ELSEVIER

European Journal of Pharmacology 348 (1998) 31–35

ejp

Short communication

5-Hydroxydecanoate selectively reduces the initial increase in extracellular K^+ in ischemic guinea-pig heart

Kenji Sakamoto, Jun Yamazaki, Taku Nagao *

Laboratory of Pharmacology and Toxicology, Graduate School of Pharmaceutical Sciences, University of Tokyo, 3-1 Hongo 7-chome, Bunkyo-ku, Tokyo 113, Japan

Received 22 December 1997; revised 18 March 1998; accepted 19 March 1998

Abstract

The aim of the present study was to determine the effect of 5-hydroxydecanoate (5-HD) on extracellular K^+ levels during global ischemia for 30 min employing K^+-sensitive electrodes in isolated guinea-pig hearts. 5-HD (100 μM) reduced the K^+ accumulation during the early period of ischemia, but did not inhibit the elevation of extracellular K^+ in the latter half of the ischemic period which was selectively enhanced by ouabain (3 μM). Thus, 5-HD appears to exert a similar mode of action as glibenclamide on extracellular K^+ accumulation in the ischemic guinea-pig hearts. The present study also strengthens the previous conclusion that an ATP-sensitive K^+ channel contributes only to the initial increasing phase of extracellular K^+ accumulation during ischemia in guinea-pig hearts. © 1998 Elsevier Science B.V.

Keywords: Ischemia, myocardial; K^+, extracellular; 5-Hydroxydecanoate; Ouabain

1. Introduction

An increase in extracellular K^+ concentration ($[K^+]_e$) during myocardial ischemia is speculated to lead to ventricular arrhythmias (Weiss and Shine, 1981). In the global ischemic model, the changes in $[K^+]_e$ show a characteristic triphasic pattern comprising an initial increasing phase, followed by a constant or falling phase, and finally a late increasing phase (Wilde et al., 1990; Sakamoto et al., 1997). Previous reports have shown that glibenclamide, an ATP-sensitive K^+ channel (K_{ATP}) blocker, reduces the initial increasing phase of $[K^+]_e$ but has no effect on $[K^+]_e$ levels during the late increasing phase (Wilde et al., 1990; Mitani et al., 1992; Sato et al., 1994). In contrast, glibenclamide has also been shown to lose its efficacy during ischemia when intracellular ADP content increases (Venkatesh et al., 1991). Glibenclamide is not a selective inhibitor of K_{ATP} as it has also been reported to inhibit Na^+–K^+ pump activity in hamster insulin-secreting cells (Ribalet et al., 1996), Cl^- channel activity in guinea-pig and canine myocytes (Yamazaki and Hume, 1997) and prevent the induction of inducible nitric oxide synthase in cultured macrophages and the anesthetized rat (Wu et al., 1995). Therefore, the study of the role of K_{ATP} on extracellular K^+ accumulation during myocardial ischemia requires further evaluation.

Sodium 5-hydroxydecanoate (5-HD), which is structurally unrelated to glibenclamide, was reported to block K_{ATP} channels in guinea-pig myocytes (Notsu et al., 1992a,b), and to be an ischemia-selective K_{ATP} blocker in dog hearts (McCullough et al., 1991). It has been reported that 5-HD antagonizes infarct size reduction of ischemic preconditioning in dogs, rabbits and rats (Auchampach et al., 1992; Hide and Thiemermann, 1996; Schultz et al., 1997). For a more accurate investigation of the role of K_{ATP} channels in myocardial $[K^+]_e$ changes during ischemia, we examined the effect of 5-HD on each of the three phases of ischemia in isolated guinea-pig hearts. To determine the selective modulation of K_{ATP} by 5-HD in the initial phase, the Na^+–K^+ pump inhibitor ouabain, known to affect both the constant and late increasing phases, was also tested.

* Corresponding author. Tel.: +81-3-3812-2111 ext. 4860; fax: +81-3-5802-2943; e-mail: tnagao@mol.f.u-tokyo.ac.jp

2. Materials and methods

2.1. Preparation

Isolated Langendorff perfused heart preparations were used in all the experiments. Male guinea-pigs weighing 300–450 g (Nihon Ikagaku, Tokyo, Japan) were heparinized (1000 U/kg, i.p.) and killed by decapitation. Their hearts were rapidly excised and immersed in ice-cold modified Krebs–Henseleit buffer solution (KHB), comprising (in mM) NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 10, in an atmosphere of 95% O_2–5% CO_2. After contraction had ceased, the hearts were cannulated via the aorta, perfused retrogradely at a constant pressure of 60 mmHg, placed in the chamber where temperature and humidity were controlled to maintain the cardiac surface at 37°C, as described by Du Toit and Opie (1992).

Aortic pressure was monitored by connecting a pressure transducer (Model 800, Bentrey Transtec, CA) to the aortic cannulae. In order to measure the left ventricular pressure, a latex balloon filled with distilled water was inserted via the left atrium into the left ventricular cavity and inflated so that the left ventricular end-diastolic pressure was adjusted to 5–10 mmHg. The balloon was connected to a pressure transducer (MPU-0.5, Toyo Baldwin, Tokyo, Japan). The transducers were connected to carrier amplifiers (AP-621G, Nihon Kohden, Tokyo, Japan) to obtain pressure pulses. The first derivative of the left ventricular pressure (LV dP/dt) was calculated by pressure processor (EQ-601G, Nihon Kohden). Heart rate was recorded by a heart rate counter (AT-601G, Nihon Kohden) and triggered by the left ventricular pressure pulses. The left ventricular developed pressure was calculated as the difference between the left ventricular systolic and diastolic pressures. The coronary flow was measured with a blood flow transducer (FF-040T, Nihon Kohden) connected to an electromagnetic flowmeter (MFV-2100, Nihon Kohden). $[K^+]_e$ was measured by employing ion-selective electrodes, as described elsewhere (Hill et al., 1978, Sakamoto et al., 1997). Briefly, a polyvinyl chloride (PVC) tube (o.d. 1.5 mm, i.d. 1.0 mm) was pulled in a flame and attached to a stainless steel wire (diameter 0.2 mm) with PVC dissolved in tetrahydrofuran. The tube was cut to make the inner tip diameter of the electrode about 0.1 mm. The K^+ electrode was filled with 500 mM KCl solution and sealed with a membrane made from dried tetrahydrofuran solution containing (mg/ml) valinomycin 4, potassium tetrakis (4-chlorophenyl) borate 1, dibutyl sebacate 70 and PVC (high-molecular weight) 25. The reference electrode was placed in the right ventricle. K^+-selective and reference electrode was connected to a high-impedance amplifier (MEZ-7200, Nihon Kohden) by an Ag–AgCl wire. The voltage difference was amplified with a bioelectric amplifier (AB-621B, Nihon Kohden) and displayed by a pen recorder (FBR-252A, Toa Electric, Tokyo, Japan).

2.2. Experimental groups

The four experimental groups employed were: control ($n = 6$), 10 μM 5-HD (Research Biochemical International, Natick, MA)-treated ($n = 4$), 100 μM 5-HD-treated ($n = 6$) and 3 μM ouabain (Research Biochemical International, Natick, MA)-treated ($n = 4$). All drugs were added directly to the perfusate to produce final concentrations. The concentration of ouabain used in this study was shown not to increase the left ventricular end-diastolic pressure. All the hearts were perfused for 30 min equilibration period, after which they were perfused for 10 min in the presence or absence of drugs. This period was followed by global ischemia by clamping the aortic cannulae for 30 min.

2.3. Statistical analysis

The data are expressed as mean ± S.E.M.. One-way or two-way analysis of variance (ANOVA) followed by Dunnet's post-hoc test was used for multiple comparisons, using Super ANOVA™ (Abacus Concepts, Barkeley, CA). Differences were considered to be statistically significant when the P value was less than 0.05.

3. Results

3.1. Changes in cardiac function before ischemia

In the control group, cardiac function did not change during perfusion for 10 min with normal KHB (Fig. 1). Likewise, the addition of 10 or 100 μM 5-HD to the perfusate produced no effect on cardiac function. The addition of 3 μM ouabain significantly increased LV dP/dt and heart rate, but did not affect the other parameters (Fig. 1).

3.2. Changes in extracellular K^+ during ischemia

In control hearts, the change in $[K^+]_e$ during 30 min of ischemia showed the typical triphasic pattern made up of an initial rapid rise, followed by a constant phase, and finally a late increasing phase (Fig. 2). The level of $[K^+]_e$ rose rapidly from its pre-ischemic value of 5.9 mM to 12.0 ± 0.8 mM 6 min after the onset of ischemia (the initial increasing phase). Thereafter, $[K_+]^e$ was maintained at a constant level for 6 to 13 min (the constant phase), and then rose continuously up to 30 min (the late increasing phase) reaching 25.1 ± 0.9 mM.

In 10 μM 5-HD-treated hearts, the rise of $[K^+]_e$ during the initial phase was significantly reduced when compared to the control group. The level of $[K^+]_e$ 6 min after the onset of ischemia was 8.9 ± 0.6 mM (Fig. 2; $P < 0.05$, one-way analysis of variance followed by Dunnet's post-hoc test). In the 100 μM 5-HD-treated group, $[K^+]_e$

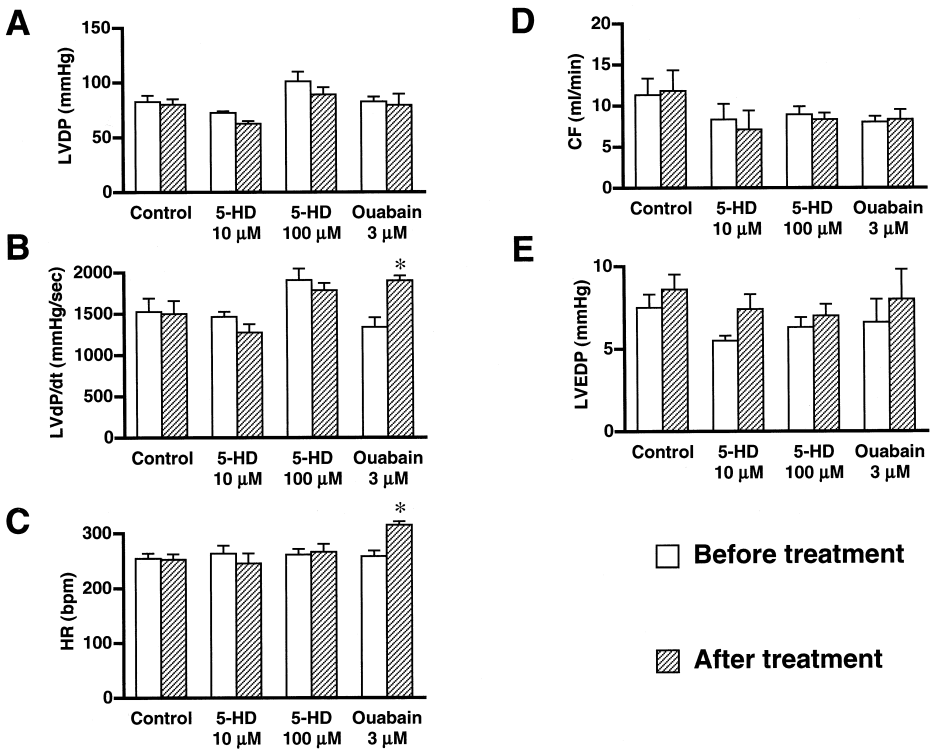

Fig. 1. Effects of 5-hydroxydecanoate (5-HD) and ouabain on the changes in the left ventricular developed pressure (A), the first derivative of the left ventricular pressure (LV dP/dt) (B), heart rate (C), coronary flow (D) and the left ventricular end diastolic pressure (E) in isolated guinea-pig hearts before ischemia. The data represent the means ± S.E.M. of 4 to 6 hearts. $* P < 0.05$, significantly different from the values before treatment.

reached a value of 8.9 ± 0.4 mM ($P < 0.05$) 6 min following the onset of ischemia and thereafter was maintained at a constant level for 6 to 11 min. This was followed by a continuous rise 30 min after the onset of ischemia reaching a final value of 23.8 ± 3.0 mM. The $[K^+]_e$ at the end of ischemia in both 5-HD-treated groups was not significantly different in comparison to the control group.

In the ouabain-treated hearts, $[K^+]_e$ rose slowly up to 5 min after the onset of ischemia (Fig. 2). The constant phase and late increasing phase developed earlier than in the control group. The late phase reached 27.1 ± 1.1 mM at 30 min.

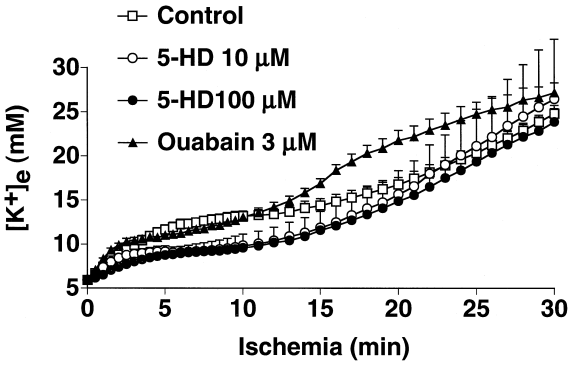

Fig. 2. Time-courses of $[K^+]_e$ changes in control, 5-hydroxydecanate (5-HD)-treated and ouabain-treated guinea-pig hearts during ischemia. The data represent the means ± S.E.M. of 4 to 6 hearts. If error bars are not shown, the S.E.M.s are included within the symbols.

3.3. The time to the onset of ischemic contracture

We defined the time to the onset of ischemic contracture as the time when the increase of the left ventricular end diastolic pressure during ischemia reached 2 mmHg. In the control group, the time to the onset of ischemic contracture was 16.8 ± 1.7 min. In the 10 and 100 μM 5-HD-treated hearts, the time to the onset of ischemic contracture slightly decreased to 13.2 ± 1.6 and 14.2 ± 0.7 min, respectively. In the 3 μM ouabain-treated hearts, the time to the onset of ischemic contracture was 11.0 ± 3.1 min. Ouabain and 5-HD did not significantly affect the time to the onset of ischemic contracture at the concentrations tested in the present study.

4. Discussion

It has been reported that 5-HD reduced the cellular K^+ loss during 5 min of ischemia in dog hearts in vivo (Miyoshi et al., 1996). However, there is little or no information available concerning the direct effect of 5-HD on the triphasic changes of $[K^+]_e$ during global ischemia in isolated hearts. In the present study, 10 or 100 μM

5-HD, concentrations which had no effect on cardiac function or coronary flow, significantly reduced the initial increasing phase but had little effect on $[K^+]_e$ in the late increasing phase. Similar findings have been previously reported with glibenclamide in our and the other laboratories (Wilde et al., 1990; Mitani et al., 1992; Sato et al., 1994). Using ouabain, a selective inhibitor of the Na^+–K^+ pump, no effect was seen on the initial increasing phase. Therefore, it appears that 5-HD has a similar mode of action as glibenclamide in blocking K_{ATP} channels during myocardial ischemia. The present study indicates that the K^+ efflux through K_{ATP} channels contributes partially to the initial increasing phase of $[K^+]_e$ during ischemia, and that the activation of these channels is not required for development of the late increasing phase. It has been reported that K_{ATP} channels in myocytes initially open and subsequently close during maintained anoxia (Thierfelder et al., 1994). Therefore, it is possible that K_{ATP} channels are activated during early ischemia, i.e., the initial increasing and the constant phase, and thereafter inactivated during the late period of ischemia.

Recently, the mitochondrial K_{ATP} channel was reported to be a target for a potassium channel opener (Garlid et al., 1996). The selective activation of the mitochondrial K_{ATP} channel by diazoxide protected against hypoxia-induced cell death and lactate dehydrogenase release, and this protection was blocked by 5-HD (Garlid et al., 1997; Liu et al., 1997). These data are consistent with the idea that the mitochondrial channels are potential targets of 5-HD. The present study does not rule out the possibility that the mitochondrial K_{ATP} channels may be a potential site of cardioprotective activity such as inhibition of ischemic contracture. However, it appears to be more appropriate to consider that 5-HD blocks the sarcolemmal K_{ATP} channels and inhibits the K^+ efflux in the present experiment, since $[K^+]_e$ is supposed primarily to reflect the activity of sarcolemmal K_{ATP} channels in ischemic hearts.

We have previously reported that the late increasing phase in $[K^+]_e$ develops after glycolytic ATP synthesis ceases (Sakamoto et al., 1997). Since the Na^+–K^+ pump has been shown to be preferentially fueled by glycolytic ATP production in sheep cardiac Purkinje cells (Glitsch and Tappe, 1993), it is possible that the reduction in Na^+–K^+ pump activity by the cessation of glycolysis contributes to the increase of $[K^+]_e$ during the late increasing phase. In a previous study, it was found that 10 μM ouabain induced a large monophasic K^+ efflux during ischemia in isolated rabbit heart (Weiss and Shine, 1982). These results may have reflected the ouabain-induced cellular injury since the pre-ischemic treatment with 10 μM ouabain developed the elevation of the left ventricular end diastolic pressure. The present study demonstrates that pre-ischemic treatment of 3 μM ouabain hastened the constant phase and the onset of the late increasing phase in $[K^+]_e$. Furthermore, this concentration did not alter pre-ischemic end diastolic pressure value or the time to the onset of ischemic contracture. It appears then, that ouabain is capable of further modifying the constant and late increasing phases, and suggests that these two phases are sensitive to a reduction of Na^+–K^+ pump activity during ischemia. By employing a more selective K_{ATP} channel blocker, the present study also confirms that K^+ efflux precedes a decrease in K^+ uptake due to a reduction in Na^+–K^+ pump activity in the ischemic heart.

In summary, these results show that 5-HD selectively reduced the initial increase in $[K^+]_e$, in a similar manner to glibenclamide. K_{ATP} contributes to the initial increasing phase of $[K^+]_e$, but does not appear to play a major role in the late increasing phase during ischemia in guinea-pig hearts. Ouabain selectively enhanced the constant and late increasing phases in $[K^+]_e$ during ischemia. Our results further strengthen the idea of a temporal difference in the contribution of different ATP-dependent K^+ efflux mechanisms to $[K^+]_e$ in the ischemic guinea-pig heart.

Acknowledgements

The authors wish to thank Dr. Tetsuro Urushidani (University of Tokyo) and Mr. Ryuichi Sato for helpful discussions. We thank Drs. Victor Ruiz-Velasco and Helen Mason (University of Nevada Reno) for critical reading of the manuscript. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

References

Auchampach, J.A., Grover, G.J., Gross, G.J., 1992. Blockade of ischemic preconditioning in dogs by the novel ATP dependent potassium channel antagonist sodium 5-hydroxydecanoate. Cardiovasc. Res. 26, 1054–1062.

Du Toit, E.F., Opie, L.H., 1992. Modulation of severity of reperfusion stunning in the isolated rat heart by agents altering calcium flux at onset of reperfusion. Circ. Res. 70, 960–967.

Garlid, K.D., Paucek, P., Yarov-Yarovoy, V., Sun, X., Schindler, P.A., 1996. The mitochondrial K_{ATP} channel as a receptor for potassium channel openers. J. Biol. Chem. 271, 8796–8799.

Garlid, K.D., Paucek, P., Yarov-Yarovoy, V., Murray, H.N., Darbenzio, R.B., D'Alonzo, A.J., Lodge, N.J., Smith, M.A., Grover, G.J., 1997. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K^+ channels. Possible mechanism of cardioprotection. Circ. Res. 81, 1072–1082.

Glitsch, H.G., Tappe, A., 1993. The Na^+/K^+ pump of cardiac Purkinje cells is preferentially fuelled by glycolytic ATP production. Pflüg. Arch. 422, 380–385.

Hide, E.J., Thiemermann, C., 1996. Limitation of myocardial infarct size in the rabbit by ischaemic preconditioning is abolished by sodium 5-hydroxydecanoate. Cardiovasc. Res. 31, 941–946.

Hill, J.L., Gettes, L.S., Lynch, M.R., Hebert, N.C., 1978. Flexible valinomycin electrodes for on-line determination of intravascular and myocardial K^+. Am. J. Physiol. 235 (Heart Circ. Physiol. 4), H455–H459.

Liu, Y., O'Rourke, B., Romashko, D., Marban, E., 1997. Mitochondrial K_{ATP} channel as novel effectors of cardioprotection. Circulation 96 (Suppl.), I-72 (abstract).

McCullough, J.R., Normandin, D.E., Conder, M.L., Sleph, P.G., Dzwonczyk, S., Grover, G.J., 1991. Specific block of the anti-ischemic actions of cromakalim by sodium 5-hydroxydecanoate. Circ. Res. 69, 949–958.

Mitani, A., Kinoshita, K., Fukamachi, K., Sakamoto, M., Kurisu, K., Tsuruhara, Y., Fukumura, F., Nakashima, A., Tokunaga, K., 1992. Effect of glibenclamide and nicorandil on cardiac function during ischemia and reperfusion in isolated perfused rat heart. Am. J. Physiol. 261 (Heart Circ. Physiol. 30), H1864–H1871.

Miyoshi, S., Miyazaki, T., Moritani, K., Ogawa, S., 1996. Different responses of epicardium and endocardium to K_{ATP} channel modulators during regional ischemia. Am. J. Physiol. 271 (Heart Circ. Physiol. 40), H140–H147.

Notsu, T., Ohhashi, K., Tanaka, I., Ishikawa, H., Niho, T., Fukutake, K., Mizota, M., 1992a. 5-Hydroxydecanoate inhibits ATP-sensitive K^+ channel in guinea-pig single ventricular myocytes. Eur. J. Pharmacol. 220, 35–41.

Notsu, T., Tanaka, I., Takano, M., Noma, A., 1992b. Blockade of the ATP-sensitive K^+ Channel by 5-hydroxydecanoate in guinea pig ventricular myocytes. J. Pharmacol. Exp. Ther. 260, 702–708.

Ribalet, B., Mirell, C.J., Johnson, D.G., Levin, S.R., 1996. Sulfonylurea binding to a low-affinity site inhibits the Na/K-ATPase and the K_{ATP} channel in insulin-secreting cells. J. Gen. Physiol. 107, 231–241.

Sakamoto, K., Yamazaki, J., Nagao, T., 1997. Diltiazem inhibits the late increase in extracellular potassium by maintaining glycolytic ATP synthesis during myocardial ischemia. J. Cardiovasc. Pharmacol. 30, 424–430.

Sato, R., Yamazaki, J., Sakamoto, K., Nagao, T., 1994. Differential effects of nifedipine, verapamil and diltiazem on extracellular potassium and pH during global ischemia in isolated guinea-pig hearts. Jpn. J. Pharmacol. 64 (Suppl. I), 260P (abstract).

Schultz, J.E., Qian, Y.Z., Gross, G.J., Kukreja, R.C., 1997. The ischemia-selective K_{ATP} channel antagonist, 5-hydroxydecanoate, blocks ischemic preconditioning in the rat heart. J. Mol. Cell. Cardiol. 29, 1055–1060.

Thierfelder, S., Doepner, B., Gebhardt, C., Hirche, H., Benndorf, K., 1994. ATP-sensitive K^+ channels in heart muscle cells first open and subsequently close at maintained anoxia. FEBS Lett. 351, 365–369.

Venkatesh, N., Lamp, S.T., Weiss, J.N., 1991. Sulfonylureas, ATP-sensitive K^+ channels, and cellular K^+ loss during hypoxia, ischemia, and metabolic inhibition in mammalian ventricle. Circ. Res. 69, 623–637.

Weiss, J., Shine, K.I., 1981. Extracellular potassium accumulation during ischemia: implications for arrhythmogenesis. J. Mol. Cell. Cardiol. 13, 699–704.

Weiss, J., Shine, K.I., 1982. Extracellular K^+ accumulation during myocardial ischemia in isolated rabbit heart. Am. J. Physiol. 242 (Heart Circ. Physiol. 11), H619–H628.

Wilde, A.A.M., Escande, D., Schumacher, C.A., Thuringer, D., Mestre, M., Fiolet, J.W.T., Janse, M.J., 1990. Potassium accumulation in the globally ischemic mammalian heart. Circ. Res. 67, 835–843.

Wu, C.C., Thiemermann, C., Vane, J.R., 1995. Glibenclamide-induced inhibition of the expression of inducible nitric oxide synthase in cultured macrophages and in the anaesthetized rat. Br. J. Pharmacol. 114, 1273–1281.

Yamazaki, J., Hume, J.R., 1997. Inhibitory effects of glibenclamide on cystic transmembrane regulator, swelling-activated, and Ca^{2+}-activated Cl^- channels in mammalian cardiac myocytes. Circ. Res. 81, 101–109.