Isoproterenol Changes the Relationship between Cytosolic Ca^{2+} and Contraction in Guinea Pig Taenia Caecum

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ABSTRACT—To determine the role of β-adrenoceptors in the regulation of intestinal smooth muscle, the action of isoproterenol (ISO) on cytoplasmic Ca^{2+} level ($[Ca^{2+}]_{cyt}$) and mechanical activity in the isolated guinea pig taenia caecum was examined. Spontaneous changes in $[Ca^{2+}]_{cyt}$ and contraction were inhibited by ISO (0.1–1 μM) without changing resting $[Ca^{2+}]_{cyt}$. ISO more strongly inhibited the histamine-induced contraction than the high K^+-induced contraction. ISO inhibited muscle tension more strongly than $[Ca^{2+}]_{cyt}$ stimulated by high K^+ and thus shifted the $[Ca^{2+}]_{cyt}$-tension curve to the lower-right. In the muscle stimulated by histamine, on the other hand, ISO inhibited both $[Ca^{2+}]_{cyt}$ and tension. Salbutamol, a β_2-selective agonist, showed similar effects as ISO on spontaneous, high K^+- and histamine-stimulated $[Ca^{2+}]_{cyt}$ and tension. Stimulation of β-adrenoceptors by ISO increased cyclic AMP content without changing cyclic GMP content. These results suggest that activation of β_2-adrenoceptors by ISO inhibits the contractions by two mechanisms of action: decrease in Ca^{2+} sensitivity of contractile elements in the muscle stimulated by K^+-depolarization and decrease in $[Ca^{2+}]_{cyt}$ in the muscle stimulated by histamine. These effects may be mediated by cyclic AMP.

Keywords: Isoproterenol, Cytosolic Ca^{2+}, Cyclic AMP, Relaxation, Smooth muscle (intestinal)

The effects of catecholamines on gastrointestinal smooth muscle are mediated by α- and β-adrenoceptors (1). These adrenoceptors activate different mechanisms (2, 3). Activation of β-adrenoceptors is characterized by an inhibition of spontaneous spike activity which occurs with a small hyperpolarization in guinea pig taenia. The mechanism underlying the β-action is supposed to be the increase in K^+ conductance or activation of an electrogenic Ca^{2+}-extrusion pump. In addition, the activation of Ca^{2+} sequestration may also be responsible for the β-action (cf. 2, 3). It has been demonstrated with fluorescent Ca^{2+}-indicators that activation of β-adrenoceptors reduces cytosolic Ca^{2+} levels ($[Ca^{2+}]_{cyt}$) and tension in gastrointestinal muscles (4, 5), possibly by cyclic AMP-dependent mechanisms.

Although the rise in $[Ca^{2+}]_{cyt}$ initiates contractions in smooth muscle, there is not a simple relationship between $[Ca^{2+}]_{cyt}$ and tension (6, 7). Several agonists have been shown to alter the sensitivity of the contractile apparatus to Ca^{2+}. For example, high K^+ induces less contractile tension for a given increase in $[Ca^{2+}]_{cyt}$ than agonists such as norepinephrine in ferret portal vein (8); norepinephrine, prostaglandins and endothelin-1 in rat aorta (9–11); thromboxane analogues and phenylephrine in rabbit pulmonary artery (12); carbachol in canine trachea (13, 14); and pilocarpine in ileum (15). Furthermore, several reports suggested that receptor-agonists induce greater myosin light chain phosphorylation than high K^+ at a given $[Ca^{2+}]_{cyt}$ (13, 16, 17). A decrease in Ca^{2+}-sensitivity due to cyclic AMP and cyclic GMP has also been reported (18–21). Therefore, it is possible that modulation of smooth muscle contraction by catecholamines is due to the decrease in $[Ca^{2+}]_{cyt}$ and Ca^{2+} sensitivity. To determine the mechanism of β-action in gastrointestinal muscles, we examined the effects of ISO on $[Ca^{2+}]_{cyt}$ and muscle tension in guinea pig taenia caecum.

MATERIALS AND METHODS

Male guinea pigs weighing approximately 300 g were

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killed by a blow on the neck and exsanguination, and the taenia caecum was isolated promptly. Strips of taenia caecum approximately 1-mm wide and 7-mm long were prepared.

The normal physiological salt solution (PSS) contained: 136.9 mM NaCl, 5.4 mM K^-, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 23.8 mM $NaHCO_3$, 0.01 mM ethylenediamine tetraacetic acid (EDTA), and 5.5 mM glucose. High K^+ solution was made by substituting NaCl with equimolar KCl in PSS. These solutions were saturated with a 95% O_2 and 5% CO_2 mixture at 37℃ and pH 7.4.

$[Ca^{2+}]_{cyt}$ was measured simultaneously with muscle contraction in fura-2-loaded taenia caecum, as reported previously (9, 22, 23). Muscle strips were treated with 10 μM acetoxymethyl ester of fura-2 (fura-2/AM) for 3−5 hr at room temperature. The non-cytotoxic detergent, cremophor EL (0.04%), was added to increase the solubility of fura-2/AM. Diisopropyl fluorophosphate (DFP, 0.1 μM) was added to inhibit the hydrolysis of fura-2/AM by cholineesterases in the extracellular space. Muscle strips were rinsed with normal PSS at 37℃ for 30 min after fura-2 loading to remove unhydrolyzed fura-2/AM. Preliminary experiments confirmed that fura-2 loading did not affect the contractile responses to K^+ or histamine. Experiments were performed with an apparatus designed to simultaneously measure both fura-2-Ca^{2+} fluorescence and contractile tension (CAF-100, Japan Spectroscopic, Tokyo, Japan). The muscle strip was held horizontally in a 10-ml organ bath at 37℃. One end of the muscle strip was connected to a strain gauge transducer (Toyo Boldwin, Tokyo, Japan) to monitor the mechanical activity. A resting tension of 2 mN were initially applied. The muscle strips was illuminated alternately (48 Hz) with 340 nm and 380 nm light. The light emitted from the muscle strip was collected by a photomultiplier through a 500-nm filter.

Absolute Ca^{2+} concentrations were not calculated in the present experiments because a large part of the intracellular fura-2 binds to soluble proteins and the dissociation constant of fura-2 and the characteristics of fura-2 fluorescence may differ from those obtained in vitro (23).

Adenosine 3':5'-cyclic monophosphate (cyclic AMP) content in taenia was measured by radioimmunoassay (19). After the incubation, the muscle strips were frozen in liquid nitrogen and homogenized in 6% trichloroacetic acid solution. Trichloroacetic acid in the supernatant after centrifugation was removed by washing with water-saturated ether. Cyclic AMP was succinylated and assayed by a competitive radioimmunoassay with [^{125}I]-succinyl cyclic AMP-tyrosinemethyl-ester. Radioactivity was counted with an Auto-Gamma Counter (Packard, U.S.A.). Tissue guanosine 3':5'-cyclic monophosphate (cyclic GMP) was measured with [^{125}I]-succinyl cyclic GMP-tyrosinemethyl-ester.

The following drugs and chemicals were used: histamine dihydrochloride, isoproterenol bitartrate (Wako Pure Chemicals, Osaka, Japan), phentolamine mesylate (Ciba-Geigy Japan, Osaka, Japan), cremophor EL (Nacalai Tesque, Kyoto, Japan), isobutylmethylxanthine (IBMX), salbutamol hemisulphate, DFP, metapronol tartrate, verapamil hydrochloride (Sigma Chemicals, St. Louis, USA), EDTA, EGTA and fura-2/AM (Dojindo Laboratories, Kumamoto, Japan).

The results of the experiments are expressed as the mean±S.E.M. Student's *t*-test was used for statistical analysis of the results.

RESULTS

Changes in $[Ca^{2+}]_{cyt}$ and muscle tension

Figure 1 shows the changes in muscle tension and fura-2-Ca^{2+} fluorescence induced spontaneously or by the addition of high K^+ solution or histamine in the taenia caecum. Spontaneous increases in muscle tension followed increases in F340, decreases in F380 and increases in the F340/F380 ratio, indicating a rise in $[Ca^{2+}]_{cyt}$. Addition of 43.9 mM K^+ or 0.3 μM histamine induced an initial transient increase followed by a sustained increase in $[Ca^{2+}]_{cyt}$ and muscle tension. During stimulation with 0.3 μM histamine, $[Ca^{2+}]_{cyt}$ and muscle tension sometimes changed to rhythmic oscillations. Higher concentration of histamine induced sustained contractions for at least 30 min.

Effects of ISO on spontaneous activities

Figure 2 shows the inhibitory effect of 1 μM ISO on the spontaneous rhythmic changes in $[Ca^{2+}]_{cyt}$ and muscle tension. Experiments were performed in the presence of 10 μM phentolamine to avoid the α-adrenergic effects. ISO inhibited the rhythmic changes in $[Ca^{2+}]_{cyt}$ and contractions without changing basal $[Ca^{2+}]_{cyt}$. Addition of EGTA decreased $[Ca^{2+}]_{cyt}$ below the resting level. However, EGTA did not change the resting tone of the muscle because the muscle did not have an active tension in the resting state. Salbutamol (1 μM), a selective β_2-agonist, also inhibited the spontaneous rhythmic responses. The effects of ISO and salbutamol were inhibited by 3 μM propranolol (data not shown).

Effects of ISO on high K^+- and histamine-induced responses

Figure 3 shows the concentration-response relationship of the inhibitory effects of ISO on high K^+ (25.4, 43.9 and 65.4 mM)- and histamine (3 μM)-induced contractions. ISO more strongly inhibited histamine-induced contraction than high K^+-induced contraction from 25.4 mM to

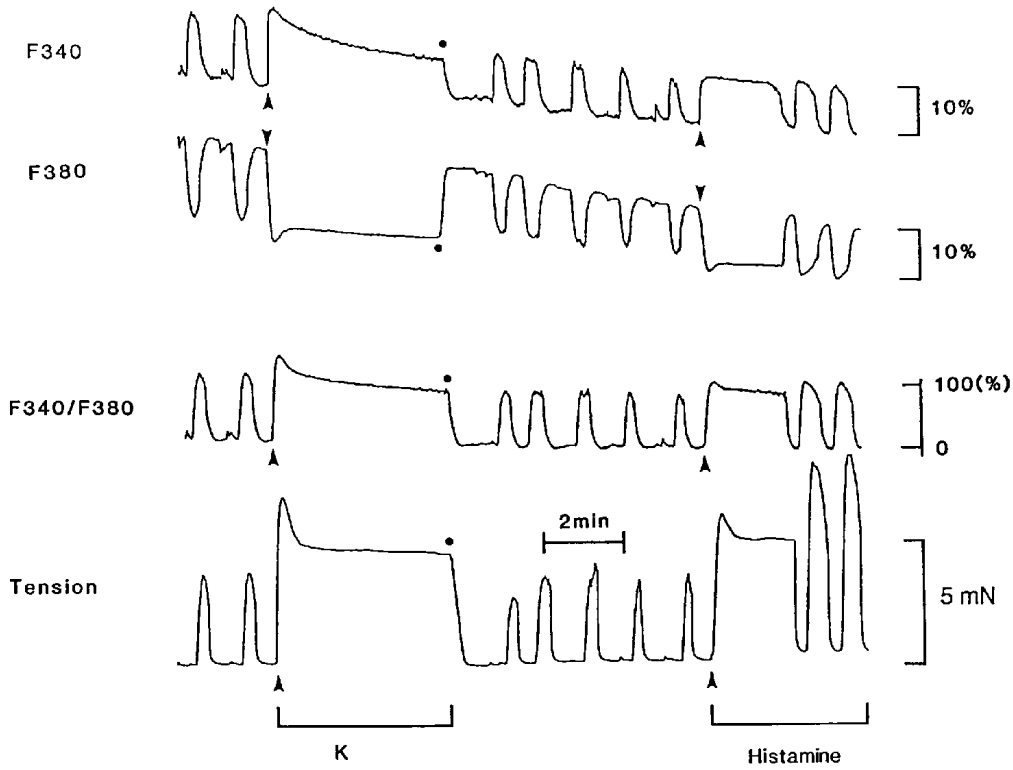

Fig. 1. Changes in fura-2-Ca^{2+} fluorescence and muscle tension induced spontaneously or by high K^+ solution (43.9 mM) and histamine (0.3 μM) in guinea pig taenia caecum. F340: the 500 nm emission at 340 nm excitation. F380: 500 nm emission at 380 excitation. F340/F380: an indicator of $[Ca^{2+}]_{cyt}$. Changes in F340 and F380 are shown by relative fluorescent intensity. 100% represents the high K^+-stimulated sustained increase in steady state $[Ca^{2+}]_{cyt}$.

43.9 mM or 65.4 mM. The inhibitory effect of ISO was reduced by raising the K^+ concentration.

As shown in Fig. 4A, high K^+ (43.9 mM) increased both $[Ca^{2+}]_{cyt}$ and muscle tension. ISO (1 μM) inhibited the high K^+-induced contraction by 34.2±4.2% (n=10) with little effect on $[Ca^{2+}]_{cyt}$ (decreased by 1.6±1.0%, n=10). The inhibitory effect of ISO was antagonized by 1 μM propranolol but not by 10 μM phentolamine or 10 μM metapronol, a selective antagonist of β_1-adrenoceptors. Salbutamol (10 μM) inhibited the high K^+-induced contraction by 60.1±2.5% (n=8) with a decrease in $[Ca^{2+}]_{cyt}$ by 12.3±1.9% (n=8). These effects were antagonized by 3 μM propranolol (data not shown). Addition of verapamil (10 μM) decreased the remaining $[Ca^{2+}]_{cyt}$ and muscle tension to their respective resting level.

Figure 4B shows the inhibitory effect of ISO on the histamine-induced changes in $[Ca^{2+}]_{cyt}$ and contraction. ISO (1 μM) inhibited $[Ca^{2+}]_{cyt}$ and tension to their respective resting level, although rhythmic changes were sometimes observed even in the presence of ISO. A higher concentration of ISO (10 μM) completely inhibited the rhythmic changes (data not shown). The effect of ISO was antagonized by 0.1 μM propranolol but not by 1 μM prazosin. Salbutamol (10 μM) inhibited the histamine-induced contraction by 70.7±2.6% (n=8) with a decrease in $[Ca^{2+}]_{cyt}$ by 32.5±8.2% (n=8). These effects were completely antagonized by 3 μM propranolol (data not shown).

Effects of ISO on $[Ca^{2+}]_{cyt}$-tension relationship

Figure 5A shows that K^+ (7, 9, 12, 15, 18, 25 and 40 mM) induced concentration-dependent increases in $[Ca^{2+}]_{cyt}$ and tension. Relationship between $[Ca^{2+}]_{cyt}$ and muscle tension suggests that there is a threshold $[Ca^{2+}]_{cyt}$ for contraction (approximately 30% $[Ca^{2+}]_{cyt}$). When $[Ca^{2+}]_{cyt}$ increased above this level, there was a positive correlation between $[Ca^{2+}]_{cyt}$ and tension. Figure 5A also shows the effects of 1 μM ISO on $[Ca^{2+}]_{cyt}$ and muscle tension stimulated by K^+. ISO inhibited the high K^+ (25 and 40 mM)-induced contraction without changing $[Ca^{2+}]_{cyt}$, whereas $[Ca^{2+}]_{cyt}$ stimulated by 18 mM or lower concentration of K^+ was decreased. Thus ISO shifted the $[Ca^{2+}]_{cyt}$-tension curve to the lower-right and increased the threshold $[Ca^{2+}]_{cyt}$ for contraction, suggesting that ISO decreased the Ca^{2+} sensitivity of contractile ele-

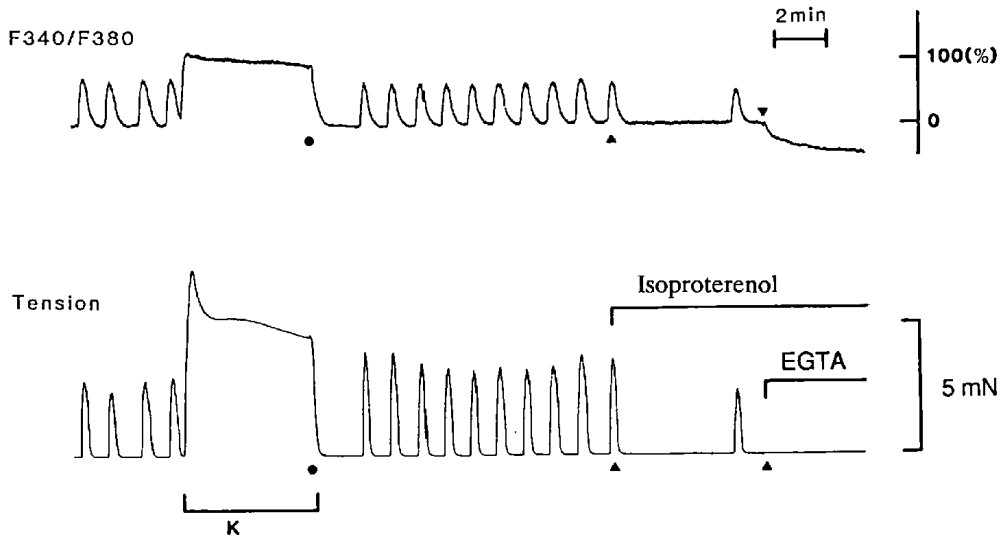

Fig. 2. The effect of ISO (1 μM) on $[Ca^{2+}]_{cyt}$ (upper trace) and muscle tension (lower trace) in spontaneously active taenia. Experiments were performed in the presence of 10 μM phentolamine to inhibit α-adrenergic action. 100% represents the steady state $[Ca^{2+}]_{cyt}$ in the presence of 43.9 mM K^+. ISO (1 μM) inhibited rhythmic increases in $[Ca^{2+}]_{cyt}$ and tension without changing basal $[Ca^{2+}]_{cyt}$. EGTA (4 mM) decreased basal $[Ca^{2+}]_{cyt}$ below the resting level with no further decrease in muscle tension.

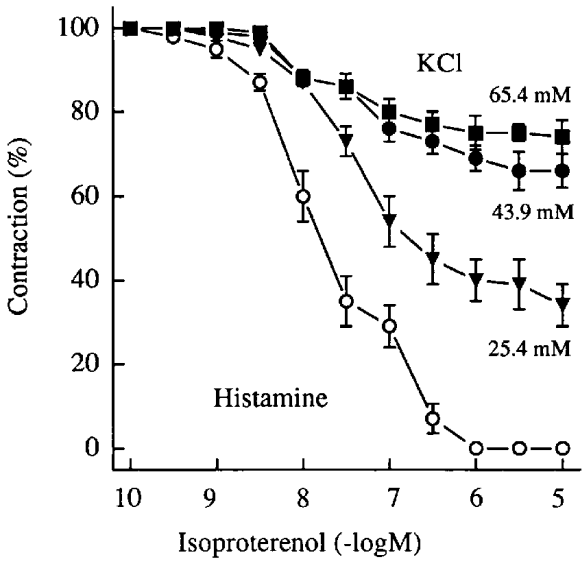

Fig. 3. Concentration-response relationship for the inhibitory effects of ISO on contraction induced by K^+ (43.9 mM) and histamine (3 μM). ISO (0.1 nM – 10 μM) was cumulatively added after the contractions reached a steady state.

ments.

As shown in Fig. 5B, histamine (0.01, 0.03, 0.1, 0.3, 1 and 3 μM) increased $[Ca^{2-}]_{cyt}$ and tension in a concentration-dependent manner. ISO (1 μM) inhibited the histamine-stimulated $[Ca^{2+}]_{cyt}$ and muscle tension without changing the threshold $[Ca^{2+}]_{cyt}$ for contraction.

Effects on cyclic nucleotides contents

As shown in Fig. 6, ISO (1 μM) increased cyclic AMP content by 50% ($P<0.05$). IBMX (0.5 mM), an inhibitor of phosphodiesterase, increased the cyclic AMP content by 100% ($P<0.05$). In the presence of IBMX, ISO (0.1 and 1 μM) more greatly increased cyclic AMP content (Fig. 6). IBMX also increased the cyclic GMP content from 94.0 ± 12.2 to 182 ± 10.5 pmole/g tissue ($P<0.05$, $n=5$ each). ISO (0.1 and 1 μM) did not increase the cyclic GMP content, in the presence of IBMX (control, 181.9 ± 10.5 pmole/g tissue; 0.1 μM ISO, 150.4 ± 12.0 pmole/g tissue; 1 μM ISO, 160.9 ± 17.8 pmole/g tissue).

DISCUSSION

ISO inhibited the contractions induced spontaneously or by stimulation with K^+ or histamine in the isolated guinea pig taenia caecum. The inhibitory effect of ISO was mediated by β-adrenoceptors since the effect was antagonized by propranolol but not by phentolamine or prazosin. The β_2-selective agonist, salbutamol, showed similar effects as ISO, suggesting that β_2-adrenoceptors are responsible for the inhibitory effect of ISO.

The inhibitory effects of ISO were stronger on the contraction induced by histamine than that induced by high K^+. Furthermore, the inhibitory effect of ISO was attenuated when the K^+ concentration was increased. The effects of ISO on $[Ca^{2+}]_{cyt}$-tension relationship in the presence of histamine showed that the inhibitory effect mediated by β_2-adrenoceptor is mainly due to the

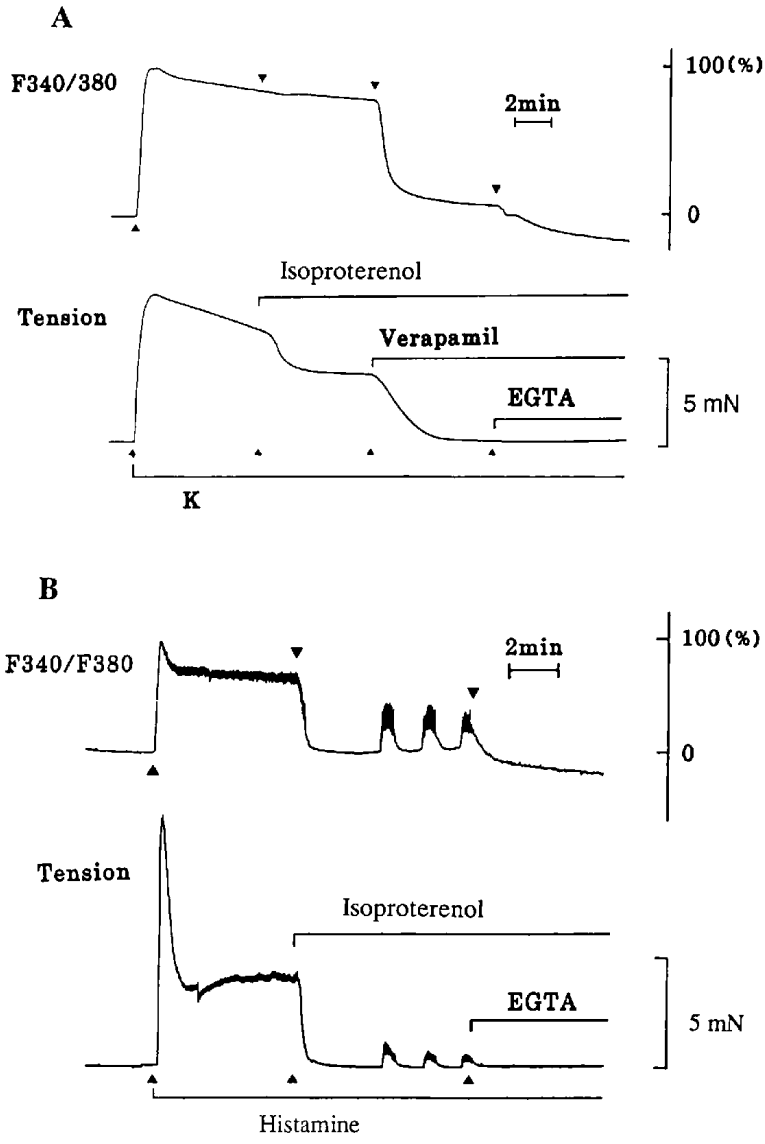

Fig. 4. Effects of ISO on high K^+ (A)- and histamin (B)-stimulated $[Ca^{2+}]_{cyt}$ (upper trace) and muscle tension (lower trace). 100% represents the 43.9 mM K^+- or histamine-induced increases in $[Ca^{2+}]_{cyt}$ before addition of ISO. In panel A, after the high K^+- stimulated $[Ca^{2+}]_{cyt}$ and muscle tension reached a steady state level, 1 μM ISO and 10 μM verapamil were sequentially added. ISO partially inhibited contraction without changing $[Ca^{2+}]_{cyt}$. Verapamil (10 μM), on the other hand, inhibited $[Ca^{2+}]_{cyt}$ and tension to the resting level. In panel B, when the $[Ca^{2+}]_{cyt}$ and muscle tension induced by histamine (3 μM) reached a steady state level, 1 μM ISO and 10 μM verapamil were sequentially added. ISO (1 μM) inhibited $[Ca^{2+}]_{cyt}$ and contraction to the resting levels.

decrease in $[Ca^{2+}]_{cyt}$, although this was not the case with the high K^+-induced contraction. In smooth muscles, ISO may decrease $[Ca^{2+}]_{cyt}$ by the following two mechanisms: i) direct inhibition of voltage-dependent Ca^{2+} channels and ii) indirect inhibition of Ca^{2+} channels following the activation of voltage- or Ca^{2+}-dependent K^+ channels. The first possibility may be less likely since ISO did not reduce the high K^+ induced increases in $[Ca^{2+}]_{cyt}$, which is readily inhibited by L-type Ca^{2+} channel blockers (24). A probable site of action of ISO is K^+ channels; an increase in K^+ current hyperpolarizes the membrane. Higher concentrations of K^+ may attenuate the membrane hyperpolarization due to opening K^+ channels also by decreasing the transmembrane K^+ gradient. This may be the reason why the inhibitory effects of ISO on $[Ca^{2+}]_{cyt}$ is less in the presence of high K^+. Inhibition of spontaneous rhythmic changes in $[Ca^{2+}]_{cyt}$ and muscle tension may be due to the inhibition of pace maker activity

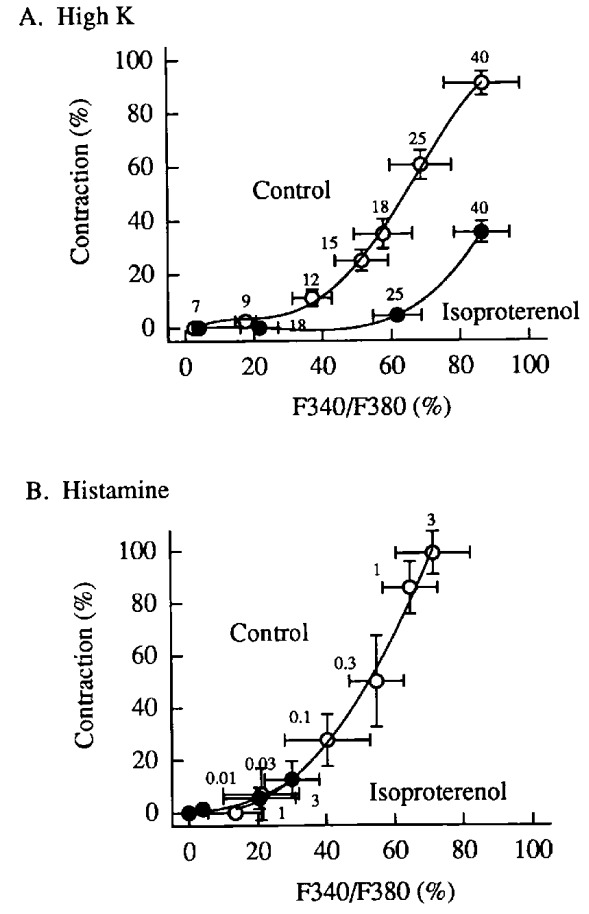

Fig. 5. Effect of ISO (1 μM, solid circles) on the relationship between $[Ca^{2+}]_{cyt}$ (abscissa) and muscle tension (ordinate) in the presence of various concentrations of K^+ (7, 9, 12, 15, 18, 25 and 40 mM) or histamine (0.01, 0.03, 0.1, 0.3, 1 and 3 μM). 100% represents 43.9 mM K^+-induced increases in $[Ca^{2+}]_{cyt}$ and muscle tension measured before cumulative addition of the stimuli. Each point represents the mean of 6 to 8 experiments, and S.E.M. is shown by vertical and horizontal bars.

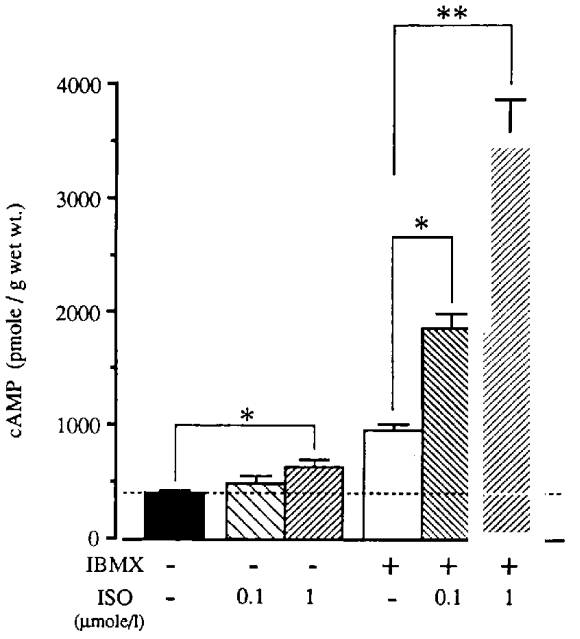

Fig. 6. Effects of ISO on cyclic AMP content. Muscles were treated with ISO (0.1 and 1 μM) for 10 min with or without IBMX (0.5 mM). *, **: Significantly different from each respective control (without ISO) with $P<0.05$ and $P<0.01$, respectively. Each point represents the mean of 5 to 6 experiments, and the S.E.M. is shown by vertical bars.

by membrane hyperpolarization and/or the inhibition of Ca^{2+} influx by the indirect inhibition of voltage dependent Ca^{2+} channels. It has been reported that the open probability of Ca^{2+}-activated K^+ channels are increased by the catalytic subunit of protein kinase A in tracheal (25) and colonic (26) smooth muscle cells. It is therefore possible that the increase in cyclic AMP production in response to ISO might activate K^+ channels via protein kinase A-mediated protein phosphorylation.

The second possibility for the ISO-induced decrease in histamine-stimulated $[Ca^{2+}]_{cyt}$ is the cyclic AMP-mediated inhibition of signal transduction (27). The inhibition by forskolin and dibutyryl cyclic AMP of phosphatidylinositol hydrolysis has been demonstrated in tracheal (28) and vascular smooth muscles (29). Activation of protein kinase A by forskolin in canine colonic smooth muscle desensitizes muscarinic receptors and inhibits phosphatydil-inositol turnover (30).

It is also possible that the reduction in $[Ca^{2+}]_{cyt}$ caused by ISO could be related to an enhancement in Ca^{2+} uptake into sarcoplasmic reticulum (3). However, this mechanism may not play a major role because ISO showed little effect on $[Ca^{2+}]_{cyt}$ in muscles depolarized with K^+. The decrease in $[Ca^{2+}]_{cyt}$ caused by ISO was not observed under the resting condition.

The inhibitory effects of ISO do not appear to be limited to a decrease in $[Ca^{2+}]_{cyt}$. Activation of β-adrenoceptors inhibited high K^+-induced contractions with little, if any, decrease in $[Ca^{2+}]_{cyt}$, suggesting that ISO decreased the Ca^{2+} sensitivity of regulatory or contractile elements to Ca^{2+}. Elevation of cyclic AMP inhibits contraction by a decrease in Ca^{2+} sensitivity in vascular (18–21, 31) and tracheal smooth muscle (14). Histamine-induced contraction was inhibited more strongly than high K^+-induced contraction possibly because the effect of ISO to inhibit the signal transduction is stronger than its effect to decrease Ca^{2+} sensitivity.

In rat gastric smooth muscle, vasoactive intestinal peptide (VIP) increased both cyclic AMP and cyclic GMP contents (32). The agents that increase cyclic AMP (ISO, VIP, CGRP and forskolin) and cyclic GMP (sodium

nitroprusside) in gastric muscle produced inhibition of electrical slow waves, Ca^{2+} transients, mechanical activities and Ca^{2+} sensitivity of contractile elements (5, 33). Thus, it is possible that not only cyclic AMP but also cyclic GMP may contribute to the inhibitory effects of ISO in taenia. Measurements of cyclic nucleotides showed that ISO increased cyclic AMP but not cyclic GMP content in the taenia either in the presence or absence of IBMX, supporting the suggestion that the effects of ISO are mediated by cyclic AMP.

In summary, activation of β_2-adrenoceptors in guinea pig taenia caecum inhibited the contraction induced by high K^+-depolarization mainly by decreasing the Ca^{2+} sensitivity of contractile elements and inhibited the histamine-induced contraction mainly by decreasing $[Ca^{2+}]_{cyt}$.

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