Influence of Urethane Anesthesia and Abdominal Surgery on Gastrointestinal Motility in Rats

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The influence of various experimental treatments on propulsive gastrointestinal motility was examined in rats, using inulin as a marker. In unanesthetized rats without any surgery, the distribution center of inulin, which was expressed as a dimensionless distance normalized by the length of the small intestine, reached 0.587 down the intestinal tract from the pylorus 60 min after the intragastric administration of inulin. However, the distribution center of inulin moved only 0.061 in 60 min in unanesthetized rats with minimal abdominal surgery for intraduodenal administration of inulin, and did not move in unanesthetized rats with cannuulas for perfusion or in the rats anesthetized with urethane and treated with abdominal surgery. In urethane-anesthetized rats without surgery, almost 100% of the inulin was recovered from the stomach 60 min after intragastric administration. These results suggest that gastrointestinal motility is extensively suppressed by minimal abdominal surgery as well as by anesthesia.

Keywords intestinal motility; rat; inulin; urethane; anesthesia; abdominal surgery

Various experimental treatments, such as anesthesia and surgery, are widely used in laboratory animal experiments. Although these treatments are necessary to carry out experiments, they have negative aspects. They could affect various physiological factors, and could be a source of interlaboratory variation in experimental data. We are largely aware of that possibility. However, such influence of experimental treatments on physiological factors has not been a subject of extensive assessment.

We recently reported that anesthetic regimens increase the aqueous diffusional resistance (or the resistance of the unstimulated water layer) and decrease the intestinal membrane permeability by passive and carrier-mediated intestinal transport. And anderson et al. also reported a similar increase in aqueous diffusional resistance in anesthetized rats. Although reduced intestinal motility by anesthesia might be involved in the increase in aqueous diffusional resistance, there has been no quantitative information about the effect of anesthesia on intestinal motility, or about the motility state under those experimental conditions. We also wondered if intestinal motility might be involved in the change in intestinal membrane permeability.

In the present study, we compared, using inulin as a nonabsorbable marker, propulsive gastrointestinal motility in rats subjected to various experimental treatments to clarify the influence of those treatments and to help interpret the results of intestinal absorption experiments. Propulsive motility, which is measured by transit of a marker, should be a better index of motility in gastrointestinal drug disposition or absorption studies than contractive motility, which is often measured in physiological studies as the changes in luminal pressure, since the gastrointestinal transit of a nonabsorbable marker is considered to represent that of gastrointestinal contents and most drugs.

MATERIALS AND METHODS

Chemicals \([^{3}H(G)]\)inulin (15.8 GBq/g) and Biofluor, a scintillation cocktail, were purchased from DuPont-NEN Co. (Boston, MA, U.S.A.). All other chemicals were of analytical grade and commercially obtained.

Measurement of Propulsive Gastrointestinal Motility

Male Wistar rats, weighing about 300 g, were fed ad libitum and used without fasting, and the gastrointestinal distribution profile of inulin 60 min after administration was compared with the initial distribution profile. All experiments were started between 10:00 a.m. and 12:00 noon.

Unanesthetized Rats without Surgery (Treatment 1): Gastrointestinal distribution profiles of \([^{3}H]\)inulin after intragastric administration (26 kBq/0.1 ml saline/rat) to rats were taken from our previous report, in which the distribution profiles were presented by dividing the small intestine into 10 equal segments.

Unanesthetized Rats with Minimal Abdominal Surgery (Treatment 2): Intestinal distribution profiles of inulin were taken from our previous report, in which \([^{3}H]\)inulin (13 kBq/0.1 ml saline/rat) was injected into the duodenal lumen of rats using a needle through a small abdominal incision under light ether anesthesia. The rats regained consciousness in a few minutes after administration. The distribution profiles of inulin were presented by dividing the small intestine into 10 equal segments.

Unanesthetized Rats with Cannulas for Perfusion (Treatment 3): This procedure was done to mimic the condition of intestinal perfusion without anesthesia. The midgut of the rat was cannulated to make a 10-cm segment under light ether anesthesia as described in our previous report, though the intestinal lumen was not washed. Right after regaining consciousness, the rat received an intraluminal injection of \([^{3}H]\)inulin (19 kBq/0.05 ml saline/rat) through the cannula, and left free in a cage at the ambient temperature of 25 °C. At the end of experiment, the intestinal segment was isolated under ether anesthesia and divided into 5 equal segments. Each segment was longitudinally cut open, and washed in 5 ml of saline. A 50 μl aliquot of the sample was placed in a counting vial, to which was added 2 ml of Biofluor, a
scintillation cocktail, to determine its radioactivity with a liquid scintillation counter (LSC-1000, Aloka Co., Tokyo).

Anesthetized Rats with Abdominal Surgery (Treatment 4): This procedure was done to mimic the condition of perfusion under anesthesia.\(^1,2\) The rats were anesthetized with urethane (1.125 g/kg, i.p.). The abdomen of the rat was cut open, \(^{3}H\)Inulin (19 kBq/0.05 ml saline/rat) was injected into the lumen of the midgut approximately 30 cm below the duodenal-duodenal flexure using a needle, a piece of thread was loosely tied around the injection site so that it could be easily located at the end of experiment, and the abdomen was sutured. A heat lamp was used to maintain the rectal temperature at 37°C. At the end of experiment, a 14 cm intestinal segment was isolated and divided into 7 equal segments so that the injection site was located at the fourth segment. Each segment was processed in the same way as the samples from unanesthetized rats with cannulas (treatment 3).

Anesthetized Rats without Surgery (Treatment 5): The rats were anesthetized with urethane (1.125 g/kg, i.p.), and intragastrically given \(^{3}H\)Inulin (7 kBq/0.1 ml saline/rat). A heat lamp was used to maintain the rectal temperature at 37°C. At the end of the experiment, the stomach and duodenum were isolated, cut open and washed in 10 and 5 ml, respectively, of saline. A 50 µl aliquot of the sample was taken to determine the radioactivity as described above.

Although the amount and concentration of administered \(^{3}H\)Inulin was varied among treatments, they did not exceed 1.65 µg (0.33 mmol)/rat or 4.8 µM, and were low enough to expect no effect on the gastrointestinal motility, for example, osmotically.

**Estimation of Distribution Center and Mean Transit Velocity** The distribution center in the small intestine (DC) was calculated as follows:

\[
DC = \frac{\sum FR_i \cdot x_i}{FR_{\text{a}}}
\]

where \(FR_i\) is the remaining fraction in the ith segment, and \(x_i\) is the dimensionless distance of the center of the ith segment from the pylorus, which is normalized by the length of the small intestine, and \(FR_{\text{a}}\) is the total remaining fraction in the small intestine. Since the residence time in the small intestine is varied for solute molecules in the oral administration experiments because of continuing gastric emptying, the mean residence time (MRT) was estimated as follows:

\[
MRT = \frac{\int_0^T FR_{\text{a}}(t) dt}{FR_{\text{a}}(T)} = \frac{T}{1 - e^{-k_{\text{a}} T}}
\]

where

\[
FR_{\text{a}} = 1 - e^{-k_{\text{a}} t}
\]

The \(k_{\text{a}}\) is the gastric emptying rate constant of 0.025 min\(^{-1}\) from our previous report.\(^3\) The \(T\) is the experimental period of 60 min. The \(MRT\) is equal to the experimental period of 60 min in the other experiments. The mean transit velocity (MTV) was estimated as follows:

\[
MTV = \frac{DC_{60} - DC_0}{MRT}
\]

where \(DC_{60}\) and \(DC_0\) are the distribution center 60 min after administration and the initial distribution center, respectively, and \(L\) is the length of the small intestine. An average \(L\) value of 90 cm was taken from our previous report.\(^4\)

**RESULTS AND DISCUSSION**

Figure 1 shows the distribution profiles of inulin in the gastrointestinal tract at 0 min (panels A) and 60 min (panels B) after administration under various experimental treatments in rats. The values of the distribution center (DC), mean residence time (MRT) and mean transit velocity (MTV) were compared for different treatments.

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**Fig. 1. Gastrointestinal Distribution Profiles of Inulin under Various Experimental Treatments in Rats**

Initial distribution profiles, panels A; the distribution profiles at 60 min, panels B. Treatments: unanesthetized rats without surgery (treatment 1), 1A and 1B; unanesthetized rats with minimal abdominal surgery (treatment 2), 2A and 2B; unanesthetized rats with cannulas for perfusion (treatment 3), 3A and 3B; the rats anesthetized with urethane and treated with abdominal surgery (treatment 4), 4A and 4B. Data are represented as the mean with S.E. (n=5 for 1A and 1B, and 3 for the others). S, D, J, M and I represent stomach, duodenum, jejunum, midgut and ileum, respectively. FR and x represent the remaining fraction and the dimensionless distance from the pylorus, respectively, which was normalized by the length of the small intestine. The arrows indicate the site of administration.
velocity (MTV) were estimated from those profiles and are summarized in Table I. In unanesthetized rats without any surgical operation (treatment 1), 75% of the inulin was emptied from the stomach by 60 min after intragastric administration, and the DC reached the lower midgut at the dimensionless distance of 0.587 from pylorus. However, only a small downward shift of 0.061 in the DC was observed 60 min after intrajejunal administration in unanesthetized rats with minimal abdominal surgery (treatment 2), and no movement in the DC was observed in unanesthetized rats with cannuas (treatment 3) or in the rats anesthetized with urethane and treated with abdominal surgery (treatment 4). In urethane-anesthetized rats without surgery (treatment 5), inulin was almost completely (92 ± 8%) recovered from the stomach 60 min after intragastric administration (not shown in Fig. 1), suggesting insignificant gastric emptying or gastrointestinal motility. These results suggest that even a minimal abdominal surgery in unanesthetized rats, as well as anesthesia with urethane, almost completely inhibits the propulsive gastrointestinal motility.

The result that propulsive motility was similarly suppressed in unanesthetized rats with cannuas and in anesthetized rats may suggest that intestinal motility was not involved in the previous observation in perfusion studies of the lower intestinal membrane permeability or the higher aqueous diffusional resistance in anesthetized rats compared to unanesthetized rats. The difference in intestinal membrane permeability may be due mainly to the direct effect of anesthetics on the intestinal membrane and carriers. The difference in aqueous diffusional resistance may be due mainly to the differences in the arrangement of the perfused segment and resultant luminal flow conditions. The perfused segment was laid, without kinking, on a flat plate outside the abdominal cavity to maintain the laminar flow in anesthetized rats, while the perfused segment was returned inside the abdominal cavity in unanesthetized rats, presumably causing turbulent luminal flow and thereby reducing aqueous diffusional resistance. However, although the propulsive motility, which is induced by peristaltic motility, pendular motility and segmenting movement, was found to be similarly suppressed under those conditions, some peristaltic motility was observed in perfusion studies under anesthesia, and, hence, a potential difference in peristaltic motility and its involvement in the differences in the intestinal membrane permeability and the aqueous diffusional resistance cannot be excluded. Thus, recently suggested differences in the aqueous diffusional resistance for different anesthetic regimens may be due to differences in the peristaltic motility.

The suppression of gastrointestinal motility by abdominal surgery coincides with the postoperative ileus and gastrointestinal dysfunction induced by stress. The corticotropin-releasing factor (CRF) in the central nervous system has recently been suggested to be a major mediator of the inhibition of gastrointestinal motility. The peripheral CRF, capsaicin-sensitive afferent neurons and increased sympathetic activity have also been suggested to be involved in the inhibitory effect.

Significant propulsive gastrointestinal motility, in which the tip of charcoal distribution traversed about 50% of the entire small intestine in 30 min after intragastric administration, was reported in rats anesthetized with pentobarbitone while we did not see significant propulsive motility in rats anesthetized with urethane. Thus, gastrointestinal motility can be affected differently by different anesthetics.

The rats were not fasted in this study. Although gastrointestinal motility may be increased by fasting the rats, it is highly possible that urethane anesthesia and abdominal surgery would extensively reduce the gastrointestinal motility in fasted rats as well.

In conclusion, the present study showed that gastrointestinal motility is extensively suppressed by only a minimal abdominal surgery as well as by anesthesia with urethane. This should be valuable information when considering the potential involvement of intestinal motility in intestinal drug absorption. All abdominal surgery should be avoided in in vivo oral absorption studies, because it may lead to a slower absorption due to a delayed gastric emptying. If surgery is unavoidable, the results should be carefully interpreted by examining the gastrointestinal motility state.

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