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Salicylaldoxime blocks K^+ and Ca^{2+} currents in rat cardiac myocytes

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Abstract

The effects of salicylaldoxime, 2-(OH)C_6H_4CH = NOH, on the action potential duration, transient outward K^+ current and slow inward Ca^{2+} current were studied in isolated rat ventricular myocytes. The application of salicylaldoxime (0.1–2.0 mM) reversibly increased the action potential duration and reduced in a dose-dependent manner both the transient outward K^+ and the slow inward Ca^{2+} currents. The effect of salicylaldoxime on these two ionic currents was similar to that of 2,3-butanedione monoxime, but was about ten times more potent. Compounds which block both K^+ and Ca^{2+} currents may represent a new type of Class III antiarrhythmic agent which counteracts arrhythmias initiated by re-entry with reduced proarrhythmic risk via triggered activity.

Keywords: Antiarrhythmic; Action potential; Ca^{2+} current; K^+ current; Oxime; Cardiac myocyte

1. Introduction

Agents that modulate cardiac K^+ channels have stimulated interest because of their potential as Class III antiarrhythmic agents. The antiarrhythmic effects of these compounds result from a prolongation of the cardiac action potential and a concomitant increase in the refractory period of the myocardium. Unfortunately, many Class III agents, which block the delayed rectifier K^+ current, can produce a distinct ventricular arrhythmia known as 'torsade de pointes'. This appears to be related to the ability of these Class III agents to induce early after-depolarizations due to incomplete repolarization (Sanguinetti, 1992).

It has been reported that the human heart has a larger than expected transient outward K^+ current and that this might provide a target for a new type of antiarrhythmic drug (Näbauer et al., 1992; Wettwer et al., 1992). An increased density of transient outward K^+ channels has been shown in hypertrophic feline right ventricle. This could explain the alteration in the right ventricular action potential observed under hypertrophic conditions (Ten Eick et al., 1993).

Coronary occlusion and reperfusion is accompanied by electrophysiological heterogeneity in the myocardium. The differential sensitivity of the epicardium and the endocardium to stimulated ischemia has been attributed to an increase in intracellular Ca^{2+} concentration after reperfusion and a more prominent epicardial transient outward K^+ current. Such a heterogeneous response can give rise to extrasystolic activity via a phase 2 re-entry mechanism. This suggests that the likelihood of sudden death in patients with myocardial ischemia might be decreased by a blocker selective for the transient outward K^+ current (Di Diego and Antzelevitch, 1994).

2,3-Butanedione monoxime has been shown to have a number of interesting effects (Sellin and McArdle, 1994), among them is a blockade of cardiac ionic currents (Coulombe et al., 1990). Therefore, we tested another oxime-containing compound, salicylaldoxime, for similar effects. A preliminary report of these findings has been published elsewhere (Karhu et al., 1994).

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2. Materials and methods

2.1. Solutions (in mM)

Solution 1 (Tyrode): NaCl 144, NaH_2PO_4 0.4, KCl 5.4, $MgCl_2$ 1.0, $CaCl_2$ 2.0, glucose 10, Hepes 10, pH 7.4 (adjusted with NaOH).

Solution 2 (pipette solution for action potentials and transient outward K^+ currents): KCl 50, K-glutamate 60, $MgCl_2$ 5, $CaCl_2$ 1.0, EGTA 14, K_2ATP 5, Hepes 5, pH 7.2 (adjusted with KOH).

Solution 3 (suffusate for transient outward K^+ current recording): *N*-methyl-D-glucamine 142, $MgCl_2$ 1.0, $CaCl_2$ 2.0, CsCl 5.0, glucose 10, Hepes 10, nifedipine 5 μM, pH 7.4 (adjusted with HCl). The Na^+ current was eliminated by substituting *N*-methyl-D-glucamine for NaCl and the Ca^{2+} and inward rectifier K^+ channels were blocked by nifedipine and CsCl, respectively.

Solution 4 (pipette solution for slow inward Ca^{2+} current recording): CsCl 110, tetraethylammonium-Cl 20, $MgCl_2$ 5, K_2ATP 5, glucose 10, EGTA 15, Hepes 10, pH 7.2 (adjusted with CsOH).

Solution 5 (suffusate for slow inward Ca^{2+} current recording): *N*-methyl-D-glucamine 105, tetraethylammonium-Cl 20, $MgCl_2$ 1.0, $CaCl_2$ 2.0, glucose 10, 4-aminopyridine 4.0, Hepes 10, pH 7.4 (adjusted with HCl). The K^+ currents were eliminated by substituting CsCl for KCl in intracellular solution and adding 20 mM teraethylammonium both inside and outside. The Na^+ current was eliminated by using *N*-methyl-D-glucamine for NaCl.

All solutions were filtered through a 0.2 μm Millipore filter. Salicylaldoxime was purchased from Aldrich (Steinheim, Germany).

2.2. Cell isolation

Rat ventricular myocytes were enzymatically isolated using a modification of a procedure of Campbell et al. (1993). Male or female rats, 6–12 weeks old, were anesthetized by i.p. injection of pentobarbital sodium (50 mg/kg, Mebunat, Orion, Espoo, Finland). The chest was opened, the aorta was cannulated in situ and the heart was dissected out. The heart was then perfused via a Langendorff apparatus with a flow rate of 5–10 ml/min. The perfusion solutions were oxygenated and maintained at 37° C. The heart was first perfused for 5 min with Tyrode (Solution 1). The perfusion was then changed for a nominally Ca^{2+}-free Tyrode for 5 min followed by a 15–20-min perfusion with a low-Ca^{2+} (50 μM) Tyrode containing 1 mg/ml collagenase type IA (Sigma, St. Louis, MO, USA). After a final 5 min perfusion with 0.2 mM-Ca^{2+} Tyrode solution, the left ventricle was cut into small pieces. The pieces were put into a dish and the cells were dispersed by gentle shaking. The Ca^{2+} concentration was then raised to 1.0 mM and the cells were stored in an Erlenmeyer beaker and oxygenated at room temperature until used. The cells were viable for 10–14 h after isolation.

2.3. Electrophysiology

The myocytes were studied using the gigaseal patch clamp technique in the whole cell recording configuration (Hamill et al., 1981). Electrodes were fabricated from thick-walled borocilicate glass (Clark Electromedical Instruments, UK) using a pipette puller (model P80/PC, Sutter Instruments Co., CA, USA). When filled with recording solutions the resistances were 3–8 MΩ. The cells were placed into a small recording chamber mounted on a modified stage of an inverted microscope (Axiovert 35 M, Zeiss, Germany). After the gigaseal formation the whole cell recording configuration was achieved by applying a brief zap pulse or by a gentle suction. Membrane currents and action potentials were recorded using a CV-4 1/100 headstage and an axopatch-1 D amplifier (Axon Instruments, Foster City, CA, USA) and filtered at 2 kHz. Data were digitalized using an A/D board (Labmaster TL-1 DMA, Axon Instruments) in a 486 microcomputer running a pCLAMP version 5.5 software (Axon Instruments) which included user-defined stimulus protocols, data acquisition and analysis programs.

Action potentials were recorded in current clamp mode. In this configuration, the resting potential of all cells was set to −80 mV to exclude any effects on action potential duration due to variability of initial resting potentials. Action potential duration was defined as the time in ms from the initial sharp upstroke to the point of repolarization where the potential reached −65 mV (Coulombe et al., 1990). In the whole cell patch clamp configuration, it is difficult to distinguish between the stimulus artefact and the fast upstroke phase of the action potential. However, the increase in action potential duration with salicylaldoxime was sufficiently large to exclude this as a complicating factor.

Current-voltage curves for the transient outward K^+ current were obtained using a holding potential of −80 mV and voltage steps in 20 mV increments up to +80 mV. The effect of salicylaldoxime on the transient outward K^+ current was studied using a holding potential of −80 mV and single command voltages to +80 mV. Current-voltage curves for the slow inward Ca^{2+} current were obtained using a holding potential of −60 mV and voltage steps in 20 mV incements up to +60 mV. The effect of salicylaldoxime on the slow inward Ca^{2+} current was studied by using a holding potential of −60 mV and single command voltages to 0 mV. The stimulus frequency in the action potential measurements was 1 Hz and in the current measurements 0.2

Hz. Leakage currents were compensated by an on-line protocol in CLAMPEX program. When different concentrations of salicylaldoxime were studied, the solution in the chamber was changed by suffusion at a flow rate of 1.1 ml/min.

2.4. Data analysis

The data analysis were done using Origin computer program (Microcal Software, California, USA) to fit the dose-response curves, and the analysis of variance for repeated measures with Student-Newman-Keuls test as a follow-up to compare control and drug treatment groups at the significance level of $P < 0.05$. Unless otherwise indicated, the results are expressed as means ± S.D. Statistical tests were performed with Primer (McGraw-Hill, USA) and CoStat (Cotlort Software, USA) computer programs.

The dose-response curves were fit by using a non-linear least squares method to a modified Michaelis-Menten equation (Arena and Kass, 1988):

$$R = 1/(1 + K/c), \quad (1)$$

where R is the percentage change from control (relative response), K is the salicylaldoxime concentration for half-maximal effect (ED_{50}) and c is the salicylaldoxime concentration.

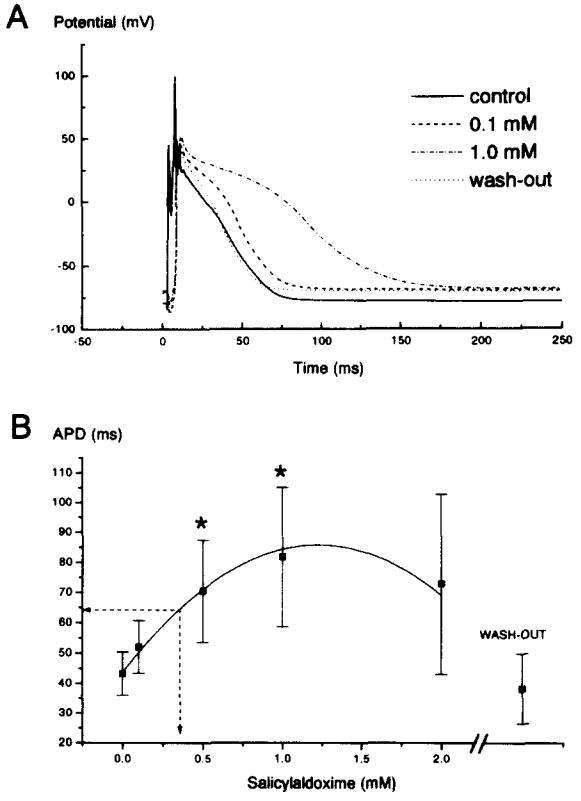

Fig. 1. (A) The effect of 0.1 and 1.0 mM of salicyladoxime on action potential duration recorded in current clamp mode from a single rat ventricular myocyte. The resting potential was set at −80 mV and a stimulus frequency of 1 Hz. (B) A dose-response curve of action potential duration vs. salicylaldoxime concentration for five cells (means ± S.E.M.). The EC_{50} for the prolongation effect was 350 μM (broken lines and arrows). Statistical significance at the $P < 0.05$ level is indicated by an asterisk. In both (A) and (B) the suffusate was solution 1 and the recording microelectrode contained solution 2.

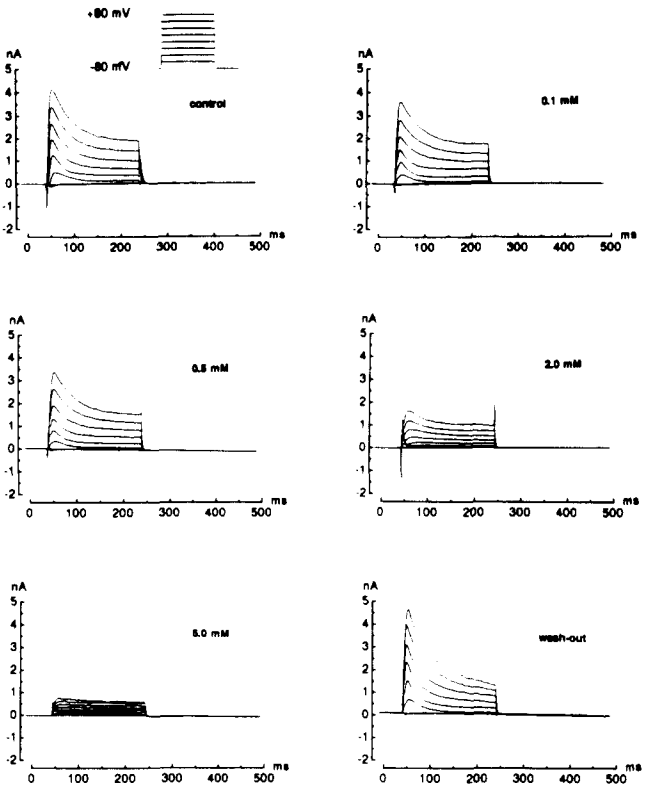

Fig. 2. The effect of various concentrations of salicylaldoxime on the transient outward K^+ current recorded from one rat ventricular myocyte. The holding potential was −80 mV with step commands of 20 mV to +80 mV (insert). The suffusate was solution 3 and the recording microelectrode contained solution 2.

3. Results

Quiescent, Ca^{2+}-tolerant ventricular myocytes having resting membrane potentials of −63 to −73 mV were used in this study. The selected cells exhibited action potentials, and those currents in voltage clamp (fast Na^+, slow Ca^{2+} and transient and sustained K^+ currents) that belong to the well characterized repertoire of ventricular myocytes (Varro and Papp, 1992). From these cells, action potentials, transient outward K^+ and slow inward Ca^{2+} currents were recorded in the presence of various concentrations of salicylaldoxime.

3.1. Effect of salicylaldoxime on action potentials

The control values for action potential duration were from 26 to 61 ms and the amplitude of the control action potential varied from 95 to 125 mV. Salicylaldoxime produced a dose-dependent and reversible prolongation of action potential duration (Fig. 1A). From a control value of 43 ± 16 ms ($n = 5$), duration increased to 52 ± 19 ms at 0.1 and 74 ± 30 ms ($P < 0.05$) at 0.5 mM salicylaldoxime (Fig. 1B). The EC_{50} for action potential prolongation was calculated to be 0.35 mM. At 1 mM, action potential duration reached a maximum of 82 ± 52 ms ($P < 0.05$). At 2.0 mM duration began to decrease, probably due to multiple effects of salicylaldoxime. This was apparent at 5 mM, which reduced action potential amplitude by up to 50%.

3.2. Effect of salicylaldoxime on the transient outward K^+ current

The presence of a prominent transient outward K^+ current in rat ventricular myocytes (Josephson et al., 1984) suggested that salicylaldoxime might prolong action potential duration by blocking this current. Fig. 2 shows the effect of various salicylaldoxime concentrations on the transient outward K^+ current in one rat ventricular myocyte. The peak of the transient component had a value of 3.0 ± 0.9 nA and declined to a maintained current (at 255 ms) of 1.6 ± 0.3 nA, when a voltage step from -80 mV to $+80$ mV ($n = 5$) was used. Addition of salicylaldoxime in concentrations of 0.1, 0.5 and 2.0 mM significantly ($P < 0.05$) reduced the peak current by $11 \pm 19\%$, $30 \pm 9\%$ and $63 \pm 6\%$, and the maintained current by $4 \pm 10\%$, $24 \pm 6\%$ and $55 \pm 12\%$, respectively ($n = 5$). The block was nearly total at 5 mM. However, the effect was completely reversible within several minutes after washout began. The dose-response curve of the effect of salicylaldoxime on the transient outward K^+ current is presented in Fig. 3A. The K value of salicylaldoxime on the transient outward K^+ current was found to be 0.97 mM. The current-voltage curves (peak current versus membrane potential) for different concentrations of salicylaldoxime are presented in Fig. 3B. Salicylal-

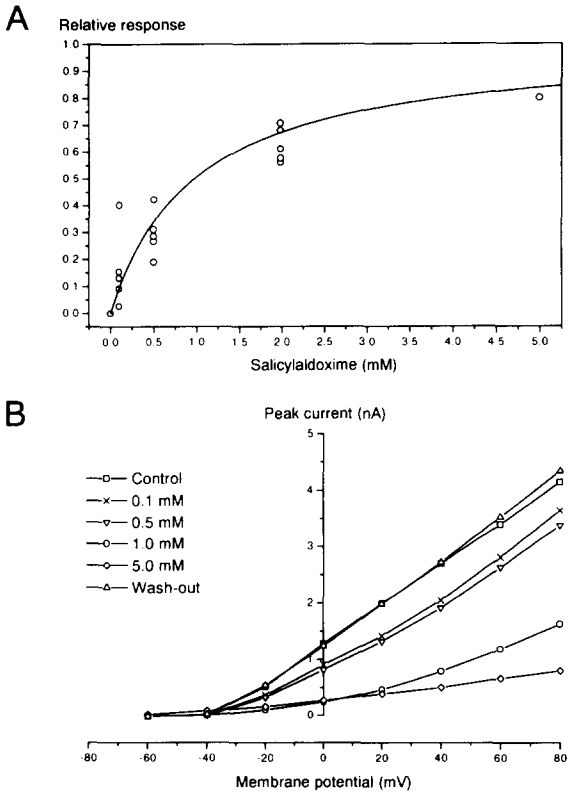

Fig. 3. (A) A dose-response curve for the effect of various concentrations of salicylaldoxime on the transient outward K^+ current. The relative response is a measurement of the % change from the control value. Each open circle represents one cell. The EC_{50} was 0.97 mM. (B) A current-voltage plot of the peak transient outward K^+ current recorded from one rat ventricular myocyte. In both (A) and (B) the suffusate was solution 3 and the recording microelectrode contained solution 2.

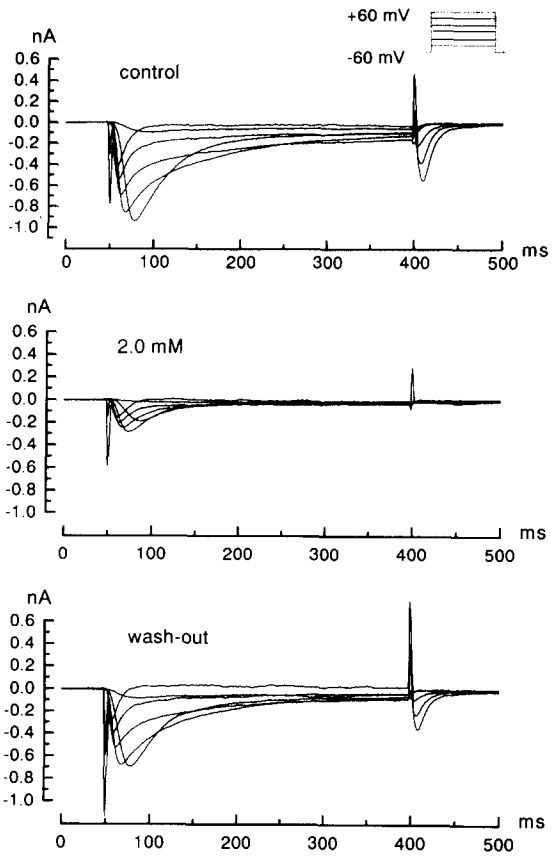

Fig. 4. The effect of 2.0 mM salicylaldoxime on slow inward Ca^{2+} currents recorded from one rat ventricular myocyte. The holding potential was -60 mV with a step command of 20 mV to $+60$ mV (insert). The suffusate was solution 5 and the recording microelectrode contained solution 4.

doxime had a voltage-dependent effect at 1 mM, but less so at lower concentrations.

3.3. Effect of salicylaldoxime on the slow inward Ca^{2+} current

A previous study using another oxime-containing compound, 2,3-butanedione monoxime, showed an effect on both the transient outward K^+ current and the slow inward Ca^{2+} current (Coulombe et al., 1990). To determine if salicylaldoxime had similar multiple effects on ion channels, we measured the slow inward Ca^{2+} current. A representative family of slow inward Ca^{2+} currents are shown in Fig. 4. Salicylaldoxime reduced both the time-dependent and maintained components of the slow inward Ca^{2+} current in a dose-dependent manner. There was a reduction in peak current beginning at 0.1 mM ($-10 \pm 15\%$), continuing at 0.5 mM ($-26 \pm 13\%$), 1.0 mM ($-49 \pm 14\%$), and 2.0 mM ($-78 \pm 10\%$). In contrast to the transient outward K^+ current, the recovery of the slow inward Ca^{2+} current after exposure to salicylaldoxime was usually not complete, varying from 53% to 87% of the control value.

The dose-response curve of the effect of salicylaldoxime on the peak slow inward Ca^{2+} current is shown in Fig. 5A. When the results are fitted by Eq. (1) the value of K (i.e. ED_{50}) is 0.66 mM. The current-voltage curves for the slow inward Ca^{2+} current are shown in Fig. 5B. In contrast to the transient outward K^+ current, the effect of salicylaldoxime on the slow inward Ca^{2+} was not voltage-dependent at 2 mM.

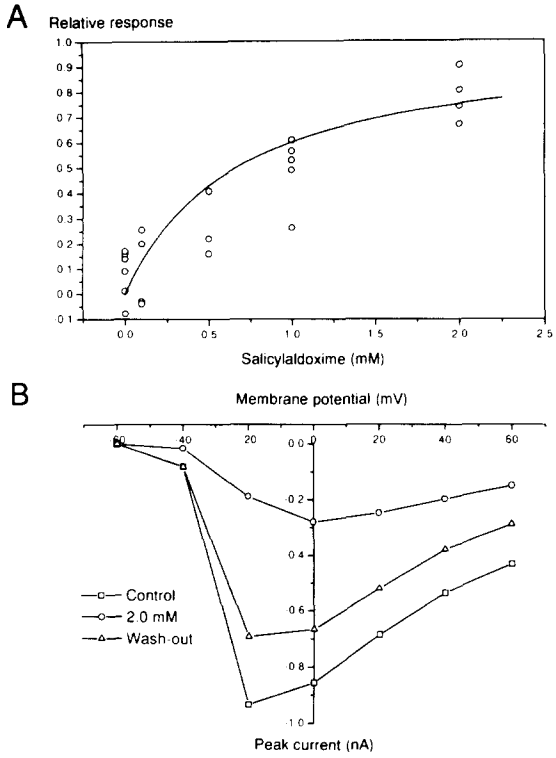

Fig. 5. (A) Dose-response curve for the effect of various concentrations of salicylaldoxime on the slow inward Ca^{2+} current. The relative response is a measurement of the % change from the control value. Each open circle represents one cell. The EC_{50} was 0.66 mM. (B) A current-voltage plot of the peak slow inward Ca^{2+} current from one rat ventricular myocyte. In both (A) and (B) the suffusate was solution 5 and the recording microelectrode contained solution 4.

4. Discussion

We have shown that salicylaldoxime, applied as concentrations ranging from 0.1 mM to 2 mM, is able to prolong action potential duration and block dose dependently the transient outward K^+ current and the slow inward Ca^{2+} current in isolated rat cardiac myocytes. Both currents were affected to a similar extent. However, in some cases the blockade of the transient outward K^+ was voltage-dependent while the Ca^{2+} was not. The increase in action potential duration can easily be understood to result from the block of the transient outward K^+ current, because this current determines the speed of early repolarization. The latter has been shown to be a main determinant of the sequence of activation and inactivation of the other voltage-dependent channels sustaining the plateau depolarization. At the same time salicylaldoxime reduces the slow inward Ca^{2+} current which also influences the plateau. In our studies of peak current, the K^+ current was clearly larger than the Ca^{2+} current. Because the inactivation of the transient outward K^+ current is fairly slow (time-constant of inactivation is about 40 ms), the prolongation of the action potential, albeit variable at high salicylaldoxime concentrations, could be explained by the blockade of the transient outward K^+ current. With high salicylaldoxime concentrations the action potential duration varied considerably, probably due to its effect on the Ca^{2+} current. It is possible that 5.0 mM salicylaldoxime blocks Na^+ current because such concentrations decreased the action potential amplitude by about 50%. We showed a difference in the washout characteristics of salicylaldoxime between the transient outward K^+ and the slow inward Ca^{2+} currents. However, the block of the slow inward current may also be reversible as in the case of transient outward current, but, because of the quick rundown of Ca^{2+} currents (Coulombe et al., 1990), the complete recovery of the slow inward Ca^{2+} current cannot be seen under these experimental conditions.

The effects of salicylaldoxime we have observed are similar to the results obtained for another oxime-containing compound, 2,3-butanedione monoxime (Coulombe et al., 1990). It also prolonged action potential duration and blocked both the transient outward K^+

current and the slow inward Ca^{2+} current. However, in these respects salicylaldoxime is at least 10 × more potent, perhaps due to its more lipophilic structure. 2,3-Butanedione monoxime has been shown to have a number of effects in addition to those on ionic currents (Sellin and McArdle, 1994). It is possible that salicylaldoxime could also have a number of other effects which were not investigated here.

The potential importance of the transient outward K^+ current in the origin of ventricular arrhythmias and as a target for Class III antiarrhythmic drugs has been suggested (Ten Eick et al., 1993; Wettwer et al., 1992; Näbauer et al., 1992; Karhu et al., 1994; Sanguinetti, 1992). It is well-known that transient coronary artery occlusion can lead to malignant ventricular arrhythmias during ischemia and reperfusion (Ferrier et al., 1985; Janse and Kleber, 1981; Penkoske et al., 1978). One prominent ischemia and reperfusion-induced alteration is an increase in the electrophysiological heterogeneity between the epicardium and the endocardium (Gilmour and Zipes, 1980; Kimura et al., 1986; Boineau and Cox, 1973; Scherlag et al., 1974; Ruffy et al., 1979). A rise in intracellular Ca^{2+} during reperfusion can produce delayed afterdepolarizations (Marban et al., 1989; Kihara et al., 1989; Corr and Witkowski, 1983; Antzelevitch and Sicouri, 1994). The large epimyocardial transient outward K^+ current may contribute to this electrical inhomogeneity (Litovsky and Antzelevitch, 1988, Litovsky and Antzelevitch, 1989) and to the genesis of ventricular arrhythmias via phase 2 re-entry. The finding that blockers of this current such as 4-aminopyridine can reverse this high Ca^{2+}-induced heterogeneity suggests that compounds which block the transient outward K^+ current could exert an antiarrhythmic effect (Di Diego and Antzelevitch, 1994).

Recently, a potential new antiarrhythmic drug, which blocks K^+ and Ca^{2+} currents has been shown to be effective against arrhythmias initiated by both re-entry and triggered activity (Bril et al., 1994). Compounds with this profile, like salicylaldoxime, may represent a novel Class III antiarrhythmic effect to counteract re-entry arrhythmias but with a reduced proarrhythmic risk.

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References

Antzelevitch, C. and S. Sicouri, 1994, Clinical relevance of cardiac arrhythmias generated by afterdepolarizations: the role of M cells in the generation of U waves, triggered activity and Torsades de Pointes, J. Am. Coll. Cardiol. 23, 259.

Arena, J. and R. Kass, 1988, Block of heart potassium channels by clofilium and its tertiary analogs: relationship between drug structure and type of channel blocked, Mol. Pharmacol. 34, 60.

Boineau, J.P. and J.L. Cox, 1973, Slow ventricular activation in acute myocardial infarction: a source of reentrant premature ventricular contractions, Circulation 48, 702.

Bril, A., B. Gout, M. Bonhomme, L. Landais, J.F. Faivre, P. Linee, R.R. Ruffolo and R.H. Poyser, 1994, Prevention by BRL-32872, a novel class III antiarrhthmic agent with calcium antagonist properties, of arrhythmias induced by re-entry without inducing triggered activities, Eur. Heart J. 15 (Suppl.), 189.

Campbell, D.L., R.L. Rasmussen, Y. Qu and H.C. Strauss, 1993, The calcium-independent transient outward potassium current in isolated ferret right ventricular myocytes. (I. Basic characterization and kinetic analysis), J. Gen Physiol. 101, 571.

Corr, P.B. and F.X. Witkowski, 1983, Potential electrophysiologic mechanismfor disrhythmias associated with reperfusion of ischemic myocardium, Circulation 68, I16.

Coulombe, A., I.A. Lefevre, E. Deroubaix, D. Thuringer and E. Coraboeuf, 1990, Effect of 2,3-butanedione monoxime on the slow inward and transient outward currents in rat ventricular myocytes, J. Mol. Cell. Cardiol. 22, 921.

Di Diego, J.M. and C. Antzelevitch, 1994, High $[Ca^{2+}]_o$-induced electrical heterogeneity and extrasystolic activity in isolated canine ventricular epicardium. Phase 2 reentry, Circulation 89, 1839.

Ferrier, G.R., M.P. Moffat and A. Lukas, 1985, Possible mechanisms of ventricular arrhythmias elicited by ischemia followed by reperfusion: studies on isolate canine ventricular tissue, Circ. Res. 56, 184.

Gilmour, R.F. and D.P. Zipes, 1980, Different electrophysiological responses of canine endocardium and epicardium to combined hyperkalemia, hypoxia and acidosis, Circ. Res. 46, 814.

Hamill, O.P., A. Marty, E. Neher, B. Sakmann and F.J. Sigworth, 1981, Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches, Pflügers Arch. 391, 85.

Janse, M.J. and A.G. Kleber, 1981, Electrophysiological changes and ventricular arrhythmias in the early phases of regional myocardial ischemia, Circ. Res. 49, 1069.

Josephson, I.R., J. Sanchez-Chapula and A.M. Brown, 1984, Early outward current in rat single ventricular cells, Circ. Res. 54, 157.

Karhu, S., M. Weckström, T. Kivistö and L.C. Sellín, 1994, Salicylaldoxime blocks the transient outward K^+ current in rat ventricular myocytes, Biophys. J. 66 (2), A63.

Kihara, Y., W. Grossman and J.P. Morgan, 1989, Direct measurement of changes in intracellular calcium transients during hypoxia, ischemia and reperfusion in the intact mammalian heart, Circ. Res. 65, 1029.

Kimura, S., A.L. Bassett, T. Kohya, P.L. Kozlovskis and R.J. Myerburg, 1986, Simultaneous recording of action potentials from endocardium and epicardium during ischemia in the isolated cat ventricle. Relation of temporal electrophysioogic heterogeneities to arrhythmias, Circulation 74, 401.

Litovsky, S.H. and C. Antzelevitch, 1988, Transient outward current prominent in canine ventricular epicardium but not endocardium, Circ. Res. 62, 116.

Litovsky, S.H. and C. Antzelevitch, 1989, Rate dependence of action potential duration and refractoriness in canine ventricular endocardium differs from that of epicardium: the role of transient outward current, J. Am. Coll. Cardiol. 14, 1053.

Marban, E., Y. Koretsune, M. Corretti, V.P. Chacko and H. Kusuoka, 1989, Calcium and its role in myocardial cell injury during iscemia and reperfusion, Circulation 80 (Suppl. IV), 17.

Näbauer, M., D.J. Beuckelmann and E. Erdmann, 1992, Presence of a large transient outward current in human ventricular myocytes, Circulation (Suppl.) 86 (4), I-617.

Penkoske, P.A., B.E. Sobel and P.B. Corr, 1978, Disparate electrophysiological alterations accompanying dysrhythmia due to coronary occlusion and reperfusion in the cat, Circulation 58, 1023.

Ruffy, R., E.D. Lovelace, S.B. Knoebel, T.M. Mueller and D.P. Zipes, 1979, Relationship between changes in left ventricular bipolar electrograms and regional myocardial blood flow during acute coronary acclusion in the dog, Circ Res. 45, 764.

Sanguinetti, M.C., 1992, Modulation of potassium channels by antiarrhythmic and antihypertensive drugs, Hypertension 19, 228.

Scherlag, B.J., N. El-Sherif, R. Hope and R. Lazzara, 1974, Characterization and localization of ventricular arrhythmias resulting from myocardial ischemia and infarction, Circ. Res. 35, 372.

Sellin, L.C. and J.J. McArdle, 1994, Multiple effects of 2,3-butanedione monoxime, Pharmacol. Toxicol. 74, 305.

Ten Eick, R.E., K. Zhang, R.D. Harvey and A.L. Bassett, 1993, Enhanced functional expression of transient outward current in hypertrophied feline myocytes, Cardiovasc. Drugs Ther. 7, 611.

Varro, A. and J.G. Papp, 1992, The impact of single cell voltage clamp on the understanding of the cardiac ventricular action potential, Cardioscience 3, 131.

Wettwer, E., J. Gath, G. Hauser, T. Mewes, J.C. Reidmeister and U. Ravens, 1992, Transient outward current in human and rat ventricular cardiomyocytes, Circulation (Suppl.) 86 (4), I-617.