., Tosoh, Tokyo, Japan) at 35°C. The chromatographic system consisted of a Model LC-9A pump (Shimadzu, Tokyo, Japan), a Model 7125 injector valve (Rheodyne, CA, U.S.A.) equipped with a 10 µl loop and a fluorescence detector (RF-535, Shimadzu, Tokyo, Japan) with

* To whom correspondence should be addressed.

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excitation at 490 nm and emission at 515 nm. The mobile phase was a mixture of 50 mm phosphate buffer (pH 8.2)–acetonitrile (90: 10, v/v). The flow-rate was 0.5 ml/min. Analytical limit was 1 ng/ml.

**Distribution of Sodium Caprate into Rectal Tissue**

Male Wistar rats (280—300 g) were fasted for 24 h prior to the experiment but water was given freely. They were anaesthetized using ether and held supine on a surface kept at 39 ± 1 °C to maintain their body temperature above 36 °C. The rectum was exposed by an abdominal incision and a ligature was tied around the large intestine approximately 6 cm from the anus. The anus was closed with surgical adhesive (Aronalpha A, Sankyo Co. Ltd., Tokyo, Japan) to prevent leakage of the solution. A buffered solution containing sodium caprate (100 mm), with or without Gln (100 mm), was administered (0.1 ml) by microsyringe into the rectal loop. At 20 min after administration, the rectal loop was removed. Rectal contents were collected and the inside of the loop was washed with saline. The rectal contents and washings were combined, and saline was added to the combined solution to make 10 ml. The solution was analysed to determine the remaining sodium caprate in the loop, then the rectal segment was weighed. The rectum was homogenized for 2 min at 0 °C in 3 volumes acetonitrile using a Polytron homogenizer (PT 10-35, Kinematica, Luzern, Switzerland). The homogenate was centrifuged for 15 min at 3000 rpm and the supernatant was separated. The supernatant was analysed to determine sodium caprate in the rectal tissue. The sodium caprate concentration was determined by the method of Wildgrube et al. with a slight modification.

**Statistical Analysis**

All the data were represented as the mean ± S.D. Data were analysed for statistical significance by the two-tailed Dunnett’s multiple comparison test or Student’s t-test after analysis of variance.

**RESULTS**

**Influence of Amino Acid on Rectal Contraction Induced by Sodium Caprate**

The effects of Gln, Arg or Met on rectal contraction patterns induced by sodium caprate were examined. Figure 1 shows typical examples after rectal administration of aqueous 100 mm sodium caprate, with or without Gln. High-amplitude rectal contractions (pressure of more than 50 mmHg lasting for at least 10 s) were observed within 5 min of administration of sodium caprate alone. However, on incorporation of Gln, no strong contraction was observed. As shown in Fig. 2, the total intensity of rectal contractions after administration of sodium caprate (100 mm) alone was 1226.6 ± 621.0 mmHg·s (mean ± S.D., n = 4), and the total intensity of rectal contractions was dramatically reduced by the addition of Gln (0.0 ± 0.0 mmHg·s), Arg (154.3 ± 308.6 mmHg·s) and Met (0.0 ± 0.0 mmHg·s) (mean ± S.D., n = 4). All the values were significant (p < 0.01).

The effects of various concentrations of Gln (0, 50, 62.5, 75, 87.5, 100 and 200 mm) on the intensity of rectal contraction induced by 100 mm sodium caprate were examined (Fig. 3). The results were 1205.3 ± 529.0, 1234.3 ± 330.8, 670.9 ± 793.4, 308.7 ± 413.0, 226.3 ± 452.5, 194.6 ± 389.2 and 267.1 ± 534.3 mmHg·s (mean ± S.D., n = 4), respectively. The intensity of rectal contraction induced by 100 mm sodium caprate was mitigated, depending on the concentration of Gln, although a significantly statistical difference by Dunnett’s multiple comparison test (p < 0.05) was observed only at 100 mm Gln. On the other hand, no reducing effect of amino acid on rectal contraction caused by glycercin was observed (data not shown). This finding suggests that the irritation mechanisms of glycercin and sodium caprate differ. A mechanism for sodium caprate will be described later.

**Influence of Amino Acid on the Absorption-Promoting Potency of Sodium Caprate for Rectal Absorption of 6-CF**

The rectal absorption-promoting effect of sodium caprate on 6-CF administered with amino acids was examined in rats. Figure 4 shows the effect of sodium caprate, with or without Gln, on the blood concentration-
Fig. 3. Effects of Various Concentrations of Gln on Rectal Contraction after Administration of Sodium Caprate (100 mm) in Rats

The intensity of the contraction was assessed by the sum of peak areas of all strong contractions for a 20 min period after administration of a test solution. A strong contraction was defined as an increase in pressure of > 50 mmHg lasting for at least 10 s. Each column represents the mean ± S.D. for 4 rats. a) *p* < 0.05 (Dunnett's multiple comparison test) compared with the group receiving sodium caprate alone.

Fig. 4. Effect of Sodium Caprate, with or without Gln, on the Blood Concentration–Time Curve of 6-CF in Rats

The test solutions (0.1 ml) were administered into the rectum through tubing inside the balloon catheter. ◀, 0.1% (w/v) 6-CF; ●, 0.1% (w/v) 6-CF + 100 mm sodium caprate; □, 0.1% (w/v) 6-CF + 100 mm sodium caprate + 10 mm Gln. Each point represents the mean ± S.D. for 4 rats.

time curve of 6-CF. Blood levels of 6-CF were low after administration of the 0.1% (w/v) 6-CF alone in rats. However, the blood levels of 6-CF increased following concurrent administration of 100 mm sodium caprate with 6-CF, irrespective of the presence or absence of Gln. The area under the curve (AUC₀−₁₂₀ min) of 6-CF (mean ± S.D., n = 4) after administration of sodium caprate, with or without Gln (100 mm), was 5437.4 ± 508.7 ng min/ml and 5150.2 ± 831.7 ng min/ml, respectively. Furthermore, the absorption-promoting potency of sodium caprate for rectal absorption of 6-CF was not influenced by the concurrent administration of Arg or Met, as was the case with Gln. The absorption-promoting potency of sodium caprate for rectal absorption of 6-CF was maintained even following concurrent administration of amino acids.

**Distribution of Sodium Caprate into Rectal Tissue**

To clarify the reasons for the protective effect of amino acids against rectal contraction caused by sodium caprate in the experiments above, the amount of sodium caprate in the rectal loop and tissue was measured in rats 20 min after administration of sodium caprate (100 mm), with or without Gln (100 mm). As shown in Fig. 5, the amount of sodium caprate in the rectal loop after administration of sodium caprate, with and without Gln, was 1.83 ± 0.29 μmol/g (mean ± S.D., n = 5) and 2.01 ± 0.35 μmol/g (n = 3) respectively. The amount of sodium caprate in the rectal loop was not influenced by addition of Gln. On the other hand, the amount of sodium caprate in rectal tissue after administration of sodium caprate, with and without Gln, was 2.27 ± 0.61 μmol/g (mean ± S.D., n = 5) and 4.00 ± 0.32 μmol/g (n = 3), respectively. The amount of sodium caprate in rectal tissue was significantly reduced by the presence of Gln in comparison with sodium caprate alone (*p* = 0.004).

**DISCUSSION**

Several studies on enhanced rectal absorption of water-soluble and poorly lipophilic drugs by various absorption promoters have been reported. In addition, mucosal damage caused by absorption promoters has been reported. Sodium caprate has been reported to cause a sensation of defecation after rectal administration of suppositories in human subjects. Murakami et al. reported the presence of a good correlation between the absorption potency of bile salts and hemolytic activity. On the other hand, Takagi et al. reported a favorable effect of Gln on the healing of stress-induced gastric lesions in rats in comparison with other amino acids. Lim et al. also reported that Met and histidine reduced the damages to the gastric mucosa induced by oral administration of aspirin. Hitherto, no studies have been reported on the protective effect against the rectal irritation (sensation of defecation) induced by absorption promoters. Previously, we reported that rectal contraction in conscious rats may predict the sensation of defecation in human subjects and an intensity of rectal contraction greater than 500 mmHg•s or so in rats could cause a sensation of defecation in human subjects. Some amino acids, such as Gln, Arg and Met, reduced the rectal contraction caused by sodium caprate without influencing its promoting action in rats (Figs. 2 and 4).

To clarify the reasons for the protective effect of Gln against the rectal contraction caused by sodium caprate,
the amount of sodium caprate in the rectal loop and tissue was measured after administration of sodium caprate, with or without Gln. The amount of sodium caprate remaining in the rectal loop was not influenced by addition of Gln. However, the amount of sodium caprate in rectal tissue was significantly decreased by the presence of Gln \( (p = 0.004) \) (Fig. 5). The decrease in the amount of sodium caprate in rectal tissue may be reflected in the reduction in rectal irritation. The mechanism governing the effects of amino acids on rectal irritation is still uncertain. The decreased effect of amino acids on rectal irritation caused by sodium caprate may be associated with changes in the affinity of sodium caprate for rectal tissues and/or in metabolic reactions of sodium caprate in the presence of amino acids.

These findings suggest that the concurrent incorporation of amino acids in rectal preparations containing sodium caprate as an absorption enhancer is very beneficial for reducing undesirable side-effects.

REFERENCES