Susceptibility to Adenosine Agonists of Giant Migrating Contractions Induced by Glycerol Enema in Anesthetized Rats

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ABSTRACT—The present study examined whether adenosine agonists influence the occurrence of giant migrating contractions (GMCs) induced by glycerol enema (65%, 2 ml/kg) in rats. Catheter pressure transducers were used to measure the colonic luminal manometric alterations. The adenosine A1 agonists (2S)-N6-(2-endo-norbornyl)adenosine ((S)-ENBA) (10 µg/kg, i.v.) and N6-cyclohexyladenosine (30 µg/kg, i.v.) abolished the GMCs, whereas the adenosine A2 agonist 2-[p-(2-carboxyethyl)phenethylamino]-5'-N-ethylcarboxamidoadenosine (CGS 21680) (30–300 µg/kg, i.v.) failed to influence the GMCs. The suppressive action of (S)-ENBA on the GMCs was entirely counteracted by the peripheral adenosine antagonist 8-(p-sulfophenyl)theophylline (10 mg/kg, i.v.). The present observations suggest that the adenosine A1 agonist suppresses the GMCs via peripheral adenosine receptors.

Keywords: Adenosine agonist, Glycerol, Giant migrating contraction

Colonic propagating contractions with high amplitude and long duration are referred to as giant migrating contractions (GMCs), which have been reported to be closely associated with the provocation of urgency and defecation (1, 2). Glycerol enema, which is clinically used for the treatment of chronic constipation and for colonic lavage prior to colonoscopy procedures, has been known to elicit GMCs (3). We have previously reported that glycerol enema induced reproducible GMCs, which were abolished by hexamethonium, clonidine or lidocaine and were prominently inhibited by atropine, in anesthetized rats (4).

Recently, considerable interest has been focused on the physiological role of adenosine as an inhibitory neuro-modulator. In view of the inhibitory action of adenosine on the release of principal excitatory neurotransmitters such as acetylcholine and tachykinins from enteric neuronal synaptosomes (5, 6), it is expected that exogenously administered adenosine or adenosine agonists exert inhibitory effects on gastrointestinal motility. In fact, it was reported that adenosine inhibited vagally-stimulated gastric motility in rabbits (7), and that adenosine agonists, by acting peripherally, suppressed the interdigestive migrating myoelectrical complex in the rat small intestine (8). As for the colon, Tonini et al. (9) described that ATP inhibited the propulsive contractions in the isolated rabbit colon. However, the in vivo effect of adenosine or adenosine agonists on colonic motility has not been reported prior to the present study.

The purpose of this study was to clarify the effect of adenosine agonists on the occurrence of GMCs induced by glycerol enema in anesthetized rats. To discriminate the subtype of adenosine receptors involved, we employed the following selective adenosine agonists (10, 11): the potent adenosine A1 agonist (2S)-N6-(2-endo-norbornyl)adenosine ((S)-ENBA), the less potent adenosine A1 agonist N6-cyclohexyladenosine (CHA) and the adenosine A2 agonist 2-[p-(2-carboxyethyl)phenethylamino]-5'-N-ethylcarboxamidoadenosine (CGS 21680).

The measurement and analysis of the colonic motor activity after glycerol enema were performed according to our previous method (4). Briefly, male Wistar strain rats (Charles River Japan, Inc., Atsugi), weighing 300–450 g and fasted overnight, were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and ketamine (10 mg/kg, i.p.). Three catheter pressure transducers (SPC-350, 5 French; Millar Instrument, Inc., Houston, TX, USA) were then inserted through the anus into the descending colon, so that the sensors of the catheters were positioned 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 (Linearcord er WR-3701; Graphtec Corp., Tokyo). Forty minutes
Fig. 1. Representative manometric alterations induced by glycerol enema (65%, 2 ml/kg) (A) and its susceptibility to the adenosine agonists (S)-ENBA (10 pg/kg, i.v.) (B), CHA (30 pg/kg, i.v.) (C) and CGS 21680 (300 pg/kg, i.v.) (D) in the rat colon. Colonic luminal pressure changes were monitored at three recording sites of the descending colon, 4 cm, 6 cm and 8 cm from the anal verge. Examples of giant migrating contractions are indicated by the arrows in the figure (A).
after the anesthesia, glycerol aqueous solution (65%, 2 ml/kg) prewarmed at 37°C 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. The adenosine agonist, (S)-ENBA (0.3 – 10 µg/kg), CHA (1 – 30 µg/kg) or CGS 21680 (30 – 300 µg/kg), was administered (1 ml/kg, i.v.) 10 min before the glycerol enema. The adenosine antagonist 8-(p-sulfophenyl)theophylline (8-SPT) was administered (2 ml/kg, i.v.) 5 min before the glycerol enema. The intraperitoneal administration of the drug was carried out into the femoral vein.

To quantitatively evaluate the drug effect on glycerol-induced manometric alterations, giant contractions (GCs) and GMCs were counted during 30 min from the beginning of the glycerol enema: If the product of the amplitude and duration of each wave was more than 1,000 mmHg·sec, the wave was regarded as a GC. When the total products of amplitude and duration of sequentially propagating waves over the 3 adjacent recording sites exceeded 3,000 mmHg·sec, the waves were interpreted as one GMC. Animals were used only once for the evaluation. All the values were expressed as means±S.E.M., and a P value less than 0.05 was considered statistically significant.

(S)-ENBA, CHA, CGS 21680 hydrochloride and 8-SPT were purchased from Research Biochemicals International (Natick, MA, USA); glycerol was from Kanto Chemical (Tokyo). CHA and CGS 21680 were dissolved in saline; 8-SPT was dissolved in distilled water. (S)-ENBA was dissolved in dimethylsulfoxide (DMSO) (10 mg/ml) and then diluted with saline. The solvent DMSO, whose final concentration was 0.1% or lower, had no effect on the glycerol-induced manometric alterations in the preliminary experiment.

A representative tracing of the manometric alterations induced by glycerol enema (65%, 2 ml/kg) is shown in Fig. 1A, in which conspicuous GCs and GMCs were observed. The occurrence of GCs and GMCs was abolished by (S)-ENBA (10 µg/kg, i.v.) (Fig. 1B) or CHA (30 µg/kg, i.v.) (Fig. 1C), but was not substantially influenced by CGS 21680 (300 µg/kg, i.v.) (Fig. 1D). Interestingly, in the tracing shown in Fig. 1B, spontaneously occurring GCs were by chance observed at 4 cm prior to the glycerol enema. These GCs were also abolished by (S)-ENBA. Another interesting event is that CGS 21680 (300 µg/kg, i.v.) per se elicited manometric alterations, which were not so intense as those induced by glycerol enema (Fig. 1D). Since the stimulation of adenosine A2 receptors on the smooth muscle cells causes relaxation of the rat colon strip (12), we suspect that CGS 21680 elicited the manometric alterations not by directly acting on the smooth muscle cells, but perhaps by acting on the enteric neurons.

Figure 2 summarizes the effects of adenosine agonists on the numbers of GCs and GMCs occurring during 30 min from the beginning of glycerol enema (65%, 2 ml/kg). Both (S)-ENBA (0.3 – 10 µg/kg, i.v.) and CHA (1 – 30 µg/kg, i.v.) unequivocally and dose-dependently decreased the numbers of GCs and GMCs. In contrast, CGS 21680 (30 – 300 µg/kg) did not exhibit any significant difference in the numbers of GCs or GMCs in comparison with those in the saline-treated control. The suppressive action of (S)-ENBA, which has higher affinity to adenosine A1 receptors than CHA (10), was more
prominent than that of CHA. Thus, it is suggested that the suppression of GCs and GMCs is associated with the stimulation of adenosine \(A_1\) receptors. The slight suppressive action of CGS 21680 is likely to be ascribed to the lower affinity of this drug to adenosine \(A_1\) receptors (11).

In the next experiment, we employed the adenosine antagonist 8-SPT to investigate the possible involvement of adenosine receptors in the suppressive action of (S)-ENBA on the occurrence of GCs and GMCs. Table 1 shows the numbers of GCs and GMCs occurring during 30 min from the beginning of glycerol enema (65\%, 2 ml/kg) in rats, which were pretreated with (S)-ENBA (10 \(\mu g/ kg\)) or its vehicle (0.1\% DMSO) followed by 8-SPT (10 mg/kg) or its vehicle (distilled water). The conspicuous GCs and GMCs occurred in rats treated with vehicles only, whereas no GC or GMC was observed in rats treated with (S)-ENBA and vehicle. However, there was no statistical difference in the numbers of GCs and GMCs between the group treated with vehicles only and the group treated with (S)-ENBA and 8-SPT, indicating that the suppressive action of (S)-ENBA was completely counteracted by 8-SPT. These observations suggest that adenosine \(A_1\) agonists suppress the occurrence of GCs and GMCs via adenosine receptors. The responsible receptors probably exist peripherally because 8-SPT is a polar adenosine antagonist, which hardly crosses the blood brain barrier (13).

It is known that adenosine inhibits the release of biologically-labeled \([\text{H}]\)acetylcholine from the synaptosomal fraction of guinea pig ileal myenteric plexus (5). Assuming that the similar reduction of acetylcholine release is elicited in the rat colon as well as in the guinea pig ileum, this reduced release of acetylcholine might be involved in the suppressive action of adenosine \(A_1\) agonists. This interpretation coincides with our previous finding (4) that the principal neuronal pathway involved in the occurrence of GCs and GMCs in rats is cholinergic. Since the stimulation of adenosine \(A_1\) receptors on the smooth muscle cells causes contraction of the smooth muscle preparation of rat colon (14), the direct action of adenosine \(A_1\) agonists on the smooth muscle cells is unlikely to contribute to the suppression of GCs and GMCs.

In regard to the pathological significance of GMCs in colonic diseases, Karaus and Sarna (1), by observing GMCs in dogs, framed the hypothesis that too frequent occurrence of GMCs may result in rapid propulsion and diarrhea. This hypothesis was afterward substantiated in a clinical study, in which the increase in the number of GMCs was demonstrated in patients with functional diarrhea (2). Considering this clinical observation, the present study suggests the possibility that adenosine \(A_1\) agonists could inhibit diarrhea in patients with conspicuous GMCs. Indeed, Tucker et al. (15) previously found that the adenosine \(A_1\) agonist CHA suppressed morphine-withdrawal diarrhea in mice. Likewise, we observed that the adenosine agonist (S)-ENBA suppressed diarrhea provoked by 16,16-dimethylprostaglandin E2 in rats (A. Tomaru et al., unpublished data). However, clinical use of adenosine \(A_1\) agonists as anti-diarrheal drugs may be limited because of the well-known adverse cardiovascular reactions induced by these compounds including negative cardiac inotropy, chronotropy and dromotropy.

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