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Verrucotoxin inhibits K_{ATP} channels in cardiac myocytes through a muscarinic M_3 receptor-PKC pathway

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Abstract

Verrucotoxin is the major component of venom from the stonefish (*Synanceia verrucosa)*. Stings from the dorsal spines of the stonefish produce intensive pain, convulsions, hypotension, paralysis, respiratory weakness and collapse of the cardiovascular system, occasionally leading to death. It has been reported that verrucotoxin might modulate ATP-sensitive K^+ (K_{ATP}) current in frog atrial fibers. However, the mechanism by which verrucotoxin acts on K_{ATP} current remains unclear. In this study, we examined whether verrucotoxin inhibited K_{ATP} current in guinea pig ventricular myocytes, using the patch clamp method. Verrucotoxin suppressed K_{ATP} current induced by pinacidil (K_{ATP} channel opener) in a concentration-dependent manner, with a half maximum concentration of 16.3 μg/ml. The effect of verrucotoxin on K_{ATP} current was suppressed by atropine (1 μM), a muscarinic receptor antagonist, or by 4-diphenylacetoxy-*N*-methylpiperidine (100 nM), a muscarinic M_3 receptor antagonist. Furthermore, the effect of verrucotoxin on K_{ATP} current was attenuated by the protein kinase C (PKC) inhibitor chelerythrine (10 μM) and calphostin C (10 μM), yet not by the cAMP-dependent protein kinase (PKA) inhibitor H-89 (0.5 μM). These results suggest that verrucotoxin inhibits K_{ATP} current through the muscarinic M_3 receptor-PKC pathway. These findings enhance our understanding of the toxic effects of verrucotoxin from the stonefish.

Keywords: K_{ATP}; Muscarinic M_3 receptor; PKC; Verrucotoxin

1. Introduction

ATP-sensitive K^+ (K_{ATP}) channels have been identified in a number of tissues and cells, including guinea pig ventricular myocytes (Noma, 1983; Munemori et al., 1996; Paajanen and Vornanen, 2002). Activation of K_{ATP} channels hyperpolarizes the membrane potential when ATP level decreases in cells (ATP IC_{50} 50–100 μM). Although most of the channels are closed in the physiological concentration range of ATP, it has been reported that K_{ATP} channels have a key role in physiological, as well as in cardiovascular pathophysiological conditions (Ashcroft and Gribble, 1999; Zingman et al., 2002; Seino and Miki, 2003). In such conditions, the activation of K_{ATP} channels protects the myocardium from Ca^{2+} overloading and mediates preconditioning by regulating properties of membrane potential (Jovanović et al., 1998; Jovanović and Jovanović, 2001; Budas et al., 2004; Du et al., 2006). The K_{ATP} channels also regulate insulin secretion in pancreatic β-cells (Miki et al., 1998).

K_{ATP} channels have been found to be modulated by ATP, pH, fatty acids, NO, SH-redox state, various nucleotides, G-proteins, diadenosine polyphosphates, lactate, 1,3-bisphosphoglycerate and various ligands (Kim and Clapham, 1989; Kirsch et al., 1990; Edwards and Weston, 1993; Coetzee et al., 1995; Jovanović et al., 1997; Crawford et al., 2002; Deka and Brading, 2004; Jovanović et al., 2005; Jovanović and Jovanović, 2005). Among these ligands, pinacidil, a selective activator of K_{ATP} channels in the heart with an approximate half-maximal concentration of 30 μM (Arena and Kass, 1989; Fan et al., 1990; Coetzee et al., 1995; Fujita et al., 2001), was used as a tool to investigate the blockage by compounds such as glimepiride (Lawrence et al.,

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2002). It has been reported that the regulation of K_{ATP} current involves activation of receptors including α_1 adrenoceptors (Bonev and Nelson, 1996), angiotensin II AT_1 receptors (Hayabuchi et al., 2001), adenosine receptors (Hu et al., 2003) and muscarinic receptors (Bonev and Nelson, 1993). These regulations are mediated by protein kinases such as PKA, PKC, tyrosine protein kinases, AMP-activated kinases and mitogen-activated protein kinases (Hatakeyama et al., 1995; Maulik et al., 1996; Stadnicka et al., 2002; Sukhodub et al., 2007).

Verrucotoxin is the major component of venom from the stonefish (*Synanceia verrucosa)*. Stings from the dorsal spines of the stonefish produce intensive pain, convulsions, hypotension, paralysis, respiratory weakness and collapse of the cardiovascular system, occasionally leading to death. On electrocardiograms, verrucotoxin produces an inverted T wave and marked displacement of the ST segment, suggesting that the toxin modulates ion channel activity (Saunders, 1959). It has been reported that verrucotoxin may modulate ATP-sensitive K^+ (K_{ATP}) current in frog atrial fibers (Garnier et al., 1997). However, the mechanism by which verrucotoxin acts on K_{ATP} current remains unclear. To address this question, we investigated the mechanism underlying the effect of verrucotoxin on K_{ATP} current in guinea pig single ventricular myocytes, by using the whole-cell patch clamp method.

2. Materials and methods

This study was conducted in accordance with the guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996), and permission for the study was granted by the Committee on Animal Experimentation, Kagoshima University, Japan.

2.1. Cell preparation

Single ventricular cells were obtained from adult guinea pig hearts by collagenase and protease dissociation, as described previously (Yazawa et al., 1990). In brief, an adult female guinea pig (weight 300–600 g) was anesthetized with 5% pentobarbital sodium solution (1.4 ml/kg, i.p.). Under artificial respiration, the chest was opened and the aorta was cannulated to perfuse the coronary artery (on a Langendroff apparatus). The isolated heart was first perfused with Tyrode solution at 37 °C for about 3 min, then with nominally Ca^{2+}-free Tyrode solution for 6 min, and finally with Ca^{2+}-free Tyrode solution containing collagenase (0.08 mg/ml; Yakult, Tokyo, Japan) for 10–15 min. The heart was then washed out with a high-K^+ and low-Ca^{2+} solution (storage solution), and the ventricular myocytes were dispersed and filtered through a 105 μm stainless steel mesh. In order to readily form a GΩ seal with a patch clamp pipette, the myocytes were incubated for 4–10 min with the storage solution containing both protease (NK-103, 0.04 mg/ml, Nagase, Osaka, Japan) and DNAase I (type IV, 0.02 mg/ml, Sigma, Louis, USA) at 37 °C. The cells were washed twice by centrifugation at 800 rpm for 5 min and stored at 4 °C in the storage solution.

2.2. Preparation of verrucotoxin

Stonefishes (*S. verrucosa*, 2.5–3.2 kg in weight) obtained from the sea surrounding Okinawa Islands, Japan, were anesthetized with 0.04% phenoxyethanol in seawater, and the dorsal spines were dissected with their bases. After removing the integument sheath and tissue residing in the grooves of the spines, crude venom was extracted with 150 mM NaCl. After centrifugation at 3000 ×*g* for 20 min at 4 °C, the resulting supernatant was immediately lyophilized and stored at −80 °C. The yield of crude verrucotoxin was 18–24 μg/kg fish. The freeze-dried powder was dissolved in Tyrode solution before use.

2.3. Solutions and chemicals

Tyrode solution contained (mM): NaCl, 135; KCl, 5.4; NaH_2PO_4, 0.33; $MgCl_2$, 1.0; glucose, 5.5; $CaCl_2$, 1.8; HEPES 10; adjusted to pH 7.4 with NaOH. The storage solution was composed of (mM): KOH, 70; glutamic acid, 50; KCl, 40; KH_2PO_4, 20; taurine, 20; $MgCl_2$, 3; glucose, 10; HEPES, 10; EGTA, 0.5; adjusted to pH 7.2 with KOH. The pipette solution contained (mM): KOH, 120; glutamic acid, 120; KCl, 25; $MgCl_2$, 1; EGTA, 5; HEPES, 10; MgATP, 0.1; adjusted to pH 7.2 with KOH. The extracellular solution was Tyrode solution. Nisoldipine was purchased from Wako (Tokyo, Japan). Pinacidil, MgATP, *N*-[2-(*p*-bromocinnamylamino) ethyl]-5-isoquinolinesulfonamide dihydrochloride (H-89), staurosporine, chelerythrine chloride, prazosin, 8-(*p*-sulfophenyl) theophylline (8-PT), atropine, methoctramine, 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP) and carbachol were purchased from Sigma (Louis, USA). Nisoldipine, pinacidil, chelerythrine and prazosin were dissolved in dimethylsulphoxide (DMSO) solution. The final concentration of DMSO was <0.3%.

2.4. Data recording and analysis

The K_{ATP} current was recorded from single guinea pig ventricular myocytes by the whole-cell patch clamp method using a patch clamp amplifier (Nihon Kohden, Tokyo, Japan). Patch pipettes were made from thin-walled borosilicate glass with a resistance of 1–3 MΩ. The Ca^{2+} current was eliminated with 3 μM nisoldipine and Ca^{2+}-activated currents were minimized by EGTA in the pipette solution. Experiments were done at 20–25 °C. Current was sampled at 2 kHz and was filtered at 1 kHz. The capacitance and leakage current in the current traces were digitally subtracted. Data were analyzed off-line using Microsoft Excel and Origin. The percentage inhibition of pinacidil-induced K_{ATP} current by verrucotoxin was calculated according to the following equation:

$$\text{Percentage inhibition } (\%) = \left(I_{\text{pinacidil}} - I_{\text{pinacidil+verrucotoxin}}\right) / \left(I_{\text{pinacidil}} - I_{\text{bas}}\right) \times 100,$$

where $I_{\text{pinacidil}}$, $I_{\text{pinacidil + verrucotoxin}}$ and I_{bas}, were the currents recorded with pinacidil, pinacidil + verrucotoxin, and the basal (background) current, respectively. The amplitude of the

currents was calculated from the values of K_{ATP} current at the end of five consecutive pulses. Data are presented as means± S.E.M. Statistical significance was evaluated by Student's *t* test for unpaired values, and $P<0.05$ was considered to be significant.

3. Results

3.1. Effect of verrucotoxin on K_{ATP} current

To investigate whether verrucotoxin modulated K_{ATP} current in guinea pig ventricular myocytes, we examined the effects of verrucotoxin using the patch clamp method. Fig. 1A illustrates the typical time course of K_{ATP} current during the application of pinacidil and verrucotoxin, and examples of current traces (inset). About 1 min after application of pinacidil (30 μM), K_{ATP} current began to increase from 0.14 nA and reached 2.14 nA within 5 min. Subsequent application of verrucotoxin (10 μg/ml) decreased K_{ATP} current to 1.30 nA, indicating 42.0% inhibition of the pinacidil-activated K_{ATP} current. This reduction in the current was significantly different from that obtained without verrucotoxin treatment (2.8±2.3% reduction, $n=5$). On washing out verrucotoxin, K_{ATP} current recovered partially (data not shown). To examine the voltage-dependence of the verrucotoxin effect on K_{ATP} current, the current was elicited at a membrane potential ranging from −140 to 60 mV. Fig. 1B (a–c) shows superimposed current traces in response to various test potentials, under the indicated treatment conditions. The current–voltage relationships illustrated in Fig. 1B (d) showed that the inhibition by verrucotoxin of the pinacidil-induced K_{ATP} current was evident at positive potentials down to −80 mV. The percentage inhibition of K_{ATP} current by verrucotoxin was almost constant (37%, $n=4$) at potentials from −40 to 60 mV. This result suggests that the verrucotoxin effect on K_{ATP} current is voltage-independent.

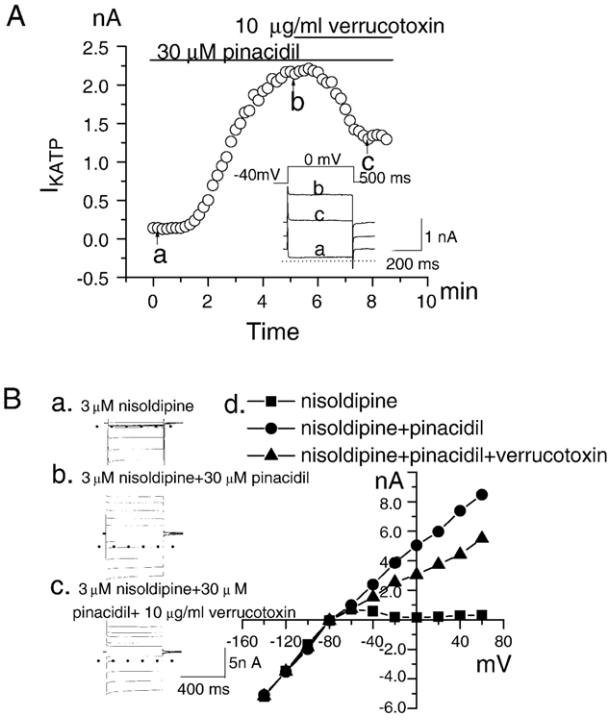

Fig. 1. Effects of verrucotoxin on pinacidil-activated KATP current in guinea pig ventricular myocytes. (A) Time course of the effect of verrucotoxin on pinacidil-activated K_{ATP} current in the presence of nisoldipine (3 μM). Holding potential was −40 mV and the test potential was 0 mV. Examples of the current traces recorded at times indicated in the graph (a–c) are shown in the inset below. Dashed line is zero current level. (B) Current–voltage relationships for the effect of verrucotoxin on K_{ATP} current in the presence of nisoldipine (3 μM) (■), nisoldipine (3 μM)+pinacidil (30 μM) (●), nisoldipine (3 μM)+pinacidil (30 μM)+verrucotoxin (10 μg/ml) (▲). Current traces for each are shown in a–c. Holding potential was −40 mV and the membrane currents were elicited at variable test potentials.

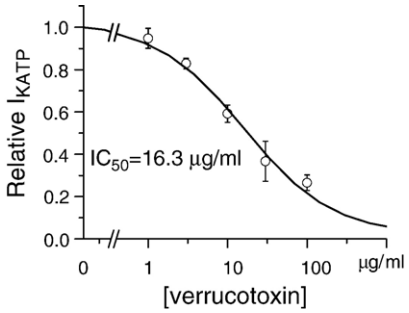

Fig. 2. Concentration–response curve for verrucotoxin-induced inhibition of K_{ATP} current in guinea pig ventricular myocytes. Currents were elicited by pulses of 500 ms duration to 0 mV from a holding potential of −40 mV. Currents were recorded at the end of the pulses. Open circles show the mean value, and standard errors are shown by bars. The continuous curve gives an IC_{50} of 16.3 μg/ml.

The concentration–response relationship between verrucotoxin and K_{ATP} current is summarized in Fig. 2. Verrucotoxin inhibited K_{ATP} current in a concentration-dependent manner in the range 1–100 μg/ml with an IC_{50} of 16.3 μg/ml and a Hill coefficient of 1.73 ($n=8$). These results suggest that verrucotoxin is a potent inhibitor of cardiac K_{ATP} current.

3.2. Verrucotoxin acts through a muscarinic M_3 receptor

To investigate the possible involvement of receptors in the inhibition of K_{ATP} current by verrucotoxin, effects of antagonists for several types of receptors on the action of verrucotoxin were tested.

In the presence of prazosin (α_1 adrenoceptor antagonist, 10 μM) or 8-PT (adenosine receptor antagonist, 10 μM), the percentage inhibition of K_{ATP} current produced by verrucotoxin (10 μg/ml) was 40.0±8.7% ($n=6$) or 38.9±6.7% ($n=4$), respectively (Fig. 3C). These values were not significantly different from that obtained without these drugs, suggesting that verrucotoxin-induced inhibition of K_{ATP} current is not mediated by activation of the α_1 adrenoceptor or the adenosine receptor.

In the presence of atropine (a muscarinic antagonist, 1 μM), pinacidil-activated K_{ATP} currents from 0.17 nA to 9.97 nA. Subsequent application of verrucotoxin (10 μg/ml) decreased the current to 8.50 nA, indicating 14.9% inhibition of the pinacidil-activated K_{ATP} current (Fig. 3A). The reduction by atropine of verrucotoxin-induced inhibition on K_{ATP} current was observed in three further experiments (Fig.

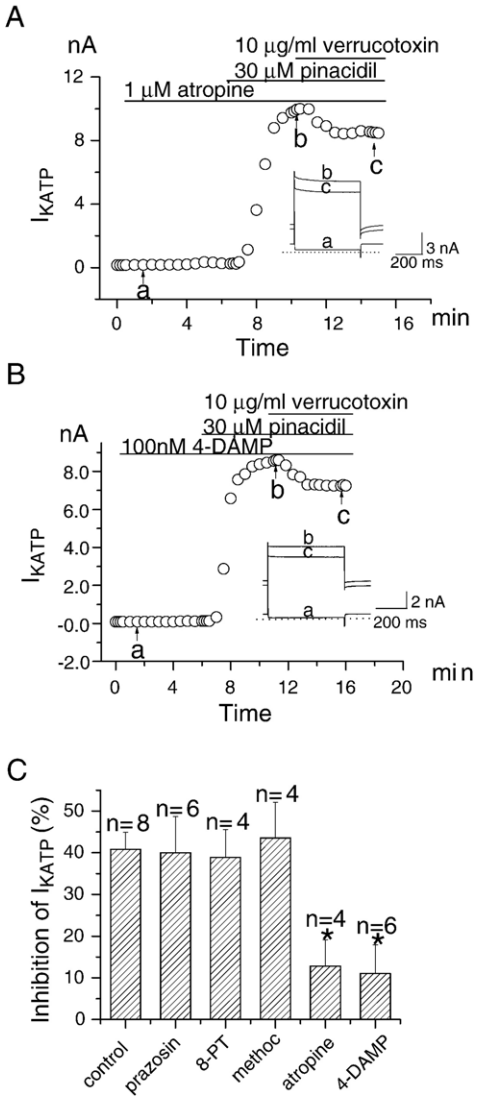

Fig. 3. Effect of receptor-mediated modulation by verrucotoxin on K_{ATP} current. Time course of the effect of verrucotoxin on pinacidil-activated K_{ATP} current in the presence of 1 μM atropine (A) and 100 nM 4-DAMP (B). K_{ATP} current amplitude was measured at the end of 500 ms test pulses at 0 mV from the holding potential of −40 mV. Inset shows example current traces at the times indicated on the graph (a–c). Dashed lines indicate zero current level. (C) Comparison of receptor-mediated effect of verrucotoxin on K_{ATP} channels in the presence of antagonists for α1 adrenoceptors (prazosin, 10 μM), adenosine receptors (8-PT, 10 μM), muscarinic receptors (atropine, 1 μM), muscarinic M_2 receptors (methoctramine; methoc, 5 μM) and muscarinic M_3 receptors (4-DAMP, 100 nM). The bars indicate means±S.E.M. Number of measurements at each inhibitor is indicated above the bars. Significant differences were evaluated using the Student's unpaired *t* test, and differences with $P<0.05$ are designated by *.

3C). This result suggested that verrucotoxin-induced inhibition of K_{ATP} current involves muscarinic receptors. Consistently, the muscarinic receptor agonist carbachol (10 μM) reduced the verrucotoxin-induced inhibition of K_{ATP} currents by 80.5% (data not shown).

To identify the subtype of muscarinic receptor for the effect of verrucotoxin on K_{ATP} currents, the selective muscarinic M_2 receptor antagonist methoctramine and muscarinic M_3 receptor antagonist 4-DAMP were tested. In the presence of methoctramine (5 μM), verrucotoxin (10 μg/ml) inhibited K_{ATP} current by 43.6±8.6% ($n=6$, Fig. 3C), which was not significantly different from that observed without the drug. On the other hand, 4-DAMP (100 nM) had a different effect. As shown in Fig 3B, application of verrucotoxin (10 μg/ml) in the presence of 4-DAMP, produced only 15.2% inhibition of the pinacidil-activated K_{ATP} current. The mean value of the inhibition with five other experiments was 11.0±6.9% in the presence of 4-DAMP, which was significantly lower than that obtained without 4-DAMP ($n=6$, Fig. 3C). In addition, no significant difference was observed between the effect of atropine and that of 4-DAMP on the action of verrucotoxin. Taking these results together, it is suggested that verrucotoxin inhibits K_{ATP} current via stimulation of the muscarinic M_3 but not M_2 receptor.

3.3. Verrucotoxin-induced inhibition of K_{ATP} current involves classical PKC and/or novel PKC

To investigate the signaling pathway involved in the verrucotoxin-mediated inhibition of K_{ATP} current, we examined the effects of a PKA inhibitor H-89 or different PKC inhibitors staurosporine, chelerythrine and calphostin C on the inhibitory action of verrucotoxin on K_{ATP} currents. In the presence of staurosporine (0.6 μM), verrucotoxin (10 μg/ml) inhibited K_{ATP} currents by only 10.9% (Fig. 4A). The mean inhibition with four other experiments was 13.1±1.3% ($n=5$, Fig. 4E), a significantly smaller value than that obtained without staurosporine. Staurosporine is a nonselective PKC inhibitor with an IC_{50} of 30 nM, and it blocks not only PKC, but also other kinases, including PKA, at high concentrations (Herbert et al., 1990). Thus, the result suggests that the verrucotoxin-induced inhibition of K_{ATP} current involved activation of PKC and/or other kinases.

H-89 is a selective inhibitor of PKA, with an IC_{50} of 50 nM (Chijiwa et al., 1990). In the presence of H-89 (500 nM), verrucotoxin (10 μg/ml) inhibited K_{ATP} current by 40.3% (Fig. 4B). This value was not statistically significant compared with the control suppression, indicating that the verrucotoxin-induced inhibition of K_{ATP} current was not mediated by the activation of PKA.

The above results suggest that verrucotoxin-induced inhibition of K_{ATP} current might involve activation of PKC. We examined which subtype of PKC was involved in the verrucotoxin-induced inhibition of K_{ATP} current, by using subtype-selective PKC inhibitors. The benzophenanthridine alkaloid chelerythrine is an inhibitor of cPKC and δ-type nPKC, with an IC_{50} of 0.66 μM (Herbert et al., 1990). In the presence of chelerythrine (10 μM), verrucotoxin (10 μg/ml) inhibited K_{ATP} current by 19.7±3.9% ($n=11$, Fig. 4C and E), a smaller extent than the control value of 41%. This result suggests that verrucotoxin-induced inhibition of K_{ATP} current involves the activation of a chelerythrine-sensitive subtype of PKC.

Calphostin C is another inhibitor that inhibits cPKC and most species of nPKC, with an IC_{50} of 0.05 μM (Kobayashi et al., 1989; Hofmann, 1997). In the presence of calphostin C (10 μM), verrucotoxin (10 μg/ml) inhibited K_{ATP} current by 12.0±3.9% ($n=4$, Fig. 4D and E), a smaller value than

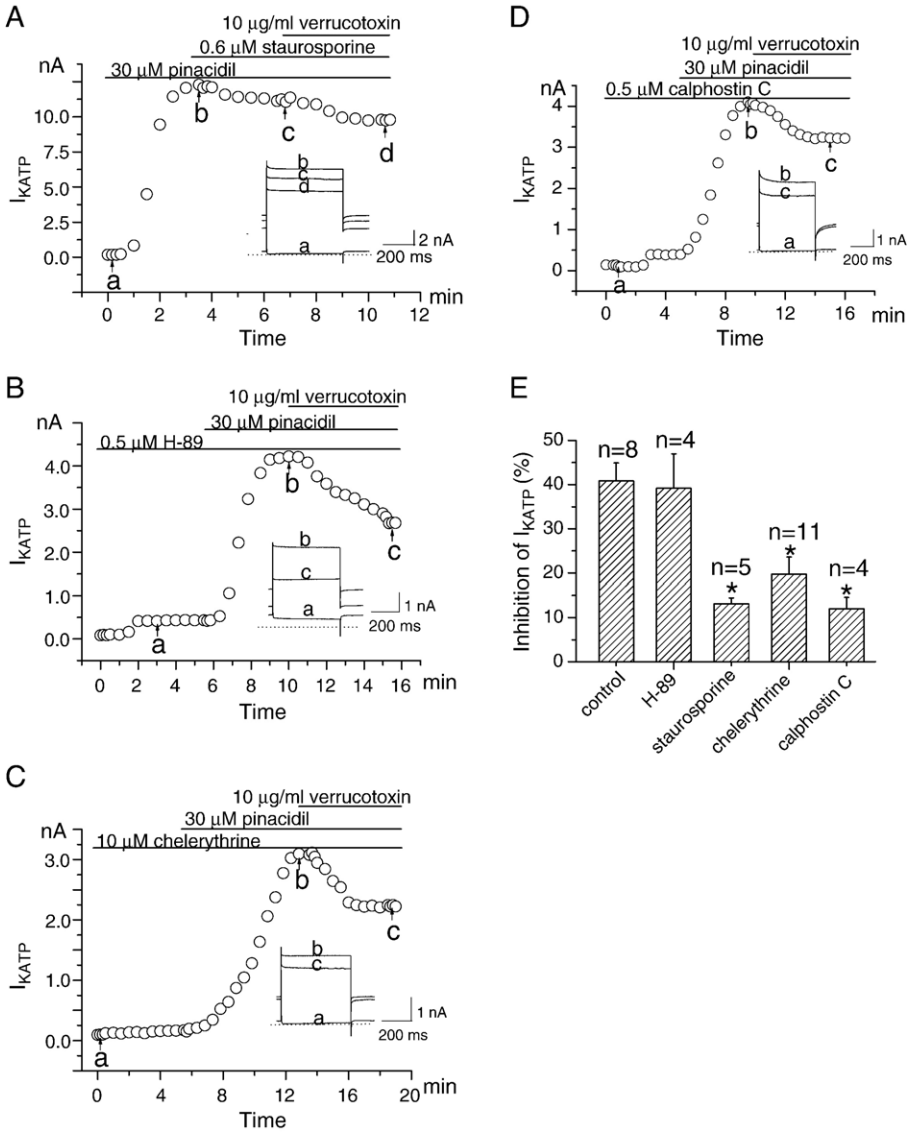

Fig. 4. Effect of kinase-mediated modulation of K_{ATP} current by verrucotoxin. Time course of the effect of verrucotoxin on pinacidil-activated K_{ATP} current in the presence of 0.6 μM staurosporine (A), 0.5 μM H-89 (B), 10 μM chelerythrine (C), and 10 μM calphostin C (D). Test potential was 0 mV and holding potential was −40 mV. Insets show example current traces at the times indicated on the graph (A–D). Dashed lines indicate zero current level. (E) Comparison of receptor-mediated effect of verrucotoxin on K_{ATP} channels in the presence of antagonists staurosporine (0.6 μM), H-89 (0.5 μM), chelerythrine (10 μM) and calphostin C (10 μM). Bars indicate mean values±S.E.M. Number of measurements for each inhibitor is indicated above the bars. Significant differences were evaluated using the Student's unpaired *t* test, and differences with $P<0.05$ are designated by * (compared with control).

the control (Fig. 4E, $P<0.05$). These results suggest that verrucotoxin-induced inhibition of K_{ATP} current involves the activation of cPKC and/or nPKC.

4. Discussion

In this study, we investigated the effects of verrucotoxin on K_{ATP} current, and the underlying mechanisms in guinea pig cardiac ventricular myocytes. Our data suggest that verrucotoxin inhibits K_{ATP} current: (1) in a voltage-independent manner and a concentration-dependent manner with an IC_{50} of 16.3 μg/ml; and (2) through activation of the muscarinic M_3 receptor-PKC pathway.

A number of studies suggest that the activity of K_{ATP} channels is regulated by receptor-mediated modulation such as α_1 adrenoceptors (Bonev and Nelson, 1996; Haruna et al., 2002), angiotensin II AT_1 receptors (Hayabuchi et al., 2001; Thorneloe et al., 2002), and adenosine receptors (Hu et al., 2003). Results of this study support the notion that verrucotoxin-induced inhibition of K_{ATP} current is not mediated by activation of the α_1 adrenoceptor or the adenosine receptor (Fig. 3C). Although the muscarinic M_2 receptor is the major subtype expressed in the heart, it has been reported that the M_3 subtype is also expressed in the mammalian heart (van Zwieten and Doods, 1995; Shi et al., 1999; Wang et al., 1999, 2004). It has been reported that K_{ATP} current can be inhibited by

stimulation of muscarinic receptors in urinary bladder smooth muscle, and muscarinic M_3 receptors in rabbit esophageal smooth muscle (Bonev and Nelson, 1993; Hatakeyama et al., 1995). Results of this study support the notion that a similar signaling pathway is present for the cardiac K_{ATP} channel, and that verrucotoxin acts on this pathway. Not only atropine, a broad muscarinic receptor antagonist, but also 4-DAMP, a specific muscarinic M_3 receptor antagonist, markedly suppressed the effect of verrucotoxin on K_{ATP} current (Fig. 3). Therefore, it is strongly suggested that verrucotoxin inhibits K_{ATP} current through stimulation of the muscarinic M_3 receptor.

It has been reported that K_{ATP} currents are subject to dual modulation by PKC and PKA (Bonev and Nelson, 1996; Hayabuchi et al., 2001). It has also been reported that other protein kinases such as tyrosine protein kinase, mitogen-activated protein kinases and AMP-activated kinases are involved in the modulation of K_{ATP} channels (Hatakeyama et al., 1995; Maulik et al., 1996; Stadnicka et al., 2002; Sukhodub et al., 2007). Among these kinases, PKC has been reported to inhibit K_{ATP} channels in kidney cells (Wang and Giebisch, 1991), insulin-secreting cells (Wollheim et al., 1988), urinary bladder smooth muscle (Bonev and Nelson, 1993), and cardiac myocytes (Hu et al., 2003). Isozymes of PKC are classified into three subtypes: (1) classical PKCs (cPKC), which require diacylglycerol, phosphatidylserine and Ca^{2+} for their activation; (2) novel PKCs (nPKC), whose activation depends on diacylglycerol, but does not require Ca^{2+}; and (3) atypical PKCs (aPKC), whose regulation is not fully understood, but is probably independent of Ca^{2+} and diacylglycerol. In this study, the inhibitory action of verrucotoxin on K_{ATP} current was strongly attenuated by chelerythrine, an inhibitor of cPKC and δ-type nPKC, and calphostin C, an inhibitor of cPKC and most species of nPKC. Considering that nPKC (δ) are abundant, and cPKC (α and β) and aPKC (ζ) are not abundant in the heart (Wetsel et al., 1992), it is suggested that cPKC and/or δ-type nPKC play a major role in the inhibition of K_{ATP} channel by verrucotoxin in cardiac myocytes. However, the possibility of a small contribution from other types of PKC is not excluded by the results of this study. Since atropine and staurosporine failed to abolish the effect of verrucotoxin on K_{ATP} current, it is also possible that other signaling pathways (e.g. angiotensin II AT1 receptors) might be involved in the action of verrucotoxin, though the extent may be less than that mediated by muscarinic receptors.

It is reported that verrucotoxin also stimulates β-adrenoceptors and increases Ca^{2+} current in frog atrial myocytes (Sauviat et al., 1995). A similar action of verrucotoxin as a β-adrenoceptor agonist has recently been confirmed in guinea pig ventricular myocytes (Yazawa et al., 2004). The present study suggests that verrucotoxin may also act as a muscarinic receptor agonist. If so, it should be emphasized that verrucotoxin can stimulate both β-adrenoceptors and muscarinic receptors. Verrucotoxin is a glycoprotein with a molecular weight of 32 kDa comprising two subunits. Although the amino acid sequence of the β subunit has been reported (Ueda et al., 2006), the structure of the α subunit is still unknown. It is interesting to speculate that different region of verrucotoxin might bind to and stimulate different receptors. Alternatively, verrucotoxin might be composed of isoforms, each of which stimulates different types of receptor. Further study is required to investigate the structure and function of verrucotoxin.

In frog atrial fibers, it has been reported that verrucotoxin shortens the duration of the plateau and the repolarizing phases of action potentials. Glibenclamide, a K_{ATP} channel inhibitor, reverses the verrucotoxin-induced shortening of the action potential repolarizing phase. These results suggest that verrucotoxin might activate K_{ATP} channels in frog atrial heart muscle (Garnier et al., 1997). Although a reason for the discrepancy of the verrucotoxin effect between guinea pig and frog heart is not clear, there might be different regulatory mechanisms of K_{ATP} channels in these species.

K_{ATP} channels are closed at the physiological condition. When the concentration of ATP decreases and Ca^{2+} concentration increases by metabolic stress such as hypoxia, K_{ATP} channels are activated in cardiac myocytes (Knopp et al., 1999; Quayle et al., 2006). The activation of K_{ATP} channels protects the myocardium from Ca^{2+} overloading and mediates preconditioning (Jovanović and Jovanović, 2001; Budas et al., 2004). Verrucotoxin can induce myocardial hypoxia and arrhythmia perhaps due to a respiratory disorder (Saunders, 1959; Breton et al., 2002). In addition, the toxin activates arrhythmogenic Ca^{2+} currents and prevents the activation of cardioprotective K_{ATP} currents, and thereby promotes a collapse of cardiovascular system.

In summary, to our knowledge, this study has shown for the first time that verrucotoxin inhibits K_{ATP} channels, in a concentration-dependent manner with an IC_{50} of 16.3 μg/ml, in guinea pig ventricular myocytes. Verrucotoxin seems to stimulate the muscarinic M_3 receptor, activate PKC and presumably phosphorylate the channel protein.

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