Colonic Giant Migrating Contractions Induced by Glycerol Enema in Anesthetized Rats

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Received August 11, 1993 Accepted October 18, 1993

ABSTRACT—Colonic motility was measured with three catheter pressure transducers that were inserted into the descending colon at the distance of 4 cm, 6 cm and 8 cm from the anal verge in anesthetized rats. Colonic infusion of glycerol (65%, 2 ml/kg) induced large phasic pressure changes with high amplitude and long duration. Some of the pressure changes propagated over all the three recording sites, appearing to be equivalent to giant migrating contractions. These glycerol-induced large propulsions were abolished by lidocaine (5%, 2 ml/kg, intracolon), hexamethonium (10 mg/kg, i.v.) or clonidine (30 µg/kg, i.v.); and they were almost entirely suppressed by atropine (3 mg/kg, i.v.), suggesting the principal involvement of the cholinergic neural pathway.

Keywords: Giant migrating contraction, Glycerol, Colon (rat)

Colonic propagating contractions with high amplitude and long duration are referred to as giant migrating contractions (GMCs), which are closely associated with the provocation of urgency and defecation (1, 2). Recently, Bazzocchi et al., using both the manometric and the scintigraphic method simultaneously, demonstrated that these colonic contractions are the motor equivalents of colonic peristalsis or the rapid and massive movements of the colonic contents (2). On the other hand, glycerol enema that is clinically used for the treatment of chronic constipation (3) or paralytic ileus (4) has been known to induce GMCs (5-7). It is reported that lidocaine suppresses the occurrence of GMCs after glycerol enema in humans (5, 7). However, the drug susceptibility of GMCs in rats has not been reported prior to the present study. In the present study, we measured the manometric alteration of colonic motor activity after glycerol enema and observed, for the first time, reproducible GMCs in rats. Effects of hexamethonium, atropine and clonidine, in addition to lidocaine, on the occurrence of GMCs were determined in our evaluating system.

Male Wistar rats (Charles River Japan, Inc., Kanagawa), weighing 300–400 g and fasted overnight, were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and ketamine (10 mg/kg, i.p.) and laid supine on the wood board. Three catheter pressure transducers (SPC-350, 5 French; Millar Instrument, Inc., Houston, TX, USA) were inserted into the descending colon at the distance of 4 cm, 6 cm and 8 cm from the anal verge. Signals from the transducers were amplified through the control unit (TCB-500, Millar Instrument, Inc.) and recorded at the speed of 1 cm/min (Linearrecorder WR-3701; Graphtec Corp., Tokyo). Forty minutes after the anesthesia, glycerol aqueous solution (2 ml/kg) prewarmed at 37ºC was infused over a 1-min period into the colon through a polyethylene tube (PE-50; Clay Adams, Parsippany, NJ, USA), whose tip was positioned at the distance of 7.5 cm from the anal verge. To determine the drug susceptibility of the colonic alteration after the glycerol enema, the rats were pretreated with each drug as follows: Lidocaine aqueous solution (5%, 2 ml/kg) prewarmed at 37ºC was instilled over a 1-min period through the polyethylene tube in stalled for the glycerol enema, 10 min before the glycerol infusion. For the control, the same volume of water was instilled in the same way. Hexamethonium (10 mg/kg), atropine (0.1–3 mg/kg) or clonidine (10–30 µg/kg) was intravenously administered (1 ml/kg) 10 min before the glycerol infusion, and the effect was compared with the control in which saline (vehicle) was intravenously administered instead of the drug. Atropine sulfate (Nacalai Tesque, Kyoto), clonidine hydrochloride and lidocaine hydrochloride (Sigma, St. Louis, MO, USA), hexamethonium bromide (Methobromine®; Yamanouchi, Tokyo) and glycerol (Kanto Chemicals, Tokyo) were used.
In the preliminary experiment, rats were laparotomized to observe the exteriorized colon visually. After the glycerol enema, we recognized the occurrence of colonic propulsive contractions that were strong enough to almost occlude the colonic lumen and ascertained that the intense propulsive contractions coincided with the propagation of large phasic pressure changes by conducting the simultaneous manometric recording of the colonic lumen in the vicinity of the intense propulsive contractions.

The following criterion was adopted to judge whether the propulsion was a GMC or not: If the total product of amplitude and duration of sequentially propagating waves over the three adjacent recording sites exceeded 3,000 mmHg·sec, we interpreted the waves as one GMC. Moreover, we also judge whether each wave was a giant contraction (GC) or not: When the product of amplitude and duration of a wave was more than 1,000 mmHg·sec, we regarded the wave as a GC. The number of GCs in all three of the recording sites and that of GMCs during 30 min after the beginning of glycerol infusion were determined. Each data was expressed as the mean±S.E.M.

Typical alteration of the colonic motor activity induced by the colonic infusion of glycerol (65%, 2 ml/kg) is shown in Fig. 1. After the glycerol enema, conspicuous GCs and GMCs occurred, while spontaneous phasic pressure changes diminished. In the preliminary studies, we recognized a concentration-dependent increase in the number of GCs and GMCs with increasing glycerol concentration (15–80%, 2 ml/kg, intracolon). However, the propagating speed of GMCs from the proximal recording site (8 cm) to the distal one (4 cm) was not dependent on the glycerol concentration. The mean velocity of 71 GMCs observed after the glycerol enema was calculated to be 1.84±0.10 mm/sec. In the rats without insertion of the catheter pressure transducers but otherwise treated under the same conditions, the time lag of the onset of defeation after the glycerol enema (65%, 2 ml/kg) was determined to be 7.80±0.47 min (N = 6). The rapid evacuation of feces after the glycerol enema suggests that the GMCs observed in the present study may be the motor equivalents of colonic peristalsis.

Intracolonic instillation of lidocaine (5%, 2 ml/kg) before the glycerol enema abolished the occurrence of GMCs, and it almost entirely inhibited that of GCs (Table 1, Fig. 2). The suppressive action of lidocaine, a local anesthetic, on the occurrence of GMCs had already

![Fig. 1. Typical alteration of colonic motor activity after glycerol enema (65%, 2 ml/kg) in rats. Colonic pressure changes were monitored at three recording sites of the descending colon, at the distance of 4 cm, 6 cm and 8 cm from the anal verge. Arrows indicate examples of giant migrating contractions.](image)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>No. of GCs</th>
<th>No. of GMCs</th>
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<tbody>
<tr>
<td>Control (Water)</td>
<td></td>
<td></td>
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<tr>
<td>Lidocaine</td>
<td>5%, 2 ml/kg, intracolon</td>
<td>10.17±1.28</td>
<td>2.83±0.31</td>
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<td></td>
<td></td>
<td>0.17±0.17**</td>
<td>0.00±0.00***</td>
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<tr>
<td>Control (Saline)</td>
<td></td>
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<tr>
<td>Hexamethonium</td>
<td>10 mg/kg, i.v.</td>
<td>13.00±1.59</td>
<td>3.33±0.33</td>
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<tr>
<td></td>
<td></td>
<td>0.00±0.00***</td>
<td>0.00±0.00***</td>
</tr>
<tr>
<td>Atropine</td>
<td>0.1 mg/kg, i.v.</td>
<td>9.00±1.69</td>
<td>2.17±0.60</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>5.33±1.41</td>
<td>1.33±0.49</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3.83±1.54**</td>
<td>0.67±0.33**</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.67±0.61**</td>
<td>0.33±0.21**</td>
</tr>
<tr>
<td>Clonidine</td>
<td>10 μg/kg, i.v.</td>
<td>6.17±1.94</td>
<td>1.00±0.45*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.17±0.17**</td>
<td>0.00±0.00**</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.M. of 6 rats in individual groups except for the saline-treated control group consisting of 15 rats. **P < 0.01, ***P < 0.001 (Wilcoxon rank sum test); *P < 0.05, **P < 0.01 (Scheffe's multiple range test), significantly different from the values in the control.
been documented in humans (5, 7). It is assumed that lidocaine inhibits the contact stimulus of glycerol on the colonic lumen due to its membrane stabilizing property (8). We ascertained that the GMCs observed by us were also lidocaine-sensitive. In the present study, pretreatment with hexamethonium (10 mg/kg) abolished the occurrence of GCs and GMCs (Table 1), indicating that the nicotinic ganglionic transmission is requisite for the occurrence of GCs and GMCs. Moreover, atropine (0.1–3 mg/kg) unequivocally and dose-dependently prevented the occurrence of GCs and GMCs (Table 1, Fig. 2), suggesting that the final motor neuron to the smooth muscle is mainly cholinergic. However, even at the high dose of 3 mg/kg of atropine, about 13% of the GCs and 10% of the GMCs remained uninhibited. This incomplete suppression by atropine might imply the existence of contractile neurotransmitters other than acetylcholine, leading to the provocation of GCs and GMCs.

Our present results were consistent with the previous results of Zhu et al. (9) and Sarna et al. (10), using monkeys and dogs, respectively, in that the occurrence of GMCs was inhibited by hexamethonium. Data on the susceptibility of GMCs to atropine, however, have been contradictory. Zhu et al. (9) stated that the GMCs in monkeys elicited by arginine vasopressin were non-cholinergic because these GMCs were not affected by atropine (0.15 mg/kg, i.v.), while Sarna et al. (10) reported that the GMCs in dogs provoked by thyrotropin releasing hormone (TRH) were blocked by atropine (0.1 mg/kg, i.v.). The contradictory results on the susceptibility to atropine might be attributable to the difference of provocative stimulus, species and/or the dose of atropine used.

We observed that clonidine (30 μg/kg, i.v.) completely suppressed the occurrence of GMCs and almost entirely inhibited that of GCs (Table 1). Clonidine is known to inhibit the release of acetylcholine from intrinsic cholinergic nerve terminals in the guinea pig ileum and colon (11, 12). Therefore, it is likely that similar reduction of acetylcholine release was elicited also in the rat colon, and that this reduction was involved in the suppression of the occurrence of GCs and GMCs. From the results that clonidine completely prevented the occurrence of GMCs in comparison to the incomplete inhibitory action of atropine, it is suggested that clonidine reduced the neuronal release of not only acetylcholine but also other neurotransmitters involved in the occurrence of GMCs. This interpretation is consistent with the recent findings (13) that in the isolated rabbit colon, the atropine-resistant neurogenic contractions elicited by pelvic nerve stimulation
were completely abolished by UK14819, an $\alpha_2$-adrenoceptor agonist, and that this effect was completely reversed by idazoxan, an $\alpha_2$-adrenoceptor antagonist.

In view of the well-known anti-diarrheal activity of clonidine (14), it is noteworthy that clonidine suppressed the occurrence of GMCs. Karaus and Sarna suggested that the absence of GMCs results in poor propulsion, leading to constipation, while too frequent occurrence of GMCs results in rapid propulsion and diarrhea (1). Afterwards, this notion was substantiated clinically in patients with idiopathic chronic constipation (15) or functional diarrhea (2), in which decrease or increase in the number of GMCs was observed, respectively. Thus, suppression of the occurrence of GMCs by clonidine may in part account for its anti-diarrheal activity.

In conclusion, a simple manometric method using catheter pressure transducers enabled us to observe reproducible GMCs after the glycerol enema in rats. The principal neural pathway involved in the occurrence of glycerol-induced GMCs was assumed to be cholinergic.

Acknowledgments

Mrs. A. Tomaru and Mrs. K. Yanase are greatly appreciated for their kind assistance in collecting valuable information. We are grateful to Prof. D. Sasaki of Hirosaki University for encouragement and support.

REFERENCES