Full Paper

Inhibitory Mechanism of Monensin on High K⁺-Induced Contraction in Guinea-Pig Urinary Bladder

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Abstract. In this study, we examined the inhibitory mechanism of monensin on high K⁺-induced contraction in guinea-pig urinary bladder. The relaxant effect of monensin (0.001 – 10 μ M) was more potent than those of NaCN (100 μ M – 1 mM) and forskolin (3 – 10 μ M). Monensin (0.1 μ M), NaCN (300 μ M), or forskolin (10 μ M) inhibited high K⁺-induced contraction without decreasing [Ca²⁺]_i level. Monensin and NaCN remarkably decreased creatine phosphate and ATP contents. Monensin and NaCN inhibited high K⁺-induced increases in flavoprotein fluorescence, which is involved in mitochondrial respiration. Forskolin increased cAMP content but monensin did not. Monensin increased Na⁺ content at 10 μ M but not at 0.1 μ M that induced maximum relaxation. In the α -toxin-permeabilized muscle, forskolin significantly inhibited the Ca²⁺-induced contraction, but monensin did not affect it. These results suggest that the relaxation mechanism of monensin in smooth muscle of urinary bladder may be an inhibition of oxidative metabolism.

Keywords: monensin, urinary bladder, phosphocreatine, Na⁺ content

Introduction

Monensin is a polyether, monocarboxylic molecule used as a Na⁺ ionophore in cellular physiology and pharmacology. Some studies have shown that monensin caused cardiotoxic effects (1 – 4). Pressman and Painter (5) considered that the cardiotoxic effects of monensin are caused by an increase in intracellular sodium concentration and the resultant stimulation of sodium-calcium exchange. On the other hand, it has been reported that monensin inhibited the high K⁺- or receptor agonist-induced contraction in some smooth muscles (6 – 9).

Smooth muscles have been classified into phasic and tonic muscles on the bases of their electrophysiological and mechanical behaviors (10 – 12). Phasic smooth muscles, including ileum, urinary bladder, uterus, vas deferens, etc., produce contraction often associated with electrical spikes. Depolarization with high K⁺

elicits an initial phasic contraction followed by a sustained contraction (13). In contrast, tonic smooth muscles such as aorta and trachea generally produce contraction without electrical spikes. A high K⁺-induced depolarization elicits a sustained contraction with the slow onset of contraction. The contractile diversity among different smooth muscles appears to be attributed to variations in the dependence on oxidative metabolism (14, 15), as well as cellular protein expression (16 – 21) and electrophysiological properties (22, 23).

In guinea-pig taenia coli, which is classified as a phasic muscle, monensin inhibited the high K⁺-induced contraction by inhibiting oxidative adenosine triphosphate (ATP) production (6). It was suggested that monensin inhibited the 5-hydroxytryptamine-induced contraction due to a change in intracellular sodium concentration in canine coronary artery, which is classified as a tonic muscle (7). These data imply that mechanism of monensin-induced relaxation in phasic muscle is inhibiting oxidative ATP production and that in tonic muscle is related to Na⁺ movement.

In the present study, we attempt to characterize the

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inhibitory mechanism of monensin on high K^+ -induced contraction in guinea-pig urinary bladder, a phasic muscle. The investigation was performed by measuring muscle tension, intracellular Ca^{2+} ($[Ca^{2+}]_i$) level, phosphocreatine (PCr) and ATP contents, oxidized flavo-protein as an indicator of mitochondrial respiration, pyridine nucleotides as indicator of glycolytic activity, cAMP content, Na^+ content, and tension development of α -toxin permeabilized muscle in the urinary bladder.

Materials and Methods

Muscle preparations and tension measurement

Male guinea pigs (Hartley strain, 300–400 g; Funabashi Farm, Funabashi) were bled after stunning, and then the urinary bladder was quickly removed. Trigumun vesicae, superficial tissue, fat, and mucous layer were removed. Longitudinal strips of urinary bladder were about 10–15 mm in length and 3–5 mm in width. The muscle strips were incubated with physiological salt solution (PSS) containing 136.8 mM NaCl, 5.4 mM KCl, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 11.9 mM $NaHCO_3$, and 5.6 mM glucose. PSS was aerated with 95% O_2 and 5% CO_2 to adjust pH to 7.2 at 37°C. This study was conducted in accordance with the Guideline for the Care and Use of Laboratory Animals of Nippon Veterinary and Animal Science University.

Muscle tension was recorded isometrically. One end of each strip was bound to a glass holder and the other end was connected with a silk thread to a strain-gauge transducer (TB-611T; Nihon Kohden, Tokyo) in an organ bath containing PSS with resting tension of 3 g. The muscle strips were equilibrated for 30 min to obtain a stable contractility induced by hyperosmotically added 65 mM KCl (H-65 K^+). The Na^+ -deficient, high K^+ solution (I-154 K^+) was made by substituting an equimolar amount of K^+ for Na^+ in the PSS. As it has been reported that Na^+ -free PSS decreases utilization of extracellular glucose in guinea-pig urinary bladder (24), 5.5 mM pyruvate was added in I-154 K^+ for an energy supply. The developed tension was expressed as a percentage, by assigning the values at rest in normal PSS to be 0% and those at a steady level of contraction in H-65 K^+ or I-154 K^+ to be 100%.

Simultaneous measurement of a muscle contraction and $[Ca^{2+}]_i$ level

$[Ca^{2+}]_i$ level was measured simultaneously with a muscle contraction as reported previously (25). Muscle strips were incubated with PSS containing 5 μ M fura2/AM for overnight at 4°C. Cremophor EL (at 0.02%), a noncytotoxic detergent, was also added to

increase the solubility of fura2/AM. One end of the muscle was pinned to the bottom of the organ bath, which was filled with 8 ml of PSS, and the other end was attached to the transducer with silk thread. The muscle strip was kept horizontally in the organ bath. The muscle strip was alternately excited with light at 340 and 380 nm through a rotating filter wheel, and the 500-nm emission was measured through a band-pass filter with a fluorometer (CAF-100; Japan Spectroscopic Co., Ltd., Tokyo), and their ratio (F340/F380) was recorded as an indicator of $[Ca^{2+}]_i$. The fluorescence ratio was expressed as a percentage, by assigning the values at rest in normal PSS to be 0% and those at a steady level of contraction in isosmotically added 65 mM KCl (I-65 K^+) just before an application of each relaxant to be 100%. We determined muscle tension and $[Ca^{2+}]_i$ level 40 min after the addition of monensin and 20 min after addition of NaCN or forskolin.

Assay of creatine phosphate and adenosine triphosphate

PCr and ATP contents in the muscle strips were measured by high-performance liquid chromatography (HPLC) as reported previously (26). The muscles were incubated with H-65 K^+ for 15 min and then with monensin for 50 min, NaCN for 20 min, or forskolin for 20 min. These treatment times were chosen since the relaxation responses to these agents reached a plateau. After the incubation, the muscles were rapidly frozen in liquid nitrogen and stored at -80°C until homogenized in 6% perchloric acid (0.9 ml). The homogenate was centrifuged at $15,000 \times g$ for 5 min and the supernatant neutralized with 0.2 ml of 2 M $KHCO_3$. The neutralized extracts were centrifuged once more and 20 μ l supernatant was applied to HPLC.

The HPLC system (Shimadzu Corp., Kyoto) consisted of a pump (LC-10AT), a system controller (SCL-10A), an auto injector (SIL-10AF), a column oven (CTO-10A), and wave length-selectable detector (SPD-10Ai) set at 216 nm. Chromatography was performed by a μ RPC C2/C18 ST (4.6-mm internal diameter and 100 mm length; Amersham Pharmacia Biotech, Little Chalfont, UK) column using mobile phases of 50 mM KH_2PO_4 and 5 mM terabutylammonium hydrogen sulphate (TBAHS) (pH 6.0, buffer A), and 50 mM KH_2PO_4 , 5 mM TBAHS and 40% methanol (pH 6.0, buffer B). Flow rate was 1.0 ml/min and the elution started with 65% buffer A. Then, for 14 min, buffer B was increased at a rate of 2.5%/min. This was followed by elution with 70% buffer B for 20 min and then with 100% buffer A for 10 min. These procedures were programmed with the system controller. The sensitivity of the detector was usually set at 1.0 a.u.f.s. and the oven temperature at 40°C. PCr and ATP contents are

expressed as $\mu\text{mol/g}$ wet weight.

Simultaneous measurement of muscle contraction and oxidized flavoprotein (FPox) fluorescence or reduced pyridine nucleotide (PNred) fluorescence

FPox or PNred fluorescence was measured simultaneously with a muscle contraction as reported previously (27). The muscle strip was also kept horizontally in the organ bath, as described for the measurement of $[\text{Ca}^{2+}]_i$ level. The muscle strip was excited with light at 450 nm and the 530 nm emission was measured with a fluorometer (CAF-100) to detect FPox fluorescence. It was excited with light at 340 nm and the 470 nm emission was measured with a fluorometer to detect PNred fluorescence. FPox or PNred fluorescence was expressed as a percentage, by assigning the values at rest in normal PSS to be 0% and those at a steady level of contraction in H-65 K^+ just before an application of monensin or NaCN to be 100%. Effects of monensin and NaCN on muscle tension and FPox or PNred fluorescence were determined 40 min and 20 min after addition of agents, respectively.

Assay of cAMP content

cAMP content in the muscle strips was measured by enzyme immunoassay as reported previously (28). After incubation of the muscles with monensin for 50 min, NaCN for 20 min, and forskolin for 20 min in the presence of H-65 K^+ , the muscles were rapidly frozen in liquid nitrogen and stored at -80°C until homogenized in 6% trichloroacetic acid (0.4 ml). The homogenate was centrifuged at $3,000 \times g$ for 15 min and the supernatant was washed with 1.5 ml of water-saturated diethylether four times; the cAMP content was assayed by using an enzyme immunoassay kit (Amersham Pharmacia Biotech). cAMP contents are expressed as pmol/g wet weight.

Measurement of Na^+ content

Na^+ content in muscle strips was measured by the 'Li-method' as reported previously (6). After incubation with test solution (normal PSS plus 60 mM KCl and 0.1 or 10 μM monensin, at 37°C), the muscles were exposed to Li solution (169.5 mM LiCl, 1.5 mM CaCl_2 , 1.0 mM MgCl_2 , 20 mM HEPES, and 5.6 mM glucose) at 0.5°C and pH 7.2 for 30 min in order to remove extracellular Na^+ . The tissues were blotted between filter papers, weighed, and ashed overnight at 180°C in a quartz tube with 0.5 ml of a mixture of equal amounts of HNO_3 (61%) and HClO_4 (60%). Na^+ content in the sample was measured by flame photometer (atomic absorption photometer, Hitachi-Z8200; Hitachi, Tokyo). Na^+ content is expressed as mmol/kg wet weight.

Measurement of muscle tension in α -toxin permeabilized smooth muscle

A thin longitudinal muscle strip, 5.0 mm in length and 0.2 mm in width, was prepared from the isolated urinary bladder. The muscle strip was held horizontally in an 1-ml organ bath. One end of each strip was fixed and the other end was connected to a strain-gauge transducer. The muscle tension was isometrically recorded. Permeabilized muscle was prepared by treating the urinary bladder strips with *Staphylococcus aureus* α -toxin (80 $\mu\text{g/ml}$, 30 min), as described previously (29). The relaxing solution contained 130 mM potassium propionate, 4 mM MgCl_2 , 4 mM ATP, 20 mM tris-maleate, 2 mM creatine phosphate, 10 U/ml creatine phosphokinase, and 2 mM ethyleneglycol bis-(β -aminoethylether)- N,N,N',N' -tetraacetic acid (EGTA). A 3- μM Ca^{2+} (pCa 5.5) solution was prepared using Ca^{2+} -EGTA buffer composed of 2 mM EGTA and 1.51 mM CaCl_2 . The required Ca^{2+} -concentrations were calculated assuming the apparent binding constant of Ca^{2+} -EGTA complex to be 10^6 M^{-1} at pH 6.8 (30). The solutions were adjusted to pH 6.8 at 24°C with KOH (0.1 N).

Chemicals

Chemicals used were monensin and forskolin (Sigma-Aldrich, St. Louis, MO, USA); sodium cyanide (NaCN) (Wako Pure Chemical, Osaka); fura2/AM (Dojindo Laboratories, Kumamoto); and cremophor EL (Nacalai Tesque, Kyoto).

Statistics

Values are expressed as the mean $\pm$ S.E.M. and statistical analyses were performed by Student's *t*-test.

Results

Effects of monensin, NaCN, and forskolin on a high K^+ -induced contraction

H-65 K^+ showed a phasic contraction, following a sustained one. When the H-65 K^+ -induced contraction reached a steady level, monensin (0.001 – 10 μM) was added. Although 0.001 μM monensin had little effect, the higher concentrations inhibited the H-65 K^+ -induced contraction (Fig. 1A). The results indicate that the rate of relaxation was dependent on the concentration of monensin. NaCN (100 – 1000 μM) or forskolin (3 – 10 μM) also inhibited the H-65 K^+ -induced contraction in a concentration dependent manner (Fig. 1: B and C). The relaxant effect of monensin was more potent than those of NaCN and forskolin.

Fig. 1. Effects of monensin (A), NaCN (B), or forskolin (C) on the contraction induced by high K^+ in urinary bladder. Ordinate: relative tension. The contractions induced by high K^+ just before the application of each agent were taken as 100%. Abscissa: time (min) of exposure to each agent. Each point represents the mean of 4–8 preparations. Vertical bars indicate the S.E.M.

Effects of monensin, NaCN, or forskolin on an elevated $[Ca^{2+}]_i$ level by high K^+

The effects of monensin, NaCN, or forskolin on $[Ca^{2+}]_i$ level were measured simultaneously with muscle tension, using a fluorescent Ca^{2+} indicator, fura2. I-65 K^+ induced a muscle contraction and elevated $[Ca^{2+}]_i$ level indicated by F340/F380. Monensin ($0.1 \mu M$)

showed a significant inhibition in the I-65 K^+ -induced contraction without a decrease in $[Ca^{2+}]_i$ level ($33.4 \pm 4.3\%$ for tension, $P < 0.01$; $93.9 \pm 3.2\%$ for $[Ca^{2+}]_i$ level, no significance, 40 min after addition of monensin) (Fig. 2A).

NaCN ($300 \mu M$) decreased muscle contraction induced by I-65 K^+ significantly, but did not change $[Ca^{2+}]_i$ level ($36.5 \pm 1.1\%$ for tension, $P < 0.01$; $95.3 \pm 1.5\%$ for $[Ca^{2+}]_i$ level, no significance, 20 min after addition of NaCN) (Fig. 2B). Forskolin ($10 \mu M$) also showed a significant inhibition in the I-65 K^+ -induced contraction with an insignificant decrease in $[Ca^{2+}]_i$ level ($37.9 \pm 1.2\%$ for tension, $P < 0.01$; $93.1 \pm 2.8\%$ for $[Ca^{2+}]_i$ level, no significance, 20 min after addition of forskolin) (Fig. 2C).

Effects of monensin, NaCN, or forskolin on PCr and ATP contents

Effects of monensin ($0.01, 0.1 \mu M$), NaCN ($300 \mu M$), or forskolin ($10 \mu M$), on PCr and ATP contents are summarized in Fig. 3. Monensin and NaCN decreased PCr and ATP contents below the control in the presence of H-65 K^+ , significantly. On the other hand, forskolin did not affect PCr and ATP levels significantly.

Effects of monensin or NaCN on change of FPox or PNred fluorescence

Effects of monensin or NaCN were investigated on FPox or PNred fluorescence measured simultaneously with muscle contraction. H-65 K^+ induced a muscle contraction and elevated FPox fluorescence (Fig. 4A). Monensin ($0.1 \mu M$) inhibited the H-65 K^+ -induced muscle contraction and increase in FPox fluorescence significantly ($42.1 \pm 3.1\%$ for tension, $25.3 \pm 2.2\%$ for FPox fluorescence, $P < 0.01$, respectively) (Fig. 4A). NaCN ($300 \mu M$) also inhibited the H-65 K^+ -induced muscle contraction and increase in FPox fluorescence significantly ($43.8 \pm 1.7\%$ for tension, $30.8 \pm 2.6\%$ for FPox fluorescence, $P < 0.01$, respectively) (Fig. 4B).

On the other hand, H-65 K^+ -induced increase in PNred fluorescence was not affected by the presence of monensin or NaCN (data not shown). These results suggest that monensin inhibits mitochondrial respiration, but not glycolytic activity.

Effects of monensin or forskolin on cAMP content

Forskolin ($10 \mu M$) increased cAMP content above the control in the presence of H-65 K^+ as shown in Fig. 5 (control, 60.7 ± 5.4 pmol/g wet weight; forskolin, 118.3 ± 4.6 pmol/g wet weight). However, monensin ($0.1 \mu M$) did not change cAMP content (59.1 ± 4.0 pmol/g wet weight).

Fig. 2. Effects of monensin (A), NaCN (B), or forskolin (C) on the I-65 K⁺-induced increases in [Ca²⁺]_i level (F340/F380, upper trace) and muscle tension (lower trace). The increases in [Ca²⁺]_i level induced by I-65 K⁺ just before an application of each agent was taken as 100%. Each trace of typical results from 4 experiments.

Effects of monensin on a contraction in Na⁺-deficient, high K⁺ (I-154 K⁺) solution

Exposure of the muscle to I-154 K⁺ solution resulted in a rapid rise followed by sustained contraction reaching a steady level within 15 min. As we have reported that Na-free PSS decreases utilization of extracellular glucose in guinea-pig urinary bladder (24), 5.5 mM pyruvate was added in I-154 K⁺ for an energy supply. The sustained contraction induced by I-154 K⁺ or H-65 K⁺ was in the same magnitude (At 15 min, the I-154 K⁺-induced contraction was $104.5 \pm 6.5\%$ of H-65 K⁺-induced contraction, no significant difference). Such a sustained contraction in I-154 K⁺ was inhibited by monensin (0.1 μM) at 25–60 min, as was the case with the H-65 K⁺. However, monensin inhibited I-154 K⁺-induced contraction more rapidly than H-65 K⁺-induced contraction (Fig. 6).

Effect of monensin on Na⁺ content

Effect of monensin was investigated on the cellular Na⁺ content in H-65 K⁺-depolarized muscle. As shown in Fig. 1, 0.1 and 10 μM monensin both inhibited H-65 K⁺-induced contraction to the same degree. In the H-65 K⁺ solution, the cellular Na⁺ content did not change at 20, 40, and 60 min after application of 0.1 μM monensin. However, the cellular Na⁺ content increased at 20, 40, and 60 min after application of 10 μM monensin (Fig. 7).

Effects of monensin or forskolin on Ca²⁺-induced contractions in permeabilized muscle

As shown in Fig. 8, 3 μM Ca²⁺ induced a sustained contraction in α-toxin-permeabilized muscle. At 30 min, monensin (0.1 μM) inhibited the high K⁺-induced contraction to $43.2 \pm 5.1\%$ in the intact muscle (Fig. 1A). However, monensin (0.1 μM) did not affect

Fig. 3. Effects of monensin (0.01 and 0.1 μM) (A) and NaCN (300 μM) or forskolin (10 μM) (B) on PCr and ATP contents in the presence of high K^+ . Control: 50 min (A) or 20 min (B) after the application of high K^+ , respectively. Monensin was applied for 50 min. NaCN or forskolin was applied for 20 min. The data are expressed as the means $\pm$ S.E.M. Vertical bars indicate the S.E.M. **: Significant difference from each respective control with $P < 0.01$.

the Ca^{2+} -induced contraction for 30 min in the permeabilized muscle (Fig. 8A). At 10 min, NaCN (300 μM) inhibited the high K^+ -induced contraction to $39.0 \pm 5.3\%$ (Fig. 1B), but NaCN (300 μM) did not affect the Ca^{2+} -induced contraction for 10 min (Fig. 8B). However, forskolin (10 μM) significantly inhibited the Ca^{2+} -induced contraction to $70.8 \pm 3.1\%$ at 10 min (Fig. 8C).

Discussion

There are two relaxing mechanisms of smooth muscle by monensin: 1) a change in intracellular Na^+ content (7), 2) an inhibition of oxidative metabolism in guinea-pig taenia coli (6). The latter mechanism was based on the evidence that monensin inhibited high K^+ -induced contraction with the decreases in oxygen consumption and ATP contents (6). It has been thought that the PCr/creatine kinase system plays a role in the transport of high energy phosphates from the mitochondrial compartment to the sites of energy utilization, correlat-

Fig. 4. Effects of monensin (A) or NaCN (B) on high K^+ -induced muscle tension (lower trace) and the increases in oxidized flavo-protein (FPox) fluorescence (upper trace). The increase in FPox fluorescence induced by high K^+ just before an application of each agent was taken as 100%. Trace of typical results from 4 experiments.

ing with oxidative metabolism in mammalian smooth muscle (15, 31, 32). In the present experiments, monensin and NaCN decreased PCr and ATP contents below the control in the presence of H-65 K^+ , significantly. On the other hand, Ozaki et al. (27) reported that the high K^+ -induced contraction was accompanied by an increase in FPox fluorescence or PNred fluorescence in guinea-pig taenia coli. They suggested that the changes in FPox and PNred fluorescence represent mitochondrial respiration activity and glycolysis activity, respectively. In the present study, NaCN, an inhibitor of mitochondrial respiration, and monensin both inhibited significantly H-65 K^+ -induced contraction and increase in FPox fluorescence. These data suggest that the inhibitory mechanisms of monensin on high K^+ -induced contraction in the guinea-pig urinary bladder

Fig. 5. Effects of monensin or forskolin on cAMP contents. Preparations were precontracted with H-65 K⁺ for 20 min and were treated with monensin or forskolin. Control was treated with H-65 K⁺ for 15 min. Monensin (0.1 μ M) or forskolin (10 μ M) was applied at 50 min or 20 min. Each point represents the mean of 4 experiments. Vertical bars indicate the S.E.M. **: Significant difference from each respective control with $P < 0.01$.

Fig. 6. Effects of monensin on the contraction induced by H-65 K⁺ or I-154 K⁺ in urinary bladder. Ordinate: relative tension. The contractions induced by H-65 K⁺ (circle) or I-154 K⁺ (triangle) just before the application of each agent were taken as 100%. Abscissa: time (min) of exposure to each agent. Each point represents the mean of 5–6 preparations. Vertical bars indicate the S.E.M. **: Significant difference from each time with $P < 0.01$.

may be mainly by the inhibition of mitochondrial respiration. Recently, the urinary bladder isolated from the rat was reported to be fairly resistant to hypoxic conditions bubbled with nitrogen instead of oxygen (33). This result is different from our present data. There are some possible explanations for this difference. Presumably, the action of agents such as cyanide and monensin is somehow different from that of hypoxia, or the different species may bring about a different response. The energy metabolism related to the contractility of smooth muscle in urinary bladder remains to be clearly

Fig. 7. Effects of monensin on Na⁺ contents. Preparations were precontracted with H-65 K⁺ for 15 min and were treated with 0.1 μ M monensin (circle) or 10 μ M (triangle). Each point represents the mean of 4 experiments. Vertical bars indicate the S.E.M. *: Significant difference from each time with $P < 0.05$.

Fig. 8. Effects of monensin (A), NaCN (B), or forskolin (C) on the Ca²⁺-induced contraction in α -toxin permeabilized muscle. Each trace of typical results from 3–4 experiments. The muscles were contracted by 3 μ M Ca²⁺. After the contraction reached plateau, monensin (A), NaCN (B), or forskolin (C) was added.

elucidated.

In the present study, monensin, NaCN, and forskolin remarkably inhibited the high K^+ -induced contraction without decreasing $[Ca^{2+}]_i$ level. There are two possible explanations in this phenomenon: 1) decreasing Ca^{2+} sensitivity of the contractile protein and 2) an inhibition of oxidative metabolism. It has been suggested that cAMP and cGMP-related agents inhibited high K^+ -induced contraction by decreasing Ca^{2+} sensitivity of the contractile protein in some smooth muscles (3). In bovine trachea, it has been reported that forskolin increased cAMP content and inhibited high K^+ -induced contraction by decreasing Ca^{2+} sensitivity of the contractile protein (34). In the present experiments, forskolin also increased cAMP content and inhibited Ca^{2+} -induced contraction in α -toxin permeabilized muscle of guinea-pig urinary bladder. However, monensin did not increase cAMP content (Fig. 5). Moreover, monensin and NaCN did not inhibit Ca^{2+} -induced contraction (Fig. 8). On the other hand, it has been shown that hypoxia and oxidative metabolic inhibition reduced high K^+ -induced contraction without decreasing $[Ca^{2+}]_i$ level in some smooth muscles (35). It has been reported that cyanide inhibited high K^+ -induced contraction without changing $[Ca^{2+}]_i$ level in guinea-pig ileum (36). These data indicate that monensin-induced relaxation is not due to decreasing Ca^{2+} sensitivity of the contractile protein but due to an inhibition of oxidative metabolism.

Kishimoto et al. (6) has shown that monensin relaxed remarkably the Na^+ -deficient, high K^+ -induced contraction and monensin ($0.1 \mu M$) induced a small increase in Na^+ content in normal solution in guinea-pig taenia coli. They concluded that the action of monensin in the inhibition of high K^+ -induced contraction of the guinea-pig taenia coli is not related to the change in intracellular Na^+ concentration. In this study, monensin ($0.1 \mu M$) was similarly potent to relax the H-65 K^+ - and I-154 K^+ -induced contraction in guinea-pig urinary bladder (Fig. 6). Furthermore, monensin increased Na^+ content at $10 \mu M$ but not at $0.1 \mu M$, a concentration that induced maximum relaxation in guinea-pig urinary bladder (Fig. 7). These results suggest that the relaxation induced by a lower concentration of monensin in phasic muscles is not related to the change in intracellular Na^+ content.

In conclusion, it is suggested that monensin potently inhibited high K^+ -induced contraction due to an inhibition of oxidative metabolism in the guinea-pig urinary bladder.

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