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Effects of ATP-sensitive potassium channel openers on the contractile and phosphatidylinositol responses of the rat trachea

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Abstract

Purpose. Although ATP-sensitive potassium channel openers suppress airway smooth muscle contraction, their potencies are different and the mechanisms involved are not fully understood. We examined the effects of cromakalim and Y-26763, a novel ATP-sensitive potassium channel opener, on the contractile and phosphatidylinositol responses of the rat trachea.

Methods. Thirty-six male Wistar rats, weighing 250–350 g, were used. In the experiment on contractile response, active contraction was induced with 0.55 μM carbachol in the presence or absence of cromakalim or Y-26763. In the experiment on phosphatidylinositol response, the tracheal slices were incubated with [^{3}H]*myo*-inositol, 0.55 μM carbachol, and cromakalim or Y-26763, and the formation of [^{3}H]inositol monophosphate (IP_1), a degradation product of phosphatidylinositol response, was measured with a liquid scintillation counter. Statistical significance ($P < 0.05$) was determined by analysis of variance (ANOVA).

Results. Carbachol-induced tension was attenuated by both cromakalim and Y-26763, the latter displaying significantly greater potency. Carbachol-induced IP_1 accumulation was influenced neither by cromakalim nor by Y-26763.

Conclusion. Both cromakalim and Y-26763 have effects on airway smooth muscle relaxation. Carbachol-induced IP_1 accumulation was influenced neither by cromakalim nor by Y-26763, suggesting that phosphatidylinositol response may not be a common pathway for the effect of ATP-sensitive potassium channel openers.

Key words K_{ATP} channel openers · Phosphatidylinositol response · Muscarinic receptors · Tracheal smooth muscle

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Introduction

ATP-sensitive K^+ (K_{ATP}) channels exist in cell membranes such as smooth muscle and neurons. K_{ATP} channel openers have a wide range of effects and are theoretically useful in patients with asthma. When K_{ATP} channels are opened in airway smooth muscle cell membranes, the increase in K^+ efflux shifts the membrane potential in a hyperpolarizing direction towards the K^+ equilibrium potential. Hyperpolarization prevents Ca^{2+} entry through voltage-operated Ca^{2+} channels, resulting in airway smooth muscle relaxation [1].

The phosphatidylinositol (PI) response is important for regulation of airway smooth muscle tone. Intracellular Ca^{2+} is mainly regulated by extracellular influx through membrane-associated Ca^{2+} channels and Ca^{2+} release from intracellular stores. The former is regulated by membrane potential, and the latter is regulated by Ca^{2+} and inositol 1,4,5-trisphosphate (IP_3). IP_3 is regulated by pharmaco-enzymatic responses through receptor activation, not through electromechanical response.

There are some reports that K_{ATP} channel openers can regulate the PI response indirectly in vascular smooth muscle [2,3]. However, since the K_{ATP} channel seems not to be so important in airway smooth muscle, few studies, except for those of Challiss et al. [4] and Kamei et al. [5], have evaluated the effects of K_{ATP} channel openers on the airway smooth muscle of bovines or dogs. These authors found that K_{ATP} channel openers inhibited inositol phosphate or IP_3 accumulations. However, the inhibitory effects of K_{ATP} channel openers on carbachol (CCh)-induced contraction differ between species and with the concentration of spasmogens [5,6]. No data are available on the effects of K_{ATP} channel openers on inositol monophosphate (IP_1), a degradation product of the PI response of the rat. The present study was performed to clarify the effects of cromakalim and Y-26763, a novel K_{ATP} channel

opener, on the contractile and PI responses of the rat trachea.

Materials and methods

These studies were conducted under guidelines approved by our animal care committee. Thirty-six male Wistar rats (Charles River, Yokohama, Japan) weighing 250–350 g were used for the experiments. The rats were anesthetized with pentobarbital (50 mg·kg^{-1} intraperitoneally), and the trachea was rapidly isolated.

Contractile response

The trachea was cut into 3-mm-wide ring segments with a McIlwain tissue chopper (Mickle Laboratory Engineering, Gomshall, UK). The tracheal ring was suspended between two stainless steel hooks and placed in a 5-ml water-jacketed organ chamber (Kishimotoika, Kyoto, Japan) containing Krebs-Henseleit (K-H) solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 1.3, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11, Na_2-EDTA 0.05). The solution was continuously aerated with 95%O_2/5%CO_2 at 37°C.

Isometric tensions were measured by an isometric transducer (Kishimotoika, Kyoto, Japan), and changes in isometric force were recorded by a MacLab system (Milford, MA, USA). The resting tension was adjusted periodically to 1.5 g during the equilibration period. The ring was washed every 15 min and re-equilibrated to baseline tension for 60 min (time 0). Active contraction was induced with 0.55 μM (ED_{80}) CCh, and 30 min later cromakalim or Y-26763 was added at cumulative doses to induce relaxation.

PI response

The technique of Brown et al. was used [7]. Inositol 1,4,5-trisphosphate (IP_3) is rapidly degraded into inositol monophosphate (IP_1), which is recycled back to phosphatidylinositol (PI) via free inositol [8]. Lithium inhibits the conversion of IP_1 to inositol. In the presence of Li^+, the accumulation rate of IP_1 reflects the extent of the PI response. Thus, we measured [^{3}H]IP_1 in tracheal slices incubated with [^{3}H]*myo*-inositol (Amersham, Tokyo, Japan) (Fig. 1). The trachea was cut longitudinally and chopped into 1-mm-wide slices with a McIlwain tissue chopper. Three pieces of the tracheal slices were placed in small, flat-bottomed tubes and preincubated for 15 min in K-H solution containing 5 mM LiCl. The solution was continuously aerated with 95%O_2/5%CO_2. An aliquot of 0.5 μCi [^{3}H]*myo*-inositol was then added to each tube (final concentration, 0.1 μM in 300 μl incubation volume), and the tubes were

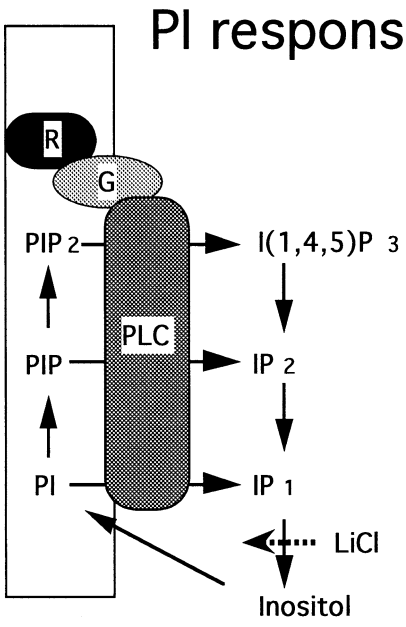

Fig. 1. PI response. *PI*, Phosphatidylinositol; *PIP*, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_2, inositol bisphosphate; IP_1, inositol monophosphate; *R*, muscarinic receptor; *G*, G-protein; *PLC*, phospholipase C

flushed with 95%O_2/5%CO_2, capped, set in a shaking bath at 37°C, and incubated for 30 min (time 0).

The slices were incubated with [^{3}H]*myo*-inositol, 0.55 μM CCh, and cromakalim or Y-26763. The tubes were reaerated with 95%O_2/5%CO_2, recapped, and reincubated. After an additional 60 min, the reaction was stopped with 940 μl chloroform : methanol (1 : 2 v/v). Chloroform and water were then added (310 μl each), and the phases were separated by centrifugation at 90 *g* for 5 min. The [^{3}H]IP_1 was separated from [^{3}H]*myo*-inositol in the water phase of 750 μl by column chromatography using Dowex AG 1-X8 resin (Bio Rad, Richmond, CA, USA) in the formate form. The [^{3}H]IP_1 formed in the tracheal slices was counted with a liquid scintillation counter and presented in becquerels (Bq).

Data were expressed as means ± SD. The results of repeated measures and multiple groups were analyzed by two-way analysis of variance. Multiple pairwise comparisons between groups were assessed by Scheffé's test. A P value < 0.05 was considered significant.

Results

Figure 2 shows a typical recording of the effects of Y-26763 on CCh-induced contraction of the rat tracheal ring. Figure 3 shows the effects of cromakalim and Y-26763 on CCh-induced contraction of the rat tracheal ring. The CCh-induced tension was 2.1 ± 0.6 g for

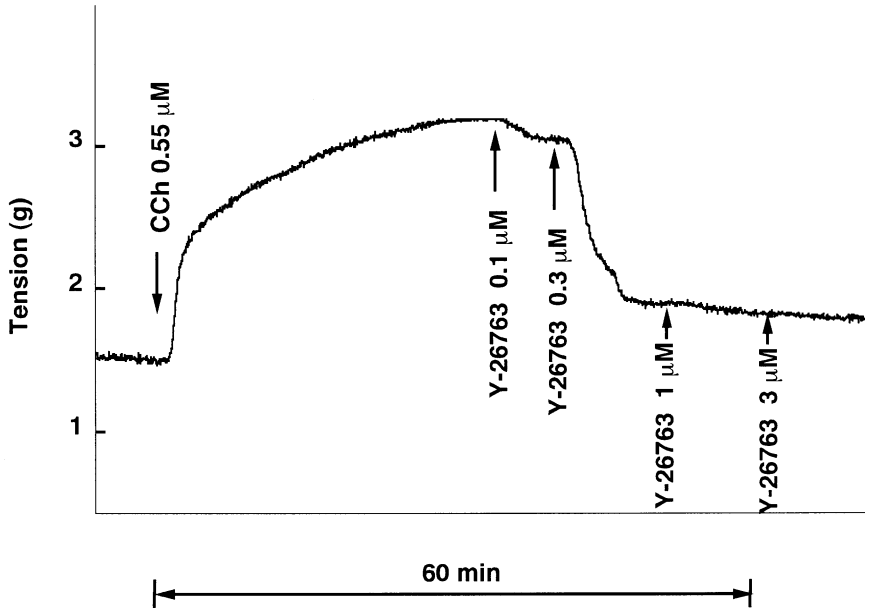

Fig. 2. A typical recording of the effects of Y-26763 on CCh-induced contraction of the rat trachea. *CCh*, Carbachol

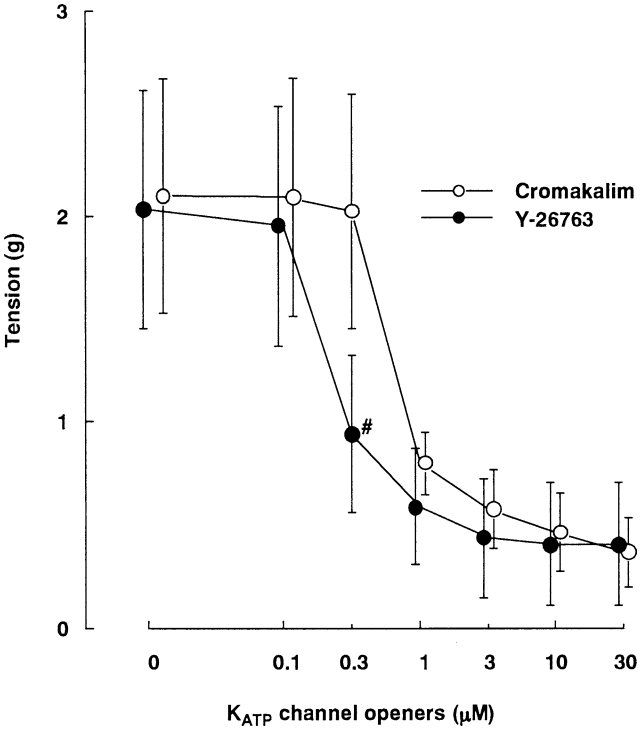

Fig. 3. Effects of cromakalim and Y-26763 on CCh-induced contraction of the rat trachea (mean ± SD, $n = 6$). # $P < 0.05$ *vs* cromakalim

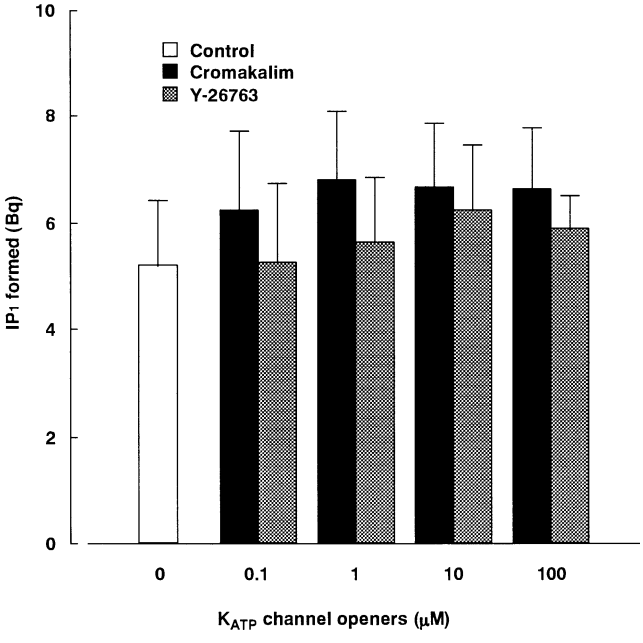

Fig. 4. Effects of cromakalim and Y-26763 on CCh-induced IP_1 accumulation in rat trachea (mean ± SD, $n = 6$–9). *IP_1*, Inositol monophosphate

cromakalim and 2.0 ± 0.6 g for Y-26763. This contraction was attenuated by both cromakalim and Y-26763; the latter displayed significantly greater potency. The doses of 50% inhibition (ID_{50}) of cromakalim was 0.8 ± 0.2 μM, whereas that of Y-26763 was 0.3 ± 0.1 μM. The maximum relaxant effects were 82% for cromakalim and 80% for Y-26763. Figure 4 shows the effects of cromakalim and Y-26763 on CCh-induced IP_1 accumulation in rat trachea. The CCh-induced IP_1 accumulation was 5.2 ± 1.3 Bq, and this accumulation was influenced neither by cromakalim nor by Y-26763.

Discussion

The main findings of the present study were that CCh-induced contraction was dose-dependently inhibited by both cromakalim and Y-26763, and that CCh-induced IP_1 accumulation was influenced neither by cromakalim nor by Y-26763.

In the airways, K_{ATP} channels exist in cell membranes, such as those of smooth muscle (postjunctional) and neurons (prejunctional). Cromakalim and Y-26763 may inhibit acetylcholine (ACh) release from postganglionic neurons, resulting in an attenuation of CCh-induced contraction. However, DMPP, a selective ganglionic nicotinic agonist, does not cause contraction of the rat tracheal ring [9]. Thus, the preparation in the present study does not appear to have contained a sufficient number of functional postganglionic cells that could be activated by the nicotinic agonists. Song et al. [10] examined the pre- and postjunctional effects of pinacidil, a K_{ATP} channel opener, in isolated bovine trachealis and found that the inhibitory effects of pinacidil on contractions of the same magnitude induced by electrical field stimulation (EFS) or exogenous ACh were not significantly different, suggesting that pinacidil had only a postjunctional effect. They concluded that pinacidil attenuated the contraction of isolated bovine tracheal smooth muscle by postjunctional mechanisms. Thus, cromakalim and Y-26763 used in the present study would affect rat tracheal smooth muscle by postjunctional mechanisms.

When K_{ATP} channels are opened in airway smooth muscle cell membranes, the membrane potential is shifted in a hyperpolarizing direction. Hyperpolarization prevents Ca^{2+} entry through voltage-operated Ca^{2+} channels, resulting in airway smooth muscle relaxation. However, Quast and Baumlin [11] reported that three to five times more cromakalim is required to induce K_{ATP} channel opening than to induce 50% relaxation in the rat portal vein and aorta. Thus, other possible mechanisms are addressed. K_{ATP} channel openers inhibit agonist-induced Ca^{2+} mobilization from the sarcoplasmic reticulum through the inhibition of 1,4,5-trisphosphate (IP_3) formation, resulting in suppression of protein kinase C activity and thereby the Ca^{2+} sensitivity of contractile structures [12]. Itoh et al. [2] found in smooth muscle cells of the rabbit mesenteric artery that the pinacidil concentration-dependently hyperpolarized the smooth muscle membrane and inhibited the increases in intracellular Ca^{2+} concentration, tension, and production of IP_3 induced by norepinephrine, and that this inhibition was reversed by glibenclamide or by the increased concentrations of KCl.

Yamagishi et al. [3] reported that hyperpolarization of the plasma membrane by K_{ATP} channel openers would inhibit IP_3 production and Ca^{2+} release from intracellular stores induced by the stimulation of the α-adrenoceptor or thromboxane A_2 receptor in canine coronary artery. Kamei et al. [5] observed the effects of KC 399, a novel K_{ATP} channel opener, on the electrical and mechanical properties of dog tracheal smooth muscle tissues and on the accumulation of second messengers. They found that in muscle tissues precontracted with CCh, KC 399 inhibited the tonic response of the contraction more effectively than the initial phasic response induced by CCh. KC399 alone had a small but significant inhibitory effect on the basal value of IP_3 production and on the CCh-induced increase in IP_3 [5]. The initial phasic response depends on muscarinic ACh receptors and Ca^{2+} mobilization from the sarcoplasmic reticulum, and IP_3 plays an important role in the PI response, whereas the tonic response depends on Ca^{2+} entry through the Ca^{2+} channels [5]. Thus, K_{ATP} channel openers would have a small effect on the PI response of airway smooth muscles.

The mechanism involved in the action of K_{ATP} channel openers on the PI response of airway smooth muscle may be as follows. K_{ATP} channel openers that decrease Ca^{2+} channel opening by membrane hyperpolarization would decrease Ca^{2+}-stimulated phospholipase C (PLC) activity and subsequently decrease the rate of CCh-induced PI response, because the PI response can also be activated by an increase in intracellular Ca^{2+}. However, in the present study, CCh-induced IP_1 accumulation was influenced neither by cromakalim nor by Y-26763. These data are inconsistent with the previous reports of inhibition of IP_3 production by K_{ATP} channel openers in the arteries and trachea. Challiss et al. [4] reported that pre-addition of lemakalim significantly inhibited the PI response to 1 μM CCh, but not to 10 μM CCh or greater, in slices of bovine tracheal smooth muscle. Since the doses of 50% effect (ED_{50}) of CCh in bovine tracheal smooth muscle is about 5.5 μM [13], the 1 μM CCh used in their study was less than the ED_{50} of CCh [4]. On the other hand, the 0.55 μM CCh used in the present study is a near-maximal dose (ED_{80}). Thus, the effect of CCh used in the present study may be similar to that of 10 μM CCh or greater in slices of bovine tracheal smooth muscle. The reason that cromakalim and Y-26763 had no influence on CCh-induced IP_1 accumulation in the present study may be as follows. A near-maximal dose of CCh may strongly induce the influx of Ca^{2+} and increase intracellular Ca^{2+} to the maximal level. This strong influx of Ca^{2+} may not be fully inhibited by cromakalim and Y-26763, resulting in the absence of any effect on Ca^{2+}-stimulated PLC activity.

In conclusion, both cromakalim and Y-26763 have effects on airway smooth muscle relaxation, and CCh-induced IP_1 accumulation was influenced neither by cromakalim nor by Y-26763, suggesting that the PI re-

sponse may not be a common mechanism involved in K_{ATP} channel opener-induced airway dilatation.

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