Effects of Adenosine on Contractile Response of Circular Muscle in Electrically Stimulated Guinea-Pig Ileum

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Abstract—The action of adenosine on the electrically induced mechanical response of circular muscle in isolated guinea-pig ileum has been investigated. Electrical stimulation (0.1 Hz) elicited the twitch response, which was completely abolished by tetrodotoxin (0.2 μM), morphine (1 μM) and atropine (0.1 μM). Adenosine (0.1–100 μM) markedly depressed the twitch response in a concentration-dependent manner, and the concentration-depression curve for adenosine was significantly shifted to the right in the presence of theophylline (30 μM). On the other hand, the contractile responses induced by acetylcholine (1–300 μM) were not affected by adenosine at all. The present investigation suggests that the twitch response is mediated through acetylcholine released from the intramural cholinergic nerves supplying the circular muscle of guinea-pig ileum, and adenosine has an inhibitory effect on the cholinergic transmission, probably via P1-purinoceptors.

Adenosine and its related compounds have been shown to exert a presynaptic inhibitory action in various tissues. Previously, we had examined the effects of 21 purine compounds on the electrically induced longitudinal contractile responses of isolated guinea-pig ileum and showed that adenosine among them markedly depressed the responses, and our results further suggested that the inhibitory action was due to reduced ACh release from the cholinergic nerve supplying the ileal longitudinal muscle layer (1, 2).

Bishop et al. (3) had reported that adenosine depressed the peristaltic reflex of rat small intestine in situ. Also, Okwuasaba et al. (4) demonstrated that adenosine and its derivatives produced inhibition of pressure-induced peristaltic activity in the guinea-pig ileum and proposed that a purine compound may be involved in descending inhibition observed during peristalsis. Generally, it is accepted that the circular muscle in the gastro-intestine plays a important role in the mingling and transit of the intraluminal contents of the intestine during the peristalsis. Furthermore, it has been suggested that the circular muscle is different from the longitudinal muscle layer in regards to the innervation (5) and electrical and mechanical properties (6–8). However, there are very few research reports about the action of adenosine and related compounds on the circular muscle of the gastro-intestine. Therefore, in the present study, we examined the effect of adenosine on the contractile response to electrical stimulation at 0.1 Hz in the circular muscle of guinea-pig ileum and discussed the action of adenosine on the cholinergic nerves supplying the circular muscle.

Materials and Methods

Guinea-pigs weighing 300–350 g were used throughout. The animals were sacrificed and bled, and a portion of the ileum near the caecum was excised. The 3–5 mm long ileal preparation was mounted horizontally in a 10 ml organ bath which contained Tyrode's solution gassed with a mixture of 95% O2 and 5% CO2 and kept at 37°C, as described
in Fig. 1. The ileal preparations were subjected to 0.5 g resting tension and allowed to stabilize for 60 min before an experiment. The mechanical responses of the circular muscle was isometrically recorded with a force-displacement transducer (Nihon Kohden, TD-170). Transmural electrical stimulation of the ileum was accomplished with two fine platinum electrodes, and the intraluminal electrode was the cathode. Rectangular pulses used were of 0.4 msec duration at the frequency of 0.1 Hz and were of a strength sufficient to give a maximal response. Adenosine and acetylcholine were cumulatively added to the organ bath, and the other drugs used were each administered as a 5 min pretreatment at the dose levels used in our previous report (2). Drugs used were obtained from the following sources: acetylcholine chloride (Daiichi), Adenosine (Sigma), Atropine sulfate (Merck), hexamethonium (Sigma), morphine hydrochloride (Takeda), tetrodotoxin (Sankyo), and theophylline (Kohjin).

Results

The effect of transmural electrical stimulation at 0.1 Hz on the circular muscle of the isolated guinea-pig ileum are shown in Fig. 2. The electrical pulses evoked a twitch response, which was similar to the response of ileal longitudinal muscle. Atropine at the concentration of 0.1 μM completely depressed the twitch response. Also, tetrodotoxin at 0.2 μM and morphine at 1 μM abolished it. Adenosine produced a concentration-dependent depression of the twitch response over the range of 0.1–100 μM (Figs. 2 and 3). The effect was relative slow in onset with maximal depression always developing within 1 min. The inhibitory effect disappeared rapidly (3–5 min) after washing the preparation. Thus, adenosine significantly reduced the contraction height by 18.8±2.5% (mean±S.E.M. n=12) at 1 μM and by 100% (n=12) at 100 μM, and the pD2 value was 5.33±0.07 (n=12). Theophylline at the concentration of 30 μM shifted the concentration-depression curve for adenosine to the right without depressing the maximal effect, and

![Fig. 1. Apparatus for the measurement of mechanical response in the isolated guinea-pig ileal circular muscle.](image)

![Fig. 2. Effect of atropine (Atr: 0.1 μM), tetrodotoxin (TTX: 0.2 μM), morphine (Mor: 1 μM) and adenosine (Ade: 10 μM) on the electrically (0.1 Hz) induced twitch response of guinea-pig ileal circular muscle.](image)

![Fig. 3. Concentration-depression curve for adenosine in the absence (Ο: n=12) and presence (●: n=5) of theophylline (30 μM). Ordinate: average % of inhibition of the twitch response produced by adenosine. Abscissa: concentration (–log M) of adenosine. Each point represents the mean±S.E.M.](image)
it significantly decreased the pD2 value to 4.94±0.08 (n=5, P<0.01). Figure 4 shows the effect of adenosine at 100 μM on the concentration-response curve for acetylcholine. Adenosine did not affect the curve in the concentration range of 1-300 aM.

Discussion
This study has shown that when the isolated guinea-pig ileum was transmurally stimulated with electrical pulses delivered at frequency of 0.1 Hz, a twitch response of the circular muscle was elicited. This response was completely abolished by atropine, morphine or tetrodotoxin and hence represented the response of the muscle to acetylcholine released from the electrically stimulated cholinergic neurons in the myenteric plexus. In the ileal longitudinal muscle, it is generally accepted that contraction induced by single low-frequency stimuli is a cholinergic response (9). In the previous report, we had shown that adenosine and its related compounds depressed the electrically-induced twitch response of the ileal longitudinal muscle of guinea-pigs (1), and the present study showed that adenosine depressed the electrically-induced twitch response of the circular muscle, concentration-dependently.

The pD2-value for adenosine of the circular muscle was 5.33±0.07, while that of the longitudinal muscle was 5.83 (2). From a comparison of the two values, it is suggested that the circular muscle cholinergic response was less sensitive to adenosine than the longitudinal muscle. Such a difference of adenosine-sensitivity between the two muscle layers may be explained by the following possibilities:

(a) It may be very difficult for adenosine to reach the action sites, since the circular muscle layer was sandwiched between the longitudinal muscle layer and the mucous layer in the small intestine.

(b) There may be a process that potently inactivates adenosine, such as degradation by adenosine deaminase and the uptake into the tissue, in the circular muscle. Harry et al. (10, 11) had suggested that a higher activity of acetylcholine esterase in the circular muscle than the longitudinal muscle might protect the cholinergic receptors. Certainly, comparing the former data on the concentration-response curve for acetylcholine in the longitudinal muscle (1) with the present data on the one for acetylcholine indicated that the sensitivity to acetylcholine in the circular muscle was less than the longitudinal muscle.

(c) The threshold of stimulation by adenosine of the action sites in the circular muscle may be higher than that of the longitudinal muscle. It is unlikely that the first possibility is responsible for the difference in adenosine sensitivity since morphine completely inhibited the twitch contraction of circular muscle at the same concentration as on the longitudinal muscle preparation (2). From these data here, we can not further discuss the significance of the difference of adenosine potency in two smooth muscle layers; however, we can at least say that this difference may have some physiological role.

Adenosine could not affect the acetylcholine-induced contractile response at all, although it depressed the electrically-induced twitch response. Previously, we had showed that adenosine at the same concentration (100 μM) depressed the maximal contractile response of longitudinal muscle to a higher concentration (100–300 μM) of acetylcholine. It is very difficult to explain these discrepancies. Probably, adenosine at
100 μM could not affect the circular smooth muscle for the reasons mentioned above. Therefore, it is likely that adenosine may act upon the postganglionic cholinergic nerves per se, reducing acetylcholine release. In the previous reports, we had shown that the inhibitory effect of adenosine on the ileal twitch response induced by electrical stimulation was due to the reduction of acetylcholine release from the intramural cholinergic nerve terminals (2).

Recently, Cooper et al. (12, 13) prepared the synaptosomes from the myenteric plexus of the guinea-pig ileum and suggested that adenosine inhibited high potassium- and DMPP-induced 3H-acetylcholine release from these preparations, and we showed that adenosine acts mainly on cholinergic nerve terminals in the ileal longitudinal muscle of guinea-pig, using the special dual organ bath (14). Probably, adenosine may affect the cholinergic nerve terminals supplying the circular muscle in the same manner as in the longitudinal muscles, and its inhibitory effect appears to be mediated through the prejunctional P1-purinoceptors described by Burnstock (15), since the inhibition was antagonized by theophylline which is a P1 antagonist. It is proposed that endogenous adenosine-related compounds may have physiological roles as modulators which control acetylcholine release in the circular muscle of guinea-pig ileum, in agreement with our suggestions (16, 17) and the other reports (4, 18).

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