Inhibitory effects of SKF96365 on the activities of \(K^+\) channels in mouse small intestinal smooth muscle cells

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ABSTRACT. In order to investigate the effects of SKF96365 (SKF), which is a non-selective cationic channel blocker, on \(K^+\) channel currents, we recorded currents through ATP sensitive \(K^+\) (\(I_{ATP}\)), voltage-gated \(K^+\) (\(I_{KV}\)) and \(Ca^{2+}\) activated \(K^+\) channels (\(I_{BK}\)) in the absence and presence of SKF in single small intestinal myocytes of mice with patch-clamp techniques. SKF (10 \(\mu M\)) reversibly abolished \(I_{ATP}\) that was induced by cromakalim (10 \(\mu M\)), which is a selective ATP sensitive \(K^+\) channel opener. These inhibitory effects were induced in a concentration-dependent and voltage-independent manner. The 50% inhibitory concentration (IC50) was 0.85 \(\mu M\), which was obviously lower than that reported for the muscarinic cationic current. In addition, SKF (1 \(\mu M\)) reversibly inhibited the \(I_{KV}\), that was induced by repetitive depolarizing pulses from −80 to 20 mV. However, the extent of the inhibitory effects was only ~30%. In contrast, SKF (1 \(\mu M\)) had no significant effects on spontaneous transient \(I_{BK}\) and caffeine-induced \(I_{BK}\). These results indicated that SKF inhibited ATP sensitive \(K^+\) channels and voltage-gated \(K^+\) channels, with the specific effect of \(K^+\) channels being more sensitive than the voltage-gated \(K^+\) channels. These inhibitory effects on \(K^+\) channels should be considered when SKF is used as a cationic channel blocker.

KEY WORDS: ATP sensitive \(K^+\) channel, \(Ca^{2+}\) activated \(K^+\) channel, intestinal smooth muscle, SKF96365; voltage-gated \(K^+\) channel

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In gastrointestinal smooth muscles, the stimulation of muscarinic receptors by cholinergic agonists, including acetylcholine, increases the intracellular concentration of \(Ca^{2+}\) ([\(Ca^{2+}\]i]), which results in a contractile response [45]. The increase in [\(Ca^{2+}\)]i is induced by the \(Ca^{2+}\) release from internal stores and \(Ca^{2+}\) entry into the cell through \(Ca^{2+}\) voltage-gated \(Ca^{2+}\) channels (\(Ca_{V1.2}\)). The activation of \(Ca_{V1.2}\) can be achieved by depolarization due to muscarinic stimulation of non-selective cationic channels [18, 23, 50]. Cationic channels are thought to consist of transient potential canonical channels 4 and 6 (TRPC4 and 6) [43], which are also permeable to \(Ca^{2+}\) and involved in \(Ca^{2+}\) entry [47]. In addition, muscarinic receptors directly or indirectly regulate the activities of \(K^+\) channels that are expressed in gastrointestinal smooth muscle cells, including ATP sensitive \(K^+\) (\(I_{ATP}\)), voltage-gated \(K^+\) (\(I_{KV}\)) and large conductance \(Ca^{2+}\) activated \(K^+\) (BK) channels, through which the electrical activities of the muscles are modulated [5, 6, 12, 14, 20].

SKF96365 (SKF) is an imidazole compound that inhibits receptor-mediated \(Ca^{2+}\) entry [27]. SKF has been reported to inhibit the opening of TRPC channels in guinea-pig [52] and mouse ileal cells [9]. Although other inhibitors of TRPC channels, such as quinine, quindine, \(La^{3+}\) and flufenamic acid, are well known [9, 15, 24], these chemicals are not specific for the TRPC channels. Therefore, in investigations of the physiological and pharmacological properties of muscarinic receptor-gated TRPC channels, SKF has been widely used as a channel inhibitor in gastrointestinal smooth muscle cells [9, 26, 52]. However, it has been reported that application of SKF evoked membrane depolarization in gastrointestinal smooth muscles [15, 51], indicating additional effects of SKF on ion channels other than TRPC channels. The depolarizing action can make it more difficult to analyze the physiological roles of TRPC channels. Schwarz et al. [36] have suggested that SKF blocks inwardly and outwardly rectifying \(K^+\) currents in endothelial cells (IC50 ≥40 \(\mu M\)). Thus, it is possible that SKF can block \(K^+\) channels that are expressed in gastrointestinal smooth muscles together with TRPC channels, thus resulting in membrane depolarization. However, the effects of SKF on the activities of the \(K^+\) channels that are expressed in gastrointestinal smooth muscle cells remain to be elucidated. In the present study, we investigated the effects of SKF on the currents through the \(I_{ATP}\) (\(I_{ATP}\)), \(I_{KV}\) (\(I_{KV}\)) and BK (\(I_{BK}\)) channels in longitudinal smooth muscle cells that were isolated from mouse small intestine, which have often been used to investigate the activation mechanisms of TRPC channels.
MATERIALS AND METHODS

The nomenclature of the ion channels was in accordance with Alexander et al. [2]. All of the animal care and experimental procedures described below complied with the guidelines of the local animal ethics committee of the Faculty of Life Sciences, Kyoto Sangyo University.

Cell preparation: Mice (hybrid of 129S4 or 129S6 and CF1) of either sex who were above 2-month-old and who weighed (mean ± standard deviation) 25.1 ± 3.0 g were sacrificed by cervical dislocation. A 15-cm-long gut segment was then removed from the region over the jejunum and ileum, except for the 2.0-cm terminal portion, and placed in physiological salt solution (PSS; for composition, see below). The gut segment was cut into 1.0- to 1.5-cm pieces, and the longitudinal muscle layer was carefully peeled off from each of the pieces. Single smooth muscle cells were isolated enzymatically from the longitudinal muscle layers as described previously [40]. The cells were suspended in PSS containing 0.5 mM CaCl2. A small aliquot was placed on coverglass and stored at 4°C for 2–7 hr until its use on the same day.

Whole-cell current recording: Whole-cell membrane current recordings were performed at room temperature (21–25°C) with standard patch-clamp techniques [11] with 4–7 MΩ patch pipettes. The current signals were amplified with a current amplifier (CEZ-2300 or 5100, Nihon Kohden Corp., Tokyo, Japan) filtered at 1 KHz and captured at a sampling rate of 4 KHz with an analog-digital converter (Digidata 1440A, Molecular Devices, LLC, Sunnyvale, CA, U.S.A.) that was interfaced with a computer (Think Centre A58 Small, Lenovo, Morrisville, NC, U.S.A.) that was running the pCLAMP program (version 10, Molecular Devices, LLC).

For the IkATP recordings, the cells were bathed in a 60-mM K+ solution and intracellularly dialyzed with a K+-rich high-BAPTA pipette solution (for compositions, see below) at a holding potential of −80 mV in order to increase the driving force of K+ and minimize the activities of the Kᵥ and BK channels [12, 20, 31]. Under these conditions, the K+ equilibrium potential (E_K) was estimated at −21 mV, and the K+ currents were inwardly activated. I_K was recorded in cells that was bathed in Ca2+-free PSS (for composition, see below) and dialyzed with the K+-rich high-BAPTA pipette solution in order to minimize the currents through the BK and CaV1.2 channels. The whole-cell voltage clamp mode was held at −80 mV, and 2 sec step pulses to 20 mV that were generated by the pCLAMP program were repeatedly applied to the cell every 20 sec to elicit I_K. When currents were recorded through I_BK, a K+-rich low EGTA pipette solution (for composition, see below) was used. The cells were bathed in PSS and held at a holding potential of 0 mV. Recordings of the muscarinic cationic channel currents (mI₉PRC) were made from cells that were bathed in Cs+-rich external solution and intracellularly dialyzed with Cs+-rich pipette solution (for compositions, see below) at a holding potential of −60 mV in order to eliminate any K+ currents.

Solutions: The PSS that was used in the experiments had the following composition (mM): NaCl, 126; KCl, 6; CaCl₂, 2; MgCl₂, 1.2; glucose, 14; and HEPES, 10.5; and the pH was adjusted to 7.2 with NaOH. The 60-mM K+ solution was made by changing the concentrations of NaCl and KCl in the PSS to 72 mM and 60 mM, respectively. If necessary, the KCl in the 60-mM K+ solution was replaced with CsCl. The Ca2+-free PSS was prepared by omitting CaCl₂ from the PSS and adding 0.5 mM EGTA. The Cs+-rich external solution had the following composition (mM): CsCl, 120; glucose, 12; and HEPES, 10; and the pH was adjusted to pH 7.4 with CsOH. The compositions of each patch-pipette solution (mM) were as follows. The K+-rich low-EGTA pipette solution contained KCl, 134; Na₂GTP, 1.0; MgCl₂, 1.2; MgATP, 1.0; glucose, 14; EGTA, 0.05; and HEPES, 10.5; and the pH was adjusted to 7.2 with KOH. For the K+-rich high-BAPTA pipette solution, CaCl₂ was removed, and 20 mM BAPTA was added. In this case, in order to adjust the total concentration of K+ to 134 mM and the pH to 7.2, the concentration of KCl was reduced to ~67 mM, and KOH was added. The Cs+-rich pipette solution contained CsCl, 80; Na₂GTP, 1.0; creatine, 5; MgATP, 1.0; glucose, 20; HEPES, 10; BPATPA; 10; and CaCl₂, 4.6 (calculated free calcium=100 nM), with the pH adjusted to 7.4 with CsOH.

 Drugs: The following drugs were used: 1-2-[4-Methoxyphenyl]-2-[3-(4-methoxyphenyl)propoxy]ethyl-1H-imidazole hydrochloride (SKF), cromakalim (Tocris Bioscience, Bristol, U.K.), carbamylcholine chloride (CCh), glibenclamide (Sigma-Aldrich Co. LLC, St. Louis, MO, U.S.A.), tetraethylammonium chloride (TEA; Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) and caffeine (Wako Pure Chemical Industries Ltd., Osaka, Japan). Cromakalim and glibenclamide were dissolved in dimethyl sulfoxide to form a stock solution and was stored at −20°C. The drug concentrations herein are expressed as the final concentrations that were applied to the cells.

Data analysis: The amplitude of I_KATP that was induced by cromakalim was measured, and the difference in the current level after the application of glibenclamide (10 μM) was calculated. In order to determine the current-voltage (I-V) relationships of I_KATP a 350-msec ramp pulse that ranged from −100 mV to 60 mV was applied to the cells before and after SKF application, and the I-V curves were constructed by subtracting the current after the application of glibenclamide from the current in the presence of cromakalim alone or with SKF [31]. The amplitude of I_K was measured by subtracting the leakage component from the outward current that was induced by the depolarizing pulses [44]. The leakage component was estimated in each experiment as follows. A 10-mV hyperpolarizing pulse was applied to the cells at the holding potential (−40 mV), and the amplitude of the current was considered the leakage current for the 10-mV step. The leakage current that corresponded to the 100-mV depolarizing step pulse was subtracted from each of the outward currents that were evoked by the depolarizing pulse. The amplitudes of the spontaneous transient outward I_BK (STOC) and caffeine-induced I_BK were calculated as the difference from the holding current. The amplitude of mI₉PRC was calculated as the difference from the holding current before the applica-
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RESULTS

Current through the Kₐtp channels in mouse small intestinal smooth muscles: The cells were dialyzed with the K⁺-rich high-BAPTA pipette solution and bathed in the 60-mM K⁺ external solution at a holding potential of −80 mV. Under these conditions, application of the Kₐtp channel opener cromakalim (10 μM) induced an inward current, as has been reported by Nuttle & Farley [31]. The current developed progressively with time and reached a plateau about 1–3 min later (Fig. 1A). Further application of the Kₐtp channel inhibitor, glibenclamide (10 μM), almost completely abolished the current. The mean amplitude of the cromakalim-induced current was −895.2 ± 66.5 pA (n=39). When the extracellular K⁺ in the bath solution was replaced with Cs⁺, the cromakalim-induced inward current was immediately abolished at −80 mV (n=5; data not shown). The I-V relationships of the cromakalim-induced current were determined by applying a voltage ramp pulse that ranged from −100 mV up to +60 mV over 350 msec (Fig. 1Ba). As shown in Fig 1Bb, the I-V curve was linear across the voltages tested. The polarity of the current was reversed at −18.8 ± 2.0 mV (n=7), which corresponded to the estimated E₉ of −21 mV, thus indicating that this current was carried through K⁺ channels. These results indicated that the cromakalim-induced current was carried through Kₐtp channels.

SKF reversibly suppressed the Kₐtp channel current (I₉ₐtp): After the cromakalim-induced I₉ₐtp reached a steady level, SKF (10 μM) was applied in the presence of cromakalim. As shown in Fig. 2A, SKF rapidly decreased the amplitude of I₉ₐtp and abolished the current within about 1 min. The time constant (τ) of the suppression (7.3 ± 1.6 sec, n=7) was significantly shorter than the value (35.6 ± 5.8 sec, n=10) in the glibenclamide-induced abolition of I₉ₐtp. The removal of SKF from the bath solution allowed the recovery of I₉ₐtp with a slower time course. Figure 2Ba shows a typical example of I₉ₐtp suppression that was induced by cumulative applications of SKF with a series of ascending concentrations (0.03–10 μM). The inhibitory effects increased with increasing SKF concentrations, and the maximal inhibition was attained at 10 μM. The mean IC₅₀ value that was estimated by the curve fitting of the data from each myocyte was 0.85 ± 0.12 μM (n=6) (Fig. 2Bb).

In order to investigate the voltage dependency of the SKF-induced I₉ₐtp suppression, the ramp pulses were applied in the absence and presence of SKF (1 μM), and the I-V curves were obtained, as shown in Fig. 2Ca. The I-V curves of I₉ₐtp remained linear, and the reversal potential was almost unchanged after the application of SKF (cromakalim alone, −18.5 ± 0.82 mV and cromakalim with SKF, −16.4 ± 0.97 mV; n=6) (Fig. 2Cb). I₉ₐtp suppression at −80, −40 and 40 mV was comparable (Fig. 2Cc). These results indicated that the SKF-induced I₉ₐtp suppression was voltage-independent.

Comparison of the SKF-induced mITRPC suppression with the I₉ₐtp suppression: The cells that were bathed in the Cs⁺-rich external solution were clamped at a voltage of −60 mV with patch pipettes that were filled with the Cs⁺-rich pipette solution in order to block any K⁺ currents ([Ca²⁺]_i buffered to 100 nM). Under these conditions, the application of CCh (100 μM) induced an inward mITRPC, as previously reported.
SKF reversibly suppressed I_Kv: The cells that were bathed in the Ca^{2+}-free PSS were clamped at a voltage of −80 mV with patch pipettes that were filled with the K⁺-rich high-BAPTA pipette solution. Under these conditions, a 2 sec-step pulse to 20 mV was repeatedly applied every 20 sec in order to elicit I_Kv. Applications of the depolarizing pulse evoked an initial transient outward current (I_KvVpeak) with an additional sustained component (I_KvVsustained), as was previously described in mouse intestinal smooth muscles [25]. As shown in Fig. 4Aa, the amplitudes of both I_KvVpeak and I_KvVsustained reduced after the application of SKF (1 µM). The I_Kv suppressions developed progressively with time and reached a plateau about 1–2 min later (Fig. 4Ab). The suppression of the I_KvVpeak had a longer time constant (τ) (66.8 ± 23.1 sec, n=8) than the I_KvVsustained suppression did (21.1 ± 5.4 sec, n=8), although the difference was not statistically significant. The mean suppressions of I_KvVpeak and I_KvVsustained by SKF (1 µM) were significantly smaller than I_KATP suppression (Fig. 4Ac). The removal of SKF allowed for a partial recovery of I_KvVpeak and I_KvVsustained with a slow time course (Fig. 4Ab). The restored I_Kv was strongly inhibited by the application of the K⁺ channel blocker TEA (30 mM).

The cumulative application of SKF (0.03–30 µM) at increasing concentrations inhibited both I_KvVpeak and I_KvVsustained in a concentration-dependent manner. The inhibitory effects reached a maximum at 30 µM (Fig. 4Ba). However, the I_KvVsustained suppressions were significantly larger than the I_KvVpeak suppressions at 3, 10 and 30 µM (Fig. 4Bb). The mean IC_{50} value that was estimated by the curve fitting of the data from each myocyte was 3.3 ± 0.24 µM in the I_KvVpeak (n=5), which was significantly higher than the value in the I_KvVsustained (2.0 ± 0.35 µM, n=5). These IC_{50} values were significantly higher than the value in the I_KATP suppression.
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Fig. 3. Effects of SKF96365 (SKF) on carbachol (CCh)-induced mITRPC.

Cells bathed in the Cs⁺-rich external solution were held under voltage clamp at −60 mV using patch pipettes filled with the Cs⁺-rich pipette solution to block any K⁺ currents. Aa and Ab show a typical current trace when SKF (10 and 30 µM) was extracellularly applied to the cell in the presence of CCh (100 µM), respectively. B shows the mean percentages of IKATP and mITRPC suppressions. Each column indicates mean ± 1 S.E.M. The numbers of cells used are presented in parenthesis. * in (B) represents significantly smaller inhibition of mITRPC induced by SKF (10 µM) relative to the IKATP one (P<0.05). # in (B) represents significantly greater inhibition of mITRPC induced by SKF (30 µM) relative to the corresponding one induced by SKF (10 µM) (P<0.05).

IKATP was less sensitive to SKF: It is well known that Ca²⁺ releases from Ca²⁺ stores can induce IKATP responses [34, 40]. Thus, under the appropriate conditions (see methods), IKATP that was evoked by the potent Ca²⁺ releaser caffeine was recorded in the presence of SKF. As shown in Fig. 5A, the STOCs that were evoked by Ca²⁺ sparks [7] occurred with various frequencies and amplitudes, as previously reported by Sakamoto et al. [33]. When [Ca²⁺]₀ was strongly chelated, the STOCs were not observed at all (see Figs. 1 and 2). The K⁺ channel blocker TEA (5 mM) almost abolished the STOCs. In the presence of TEA, the application of CCh and the subsequent application of caffeine failed to evoke the IKATP data not shown, n=3). These findings strongly indicated that the STOCs and caffeine-induced IKATP were conducted through BK channels. SKF (1 µM) had no obvious effects on the STOCs (Fig. 5A and 5B). The change rate of the STOC amplitude was similar to the time-dependent rundown that was observed in the cells without SKF (Fig. 5C). Caffeine (10 mM) consistently evoked a brief IKATP, even in the presence of SKF (1 µM) (Fig. 5B). The mean amplitude of the IKATP that was induced by caffeine did not differ significantly from the value without SKF (Fig. 5D). The higher concentration of SKF (10 µM) reduced the STOCs, but not the caffeine-induced IKATP (Fig. 5C and 5D).

DISCUSSION

Activation of the muscarinic receptors induces the opening of cationic channels comprising TRPC4 and 6 [43]. SKF has been used as an inhibitor of the TRPC channels [9, 26, 52]. However, it has been reported to inhibit other types of ion channels, including Cav1, Cav2, Cav3 [27, 37], Nav1 [13] and inwardly or outwardly rectifying K⁺ channels [36]. In this study, we investigated the effects of SKF on the activities of the KATP, KV and BK channels that are expressed in gastrointestinal smooth muscle cells.

KATP channels have been suggested to regulate electrical excitability in gastrointestinal smooth muscles [19–21]. Cromakalim induced a sustained inward current that was abolished by glibenclamide in small intestinal smooth muscles of the mouse, as has been reported by Nuttle & Farley [31]. The I-V curve of the cromakalim-induced current was almost linear, which was consistent with the results obtained for rabbit esophageal [12] and swine tracheal [31] smooth muscle cells. The reversal potential of the current was similar to the estimated EK value, and the replacement of extracellular K⁺ with Cs⁺ abolished the current, which indicated that the charge carrier of this current was K⁺. These results indicated that the cromakalim-induced current was carried through the KATP channels. The extracellular application of SKF reversibly inhibited the cromakalim-induced IKATP in a concentration-dependent manner. The IKATP was abolished at a SKF concentration of 10 µM. Although SKF (10 µM) also inhibited the CCh-induced mITRPC, the mean percentage of suppression was only about 50% in the present study, which was consistent with the IC₅₀ (6.5–14.5 µM) that has been reported for SKF-induced mITRPC suppression [52]. The mean IC₅₀ value (0.85 µM) for the SKF-induced IKATP suppression in the present study was obviously lower than the reported IC₅₀ value for the mITRPC suppression, which suggested that SKF suppressed the KATP channels with a higher affinity than that required for inhibition of TRPC channels.

The KATP channels are composed of Kir6 and SUR subunits. Two Kir6 genes (Kir6.1 and Kir6.2) and two SUR genes (SUR1 and SUR2) have been identified [1]. The pharmacological properties of the KATP channels, including the sensitivity to its channel opener and blocker, depend on the channel subunits [16, 17, 49]. KATP channels, which have been reported in pancreatic β cells, cardiac muscles and vascular smooth muscles, consist of the Kir6.2/SUR1 [10, 28], Kir6.2/SUR2A [4] and Kir6.1/SUR2B [29, 39, 49] subunits, respectively. Nakayama et al. [30] have reported the expression of Kir6.1 and SUR2B in mouse ileal smooth muscles. Quayle et al. [32] have reported that the IC₅₀ values of glibenclamide, tolbutamide, which is a selective KATP channel inhibitor, and TEA, which is a nonselective K⁺ channel blocker, were 101 nM, 351 µM and 6.2 mM, respectively, in the smooth muscles from the rabbit mesenteric artery. Taken together with our results, the order of affinity to the KATP channels appears to be glibenclamide >> SKF >> tolbutamide >> TEA. Future investigations are needed on the sensitivity of SKF to other types of KATP channels.

Because the I-V curve of the IKATP that was obtained from
the myocytes in the presence of SKF remained linear, the inhibitory effects of SKF on $I_{KATP}$ seemed to be voltage-independent. Glibenclamide inhibits the $K_{ATP}$ by occupying sulfonylurea receptors [8]. The $I_{KATP}$ abolition that was induced by glibenclamide developed more slowly than that induced by SKF (1 $\mu$M). Each column indicates mean ± 1 S.E.M. The numbers of cells used are presented in parenthesis. B (a): time courses of the change in $I_{K_{peak}}$ and $I_{K_{sustained}}$ when SKF (0.03-30 $\mu$M) was cumulatively applied. Bb: the averaged concentration-inhibition curves of $I_{K_{peak}}$ and $I_{K_{sustained}}$. Each point in Bb indicates the mean ± S.E.M. of measurements in 3–5 cells. # in (Ac) represents significantly smaller inhibition of $I_{K_{V}}$ induced by SKF (1 $\mu$M) relative to the $I_{K_{ATP}}$ one ($P<0.05$). Asterisks in (Ac) and (Bb) represent significantly greater inhibition of the $I_{K_{sustained}}$ relative to the $I_{K_{peak}}$ ($P<0.05$).

**Fig. 4.** Effects of SKF on voltage-gated K$^+$ channel currents ($I_{K_{V}}$). Cells bathed in the Ca$^{2+}$-free PSS were held under voltage clamp at −80 mV using patch pipettes filled with the K$^+$-rich high-BAPTA pipette solution. A 2 sec step pulse to 20 mV was repeatedly applied every 20 sec in order to elicit $I_{K_{V}}$. Aa: $I_{K_{peak}}$ (●) and $I_{K_{sustained}}$ (Δ) recorded in a cell when SKF (1 $\mu$M) was extracellularly applied. Ab: time courses of the change in $I_{K_{peak}}$ and $I_{K_{sustained}}$ in the cell in a plotted against time (the beginning of depolarizing pulse applications was taken as zero) when SKF (1 $\mu$M) was extracellularly applied. Points a–c in the graph correspond with actual $I_{K_{V}}$ records (a–c) in Aa. Ac shows the mean percentages of $I_{K_{ATP}}$ and $I_{K_{V}}$ suppressions induced by SKF (1 $\mu$M). Each column indicates mean ± 1 S.E.M. The numbers of cells used are presented in parenthesis. B (a): time courses of the change in $I_{K_{peak}}$ and $I_{K_{sustained}}$ when SKF (1 $\mu$M) was extracellularly applied. Bb: the averaged concentration-inhibition curves of $I_{K_{peak}}$ and $I_{K_{sustained}}$. Each point in Bb indicates the mean ± S.E.M. of measurements in 3–5 cells. # in (Ac) represents significantly smaller inhibition of $I_{K_{V}}$ induced by SKF (1 $\mu$M) relative to the $I_{K_{ATP}}$ one ($P<0.05$). Asterisks in (Ac) and (Bb) represent significantly greater inhibition of the $I_{K_{sustained}}$ relative to the $I_{K_{peak}}$ ($P<0.05$).
IK, can make it difficult to determine the sensitivity of the KV family to SKF due to the lack of commercial blockers with high selectivity. However, the IK\textsubscript{V\text{\textsuperscript{sustained}}} suppression was greater than the IK\textsubscript{V\text{\textsuperscript{peak}}} suppression with a lower IC\textsubscript{50} value (IK\textsubscript{V\text{\textsuperscript{sustained}}} ≈ 2.2 µM vs. IK\textsubscript{V\text{\textsuperscript{peak}}} ≈ 3.3 µM). Furthermore, the time constant of the IK\textsubscript{V\text{\textsuperscript{sustained}}} suppression was shorter than the value for the IK\textsubscript{V\text{\textsuperscript{peak}}} suppression. These findings may reflect decreased sensitivity of the KV\textsubscript{4} family for SKF compared with KV\textsubscript{2}. The short time course of the SKF-induced IK\textsubscript{V\text{\textsuperscript{sustained}}} suppression may imply a direct bind of SKF to the channel, as indicated in TEA or 4-Aminopyridine-induced KV\textsubscript{channel suppressions [38, 42] as well as the IK\textsubscript{ATP} suppression.

In gastrointestinal smooth muscles, BK channels are expressed and supposed to involve in a negative feedback mechanism to prevent cytosolic Ca\textsuperscript{2+} overload induced by the muscarinic Ca\textsuperscript{2+} mobilization [46]. SKF (1 µM) had no significant effects on STOCs and caffeine-induced IBK which reflected the Ca\textsuperscript{2+} release from the internal stores in the myocytes. These results indicated that BK channel is less sensitive to SKF. Taken together, SKF can inhibit K\textsubscript{ATP} > KV > BK channels in addition to TRPC channel, and the order of affinity of SKF to those channels appears to be K\textsubscript{ATP} > KV ≈ TRPCs > BK. SKF (3–50 µM) has been reported to evoke membrane depolarization in gastrointestinal muscles (15, 51). The depolarization can be explained by the inhibitory effects of SKF on those K\textsuperscript{+} channels. The depolarization can cause to underestimate a contribution of TRPC channel to mechanical and electrical responses. Thus, the inhibitory effects of those K\textsuperscript{+} channels should be considered when SKF is used as a TRPC channel blocker in gastrointestinal smooth muscles. Characterizing the SKF-induced suppression, such as IC\textsubscript{50} and voltage-dependency, and additional tests with other TRPC blockers may be necessary to assess an involvement of TRPC channel in a response.

In conclusion, SKF can suppress K\textsubscript{ATP}, KV and BK channels. The order of affinity of SKF to those channels is K\textsubscript{ATP} > KV > BK.

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