J. Pharm. Pharmacol. 1996, 46: 310–315
Received March 20, 1995
Accepted October 2, 1995

A Novel Method for the Preclinical Assessment of Rectal Irritation

YOSHIHITO KINOUCHI, YOH'ICHIRO TAKEICHI AND NOBORU YATA*

*Pharmaceutical Research Laboratory, Taiho Pharmaceutical Co., Kawauchi-Cho, Tokushima 771-01, and *Institute of Pharmaceutical Sciences, Hiroshima University, Minami-ku, Hiroshima 734, Japan*

Abstract

The purpose of this study concerns a novel method for preclinical assessment of rectal irritation caused by suppositories introduced into the rectum. 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 adjuvants in the suppository.

In control experiments (vehicle only), significant rectal contractions were not observed in a range of pH 1·5–11·0 and osmotic pressure 70–2000 mOsm kg^{-1} H_2O, respectively. On the other hand, strong contractions were observed after rectal administration of an aqueous solution of 50% glycerin, 100 mM sodium caprate or 25 mM sodium cholate. The intensity of contraction after rectal administration of sodium caprate or sodium cholate was dependent on the concentration in the dosing solution. In addition, the effect of sodium caprate and sodium cholate on rat rectal mucosa was investigated by optical light microscopy. Although slight or moderate alteration such as the presence of mucinous substance in lumen and congestion, oedema and haemorrhage of the rectal membrane 20 min after rectal administration, there was no major damage to the rectal mucosa. There was a correlation between the median score for mucinous substance in lumen and mean intensity of rectal contraction. For comparative purposes, defecating sensations, pain, itch, burning sensations, and awareness of the presence of a foreign body after administration of suppositories containing 0, 1, 2 and 4% sodium caprate were examined in eight healthy volunteers. The defecating sensation in the human subjects correlated with the intensity of rectal contraction in rats.

The results suggest that rectal contraction in conscious rats could be a useful index for prediction of a defecating sensation in man.

No suitable method has previously been reported for detecting irritation and defecating sensation in preclinical studies. In the present study, a novel animal method to detect these problems with suppositories in man is proposed, based on measuring rectal pressures.

Materials and Methods

Materials

Sodium caprate was purchased from Tokyo Kasei Kogyo Co., Japan. Sodium cholate was obtained from Wako Pure Chemical Industries Co., Japan. Witepsol H-15 (Hüla AG, Witten, Germany) was used as a suppository base. All other chemicals and solvents were of reagent grade and used without further purification.

Animal studies

Male Wistar rats, 250–300 g, were fasted for 24 h before experimentation, but were allowed free access to water. Conscious rats were individually housed in a modified metabolic cage during the experiment. Body temperature was maintained at 37 ± 1°C by heating with a 50-W incandescent lamp in a reflector suspended about 25 cm over the animal during the experiment. Rectal contraction was assessed by monitoring the rectal pressure via a silicone balloon catheter (8FR-3 cc, Nipro, Tokyo, Japan) illustrated in Fig. 1. The faeces in the rectum were evacuated by rubbing the whole rectum with fingers and the silicone balloon catheter was then inserted into the rectum approximately 2 cm above the anus. A solution (0·1 mL) was administered into the rectum through an inlet tubing inside the balloon catheter. The rectal pressure on the balloon was detected by a transducer and recorded on a polygraph (Type-360, NEC San-ei, Tokyo, Japan) 10 min before to 20 min after the administration of a test solution. A signal Processor (7T18A, NEC San-ei, Tokyo, Japan) was used for peak-area measurement. Experiments were performed in an air-conditioned room at 22–25°C and a relative humidity of 40–60%.

Correspondence: Y. Kinouchi, Pharmaceutical Research Laboratory, Taiho Pharmaceutical Co., Kawauchi-Cho, Tokushima 771-01, Japan.

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 peak areas of all strong contractions for 20 min after the administration of a test solution.

Preparation of a test solution

Influence of pH on rectal contraction in rats. The influence of the pH on the rectal contraction was examined with different pH buffer solutions. The osmotic pressure of 50 mM Tris-HCl buffer solution was adjusted to 580 mOsm kg^{-1} H_2O by adding NaCl, measured using a Fiske Osmometer-3400

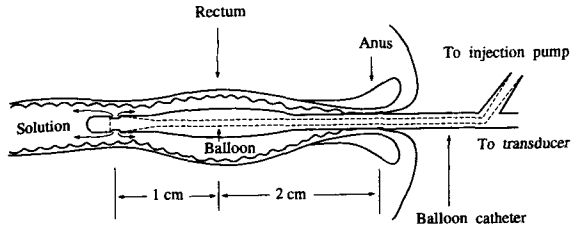

FIG. 1. Schematic diagram of the balloon catheter. A solution was administered into the rectum through an inlet tubing inside the balloon catheter. The rectal pressure on the balloon was detected by a transducer.

(Fiske Associates, Massachusetts), and the pH of the solution was adjusted to pH 1·0, 1·5, 8·0, 11·0 or 11·5 by adding 1 M HCl or NaOH (pH Meter F-16, Horiba, Tokyo, Japan).

Influence of osmotic pressure on rectal contraction. The osmotic pressure of 50 mM Tris-HCl buffer solution (pH 8·0) was adjusted to 70, 580 or 2000 mOsm kg^{-1} H_2O by adding the calculated amount of NaCl and measured with an osmometer.

Effect of the concentration of sodium caprate and sodium cholate. The test solutions were prepared by dissolving various amounts of sodium caprate or sodium cholate in a 50 mM Tris-HCl buffer, and the resulting solution was adjusted to pH 8·0 by adding 1 M NaOH. The osmotic pressure of the test solution was adjusted to 580 mOsm kg^{-1} H_2O by adding NaCl.

Effects of some drugs on rectal contraction. Sorbitan trioleate (Span 85, 20%), sodium diclofenac (2·5%), chloral hydrate (25%), aspirin (25%), indomethacin (5%), Tween 20 (20%), acetic acid (6%), citric acid (4%) and sodium caprate (3%) were suspended in miglyol 812 at a concentration shown in parentheses, and 0·1 mL was introduced into the rectum.

Measurement of the pH of the rectal fluid

The pH of the rectal fluid in male Wistar rats (250–300 g) was measured using a miniature pH electrode (o.d. 2 mm, Horiba, Tokyo, Japan) attached to a pH meter (F-16, Horiba, Tokyo, Japan). The miniature pH electrode was inserted into the rectum approximately 3 cm above the anus.

Tissue processing

The damage to rectal mucosa after rectal administration of Tris-HCl buffer, sodium caprate or sodium cholate solution into the rat rectum was examined in relation to rectal contraction. Rats were anaesthetized with ether 20 min after administration of a solution and the rectum and anus were rapidly removed, opened lengthwise, cleaned with 154 mM NaCl, and then fixed with 10% formaldehyde 100 mM phosphate buffer at pH 7·2. Specimens were examined macroscopically before being cut longitudinally in three or four sections, and were then routinely embedded in paraffin wax, and sectioned at 5-μm thickness. After haematoxylin–eosin staining, the sections were examined histologically for degeneration of the surface epithelium and cell infiltration of the submucosa.

Evaluation of rectal damage

Rectal damage was scored on pathological findings shown in Table 2. Microscopic findings were scored according to the type and degree of mucosal damage on the rectum.

Preparation of suppositories for volunteers

Sodium caprate powder was passed through a No. 100 sieve to obtain particles < 150 μm in size. Conventional suppositories (2 g) of the same appearance, and containing 0, 1, 2 and 4% sodium caprate, were prepared using the fusion method (Parrott 1973).

Subjects

Human cross-over studies were carried out on eight healthy male volunteers aged between 23 and 34 years. All gave written informed consent at the Clinical Pharmacology Center of the Daiich Hospital (Tokyo, Japan).

Irritation was evaluated in respect of five items: sensation of defecation, pain, itch, burning, and awareness of the presence of a foreign body. The suppository containers were numbered, but volunteers, doctors and nurses were unaware of the contents of the suppositories for the double blind test. One hour after defecation, a suppository was administered to each subject by a nurse, and subjects were then interviewed regarding their symptoms and scoring was made by another nurse at any time during the designated time intervals.

Statistical analysis

Data were analysed for statistical significance by Wilcoxons' rank sum test (two-tailed) for both the animal and volunteer studies. Linear correlations were determined by least regression analysis.

Results and Discussion

Assessment of rectal irritation

Effect of fasting on rectal contraction. Typical normal rectal contraction patterns in rats for 30 min, recorded using the balloon method under non-fasting and fasting for 24 h conditions, are shown in Fig. 2. An increase in pressure of

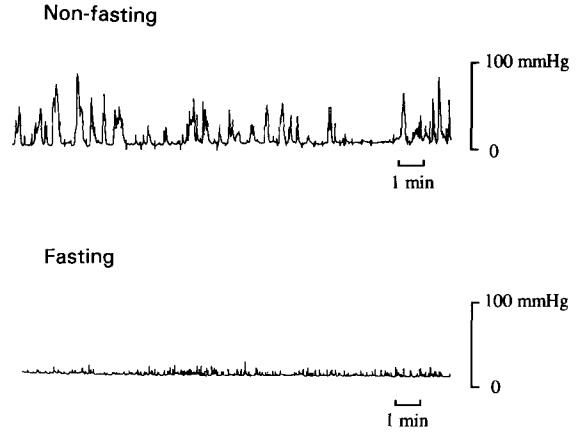

FIG. 2. Responses of rat rectal pressure under non-fasting or 24 h fasting conditions in conscious rats. The rectal pressure on the balloon was detected by a transducer and recorded on a polygraph.

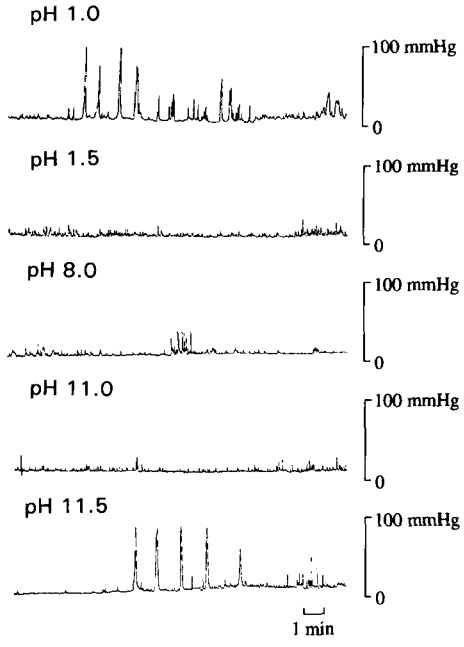

FIG. 3. Effect of pH on rectal contraction in rats. The osmotic pressure of 50 mM Tris-HCl buffer was fixed at 580 mOsm kg^{-1} H_2O. The test solutions (0·1 mL) were administered into the rectum through an inlet tubing inside the balloon catheter.

> 50 mmHg lasting for at least 10 s in the rectum was defined as a strong or high amplitude contraction. The usual non-fasting rectal contraction pattern consisted of both irregular low and high amplitude contractions. Frequent copious evacuation of stools was observed after high amplitude contractions. The rectal contraction pattern after fasting for 16 h was the same as in the non-fasting condition (data not shown). However, after 24 h fasting, no strong contraction was observed. Faeces were present in the rectum and colon in the non-fasting condition and after 16 h

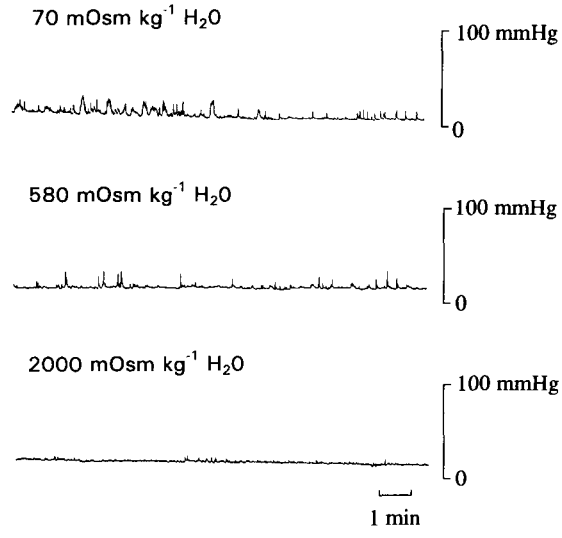

FIG. 4. Effect of osmotic pressure on rectal contraction in rats. The pH of 50 mM Tris-HCl buffer solution was fixed at pH 8·0. The test solutions (0·1 mL) were administered into the rectum through an inlet tubing inside the balloon catheter.

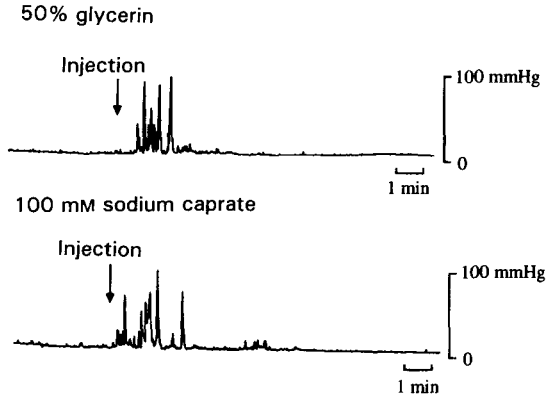

FIG. 5. Contraction–time profile after rectal administration of 50% glycerin or 100 mM sodium caprate in rats. The test solutions (0·1 mL) were administered into the rectum through an inlet tubing inside the balloon catheter.

fasting, but few were present after 24 h fasting. These findings suggest that rectal contraction patterns are associated with the presence of rectal contents; hence, rectal contraction after administration of various test solutions was carried out under 24-h fasting conditions.

Effect of pH and osmotic pressure. The effects of pH and osmotic pressure (50 mM Tris-HCl buffer) on the rectal contraction pattern were investigated. In the experiments to study pH effect, osmotic pressure was fixed at 580 mOsm kg^{-1} H_2O. For the study on the effect of osmotic pressure, the dosing solution was fixed at pH 8·0. Typical rectal contraction patterns after rectal administration of solutions at various pH values and osmotic pressures are shown in Figs 3 and 4. High-amplitude rectal contractions after administration of pH 1·0 and 11·5 buffer solutions (580 mOsm kg^{-1} H_2O) were observed within 5 min of administration. However, in a pH range of 1·5–11·0 and 70–2000 mOsm kg^{-1} H_2O, no strong contractions were observed during 20 min.

In the preliminary experiments, the rectal contraction pattern after administration of 154 mM NaCl (0·1–0·5 mL) was studied in rats. There were no differences in contraction patterns before and after the administration of each solution. Published data on the luminal pH of rectum in rat are conflicting (Crommelin et al 1979; Wu et al 1987). In the present study, the pH of the rectal fluid in rats was measured using a miniature pH electrode attached to a pH meter. The luminal pH of the rectum approximately 3 cm from the anus was pH 8·0 ± 0·1 (mean ± s.d., n = 6); therefore, in the following experiments, the solutions were adjusted to pH 8·0. The osmotic pressure of the dosing solution was adjusted to 580 mOsm kg^{-1} H_2O including the contribution by the test compound.

Influence of glycerin and sodium caprate on the rectal contraction pattern. Glycerin enemas are used to facilitate defecation and to cleanse the large intestine for medical treatment or examination. Sugimura et al (1990) classified glycerin enemas as mild irritants, and sodium caprate has also been reported to cause a sensation of defecation (Motohiro et al 1985). Thus, the effect of these irritant

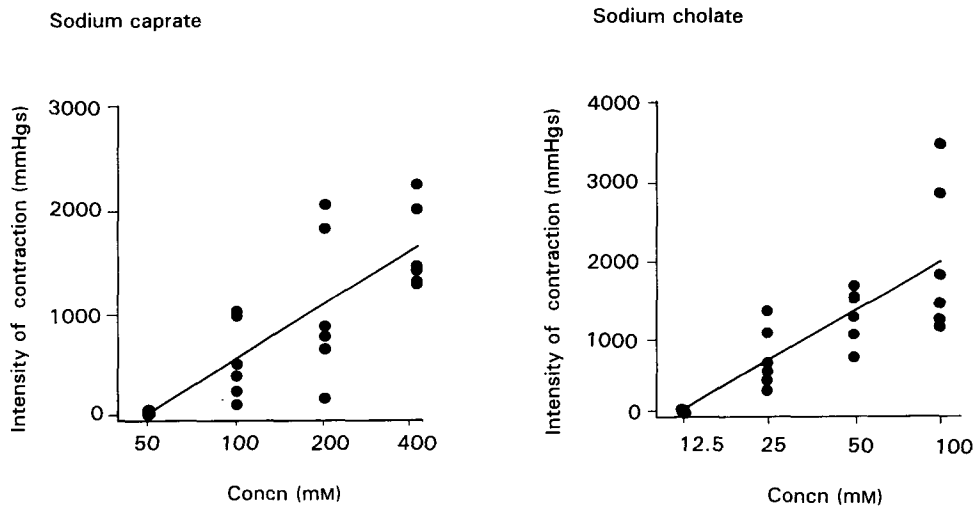

FIG. 6. Effects of concentration of sodium caprate ($r = 0{\cdot}819$, $P = 0{\cdot}0001$, $n = 24$) or sodium cholate ($r = 0{\cdot}821$, $P = 0{\cdot}0001$, $n = 24$) administered rectally on the rectal contractions of rats. The intensity of the contraction was assessed by the sum of peak areas of all strong contractions for 20 min after the administration of a test solution. The strong contraction was defined as an increase in pressure of > 50 mmHg lasting for at least 10 s.

drugs was investigated on rectal contraction patterns using the balloon method. Typical examples, after rectal administration of aqueous 50% glycerin or 100 mM sodium caprate are shown in Fig. 5. High-amplitude rectal contractions were observed within 5 min of administration. Signal patterns of rectal contraction were not exactly the same in individuals. However, total intensity of contraction as described in the experimental section was within experimental error. Intensity of the rectal contraction was examined by administering 50, 86 and 100% glycerin, and found to be 1510 ± 481, 2168 ± 76 and 3312 ± 1189 mmHg s (mean ± s.d., $n = 4$), respectively. The mean total intensity of contractions with 50, 86 and 100% glycerin was concentration-related ($r = 0{\cdot}697$, $P = 0{\cdot}012$). There are several reports concerning the sensation of defecation in patients, but the mechanism remains to be elucidated (Goligher & Hughes 1951; Scharli & Kiesewetter 1969; Sanberg 1989). However, it is assumed that the sensation of defecation correlates with rectal distention (Goligher & Hughes 1951; Scharli & Kiesewetter 1970). Defecation is generally accompanied by giant migrating contractions in the colon (Karaus et al 1987; Matsushima 1989). Our present data suggest a close association between the sensation of defecation caused by irritants in patients and the ability to cause rectal contractions in rats.

Table 1. Intensity of rectal contraction of rats after rectal administration of various drugs. Compounds were suspended in Miglyol 812, and 0·1 mL was introduced into the rectum. Each value represents the mean ± s.d. for 4–5 rats.

Compound	%	Intensity of contraction (mmHg s)
Miglyol 812		0 ± 0
Span 85	20	0 ± 0
Sodium diclofenac	2·5	371 ± 446
Chloral hydrate	25	120 ± 268
Aspirin	25	74 ± 148
Indomethacin	5	94 ± 189
Tween 20	20	543 ± 1087
PEG 400	100	1765 ± 464
Acetic acid	6	1082 ± 788
Citric acid	4	2808 ± 1281
Sodium caprate	3	1191 ± 547

Effect of drug concentration on the rectal contraction

The intensity of contraction after rectal administration of sodium caprate or sodium cholate was plotted against the concentration in the dosing solution. As shown in Fig. 6, fairly good linear relations were observed between intensity of contraction and the concentration of sodium caprate ($r = 0{\cdot}819$, $P = 0{\cdot}0001$, $n = 24$) or sodium cholate ($r = 0{\cdot}821$, $P = 0{\cdot}0001$, $n = 24$).

The rectal contraction pattern with some irritant drugs

Intensity of the rectal contraction was examined by administering 100% PEG 400 and the Miglyol suspension of some irritative compounds such as 20% Span 85, 2·5% sodium diclofenac, 25% chloral hydrate, 25% aspirin, 5% indomethacin, 20% Tween 20, 6% acetic acid, 4% citric acid and 3% sodium caprate. Results are shown in Table 1. Miglyol 812 was used as the control. Tween 20, PEG 400, acetic acid, citric acid and sodium caprate caused strong rectal contractions, with mean total intensities of contraction higher than 500 mmHg s. Span 85, sodium diclofenac, chloral hydrate, aspirin and indomethacin had no significant effect. It has been reported that Span 85, sodium diclofenac, chloral hydrate, aspirin, indomethacin, Tween 20, PEG 400, acetic acid and citric acid can damage the rectal mucosa in man and experimental animals (Ito 1980; Yaginuma et al 1981; Swift et al 1992), in agreement with our findings on Tween 20, PEG 400, acetic acid and citric acid but not on span 85, sodium diclofenac, chloral hydrate, aspirin and indomethacin.

Histopathological study of the rat rectal mucosa

Damage to the rectal mucosa after rectal administration in rats of Tris-HCl buffer, sodium caprate or sodium cholate

Table 2. Summary of pathological findings[a].

Pathological feature	Grade	Score	Control	Sodium caprate (mM) 100	200	400	Sodium cholate (mM) 25	50	100
Mucinous substance in lumen	Negative	0	5	2	1	0	1	0	0
	Focal	1	0	3	2	2	4	3	4
	Diffuse	2	0	0	2	3	0	2	1
Vascular congestion	Negative	0	5	4	5	3	5	3	1
	Focal	1	0	1	0	0	0	0	3
	Diffuse	2	0	0	0	2	0	2	1
Oedema	Negative	0	5	5	5	4	3	2	2
	Focal	1	0	0	0	1	1	1	0
	Diffuse	2	0	0	0	0	1	2	3
Haemorrhage	Negative	0	5	5	5	4	5	4	5
	Focal	1	0	0	0	1	0	1	0
	Diffuse	2	0	0	0	0	0	0	0
Erosion or ulceration	Negative	0	5	5	5	5	5	5	5
	Focal	1	0	0	0	0	0	0	0
	Diffuse	2	0	0	0	0	0	0	0

[a] Number of rats.

buffer solutions was examined in relation to rectal contraction. Table 2 shows the results of the histopathology. No damage was observed in the control group, indicating no damage by the insertion of a balloon catheter and administration of vehicle. Sodium caprate 100, 200, or 400 mM or sodium cholate solution 25, 50 or 100 mM induced mild damage, such as mucinous substance in lumen, vascular congestion, oedema and haemorrhage. The damage was always limited to the epithelium, and there were no abnormal findings in the submucosa. The increase of mucinous substance in the lumen after rectal administration of sodium caprate (100 mM, median 1, $P = 0{\cdot}067$; 200 mM, median 1, $P = 0{\cdot}024$; 400 mM, median 2, $P = 0{\cdot}007$) and sodium cholate (25 mM, median 1, $P = 0{\cdot}020$; 50 mM, median 1, $P = 0{\cdot}007$; 100 mM, median 1, $P = 0{\cdot}006$) was similar; furthermore, the median score for mucinous substance after rectal administration was dependent on the concentration of the dosage solution of sodium caprate. The median score for vascular congestion increased when 100 mM sodium cholate solutions were administered (median 1, $P = 0{\cdot}023$), with all doses of sodium caprate and sodium cholate, oedema, haemorrhage, erosion and ulceration were not significantly different from the control group. Mucinous substance in lumen may indicate rectal irritation.

Irritability of sodium caprate suppositories

Examination for sensation of defecation, pain, itch, burning and awareness of the presence of a foreign body in the rectum of healthy human subjects was investigated after administration of suppositories containing 0 (placebo), 1, 2 or 4% sodium caprate (0, 20, 40 and 80 mg). The results are summarized in Table 3. Median scores for irritation reached a maximum 15 min after administration. Placebo caused little or no irritability, the median scores for the irritation responses 15 min after administration of placebo being consistently zero. Suppositories of 4% sodium caprate increased the sensation of defecation (median scores ranging from 0 to 1, $P = 0{\cdot}03$), but 1 and 2% sodium caprate showed no significant effect (median 0, $P = 1{\cdot}00$ and median 0, $P = 0{\cdot}90$). The median score for awareness of the presence of a foreign body slightly increased when 4% sodium caprate suppositories were administered (ranging from 0

Table 3. Irritation[a] after single rectal administration of sodium caprate suppositories to eight normal volunteers.

Sodium caprate (%)		Time (min) Pre	1–15	16–30	31–60	61–90	91–120
0	Median	0	0	0	0	0	0
	Interquartile range	0	1	0	0·5	0·5	0
1	Median	0	0	0	0	0	0
	Interquartile range	0	1	0·5	0·5	0·5	0
2	Median	0	0	0	0	0	0
	Interquartile range	0	1	0	0	0	0·5
4	Median	0	1*	1**	0	0	0
	Interquartile range	0	0·5	1	1	0·5	0

[a] Score of irritative response: 0, no effect; 1, slight effect; 2, moderate effect; 3, severe effect. *$P < 0{\cdot}05$; **$P < 0{\cdot}01$ (Wilcoxon's rank sum test) compared with the placebo group.

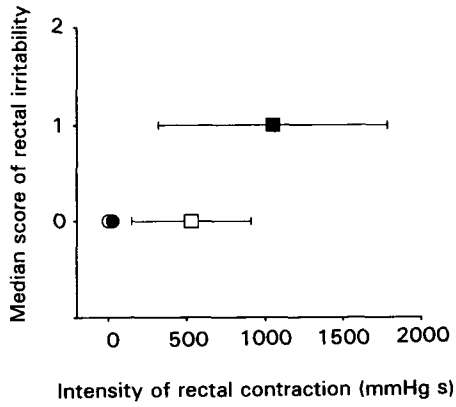

FIG. 7. Relationship between median score for the need to induce defecation in human subjects and the intensity of rectal contraction (mean ± s.d.) in rats. Sodium caprate: 0%, ○; 1%, ●; 2%, □; 4%, ■.

to 0·5, $P = 0·30$), with all doses of sodium caprate, the scores of pain, burning sensations or itch were similar to those in the control group. Our present data demonstrate that sodium caprate (80 mg) caused the sensation of defecation in volunteers, in agreement with Motohiro et al (1985) who demonstrated that sodium caprate induced the sensation of defecation depending on concentration.

No studies have been reported on the preclinical assessment of rectal irritation after administration of suppositories. To study a correlation between effects in volunteer and animal data, the median score for the sensation of defecation in volunteers was plotted against intensity of rectal contraction in rats (Fig. 7). From this figure it can be suggested that intensity of rectal contraction of more than 500 mmHg s could cause the sensation of defecation in human subjects. We conclude that rectal contraction tests in rats may predict the sensation of defecation in man.

Acknowledgements

The technical assistance of S. Hayashi and M. Abe is gratefully acknowledged.

References

Crommelin, D. J. A., Modderkolk, J., de Blaey, C. J. (1979) The pH dependence of rectal absorption of theophylline from solutions of aminophylline in situ in rats. Int. J. Pharm. 3: 299–309

Goligher, J. C., Hughes, E. S. R. (1951) Sensibility of the rectum and colon. Its role in the mechanism of anal continence. Lancet 10: 543–547

Ito, Y. (1980) A new method for testing rectal irritation following intrarectal administration of suppositories in rats, rabbits and dogs. J. Med. Soc. Toho, Japan 27: 52–73

Karaus, M., Sarna, S. K., Ammon, H. V., Wienbeck, M. (1987) Effects of oral laxatives on colonic motor complexes in dogs. Gut 28: 1112–1119

Matsushima, Y. (1989) Studies on colonic motor correlates of spontaneous defecation in conscious dogs. Jpn. J. Smooth Muscle Res. 25: 137–146

Motohiro, T., Aramaki, M., Tanaka, K., Koga, T., Shimada, Y., Tomita, N., Sakata, Y., Fujimoto, T., Nishiyama, T., Kuda, N., Ishimoto, K., Tominaga, K., Yamashita, F. (1985) Fundamental study on ceftizoxime suppository in adults and children. Jpn. J. Antibiot. 38: 3013–3056

Parrott, E. L. (1973) Student experiments in pharmaceutical technology. VII. Preparation and evaluation of suppository formulations. Am. J. Pharm. Educ. 37: 39–45

Sanberg, P. R. (1989) Neuroleptic-induced emotional defecation: effects of pimozide and apomorphine. Physiol. Behav. 46: 199–202

Scharli, A. F., Kiesewetter, W. B. (1969) Imperforate anus; anorectosigmoid pressure studies as a quantitative evaluation of postoperative continence. J. Pediat. Surg. 4: 694–704

Scharli, A. F., Kiesewetter, W. B.(1970) Defecation and continence: some new concepts. Dis. Colon Rectum 13: 81–107

Sugimura, F., Ryoh, H., Watanabe, T., Kaneda, N., Yonemitsu, K., Aoki, T., Motoki, Y., Kawamura, F., Ariga, H., Matsuo, Y., Honda, T. (1990) Comparative studies on the usefulness of phosphate versus glycerin enema in preparation for colon examinations. Gastroenterologia Japonica 25: 437–450

Swift, G. L., Arnold, J., Williams, G. T., Williams, B. D., Rhodes, J., Khan, F. (1992) A comparison of upper gastrointestinal mucosal damage by standard and delayed-release indomethacin. Aliment. Pharmacol. Ther. 6: 717–725

Yaginuma, H., Nakata, T., Toya, H., Murakami, T., Yamazaki, M., Kamada, A., Shimazu, H., Makita, I. (1981) Rectal delivery of antiinflammatory drugs. II. The influence of basic amino acid salts on rectal absorption of diclofenac. Chem. Pharm. Bull. 29: 3326–3333

Wu, W. M., Murakami, T., Higashi, Y., Yata, N. (1987) Enhancement of the rectal absorption of sodium ampicillin by *N*-acylamino acids in rats. J. Pharm. Sci. 76: 508–512