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Cromakalim-induced membrane current in guinea-pig tracheal smooth muscle cells

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Abstract

The characteristics of the cromakalim-induced membrane current were examined in single tracheal myocytes of the guinea-pig under voltage-clamp conditions. When K^+ concentrations in the pipette and bathing solutions were ~140 mM, cromakalim activated a membrane current (I_{crom}) which was inward at −60 mV and reversed at −2 mV. I_{crom} was blocked by 10 μM glibenclamide and potentiated when the ATP concentration in the pipette solution was decreased. The K_d and Hill coefficient of glibenclamide for I_{crom} block were 200 nM and 1.05, respectively. Application of the tyrosine kinase inhibitors, genistein and α-cyano-3-ethoxy-4-hydroxy-5-phenylthiomethylcinnamamid (ST638), reduced I_{crom} in a concentration-dependent manner. Daidzein, which does not inhibit tyrosine kinase, was about 10 times less effective than genistein. Herbimycin A had no effect on I_{crom}. Internal application of these inhibitors from the pipette did not affect I_{crom}. In conclusion, cromakalim is a potent activator of the ATP-sensitive K^+ channel (K_{ATP} channel) in guinea-pig tracheal myocytes. The inhibition of I_{crom} by genistein and ST638 may be due to the direct block of the channel from outside.

Keywords: Smooth muscle, Tracheal; Cromakalim; K^+ channel, ATP-sensitive; Tyrosine kinase inhibitor

1. Introduction

The ATP-sensitive K^+ channel (K_{ATP} channel), which is characterized by intracellular ATP-dependent closure of the channel, was originally described in the membrane of cardiac myocytes (Noma, 1983) and has also been found in various types of tissues including smooth muscles (Ashcroft and Aschcroft, 1990; Quayle et al., 1997). K_{ATP} channels are the targets of two classes of drugs: sulfonylurea anti-diabetic drugs such as glibenclamide and tolbutamide are potent blockers of K_{ATP} channels (Zunkler et al., 1988) and a series of vasorelaxants such as pinacidil, nicorandil and cromakalim have K_{ATP} channel opening properties (Edwards and Weston, 1993). Whole-cell ATP-sensitive K^+ channel currents ($I_{K\text{-}ATP}$) have been measured in various types of single smooth muscle cells obtained from vascular, (Clapp and Gurney, 1992; Beech et al., 1993; Quayle et al., 1994, 1995), urinary bladder (Bonev and Nelson, 1993) and urethral tissues (Teramoto and Brading, 1997). The channels opened by K^+ channel openers in smooth muscles are a subvariety of K_{ATP} channels called K_{NDP} channels, which nucleotide diphosphates effectively activate (Beech et al., 1993; Yamada et al., 1997). Opening of K_{ATP} channel induces membrane hyperpolarization, which prevents the contractile activity of smooth muscle by displacing the membrane potential away from the threshold at which voltage-dependent Ca^{2+} channels are gated (Edwards and Weston, 1993).

In guinea-pig isolated trachealis, cromakalim causes concentration-dependent suppression of spontaneous muscle tone (Allen et al., 1986; Arch et al., 1988). This effect is associated with both stimulation of $^{86}Rb^+$ efflux and the membrane hyperpolarization close to the K^+ equilibrium potential. K^+ channel blocking agents such as procaine and tetraethylammonium inhibit cromakalim-induced hyperpolarization and relaxation (Allen et al., 1986). Gliben-

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clamide also effectively antagonizes the relaxation and hyperpolarization evoked by cromakalim in guinea-pig trachealis (Murray et al., 1989). The information about electrophysiological measurements of $I_{K\text{-}ATP}$ in tracheal smooth muscle is, however, quite limited except that a benzopyran K^+ channel opener, (3*S*,4*R*)-3,4-dihydro-2,2-dimethyl-4-(2-ox-opiperidin-1-yl)-6-pentafluoroethyl-2*H*-1-benzopyran-3-ol (BRL55834) and cromakalim induce K^+ currents in bovine and swine trachea (Edwards et al., 1995; Nuttle and Farley, 1997).

It has been reported that the stimulation of c-AMP-dependent protein kinase is involved in the activation of $I_{K\text{-}ATP}$ by vasodilators and that the stimulation of protein kinase C is responsible for the suppression of $I_{K\text{-}ATP}$ by vasoconstrictors (Miyoshi et al., 1992; Bonev and Nelson, 1996; Quayle et al., 1997). These findings indicate that phosphorylation of K_{ATP} channels by protein kinases plays an important role in the regulation of K_{ATP} channel activity by various agonists. In addition, it has been shown that outward currents induced by pinacidil in rabbit portal vein are inhibited by genistein, suggesting that the activation of tyrosine kinase augments $I_{K\text{-}ATP}$ in vascular smooth muscle (Ogata et al., 1997). In contrast, it has been reported that $I_{K\text{-}ATP}$ in a rat insulinoma cell line (Harvey and Ashford, 1998) and rabbit ventricular myocytes (Nishio et al., 1999) is inhibited by the activation of tyrosine kinase. Since nonspecific effects of tyrosine kinase inhibitors on membrane ionic currents have been reported (Smirnov and Aaronson, 1995), the contribution of tyrosine kinases to the activation of $I_{K\text{-}ATP}$ by K_{ATP} channel opener still remains unclear. In the present study, we investigated (1) the presence of $I_{K\text{-}ATP}$ in guinea-pig tracheal smooth muscle cells, (2) the sensitivity of $I_{K\text{-}ATP}$ to glibenclamide and (3) the involvement of phosphorylation by tyrosine kinases in activation of $I_{K\text{-}ATP}$.

2. Materials and methods

2.1. Cell isolation

Male Hartley guinea-pigs, weighing about 200 g, were stunned by a blow to the head and killed by exsanguination. All experiments were carried out in accordance with guiding principles for the care and use of laboratory animals (the Science and International Affairs Bureau of the Japanese Ministry of Education, Science, Sports and Culture) and also with the approval of the ethics committee of Nagoya City University. The trachea was dissected out and single cells were dispersed using collagenase and papain as described previously (Henmi et al., 1995). Only relaxed spindle-shaped cells that were longer than 100 μm were used for electrical recordings. These cells showed a good contractile response to 10 μM acetylcholine or histamine.

2.2. Electrophysiological measurements

Standard whole-cell voltage-clamping (Hamil et al., 1981) was performed using a CEZ-2300 (Nihon-Koden, Japan) amplifier. The pipette resistance ranged from 2 to 4 MΩ when filled with the pipette solution. The seal resistance was approximately 30 GΩ. Series resistance was between 4 and 8 MΩ and was partly compensated. Cell-capacitance was not neutralized. Current and voltage signals were stored on videotape using a PCM system and were later captured on an IBM computer using DT2801A as an analog–digital converter and an acquisition program for precise analysis as described previously (Henmi et al., 1995). All experiments were carried out at 23 ± 2°C.

2.3. Solutions

The physiological salt solution for electrophysiological experiments contained (mM): NaCl 137, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2.2, glucose 14, HEPES 10. The pH was adjusted to 7.4 with NaOH. A solution for dispersing cells was prepared by omitting Ca^{2+} and Mg^{2+} from the physiological salt solution. The high K^+ solution to record the cromakalim-induced membrane current contained (mM): KCl 131.9, $MgCl_2$ 1.2, glucose 14, HEPES 10. The pH was adjusted to 7.4 using NaOH. The pipette solution for electrical recordings contained (mM): KCl 140, $MgCl_2$ 4, ATP-2Na 1 or 5, EGTA 10, HEPES 10. The pH was adjusted to 7.2 with KOH.

2.4. Chemicals

Cromakalim (a racemic mixture, Sigma, St. Louis, USA), glibenclamide (Sigma), tetraethylammonium chloride (Tokyokasei, Tokyo, Japan), genistein (Calbiochem, USA), daidzein (Calbiochem), α-cyano-3-ethoxy-4-hydroxy-5-phenylthiomethylcinnamamid (ST638, kindly given by Kanegabuchi Chemicals, Tokyo, Japan) and herbimycin A (Wako, Tokyo, Japan) were from the companies shown in parentheses. All these drugs except tetraethylammonium were dissolved in dimethylsulfoxide (DMSO, 100 mM as stock). Tetraethylammonium was dissolved in distilled water to make a 1 M stock solution. These solvents (DMSO and distilled water) had no effect on membrane currents and potentials when the corresponding amount was applied.

2.5. Statistics

Results are given as means ± S.E.M. and were compared using Student's *t*-test for paired or unpaired experiments for two groups. $P < 0.05$ was considered significant, indicated in the figures by an asterisk.

3. Results

3.1. ATP-sensitive K^+ currents in guinea-pig tracheal smooth muscle cells

When the pipette and bathing solution contained mainly 140 and 131.9 mM K^+, respectively, application of cromakalim in a concentration range of 1–300 μM produced an inward current in a concentration-dependent manner at a holding potential of −60 mV (Fig. 1Aa). To minimize contamination by the Ca^{2+}-dependent K^+ current, 10 mM EGTA was added to the pipette solution. The voltage-dependent Ca^{2+} current was inhibited by the addition of 0.1-mM Cd^{2+} and the removal of Ca^{2+} from the bathing solution. The amplitude of I_{crom} depended on the concentration of ATP in the pipette solution: when the ATP concentration was 1 mM, the amplitude was clearly larger than that observed in the presence of 5 mM ATP (Fig. 1Aa,b). Fig. 1B shows summarized data for the amplitude of I_{crom} plotted against the concentration of cromakalim. The response to cromakalim appeared from the concentration of 1 μM and reached the maximum at 100 μM. The averaged amplitude of inward currents elicited by 300 μM cromakalim was 186.3 ± 20.4 ($n = 6$) and 100 ± 10.1 pA ($n = 6$, $P < 0.01$) in the presence of 1 and 5 mM ATP in the pipette solution, respectively. Each set of data was fitted to the following equation;

$$I_{drug} = I_{drug\text{-}max} / \left(1 + K_d / [drug]^n\right), \quad (1)$$

where $I_{drug\text{-}max}$ is the maximum amplitude of drug-induced inward current, K_d is an apparent dissociation constant of the drug, [drug] is the concentration of the drug and n is a Hill coefficient. K_d value and n of cromakalim for the activation of the inward current at −60 mV were calculated to be 16.1 μM and 1.29, and 16.7 μM and 1.34 in the presence of 1 and 5 mM ATP in the pipette, respectively.

When the bathing solution contained 5.9 mM K^+, 30 μM cromakalim activated the outward current at a holding potential of −30 mV (27 ± 8.3 pA, $n = 7$). In Fig. 2, a ramp wave pulse which changed the membrane potential from −100 to +40 mV at the rate of 400 mV/s was applied in the absence and presence of cromakalim to obtain the current–voltage (I–V) relationship of I_{crom}. The cromakalim-sensitive current component was obtained by subtracting the I–V relationship in the presence of 30 μM cromakalim from that in its absence. The concentration of ATP in the pipette was 1 mM. In a bathing solution containing 5.9 mM K^+, I_{crom} reversed at -80 ± 3.4 mV ($E_{r\text{-}crom}$, $n = 5$). The mean slope conductance at the $E_{r\text{-}crom}$ was 880 ± 270 pS. When the bathing solution contained 131.9 mM K^+, $E_{r\text{-}crom}$ was shifted to -1.8 ± 1.7 mV ($P < 0.01$, $n = 8$) and the slope conductance at the $E_{r\text{-}crom}$

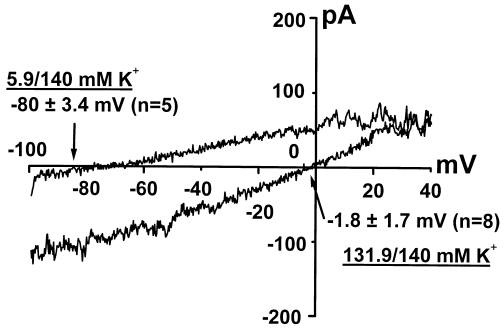

Fig. 2. Typical examples of current–voltage relationship of I_{crom}. Ramp wave pulses (400 mV/s) were applied before and during application of 30 μM cromakalim. Cromakalim-sensitive currents were obtained by subtracting the current in the presence of cromakalim from that in its absence. The pipette solution contained mainly 140 mM K^+ and 1 mM ATP. Cells were superfused with the bathing solution containing 5.9 or 131.9 mM K^+. K^+ concentrations in the bathing and pipette solutions are underlined. Arrows indicate the averaged reversal potentials. Numbers in parentheses are the number of cells used.

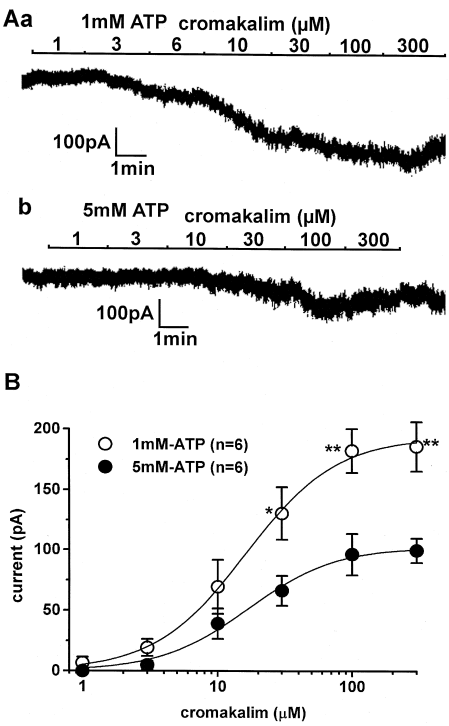

Fig.1. I_{crom} in guinea-pig tracheal smooth muscle cells. Cells were superfused with a bathing solution containing 131.9 mM K^+ (see Methods). Concentrations of K^+ and ATP in the pipette solution were 140 and 1 (Aa) or 5 (Ab) mM, respectively. Cromakalim over a concentration range of 1–300 μM was applied cumulatively to the cell, which was held at the holding potential of −60 mV. (B) Summarized data describing the concentration–response relationship of cromakalim-induced inward currents at −60 mV. Open and closed symbols show the averaged amplitude of the inward current at each concentration of cromakalim in cells which were dialyzed with pipette solution containing 1 and 5 mM ATP, respectively. Numbers in parentheses are the number of cells used. The curve was obtained by fitting an equation modified from Eq. (1) in the text to the data. K_d and Hill coefficient were calculated to be 16.1 μM and 1.29, and 16.7 μM and 1.34 in the presence of 1 and 5 mM ATP in the pipette, respectively. Asterisks indicate significant difference between 1 and 5 mM ATP (*$P < 0.05$ and **$P < 0.01$).

increased to 2500 ± 500 pS ($P < 0.05$, $n = 8$). The K^+ equilibrium potentials in these bathing solutions were calculated to be -80 and -1.4 mV, respectively. These results indicate that I_{crom} is an ATP-sensitive K^+ current ($I_{K\text{-}ATP}$).

3.2. Effects of potassium channel blockers on I_{crom}

The effects of K^+ channel blockers, glibenclamide and tetraethylammonium, on I_{crom} were examined under the conditions where ATP and K^+ concentrations in the pipette and the bathing solutions were 1 and 131.9 mM, respectively. Fig. 3A shows that the inward current elicited by 30 μM cromakalim at -60 mV was reduced by the addition of glibenclamide in a concentration-dependent manner over the range 0.03–1 μM. In the presence of 30 μM cromakalim, I_{crom} was sustained for at least 5 min without a substantial decrease. Fig. 3B indicates the summarized data of the concentration-dependent block of I_{crom} by glibenclamide. Each set of data was fitted to an equation which was modified from Eq. (1) for the blocking action, and K_d and Hill coefficient of glibenclamide were calculated to be 195 nM and 1.05, respectively. The difference in the holding current at -60 mV before application of 30 μM cromakalim from that after addition of 10 μM glibenclamide was small (2.5 ± 4.9 pA as an outward current, $n = 8$), suggesting that $I_{K\text{-}ATP}$ was absent without cromakalim in guinea-pig tracheal myocytes. In addition, application of 2 mM tetraethylammonium, a non-selective K^+ channel blocker, also reduced I_{crom} to $75 \pm 8.3\%$ of control ($n = 5$, data not shown). Fig. 3C shows the I–V relationship of the glibenclamide-sensitive current, which was obtained by using ramp wave pulses (400 mV/s). The ramp wave pulses were applied twice in the presence of 30 μM cromakalim, before and during the exposure to 10 μM glibenclamide. The E_r of the glibenclamide-sensitive current was -0.92 ± 1.2 mV ($n = 5$).

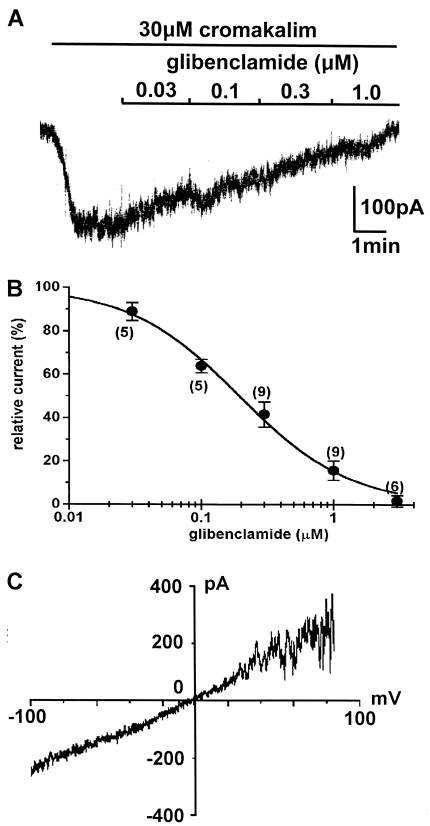

Fig. 3. Effects of glibenclamide on I_{crom} in guinea-pig tracheal smooth muscle cells. (A) After I_{crom} was activated by 30 μM cromakalim, glibenclamide over a concentration range of 0.03–1 μM was cumulatively applied. K^+ concentration in the pipette and bathing solution was 140 and 131.9 mM, respectively. The holding potential was -60 mV. (B) The concentration–response relationship of glibenclamide for I_{crom} inhibition. The amplitude of I_{crom} in the presence of glibenclamide was normalized to that in its absence and expressed as a percentage. Each point represents the mean ± S.E.M. Numbers in parentheses are the number of cells used. The curve was obtained by fitting an equation modified from Eq. (1) in the text to the data. K_d and Hill coefficient were 195 nM and 1.05, respectively. (C) A typical I–V relationship of the glibenclamide-sensitive current component obtained in the continuous presence of 30 μM cromakalim. The glibenclamide-sensitive current was obtained by subtraction of the current in the absence of 10 μM glibenclamide from that in the presence and plotted against each potential.

3.3. Effects of tyrosine kinase inhibitors on I_{crom}

Genistein and ST638 have been introduced as inhibitors of epidermal growth factor (EGF) receptor kinase, a kind of tyrosine kinase. Genistein and ST638 inhibit tyrosine kinase activity by competing with ATP for binding to tyrosine kinase (Akiyama et al., 1987) and by competing with substrate proteins (Shiraishi et al., 1987). Herbimycin A directly binds to the reactive SH groups of some types of tyrosine kinases to block their activity (Uehara et al., 1989). Fig. 4A shows effects of externally applied genistein and daidzein, an inactive analogue of genistein, on I_{crom} at -60 mV in a tracheal smooth muscle cell. The pipette solution contained mainly 1 mM ATP and 140 mM K^+. The cell was superfused with high K^+ solution that contained 131.9 mM K^+. I_{crom} was slightly reduced by addition of 40 μM daidzein or 3.7 μM genistein and more effectively by 37 μM genistein. The onset and withdrawal of the inhibition of I_{crom} by 37 μM genistein was fast (< 1 min). The effects of ST638 and herbimycin A, different types of tyrosine kinase inhibitor, on I_{crom} were also examined and are summarized in Fig. 4B. Genistein was about 10 times more effective than daidzein as a blocker of I_{crom}, whereas herbimycin A at a high concentration of 1 μM did not affect I_{crom}. ST638 was more potent than genistein, and their K_d values were calculated to be 2.3 and 17 μM, respectively, according to the modified Eq. (1). In a bathing solution containing 5.9 mM K^+, 37 μM genistein and 30 μM ST638 also inhibited the cromakalim-induced outward currents at -30 mV by approximately 80% (genistein: $79.3 \pm 4.7\%$ inhibition, $n = 7$, ST638: $88.0 \pm 6.1\%$ inhibition, $n = 6$, data not shown).

Since it is possible that tyrosine kinase inhibitors directly block K^+ channels from the outside, the effects of

internally applied tyrosine kinase inhibitors on I_{crom} were examined in the bathing solution containing 131.9 mM K^+ (Fig. 5). Tyrosine kinase inhibitors were added to the pipette solution and applied to the cytoplasm by simple diffusion after rupture of the patch membrane. Cromakalim at 30 μM was first applied just after the start of whole-cell recording (within 3 min) and the second application of 30 μM cromakalim was 20 min after the first application. The peak amplitude of I_{crom} at −60 mV induced by the first application of 30 μM cromakalim in the presence of an inhibitor in the pipette solution was 208 ± 32.3 pA and not significantly different from that in the absence of the inhibitor ($P > 0.05$). The amplitude of I_{crom} after the second application of 30 μM cromakalim was normalized to that after the first one. The concentrations of internally applied genistein (1.1 mM) and ST-638 (30 μM) were much higher than those required for maximum inhibition when applied externally (Fig. 4). These inhibitors had no significant effects on I_{crom} induced by the second application of cromakalim ($P > 0.05$, Fig. 5A). In Fig. 5B, cromakalim at 30 μM was first applied just after the start of whole-cell recording (within 3 min) and then 30 μM cromakalim was applied every 5 min after the first application. The amplitude of I_{crom} with 1.1 mM genistein was compared with that of a separate group without genistein as a time control. While the I_{crom} obtained from both groups gradually decreased in a time-dependent manner, I_{crom} was not significantly affected by intracellular genistein.

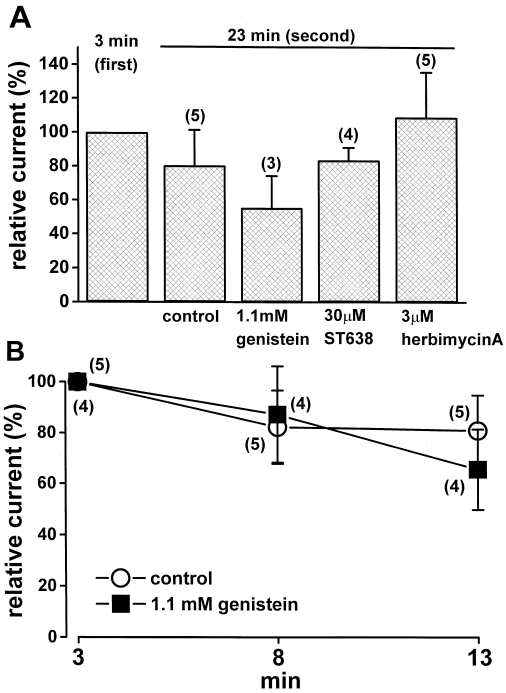

Fig. 5. Internal dialysis of cells with solution containing tyrosine kinase inhibitors. The pipette and bathing solutions contained mainly 140 and 131.9 mM K^+, respectively. The concentration of ATP in the pipette solution was 1 mM. Cells were held at −60 mV. (A) Cromakalim at a fixed concentration of 30 μM was applied 3 (first) and 23 min (second) after the establishment of the whole-cell clamp configuration. The amplitude of I_{crom} after the second application of cromakalim was normalized to that after the first application. Each column and bar represent the mean ± S.E.M., respectively. (B) Cromakalim at 30 μM was applied every 5 min after the first application (3 min after the establishment of whole-cell clamp configuration). The amplitude of I_{crom} with (closed squares) or without 1.1 mM genistein (open circles) was normalized to that after the first application of cromakalim. Numbers in parentheses show the number of cells used.

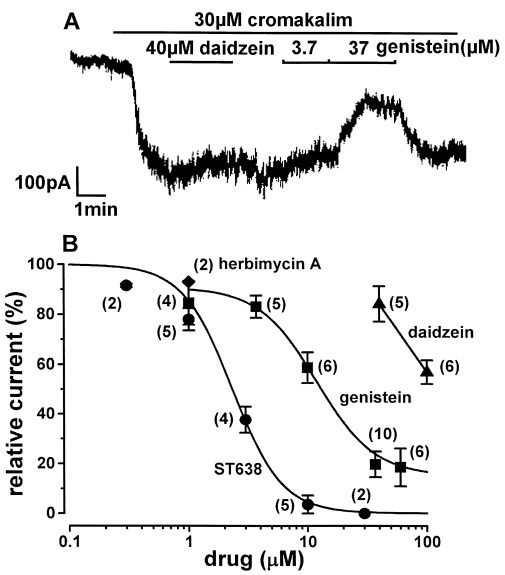

Fig. 4. Effects of externally applied tyrosine kinase inhibitors on I_{crom} in guinea-pig tracheal smooth muscle cells. (A) Effects of genistein and its inactive analogue, daidzein, on I_{crom} activated by 30 μM cromakalim. The pipette and bathing solution contained mainly 140 and 131.9 mM K^+, respectively. Concentration of ATP in the pipette solution was 1 mM. Cells were held at −60 mV. (B) Concentration–response relationships of tyrosine kinase inhibitors for the block of I_{crom}. The relative amplitude of I_{crom} in the presence of a drug to that in the absence was averaged and plotted against the concentration of the drug. Open, square and triangles indicate mean values obtained after application of ST638, genistein and daidzein, respectively. Numbers in parentheses show the number of cells used. The curve was obtained by fitting an equation modified from Eq. (1) in the text to the data. K_d and Hill coefficient of ST638 and genistein were calculated to be 2.3 μM and 2.1, and 17 μM and 1.8, respectively.

4. Discussion

The present study shows that the application of cromakalim induces I_{crom} in tracheal smooth muscle cells of the guinea-pig, which is supposed to reflect opening of K_{ATP} channels susceptible to glibenclamide. I_{crom} was suppressed by externally applied tyrosine kinase inhibitors, genistein and ST638, but not by herbimycin A. Internally applied genistein, ST638 and herbimycin A did not cause significant inhibition of I_{crom}.

The potency of cromakalim to elicit K^+ currents in tracheal smooth muscle cells of guinea-pig ($EC_{50} = 16$

μM) under the present experimental conditions appears to be comparable to that measured in vascular smooth muscles (Beech and Bolton, 1989; Okabe et al., 1990), while only few studies determined the exact potency. I_{crom} in tracheal smooth muscle cells was suppressed by glibenclamide in a concentration-dependent manner. Moreover, the concentration–response relationship of glibenclamide for the inhibition of I_{crom} is described by a one to one stoichiometric relationship (Hill coefficient: 1.05). It has been revealed that the functional K_{ATP} channel sensitive to glibenclamide consists of at least two components; an inward rectifying K^+ channel subunit (K_{IR}) and sulfonylurea receptor (Schmid-Antomarchi et al., 1987; Inagaki et al., 1995). The IC_{50} (K_d) of glibenclamide to inhibit I_{crom} in this study was 195 nM, between that reported in pancreatic β-cells (4–50 nM, Ashcroft and Aschcroft, 1990) and that in cardiac myocytes (0.3–2 μM. Ashcroft and Aschcroft, 1990). Glibenclamide inhibits cromakalim-induced K^+ currents in rabbit portal vein with a K_d of 200 nM (Beech et al., 1993) and pinacidil-induced K^+ currents in rabbit mesenteric artery with a K_d of 101 nM (Quayle et al., 1995). These results suggest that the affinity of glibenclamide for K_{ATP} channels in tracheal smooth muscle cells is similar to that in vascular smooth muscle cells.

The characteristics of K_{ATP} channels in vascular smooth muscle cells are apparently different from those of the classical one in cardiac and skeletal myocytes and pancreatic β cells, which may result from the different combination of K_{IR} 6.0 subunits (K_{IR} 6.1/6.2) and sulfonylurea receptors (sulfonylurea receptor 1, sulfonylurea receptor 2A and sulfonylurea receptor 2B) (Yamada et al., 1997; Shindo et al., 1998). In the present study, the enhancement of I_{crom} by lower ATP concentration in the pipette solution may suggest somewhat different characteristics from those of vascular K_{NDP} channels (Beech et al., 1993; Kamouchi and Kitamura, 1994), which resemble the K_{IR} 6.1/sulfonylurea receptor 2B complex (Yamada et al., 1997). Further single channel recordings and molecular analyses of K_{ATP} channels in tracheal smooth cells were not done in the present study.

It has been reported that activation of $I_{K\text{-}ATP}$ in vascular smooth muscle by calcitonin gene-related peptide or adenosine is due to the activation of adenylate cyclase and subsequent protein kinase A (Quayle et al., 1994). The suppression of $I_{K\text{-}ATP}$ by angiotensin II and vasopressin involves the stimulation of protein kinase C through pertussis toxin-insensitive G protein coupling with the receptors in vascular smooth muscle (Bonev and Nelson, 1996). These regulatory mechanisms of K_{ATP} channels by protein kinase A and C in smooth muscle are similar to those in cardiac muscle. In contrast, the mechanism for the regulation of $I_{K\text{-}ATP}$ by tyrosine kinases in rabbit portal vein myocytes (Ogata et al., 1997) appears to be completely different from those in rat insulinoma cell line (Harvey and Ashford, 1998) and rabbit ventricular myocytes (Nishio et al., 1999). $I_{K\text{-}ATP}$ induced by pinacidil in portal vein was blocked by genistein and tyrphostin B46, but not by daidzein, herbimycin A, lavendustin A and tyrphostin 23. In the insulinoma cell line, the inhibition of tyrosine kinase elicited $I_{K\text{-}ATP}$ under similar experimental conditions. In this cell, inhibition of tyrosine kinase and subsequent dephosphorylation are required for activation of the K_{ATP} channel. The inhibition of $I_{K\text{-}ATP}$ by interferon-α in ventricular myocytes was blocked by genistein and can be considered to be mediated by stimulation of tyrosine kinase (Nishio et al., 1999).

The use of K^+ channel openers in the therapy of airway hyper-reactivity and/or asthma has been attempted since an early stage in the development of the drugs (Chapman et al., 1992). It has been reported that the effective dose of K^+ channel openers for reducing airway resistance in vivo is lower than that required for the relaxation of airway smooth muscle in vitro (McCaig and DeJonckheere, 1989). A tentative explanation for this phenomenon is that the reactivity of sensory neurons in airway epithelium is reduced by low concentrations of K^+ channel openers via activation of K_{ATP} channels, which results in hyperpolarization of the neuron (Shikada and Tanaka, 1995). Quantitative analyses of the effectiveness of K^+ channel openers to hyperpolarize and subsequently to relax smooth muscle cells of hyper-reactive airway remain to be determined.

In the present study, the activation of $I_{K\text{-}ATP}$ by tyrosine kinase inhibitors as seen in insulinoma cells was not observed. Externally applied genistein and ST638 inhibited I_{crom} in guinea-pig tracheal smooth muscle cells, whereas daidzein was less effective than genistein. Herbimycin A did not affect I_{crom}. The inhibition of I_{crom} by genistein and ST638 might be due to their interaction with EGF receptor kinase in tracheal smooth muscle cells, as has been suggested in portal vein myocytes. It is, however, rather unlikely since genistein and ST638 did not significantly reduce I_{crom} even when they were added to the pipette solution at high concentrations. The inhibition of I_{crom} by genistein and ST638 may be due to the direct block of K_{ATP} channels from the outside. Moreover, the observation that the recovery of I_{crom} from the inhibition after the washout of genistein and ST638 was extraordinary fast (< 1 min) and complete provides further evidence supporting the direct block of the channel from the outside. In addition, the inhibition of voltage-dependent K^+ currents in rat and rabbit pulmonary artery myocytes by genistein and ST638 has been explained as the direct block of the channel (Smirnov and Aaronson, 1995). Genistein and ST638 blocked outward and inward I_{crom} equally, thus excluding the possibility of the entry of these drugs into the channel pore. Moreover, the site where genistein and daidzein directly block the channel in part discriminated between these drugs, which have quite similar structures. The action of genistein at a regulatory or allosteric binding site on the extracellular surface might cause the channel to block itself. The present conclusion, however, does not eliminate completely the possibility that

the phosphorylation of K_{ATP} channels by tyrosine kinase modulates channel activity.

In conclusion, cromakalim activates $I_{K\text{-}ATP}$ in tracheal smooth muscle cells of the guinea-pig, and the activation of $I_{K\text{-}ATP}$ may not involve the phosphorylation of the channel by tyrosine kinase.

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