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Effects of Nicorandil on the cAMP-Dependent Cl^- Current in Guinea-Pig Ventricular Cells

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Abstract. In guinea-pig cardiomyocytes, a cAMP-dependent Cl^- current ($I_{Cl,cAMP}$) flows through a cardiac isoform of the cystic fibrosis transmembrane conductance regulator (CFTR), which belongs to a family of the ATP-binding cassette (ABC) proteins. Although several K^+-channel openers and sulfonylurea ATP-sensitive K^+ (K_{ATP})–channel blockers reportedly inhibit $I_{Cl,cAMP}$, effects of nicorandil on the Cl^- current have not been evaluated. This study was conducted to examine the effects of nicorandil on $I_{Cl,cAMP}$ in isolated guinea-pig ventricular cells using patch clamp techniques. Nicorandil in concentrations higher than 300 μM enhanced the $I_{Cl,cAMP}$ preactivated by 0.1 μM isoproterenol. The isoproterenol-induced $I_{Cl,cAMP}$ was inhibited by 100 μM glibenclamide, but not by 100 μM pinacidil. SNAP (*S*-nitroso-*N*-acetyl-D,L-penicillamine, 10 μM), a nitric oxide (NO) donor, similarly enhanced the isoproterenol-induced $I_{Cl,cAMP}$. However, SG-86, a denitrated metabolite possessing K^+ channel–opening action, failed to enhance the Cl^- current. When the $I_{Cl,cAMP}$ was activated by 3-isobutyl-1-methylxanthine (IBMX, 30 μM), either nicorandil or SNAP failed to enhance the isoproterenol-induced $I_{Cl,cAMP}$. Thus, nicorandil enhances $I_{Cl,cAMP}$ in guinea-pig cardiomyocytes through an increase in intracellular cGMP, although direct modulation of $I_{Cl,cAMP}$ by NO cannot be completely excluded.

Keywords: cAMP-dependent Cl^- current, nicorandil, heart, nitric oxide (NO), cGMP

Introduction

It has been acknowledged that there are at least three types of Cl^- currents in cardiac cells: these include a cAMP- and protein kinase A (PKA)–dependent Cl^- current ($I_{Cl,cAMP}$, $I_{Cl,PKA}$, or $I_{Cl,CFTR}$), a calcium-activated Cl^- current ($I_{Cl,Ca}$), and a swelling-induced Cl^- current ($I_{Cl,swell}$) (1 – 3). They may modulate the electrical activity under physiological and pathophysiological conditions. Although the molecular identity of the Cl^- channels through which $I_{Cl,Ca}$ or $I_{Cl,swell}$ flows has not been established, $I_{Cl,cAMP}$ is known to be carried by a cardiac isoform of the cystic fibrosis transmembrane conductance regulator (CFTR) (4, 5). CFTR is the protein that is defective in patients with cystic fibrosis and belongs to a family of the ATP-binding cassette (ABC) proteins that have two nucleotide-binding domains and hydrolyze ATP. The ABC family includes numerous proteins such as P-glycoprotein and receptors for sulfonylurea (SUR) drugs that are clinically used for diabetes mellitus (6). Since ATP-sensitive K^+ (K_{ATP}) channels are composed of the sulfonylurea receptor (SUR1, SUR2) and the inward rectifying K^+-channel subfamily Kir6.0 (Kir6.2 or Kir6.1) (7, 8), a modulator for K_{ATP} channel may affect $I_{Cl,cAMP}$. Indeed, it was reported that potassium-channel blockers, tolbutamide and glibenclamide, inhibit cAMP-induced Cl^- current in NIH 3T3 fibroblast cells expressing CFTR (9). It is also known in cardiac cells that glibenclamide inhibits $I_{Cl,cAMP}$ in a concentration-dependent manner (10, 11). In terms of K^+-channel openers that activate K_{ATP} current, lemakalim, minoxidil, and diazoxide inhibit cAMP-induced Cl^- current in NIH 3T3 fibroblast cells expressing CFTR (9).

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Nicorandil is a clinically-available hybrid vasodilator with dual mechanism of action, one is as a K^+-channel opener and the other is a nitrate (12). It may be of interest to examine the effect of nicorandil on cardiac $I_{Cl,cAMP}$. Unexpectedly, nicorandil potentiated rather than inhibited $I_{Cl,cAMP}$ in isolated guinea-pig ventricular myocytes. We also examined synergistic effects of nitric oxide (NO) donor and SG-86, a main denitrated metabolite of nicorandil (13), in comparison with the prototype K^+-channel opener pinacidil. These data suggest that nicorandil augments cardiac $I_{Cl,cAMP}$ through increment of cGMP levels.

Materials and Methods

Cell preparations

All experiments were performed under the regulations of the Animal Research Committee of the Graduate School of Medicine, Chiba University. Single ventricular cells of the guinea-pig heart were isolated by an enzymatic dissociation method as previously described (14, 15). Guinea pigs weighing 250 – 400 g were anesthetized with pentobarbital sodium. Hearts were mounted on a Langendorff apparatus immediately after excision followed by cannulation distal to the aortic valve and retrogradely perfused at a constant pressure of 80 mmHg with heated (37°C), oxygenated buffer. The heart was perfused sequentially with the following buffers: 1) normal HEPES-Tyrode solution for 10 min, 2) nominally Ca^{2+}-free Tyrode solution for 10 min, and 3) Ca^{2+}-free Tyrode solution containing 0.1 – 0.2 mg/ml collagenase (Wako, Osaka) for 20 – 30 min. After digestion, the heart was perfused with a high-K^+ low-Cl^- solution [modified Kraft-Brühe (KB) solution]. Ventricular tissue was minced in the modified KB solution, and the pieces were gently agitated to dissociate cells. The cell suspension was stored at 4°C until use.

Solutions

The compositions of the buffers used in the experiments are as follows: 1) Normal HEPES-Tyrode solution: 143 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.33 mM NaH_2PO_4, 5.5 mM glucose, and 5 mM HEPES-NaOH (pH 7.4); 2) KB solution: 70 mM KOH, 50 mM L-glutamic acid, 40 mM KCl, 20 mM taurine, 20 mM KH_2PO_4, 3 mM $MgCl_2$, 10 mM glucose, 1 mM EGTA, and 10 mM HEPES-KOH (pH 7.4); 3) external solution for the measurement of $I_{Cl,cAMP}$: 130 mM NaCl, 5.4 mM CsCl, 1.0 mM $MgCl_2$, 2.0 mM $CoCl_2$, 1.5 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES-NaOH (pH 7.4); 4) pipette solution: 120 mM CsCl, 20 mM tetraethylammonium Cl, 5 mM ATP Mg, 5 mM EGTA, 0.2 mM GTP Na, and 5 mM HEPES-CsOH (pH 7.4). The external and internal solutions contain approximately equimolar Cl^-. To eliminate K^+ currents, external K^+ was replaced with Cs^+ and internal K^+ was replaced with Cs^+ and tetraethylammonium Cl.

Whole-cell current recording

Whole-cell membrane currents were recorded by the patch-clamp method, as previously described (14 – 16). Single ventricular cells were placed in a recording chamber (1-ml volume) attached to an inverted microscope (IMT-2; Olympus, Tokyo) and superfused with the HEPES-Tyrode solution at a rate of 3 ml/min. The temperature of the external solution was kept within 36.0 ± 1.0°C. Patch pipettes were made from glass capillaries with a diameter of 1.5 mm using a vertical microelectrode puller (PB-7; Narishige, Tokyo). They were filled with an internal solution, and their resistance was 2 – 4 megohm. After the gigaohm seal between the tip and cell membrane was established, the membrane patch was disrupted by higher negative pressure to make the whole-cell voltage-clamp mode. The electrode was connected to a patch-clamp amplifier (CEZ-2300; Nihon Kohden, Tokyo). Signals were recorded through an 1-kHz-bandwidth filter, and series resistance was compensated by 40% – 70%. Command pulse signals were generated by a 12-bit digital-to-analogue converter controlled by pCLAMP software (Axon Instrument, Inc., Foster City, CA, USA). Current signals were digitized at a rate of 2 kHz and recorded.

Membrane currents were recorded by delivering 500-ms hyperpolarizing and depolarizing pulses from a holding potential of 0 mV at a rate of 0.1 Hz. This holding potential excludes Na^+ and Ca^{2+} currents. To obtain the I-V relation, voltage pulses to potentials ranging from −80 to +80 mV were delivered, and drug effects on the membrane current were examined.

Chemicals

Drugs used in this study were as follows: nicorandil [*N*-(2-hydroxyethyl) nicotinamide nitrate] and SG-86 [*N*-(2-hydroxyethyl) nicotinamide] (Chugai Pharmaceutical Co., Tokyo); pinacidil, (−)-isoproterenol hydrochloride, 3-isobutyl-1-methylxanthine (IBMX), and SNAP (*S*-nitroso-*N*-acetyl-D,L-penicillamine), anthracene-9-carboxylic acid (Sigma-Aldrich, St. Louis, MO, USA). SNAP and isoproterenol were dissolved in distilled water to make a stock solution. Ascorbic acid was added to the stock solution of isoproterenol to retard the oxidation. IBMX and pinacidil were dissolved in 50% sulfolane and 0.1 N HCl, respectively. These stock solutions were diluted to HEPES-Tyrode solution by 1000 times. Nicorandil and SG-86 were directly dissolved in HEPES-Tyrode solution.

Statistics

All data are presented as the mean ± S.E.M. Student's *t*-test or analysis of variance (ANOVA) was used for the statistical analyses. *P*-values of <0.05 were considered statistically significant.

Results

Effects of nicorandil, glibenclamide, and pinacidil on $I_{Cl,cAMP}$

Effects of nicorandil on the isoproterenol-induced $I_{Cl,cAMP}$ were examined in guinea-pig ventricular cells in comparison with those of pinacidil and glibenclamide. Isoproterenol at a concentration of 0.1 μM activated $I_{Cl,cAMP}$, as shown in Fig. 1. In another series of experiments, we examined the concentration-dependent effects of isoproterenol on $I_{Cl,cAMP}$. It was confirmed that 0.1 μM isoproterenol submaximally activated $I_{Cl,cAMP}$ and further increase in isoproterenol concentration resulted in activation of greater $I_{Cl,cAMP}$. Addition of nicorandil enhanced rather than inhibited the preactivated $I_{Cl,cAMP}$. Nicorandil at a concentration of 1 mM significantly increased the isoproterenol-induced $I_{Cl,cAMP}$ at 80 mV by 23.1 ± 3.9% (n = 7). Nicorandil at concentrations higher than 100 μM increased the isoproterenol-activated $I_{Cl,cAMP}$ in a concentration-dependent manner (Fig. 1).

In the absence of preactivation of $I_{Cl,cAMP}$ with isoproterenol, effects of nicorandil on the membrane current were evaluated in guinea-pig ventricular cells. Nicorandil at concentrations higher than 300 μM increased the steady-state current, as shown in Fig. 2. The nicorandil-induced current in the absence of isoproterenol showed slightly outward rectification and the reversal potential was around −20 mV with use of almost symmetrical Cl^- solutions. Therefore, there might be slight contamination of other membrane current(s). Accordingly effects of anthracene-9-carboxylic acid, a specific blocker of $I_{Cl,cAMP}$ (17), on the nicorandil-induced current were evaluated. Nicorandil at a concentration of 1 mM increased the steady-state current at +80 mV from 1.77 ± 0.19 to 2.59 ± 0.40 pA/pF and addition of 300 μM anthracene-9-carboxylic acid decreased the current to 2.04 ± 0.41 pA/pF (n = 5) (Fig. 2). Thus the nicorandil-induced current was largely blocked by anthracene-9-carboxylic acid, suggesting most of the nicorandil-induced current would be $I_{Cl,cAMP}$.

As it was reported that potassium-channel blockers inhibit the cAMP-induced Cl^- current in NIH 3T3 fibroblast cells expressing CFTR (9), effects of nicorandil, glibenclamide, and pinacidil on isoproterenol-induced $I_{Cl,cAMP}$ were examined. Glibenclamide, an ATP-sensitive potassium-channel blocker, at a concentration of 100 μM markedly inhibited the isoproterenol-induced $I_{Cl,cAMP}$, as shown in Fig. 3. However, pinacidil, a potassium-channel opener, at a concentration of 100 μM hardly affected the isoproterenol-induced $I_{Cl,cAMP}$. Thus, nicorandil produced

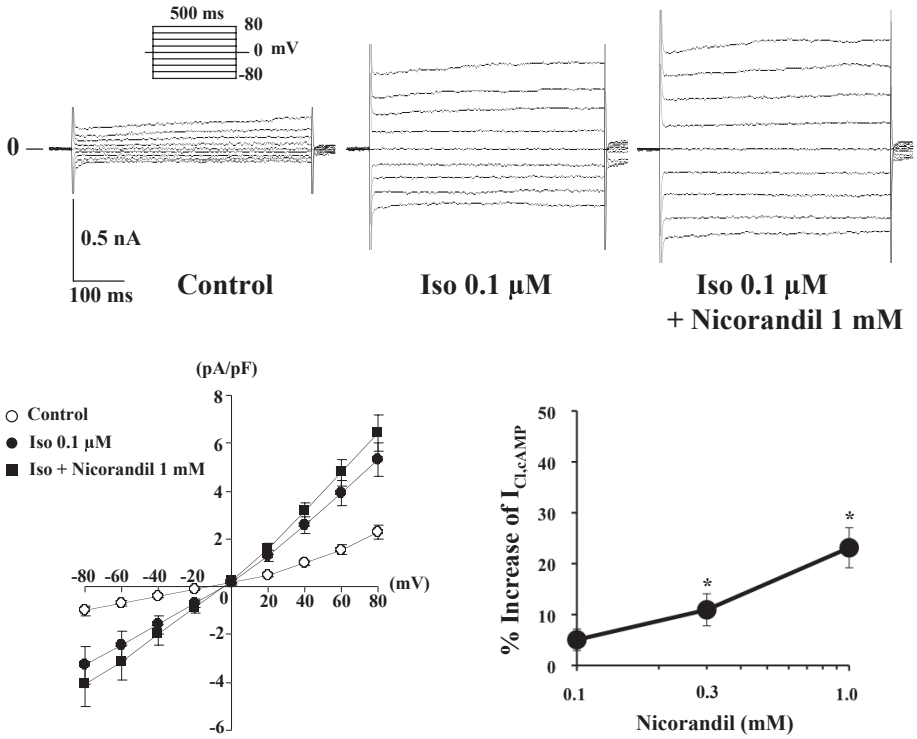

Fig. 1. Effects of nicorandil on the Cl^- current preactivated by isoproterenol in guinea-pig ventricular cells. Representative current traces before and after 0.1 μM isoproterenol (Iso) and 0.1 μM isoproterenol plus 1 mM nicorandil are shown in the upper panels. Membrane currents were elicited by 500-ms hyperpolarizing and depolarizing pulses from a holding potential of 0 mV. The pulse protocol is indicated on the inset. Current–voltage relationships for the steady state currents measured at the end of 500-ms pulses before and after drugs are indicated in the bottom left panel. Each value is the mean ± S.E.M. of 7 cells. Concentration–response relationship for the increasing effect of nicorandil on the $I_{Cl,cAMP}$ (at +80 mV) activated by 0.1 μM isoproterenol in the bottom right panel. Each point represents % increase in $I_{Cl,cAMP}$ as the mean ± S.E.M. of 5 – 7 cells. *$P < 0.05$ vs. 0.1 μM isoproterenol alone.

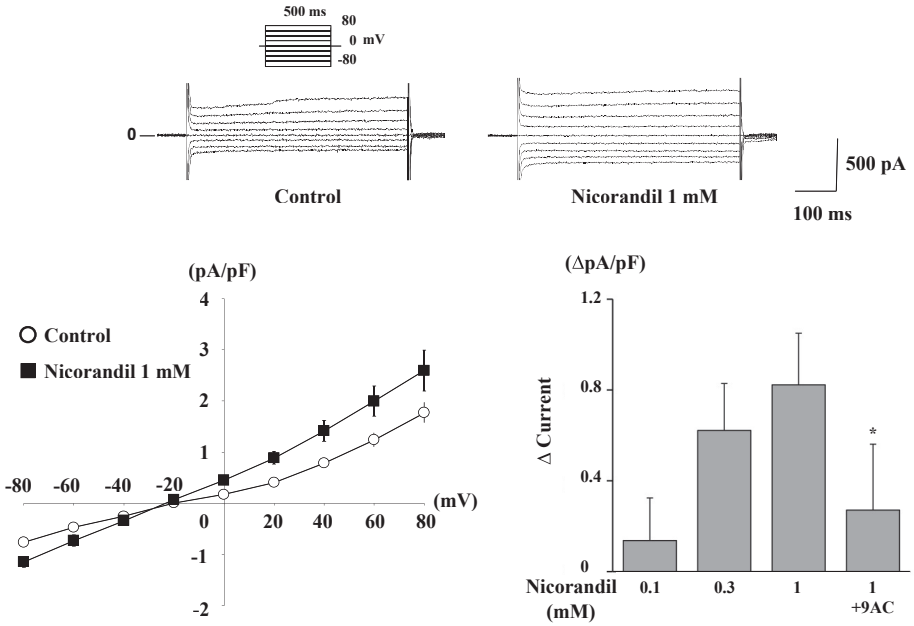

Fig. 2. Effects of nicorandil on the steady-state membrane current in the absence of isoproterenol. Nicorandil concentration-dependently increased the steady-state current in the absence of isoproterenol. The activated current was sensitive to anthracene-9-carboxylic acid (9AC, 0.3 mM). Representative current traces before (Control) and after 1 mM nicorandil are shown in the upper panels and the current-voltage relationships are shown in the bottom left panel. Each point represents the mean ± S.E.M. of 5 cells. Increases of the steady state current at +80 mV after various concentrations of nicorandil and 1 mM nicorandil plus 0.3 mM 9AC are indicated as the mean ± S.E.M. of 4 – 5 cells in the bottom right panel. $*P < 0.05$ vs. 1 mM nicorandil alone.

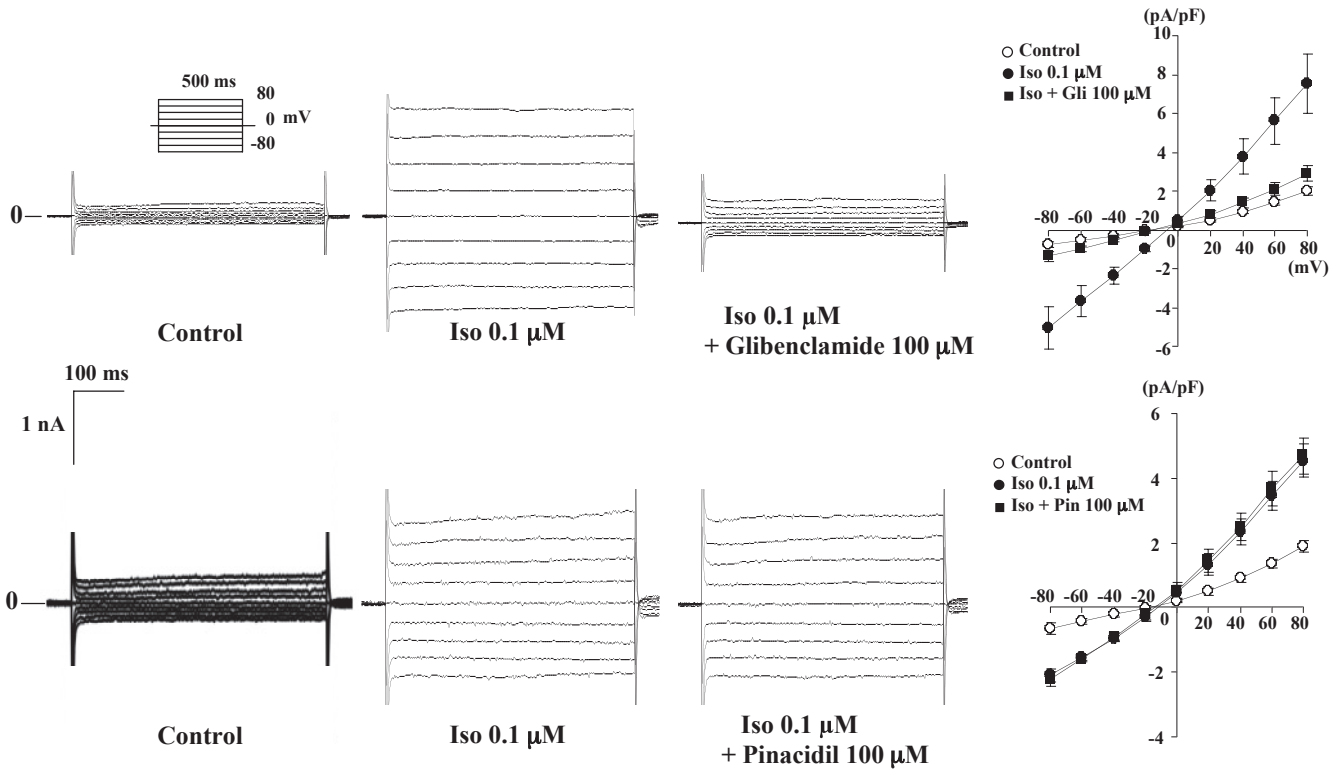

Fig. 3. Effects of glibenclamide and pinacidil on the Cl^- current activated by isoproterenol in guinea-pig ventricular cells. Membrane currents were elicited by 500-ms hyperpolarizing and depolarizing pulses from a holding potential of 0 mV. The pulse protocol is indicated on the inset. Representative current traces before and after 0.1 μM isoproterenol (Iso) and addition of 100 μM glibenclamide (Gli) (upper panels) or 100 μM pinacidil (Pin) (lower panels) are shown. Current–voltage relationships for the steady state currents are indicated in the right panels. Each value is the mean ± S.E.M. of 5 cells. Note that 100 μM glibenclamide but not 100 μM pinacidil inhibited the $I_{Cl,cAMP}$ preactivated by 0.1 μM isoproterenol.

an increasing effect on the isoproterenol-induced $I_{Cl,cAMP}$, whereas glibenclamide but not pinacidil inhibited the Cl^- current.

Effects of NO donor and SG-86 on the $I_{Cl,cAMP}$ induced by isoproterenol

Nicorandil is a unique compound that has dual mechanisms as a potassium-channel opener and as a nitrate (12). Since pinacidil did not modify isoproterenol-activated $I_{Cl,cAMP}$, the increasing effect on $I_{Cl,cAMP}$ by nicorandil may stem from an action as a nitrate. Accordingly, we examined effects of a NO donor on $I_{Cl,cAMP}$. SNAP, a stable nitrosothiol-based NO donor, was used as an NO source. Following activation with 0.1 μM isoproterenol, cells were further treated with SNAP at 10 μM. The $I_{Cl,cAMP}$ was significantly enhanced as it was by nicorandil (Fig. 4). Treatment with SNAP at 10 μM significantly increased $I_{Cl,cAMP}$ by 45.5 ± 11.7% (n = 6) at +80 mV.

In order to confirm the involvement of the nitrate-like action of nicorandil on $I_{Cl,cAMP}$, a denitrated metabolite, SG-86, was used in place of nicorandil. SG-86 has the identical chemical structure but without the side-chain of nitrite. Accordingly, SG-86 is a potent opener of K_{ATP} channels, but it does not show any action as a nitrate compound (13). SG-86 at a concentration of 1 mM hardly affected the isoproterenol-induced $I_{Cl,cAMP}$, as shown in Fig. 4, suggesting the molecular function of the nitrite moiety of nicorandil is crucial for the enhancement of $I_{Cl,cAMP}$. Since the NO-donor SNAP emulated the effect of nicorandil on $I_{Cl,cAMP}$ and the nitrite-branch of nicorandil was critical for this activity, the molecule underlying $I_{Cl,cAMP}$ enhancement might be related to NO.

Effects of nicorandil and SNAP on the $I_{Cl,cAMP}$ induced by IBMX

A typical mechanism of NO in cells as a signaling molecule is via production of cGMP. It is also known that phosphodiesterase (PDE) can be inhibited by cGMP in mammalian heart (18). It may be possible that cGMP might enhance the cAMP-mediated response on the Cl^-

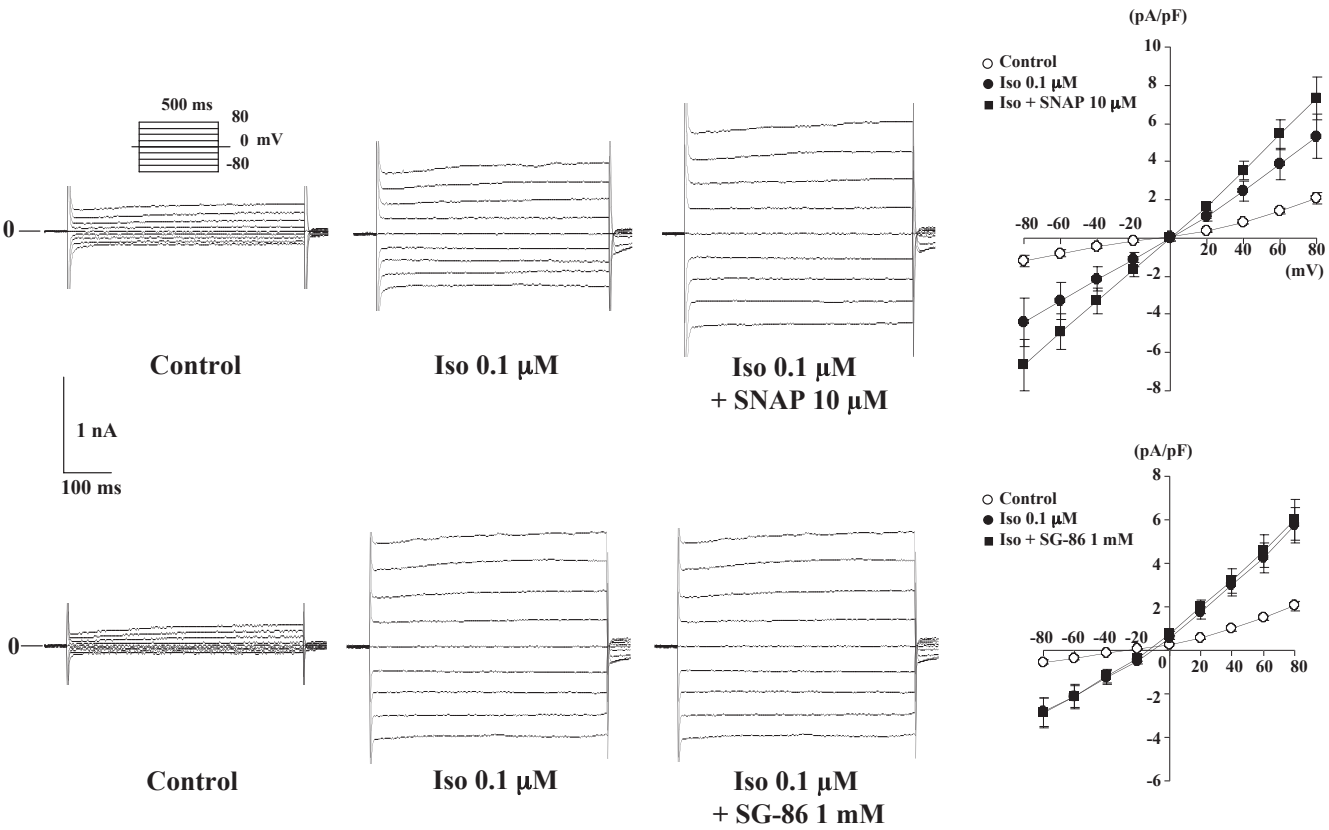

Fig. 4. Effects of SNAP (*S*-nitroso-*N*-acetyl-D,L-penicillamine) and SG-86 [*N*-(2-hydroxyethyl) nicotinamide] on the Cl^- current preactivated by isoproterenol in guinea-pig ventricular cells. Membrane currents were elicited by 500-ms hyperpolarizing and depolarizing pulses from a holding potential of 0 mV. The pulse protocol is indicated on the inset. Representative current traces before and after 0.1 μM isoproterenol (Iso) and addition of 10 μM SNAP (upper panels) or 1 mM SG-86 (lower panels) are shown. Current–voltage relationships for the steady state currents are indicated in the right panels. Each value is the mean ± S.E.M. of 5 – 6 cells. Note that SNAP but not SG-86 enhanced the $I_{Cl,cAMP}$ preactivated by 0.1 μM isoproterenol.

current via cGMP-mediated inhibition of PDE. In order to support this idea, IBMX, a broad-spectrum PDE inhibitor, was used for the experiments to activate $I_{Cl,cAMP}$. In a part of the experiments, the effects of 30 and 100 μM IBMX on the membrane current were examined. IBMX at concentrations of 30 and 100 μM increased the steady state outward current at +80 mV by 2.07 ± 0.92 and 4.40 ± 0.44 pA/pF in guinea-pig ventricular cells (n = 3). We selected 30 μM IBMX and examined effects of nicorandil and SNAP on the IBMX-induced $I_{Cl,cAMP}$ in 5 cells. Neither nicorandil nor SNAP further enhanced the IBMX (30 μM)–induced $I_{Cl,cAMP}$, as shown in Fig. 5. These results indicate that nicorandil increases cGMP level in cardiac cells, which might inhibit PDE and increase the cellular cAMP.

Discussion

Nicorandil, a hybrid coronary vasodilator with both K_{ATP} channel–activating properties and nitrate-like structure, is being used clinically for the treatment of angina pectoris (19, 20). In enzymatically-dissociated cardiac cells, nicorandil was shown to activate the K_{ATP} channel in concentrations higher than 100 μM (21, 22). It is acknowledged that cardiac and vascular K_{ATP} channels are composed of SUR2A/Kir6.2 and SUR2B/Kir6.1, respectively (15, 23 – 25). Since nicorandil shows lower affinity to SUR2A than to SUR2B (26), relatively high concentrations of nicorandil are needed to activate cardiac K_{ATP} channels. Recently it has been demonstrated that nicorandil can also activate mitochondrial K_{ATP} channels and thereby exert a cardioprotective effect (27), although the molecular identity of mitochondrial K_{ATP} channel remains elusive. Effects of nicorandil on $I_{Cl,cAMP}$, however, have not been evaluated. This study has demonstrated that nicorandil potentiated rather than inhibited $I_{Cl,cAMP}$ in isolated guinea-pig ventricular myocytes in its relatively high concentrations.

A denitrated metabolite of nicorandil, SG-86, possessing K_{ATP} channel–opening action failed to show an accelerating effect on the $I_{Cl,cAMP}$ preactivated by isoproterenol. Thus the stimulatory effect of nicorandil on

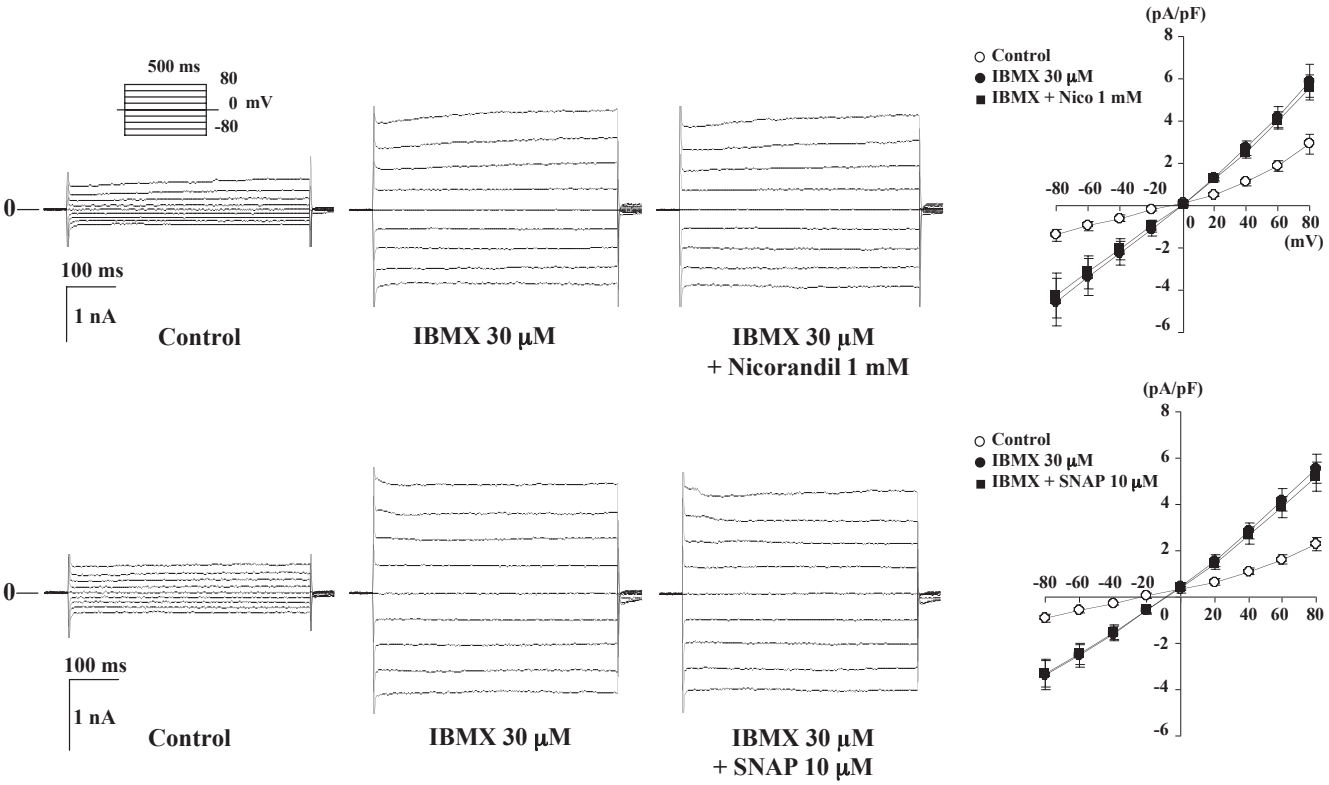

Fig. 5. Effects of nicorandil and SNAP (*S*-nitroso-*N*-acetyl-D,L-penicillamine) on the Cl^- current preactivated by IBMX (3-isobutyl-1-methylxanthine) in guinea-pig ventricular cells. Membrane currents were elicited by 500-ms hyperpolarizing and depolarizing pulses from a holding potential of 0 mV. The pulse protocol is indicated on the inset. Representative current traces before and after 30 μM IBMX and addition of 1 mM nicorandil (upper panels) or 10 μM SNAP (lower panels) are shown. Current–voltage relationships for the steady state currents are indicated in the right panels. Each value is the mean ± S.E.M. of 5 cells. Note that neither nicorandil nor SNAP further enhanced the $I_{Cl,cAMP}$ preactivated by 30 μM IBMX.

$I_{Cl,cAMP}$ might be ascribed to the nitrite–cGMP action. Indeed, in the presence of IBMX, either SNAP or nicorandil failed to enhance $I_{Cl,cAMP}$. Therefore, nicorandil might enhance $I_{Cl,cAMP}$ via cGMP production and resultant inhibition of the cGMP-inhibited PDE3, leading to the intracellular increase in cAMP. In our preliminary experiments, nicorandil at a concentration of 1 mM failed to increase the isoproterenol (0.1 μM)–induced $I_{Cl,cAMP}$ when a pipette solution containing 50 μM 8-bromo-cGMP was used (data not shown). The production of cGMP might play an important role in the nicorandil-induced enhancement of $I_{Cl,cAMP}$. It was reported that nicorandil increased intracellular cGMP in vascular tissues, although relative high concentrations of nicorandil were needed (28, 29). In terms of cGMP-mediated modulation of $I_{Cl,cAMP}$, Ono et al. (30, 31) elegantly demonstrated the enhancement of $I_{Cl,cAMP}$ by intracellular cGMP with use of intracellular dialysis and flash photolysis caged compounds. They indicated that intracellular loading of cGMP enhanced the $I_{Cl,cAMP}$ activated by submaximal dose of isoproterenol. Similar cGMP-mediated enhancement has been observed with the L-type Ca^{2+} current in cardiomyocytes (32, 33).

In the present study, nicorandil activated a steady state current in the absence of β-adrenergic stimulation. Since the nicorandil-activated current showed slightly outward rectification and the reversal potential was around −20 mV, the nicorandil-induced current might not be a pure Cl^- current and might be contaminated by other currents. However, the nicorandil-induced current was largely inhibited by anthracene-9-carboxylic acid, a specific blocker of $I_{Cl,cAMP}$ (17). Therefore, nicorandil might slightly activate $I_{Cl,cAMP}$ in the absence of β-adrenergic stimulation, although the nature of the contaminating current(s) was not identified in this study. In addition, SNAP at a concentration of 10 μM per se activated a steady state current, which was very similar to the current activated by nicorandil alone (data not shown). Recently it has been reported that NO can directly modulate the function of ion channels in cardiac cells (34) and CFTR in airway mucosal gland cells (35). Cardiac ion-channel modulation by NO and cGMP is undoubtedly complex and may be species-dependent (36).

It has been reported that sarcolemmal K_{ATP} channels can be also activated by the NO–cGMP pathway not only in vascular smooth muscle cells (37, 38) but also in cardiac cells (39, 40). The activation of cardiac K_{ATP} channels is suggested to be mediated by phosphorylation by protein kinase G (40). It has been reported that mitochondrial K_{ATP} channels can be also activated by protein kinase C (41) and NO (42). This study has demonstrated that sarcolemmal cAMP-dependent Cl^- channels can be also activated by nicorandil via the NO–cGMP pathway. Activation of sarcolemmal K_{ATP} and Cl^- channels may lead to action potential shortening, although these channels in cardiac cells are activated by relatively high concentrations of nicorandil. The therapeutic concentrations of nicorandil are assumed to be less than 10 μM (43) and much lower than the concentrations that modulate cardiac cAMP-dependent Cl^- channels.

Less is known regarding the molecular identity and physiological role of Cl^- channels compared to cation channels in the heart (1). Therefore, the pharmacological significance of nicorandil-induced enhancement of $I_{Cl,cAMP}$ cannot be determined with certainty. Recently it has been reported that activation of $I_{Cl,swell}$ and other Cl^- currents plays an important role in regulation of cell volume, and Cl^--channel blockers abolish the cardioprotective effect of ischemic preconditioning (44). Therefore, activation of Cl^- channels as well as sarcolemmal K_{ATP} channels by nicorandil may restore cell volume and afford cardioprotection during ischemia/reperfusion. In addition, activation of Cl^- channels may lead to action potential shortening, thereby lessening intracellular Ca^{2+} overload with reduction of Ca^{2+} influx during the plateau phase. However, it has been also reported that Cl^--channel blockers improved the recovery of contractile function after ischemia/reperfusion in isolated guinea-pig ventricular preparations (45). Further studies are needed to evaluate the pathophysiological significance of Cl^- channels in the heart.

In conclusion, nicorandil enhances $I_{Cl,cAMP}$ in guinea-pig cardiomyocytes through an increase in intracellular cGMP, although direct modulation of $I_{Cl,cAMP}$ by NO cannot be completely excluded.

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