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Inhibition of the Na^+/H^+ exchanger attenuates phase 1b ischemic arrhythmias and reperfusion-induced ventricular fibrillation ☆

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Abstract

The sodium–hydrogen exchanger-isotype 1 (NHE-1) plays a critical role in myocardial ischemia–reperfusion injury. While studies employing less selective sodium–hydrogen inhibitors have demonstrated antiarrhythmic activity, only one study has examined the in vivo efficacy of selective NHE-1 inhibition in a canine model of ischemia–reperfusion-induced arrhythmia. In the present study, the antiarrhythmic activity of Benzamide, *N*-(aminoiminomethyl)-4-[4-(2-furanylcarbonyl)-1-piperazinyl]-3-(methylsulfonyl), methanesulfonate (BIIB 513), a novel NHE-1 inhibitor, was examined. An in vivo canine model of myocardial ischemia–reperfusion injury in which 60 min of left anterior descending coronary artery (LAD) occlusion followed by 3 h of reperfusion was employed. BIIB 513 was infused either prior to ischemia or prior to reperfusion. Arrhythmias were quantified by single lead electrocardiogram. Infarct size, determined by triphenyltetrazolium staining, was expressed as a percent of the area-at-risk. In vivo, NHE-1 inhibition did not affect phase 1a arrhythmias, which occur within the first 10 min of occlusion, however, BIIB 513 significantly reduced the incidence of ischemia-induced phase 1b arrhythmias which occur between 10 and 30 min following occlusion and the incidence of reperfusion-induced ventricular fibrillation. Furthermore, NHE-1 inhibition significantly reduced infarct size, when the drug was administered either prior to ischemia or prior to reperfusion. NHE-1 inhibition selectively reduces both ischemia-induced phase 1b arrhythmias and reperfusion-induced ventricular fibrillation, and also markedly reduces myocardial infarct size when the drug is administered prior to ischemia or prior to reperfusion.

Keywords: Sodium–hydrogen exchanger; Arrhythmias; Myocardial infarct size

1. Introduction

1.1. General

With myocardial ischemia, mitochondrial ATP production ceases and glycolysis results in the depletion of ATP and an increase in intracellular H^+ (Dennis et al., 1991) which activates the Na^+/H^+-exchanger resulting in the extrusion of H^+ and the influx of Na^+ (Frelin et al., 1984; Lazdunski et al., 1985). Increases in intracellular Na^+ correlate with increases in intracellular Ca^{2+} via either reversal or inhibition of the Na^+/Ca^{2+} exchanger (Frelin et al., 1984; Tani and Neely, 1989, 1990). Thus, the net effect of increasing intracellular sodium is an accumulation of Ca^{2+} in the ischemic myocardium which contributes to cellular damage resulting in arrhythmias and contraction band necrosis (Steenbergen et al., 1990; Tani, 1990; Tani and Neely, 1990).

With reperfusion, extracellular H^+ rapidly decreases establishing a large intracellular to extracellular H^+ gradient. Activity of the Na^+/H^+ exchanger results in an increase in intracellular Na^+, which, via effects on the Na^+/Ca^{2+} exchanger, contributes to an abnormally large accumulation of Ca^{2+} during reperfusion (DuToit and Opie, 1992; Tani and Neely, 1989) which contributes to reperfusion arrhythmias, myocardial contracture and necrosis (Steenbergen et al., 1990). Inhibition of the Na^+/H^+ exchanger has been shown to have cardioprotective effects in a variety of in vivo and in vitro animal models (Karmazyn, 1996).

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While a number of studies have demonstrated that inhibition of the Na^+/H^+ exchanger isoform type-1(NHE-1) protects against myocardial infarction, the vast majority of these experiments have been conducted in the isolated hearts or in the isolated cardiomyocytes (Karmazyn, 1996) using less specific inhibitors of the NHE-1 isoform, the predominant isoform in cardiomyocytes (Orlowski et al., 1992).

NHE inhibition also has been reported to reduce both ischemia- and reperfusion-induced arrhythmias (Fukuta et al., 1996; Sack et al., 1994; Scholz et al., 1995; Xue et al., 1996; Yasutake et al., 1994). In dogs, ischemia-induced arrhythmias occur in two discrete phases; an early period of arrhythmias termed phase 1a occurs between 0–10 min of ischemia while a late period of arrhythmias termed phase 1b occurs from 10–30 min of ischemia (Kaplinsky et al., 1979). While the mechanisms of phase 1 arrhythmias- and reperfusion-induced arrhythmias are thought to differ, an evidence suggests a role of the sodium–hydrogen exchanger in both mechanisms (Fukuta et al., 1996; Sack et al., 1994; Scholz et al., 1995; Schomig et al., 1984, 1988; Xue et al., 1996; Yasutake et al., 1994). However, to date, the effect of selective NHE-1 inhibition on both ischemia- and reperfusion-induced arrhythmias secondary to a moderate ischemic insult has not been adequately examined in vivo and is the main objective of the present study.

2. Materials and methods

2.1. Animal welfare

All experiments were conducted in an American Association of Laboratory Animal Care (AAALAC)-approved facility and in accordance with the ''Position of the American Heart Association on Research and Animal Use'', 1984, as well as the guidelines of the Animal Care Committee of the Medical College of Wisconsin.

2.2. Materials

The previously described specific and selective NHE-1 inhibitor Benzamide, *N*-(aminoiminomethyl)-4-[4-(2-furanylcarbonyl)-1-piperazinyl]-3-(methylsulfonyl),methanesulfonate (BIIB 513) was used in the current study (Gumina et al., 1999). All reagents were obtained from Sigma Chemical (St. Louis, MO) or Gibco BRL (Gathersburg, MD) unless otherwise indicated.

2.3. Ischemia–reperfusion protocol

A standard myocardial ischemia–reperfusion protocol, on which we have previously published, was employed (Gumina et al., 1998). Briefly, adult mongrel dogs of either sex, weighing 18.5–25.3 kg, were fasted overnight, anesthetized with a combination of sodium barbital (200 mg/kg) and sodium pentobarbital (15 mg/kg), and ventilated by a respirator with room air supplemented with 100% oxygen. Arterial blood pH, P_{CO_2} and P_{O_2} were monitored at selected intervals by an automatic blood gas system (AVL 995, AVL Scientific). Aortic blood pressure and left ventricular pressure were monitored via a double-pressure transducer-tipped catheter (PC 771, Millar Instruments). Left ventricular dP/dt was recorded by electronic differentiation of the left ventricular pressure pulse, and heart rate was determined by a tachometer. The right femoral vein and artery were cannulated for drug administration and for blood gas analysis and measurement of the reference blood flow used to determine myocardial tissue blood flow. A left thoracotomy was performed at the fifth intercostal space, the lung was carefully retracted, the pericardium incised, and the heart suspended in a pericardial cradle. A proximal portion of the left anterior descending coronary artery (LAD) distal to the first diagonal branch was isolated from the surrounding tissue. A mechanical occluder was placed around the vessel to produce ischemia. A continuous electrocardiogram for monitoring arrhythmias and ventricular fibrillation was obtained via single lead electrodes placed at the apex of the heart. If the basal heart rate was < 150 beats/min, the heart was paced as previously described (Gumina et al., 1998). Hemodynamics, heart rate and left anterior descending coronary blood flow were monitored and recorded by a polygraph (Model 7, Grass Instruments). The left atrium was cannulated via the appendage for radioactive microsphere injection.

Fig. 1 shows the protocols used in this study. Dogs were assigned to one of four groups. All dogs were subjected to 60 min of left anterior descending coronary occlusion and 3 h of reperfusion. In groups 1–3, either saline (control group) or one of two doses of BIIB 513 (0.75 or 3.0 mg/kg) were infused intravenously for 15 min immediately prior to coronary artery occlusion. In group 4, one dose of BIIB 513 (3.0 mg/kg) was infused

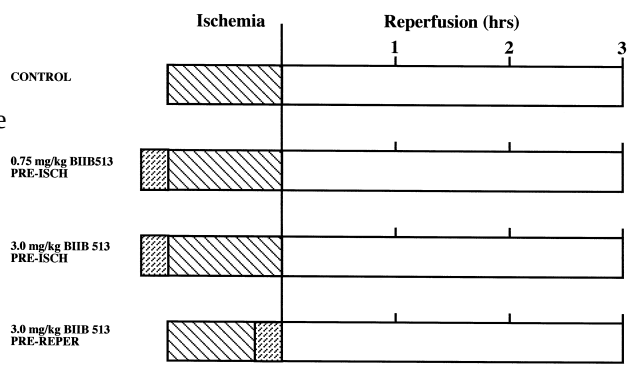

Fig. 1. Experimental protocols used to examine the effects of NHE-1 inhibition on myocardial infarct size. Dogs were assigned to one of the four groups. All animals were subjected to 60 min of LAD occlusion and 3 h of reperfusion.

intravenously for 15 min prior to reperfusion. In all groups, hemodynamic measurements and arterial blood gas analysis were obtained prior to coronary artery occlusion, at 30 min during the 60-min occlusion, and every hour following reperfusion. Regional myocardial blood flows were determined at 30 min during the 60-min occlusion period and at the end of the experiment.

At the end of the 3 h reperfusion period, the anatomic area-at-risk and the non-ischemic area were differentiated as previously described (Gumina et al., 1998). The hearts were electrically fibrillated, removed and prepared for infarct size determination and regional myocardial blood flow measurements. The left ventricle was dissected and sliced into serial transverse sections from 6 to 7 mm wide. The non-stained ischemic area and the blue-stained normal area were separated, and both regions were incubated at 37°C for 15 min in 1% 2,3,5 triphenyltetrazolium chloride (TTC) in 0.1 mol/l phosphate buffer adjusted to pH 7.4. After storage overnight in 10% formaldehyde, infarcted and noninfarcted tissues within the area-at-risk were separated and determined gravimetrically. Infarct size was expressed as a percent of the area-at-risk. Regional myocardial blood flow was measured by the radioactive microsphere technique as described previously in this laboratory (Gumina et al., 1998).

Dogs were excluded if: (1) heartworms were found after the dogs were sacrificed, (2) transmural collateral blood flow was > 0.20 ml min^{-1} g^{-1}, (3) heart rate was > 180 beats/min at the beginning of the experiment, or (4) more than three consecutive attempts were needed to convert ventricular fibrillation with low-energy direct current pulses.

2.4. Statistical analysis

All values are expressed as mean ± SEM unless otherwise noted. Differences between groups in hemodynamics and blood gases were compared by the use of a two-way (for time and treatment) analysis of variance with repeated measures. Differences between groups in tissue blood flows, area-at-risk, infarct size, the quantity of arrhythmias during ischemia, and the incidence of ventricular fibrillation during reperfusion were compared by one-way analysis of variance and comparisons between individual groups were made with a two tailed *t*-test. An analysis of covariance was used to determine whether the relation between transmural collateral blood flow and infarct size differed between the control and drug-treated groups.

3. Results

3.1. Hemodynamic and blood gas data

Table 1 summarizes the hemodynamic data. There were no significant differences within or between groups throughout the experiment with regard to heart rate, mean arterial pressure, rate–pressure product and left ventricular

Table 1
Hemodynamics in the different treatment groups

	Baseline	After 15 min drug treatment	Occlusion		Reperfusion		
			30 min	60 min	1 h	2 h	3 h
HR (beats / min)							
Control	151 ± 3	–	154 ± 4	152 ± 5	148 ± 3	146 ± 4	147 ± 3
0.75 mg/kg BIIB 513 pre-ischemic	153 ± 2	151 ± 3	154 ± 4	152 ± 4	154 ± 5	149 ± 6	152 ± 4
3 mg/kg BIIB 513 pre-ischemic	150 ± 2	150 ± 2	152 ± 2	151 ± 5	149 ± 4	151 ± 6	152 ± 6
3 mg/kg BIIB 513 pre-reperfusion	152 ± 4	–	151 ± 3	149 ± 3	154 ± 2	153 ± 3	154 ± 3
MBP (mm Hg)							
Control	98.9 ± 5.8	–	93.0 ± 4.6	88.5 ± 6.2	93.5 ± 4.8	102.0 ± 4.3	102.6 ± 4.8
0.75 mg/kg BIIB 513 pre-ischemic	104.4 ± 7.5	114.8 ± 7.1	98.0 ± 8.3	94.0 ± 5.9	103.3 ± 5.2	110.4 ± 7.0	109.7 ± 4.1
3 mg/kg BIIB 513 pre-ischemic	103.9 ± 9.1	110.7 ± 11.0	100.7 ± 8.9	101.8 ± 6.6	102.0 ± 4.8	111.9 ± 4.8	106.4 ± 5.5
3 mg/kg BIIB 513 pre-reperfusion	105.7 ± 5.0	–	105.3 ± 6.3	104.8 ± 7.7	96.5 ± 4.5	107.0 ± 4.4	106.3 ± 4.7
RPP (mm Hg min^{-1} 1000^{-1})							
Control	16.7 ± 1.2	–	16.3 ± 1.1	15.6 ± 1.4	15.7 ± 0.8	16.4 ± 0.7	16.8 ± 0.9
0.75 mg/kg BIIB 513 pre-ischemic	17.8 ± 1.8	19.3 ± 1.6	16.9 ± 1.8	15.9 ± 1.4	17.6 ± 1.0	18.3 ± 1.5	18.4 ± 0.8
3 mg/kg BIIB 513 pre-ischemic	17.4 ± 1.4	18.8 ± 1.7	17.2 ± 1.4	17.4 ± 1.1	17.2 ± 0.8	19.1 ± 0.8	18.1 ± 0.8
3 mg/kg BIIB 513 pre-reperfusion	17.4 ± 0.9	–	17.4 ± 1.1	17.1 ± 1.1	16.5 ± 0.7	18.1 ± 0.7	18.1 ± 0.8
LV dP / dt (mm Hg / s)							
Control	1931 ± 254	–	1856 ± 172	1950 ± 192	1594 ± 98	1594 ± 75	1537 ± 68
0.75 mg/kg BIIB 513 pre-ischemic	1819 ± 213	2044 ± 181	1875 ± 188	1763 ± 138	1594 ± 89	1613 ± 97	1575 ± 57
3 mg/kg BIIB 513 pre-ischemic	2156 ± 127	2381 ± 217	2231 ± 291	2175 ± 221	1819 ± 111	1988 ± 126	1743 ± 144
3 mg/kg BIIB 513 pre-reperfusion	1819 ± 100	–	1894 ± 170	1781 ± 140	1706 ± 69	1781 ± 66	1762 ± 62

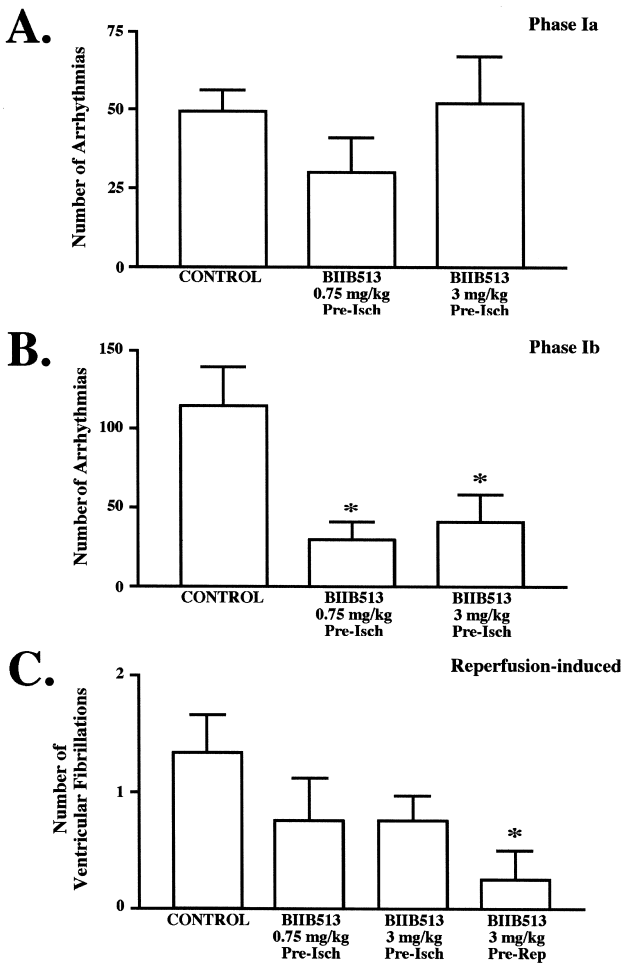

Fig. 2. Effect of NHE-1 inhibition on arrhythmias. BIIB 513 (0.75 or 3.0 mg/kg) or saline was administered either 15 min prior to the occlusion of the LAD for 60 min or BIIB 513 (3.0 mg/kg) was administered 15 min prior to the reperfusion of the LAD. The number of arrhythmias occurring per dog either during ischemia or reperfusion was quantitated. (A) Ischemia-induced phase 1a arrhythmias (0–10 min of ischemia). (B) Ischemia-induced phase 1b arrhythmias (10–30 min of ischemia). (C) Reperfusion-induced ventricular fibrillation (0–60 min of reperfusion). All values are the mean ± SEM ($N = 8$–12). $^{*}p < 0.05$ vs. the control group.

dP/dt. Blood gases were stable throughout the experiment and there were no differences between groups at any time measured.

3.2. Arrhythmia data

The incidence of ischemia-induced phase 1a and 1b arrhythmias, as well as the incidence of reperfusion-induced ventricular fibrillation, was examined. Phase 1 arrhythmias were predominantly premature ventricular contractions (PVCs). While NHE-1 inhibition with BIIB 513 had no significant effect on the number of phase 1a arrhythmias, phase 1b arrhythmias (PVCs) were reduced significantly by the administration of either 0.75 or 3.0 mg/kg of BIIB 513 ($p < 0.05$) (Fig. 2A and B). While the administration of either 0.75 or 3.0 mg/kg of BIIB 513 prior to ischemia reduced the incidence of ventricular fibrillation, the reduction observed was not statistically significant. However, administration of 3.0 mg/kg of BIIB 513 fifteen minutes prior to reperfusion resulted in a significant reduction in the incidence of ventricular fibrillation upon reperfusion ($p < 0.05$) (Fig. 2C).

3.3. Infarct size

Table 2 summarizes the effect of NHE-1 inhibition prior to ischemia or prior to reperfusion on the area-at-risk expressed as a percent of the left ventricle and infarct size expressed as a percent of the area at risk. Both pre-ischemic and pre-reperfusion administration of BIIB 513 resulted in significant ($p < 0.05$) reductions in infarct size expressed as percent of left ventricle and area-at-risk (Table 2). The reduction of infarct size was greater in the two groups treated prior to ischemia, however, only the high dose was significantly different from the group treated prior to reperfusion. There were no significant differences in left ventricular weight, area-at-risk, or area-at-risk expressed as the percent of the left ventricle between groups (Table 2). There were also no differences in transmural collateral blood flow between groups, indicating that all groups were subjected to equivalent degrees of ischemia (Table 2). However, when transmural collateral blood flows were plotted vs. infarct size, the regression lines describing this relationship in BIIB 513-treated animals were shifted down as compared to the control group (data not shown).

Table 2
Infarct size data and transmural blood flow data
All values are the mean ± SEM.

Group	n	Weight (g)			Calculated data (%)			Transmural blood flow (ml min^{-1} g^{-1}) ischemic region at 30 min occulation
		LV	AAR	IS	AAR/LV	IS/AAR	IS/LV	
Control	8	99.2 ± 6.7	32.7 ± 2.5	8.4 ± 1.1	33.1 ± 1.5	25.3 ± 2.0	8.4 ± 0.9	0.04 ± 0.01
BIIB 513 (0.75 mg/kg) PRE	8	104.7 ± 4.8	37.2 ± 2.3	3.1 ± 1.4[a]	35.8 ± 2.2	8.0 ± 3.3[a]	2.9 ± 1.3[a]	0.06 ± 0.02
BIIB 513 (3.0 mg/kg) PRE	8	96.2 ± 5.3	31.6 ± 2.5	1.6 ± 0.7[a]	32.8 ± 1.8	4.3 ± 1.7[a]	1.5 ± 0.6[a]	0.08 ± 0.01
BIIB 513 (3.0 mg/kg) POST	8	105.2 ± 6.5	35.9 ± 3.4	5.0 ± 1.4	34.3 ± 2.7	12.8 ± 2.9[a]	4.8 ± 1.2[a]	0.09 ± 0.02

[a] $p < 0.05$.

4. Discussion

4.1. General

This study examined the effects of selective NHE-1 inhibition by BIIB 513, a new selective NHE-1 inhibitor, on ischemia–reperfusion-induced arrhythmias in vivo. Inhibition of NHE-1 not only significantly reduced myocardial infarct size, but also selectively reduced ischemia-induced phase 1b arrhythmias and reperfusion-induced ventricular fibrillation. These data provide evidence that NHE-1 inhibition in vivo not only reduces myocardial infarction but also attenuates potentially lethal arrhythmias. Based upon these results, NHE-1 inhibition may prove an efficacious adjunct therapy to current reperfusion strategies that not only reduce myocardial infarction but also confer anti-arrhythmic activity.

4.2. Antiarrhythmic efficacy of NHE-1 inhibition

The underlying mechanisms of ischemia and reperfusion arrhythmias are known to be different (Corbalan et al., 1976). An adrenergic mechanism is one factor which is thought to play a role in ischemia-induced arrhythmias (Corbalan et al., 1976; Penny, 1984; Schomig and Richardt, 1990; Schomig et al., 1984, 1988). During myocardial ischemia, locally mediated mechanisms result in neuronal release and extracellular accumulation of catecholamines within the ischemic area (Schomig et al., 1984). Previous studies have demonstrated that the inhibition of NHE activity markedly attenuates ischemia-induced catecholamine release (Schomig et al., 1988) and we postulate that the inhibition of ischemia-induced catecholamine release may contribute to the protective effects of NHE inhibition (Yasutake et al., 1994). However, it is recognized that this hypothesis is purely speculative and that further experiments in which catecholamine release is measured are necessary to directly test this theory. Another possibility is that NHE-1 inhibition may directly reduce intracellular sodium and calcium overload (Karmazyn, 1996) and reduces the incidence of ischemia-induced arrhythmias by this mechanism.

Previously, NHE inhibition was shown to reduce PVCs and the incidence of ventricular fibrillation during the 30 min of coronary artery ligation in rats (Scholz et al., 1995). Recently, it has been reported that in dogs, NHE inhibition with HOE 642 did not decrease the number of PVCs during ischemia (Xue et al., 1996). In contrast, the current study in dogs demonstrates a reduction in ischemia-induced PVCs.

4.3. Effect of NHE inhibition on reperfusion-induced ventricular fibrillation

NHE-1 inhibition not only diminished ischemia-induced arrhythmias, but also significantly reduced the incidence of reperfusion-induced ventricular fibrillation. This reduction in the incidence of ventricular fibrillation was more pronounced when BIIB 513 was administered prior to reperfusion. Similar results have been reported in a canine model using the NHE-1 inhibitor HOE 642 (Xue et al., 1996). Reperfusion-induced arrhythmias are postulated to be related to increased automaticity secondary to the increase of intracellular calcium that occurs during ischemia and reperfusion (Steenbergen et al., 1990; Tani and Neely, 1989) as well as Ca^{2+} oscillations (Ladilov et al., 1995). As discussed above, NHE inhibition prevents Ca^{2+} accumulation (Steenbergen et al., 1990; Tani and Neely, 1989), decreases Ca^{2+} oscillations in isolated myocytes (Ladilov et al., 1995), and prevents hypercontraction in isolated cardiomyocytes (Gumina et al., in press; Ladilov et al., 1995). In addition, inhibition of NHE-1 has been shown to significantly reduce reperfusion-induced ventricular fibrillation in isolated rat hearts (Scholz et al., 1995; Yasutake et al., 1994) and in vivo in the rat heart (Scholz et al., 1995; Xue et al., 1996), porcine heart, (Fukuta et al., 1996; Sack et al., 1994), and canine heart (Xue et al., 1996). Based upon the attenuation of hypercontracture observed in isolated myocytes (Gumina et al., in press), we postulate that NHE-1 inhibition via BIIB 513 reduces Ca^{2+} accumulation and/or oscillations, which results in the observed reduction of reperfusion-induced ventricular fibrillation. Furthermore, neutrophil activity which has been shown to contribute to reperfusion-induced arrhythmias (Dhein et al., 1995) is also attenuated by NHE-1 inhibition with BIIB 513 (Gumina et al., in press). Thus, the observed reduction in reperfusion-induced ventricular fibrillation may be due, in part, to inhibitory effects of BIIB 513 on neutrophil activity. Again, this hypothesis has not been directly tested and awaits further experimentation.

4.4. Conclusions

Pharmacological inhibition of the sodium–hydrogen exchanger isotype 1 with BIIB 513 significantly reduced ischemia-induced arrhythmias and reperfusion-induced ventricular fibrillation. A reduction in Ca^{2+} overload may be the final common pathway by which NHE-1 inhibition reduces both ischemia and reperfusion induced arrhythmias in vivo. NHE-1 inhibition may prove an efficacious adjunct therapy to current treatment strategies of myocardial infarction that not only reduces infarct size but also confers antiarrhythmic activity.

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References

Corbalan, R., Verrier, R., Lown, B., 1976. Differing mechanisms for ventricular vulnerability during coronary artery occlusion and release. Am. Heart J. 92, 223–230.

Dennis, S., Gevers, W., Opie, L., 1991. Protons in ischemia: where do they come from; where do they go to? J. Mol. Cell. Cardiol. 23, 1077–1086.

Dhein, S., Schott, M., Gottwald, E., Muller, A., Klaus, W., 1995. The contribution of neutrophils to reperfusion arrhythmias and a possible role for antiadhesive pharmacological substances. Cardiovasc. Res. 30, 881–889.

DuToit, E., Opie, L., 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.

Frelin, C., Vigne, P., Lazdunski, M., 1984. The role of Na^+/H^+ exchange system in cardiac cells in relation to the control of internal Na^+ concentration. A molecular basis for the antagonistic effect of ouabain and amiloride on the heart. J. Biol. Chem. 259, 8880–8885.

Fukuta, M., Wakida, Y., Uesugi, M., Kobayashi, T., 1996. Role of Na^+–H^+ exchange on reperfusion related myocardial injury and arrhythmias in an open-chest swine model. Pacing Clin. Electrophys. 19, 2027–2032.

Gumina, R., Mizumura, T., Beier, N., Schelling, P., Schultz, J., Gross, G., 1998. A new Na^+/H^+ exchange (NHE-1) inhibitor, EMD 85131, limits infarct size in dogs when administered prior to or after coronary artery occlusion. J. Pharmacol. Exp. Ther. 286, 175–183.

Gumina, R., Buerger, E., Eickmeier, C., Daemmgen, J., Gross, G., 1999. Inhibition of the Na^+/H^+ exchanger confers greater cardioprotection against 90 minutes of myocardial ischemia than ischemic preconditioning in dogs. Circulation 100, 2519–2526.

Gumina, R., Auchampach, J., Wong, R., Buerger, E., Eickmeier, C., Moore, J., Daemmgen, J., in press. Na^+/H^+ exchange inhibition-induced cardioprotection in dogs: effects on neutrophils versus cardiomyocytes.

Kaplinsky, E., Ogawa, S., Blake, C., Dreifus, L., 1979. Two periods of early ventricular arrhythmias in the canine acute infarction model. Circulation 60, 397–404.

Karmazyn, M., 1996. The sodium–hydrogen exchange system in the heart: its role in ischemic and reperfusion injury and therapeutic implications. Can. J. Card. 12, 1074–1082.

Ladilov, Y., Siegmund, S., Piper, H., 1995. Protection of reoxygenated cardiomyocytes against hypercontracture by inhibition of Na^+/H^+ exchange. Am. J. Physiol. 268, H1531–H1539.

Lazdunski, M., Frelin, C., Vigne, P., 1985. The sodium/hydrogen exchange system in cardiac cells: its biochemical and pharmacological properties and its role in regulating internal concentrations of sodium and internal pH. J. Mol. Cell. Cardiol. 17, 1029–1042.

Orlowski, J., Kandasamy, R., Schull, G., 1992. Molecular cloning of putative members of the Na^+/H^+ exchanger gene family — cDNA cloning, deduced amino acid sequence, and mRNA tissue expression of the rat Na^+/H^+ exchanger NHE-1 and two structurally related proteins. J. Biol. Chem. 267, 9331–9339.

Penny, W., 1984. The deleterious effects of myocardial catecholamines on cellular electrophysiology and arrhythmias during ischaemia and reperfusion. Eur. Heart J. 5, 960–967.

Sack, S., Mohri, M., Schwarz, E., Arrar, M., Schaper, J., Ballagi-Pordany, G., Scholz, W., Lang, H., Scholkens, B., Schaper, W., 1994. Effects of a new Na^+/H^+ antiporter inhibitor on postischemic reperfusion in pig heart. J. Cardiovasc. Pharmacol. 23, 72–78.

Scholz, W., Albus, U., Counillon, L., Gogelein, H., Lang, H., Linz, W., Weichert, A., Scholens, B., 1995. Protective effects of HOE 642, a selective sodium–hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. Cardiovasc. Res. 29, 260–268.

Schomig, A., Richardt, G., 1990. The role of catecholamines in ischemia. J. Cardiovasc. Pharmacol 16 (Suppl. 5), S105–S110.

Schomig, A., Dart, A., Dietz, R., Mayer, E., Kuebler, W., 1984. Release of endogenous catecholamines in the ischemic myocardium of the cat. Part A: locally mediated release. Circ. Res. 55, 689–696.

Schomig, A., Kurz, T., Richardt, G., Schomig, E., 1988. Neuronal sodium homeostasis and axoplasmic amine concentration determine calcium-independent noradrenaline release in normoxic and ischemic rat heart. Circ. Res. 63, 214–222.

Steenbergen, C., Perlman, M., London, R., Murphy, E., 1990. Correlation between cytosolic free calcium, contracture, ATP, and irreversible ischemic injury in perfused rat heart. Circ. Res. 66, 135–142.

Tani, M., 1990. Mechanisms of Ca^{2+} overload in reperfused ischemic myocardium. Annu. Rev. Physiol. 52, 543–559.

Tani, M., Neely, J., 1989. Role of intracellular Na^+ in Ca^{2+} overload and depressed recovery of ventricular function of reperfused ischemic rat hearts. Possible involvement of H^+–Na^+ and Na^+–Ca^{2+} exchange. Circ. Res. 65, 1045–1056.

Tani, M., Neely, J., 1990. Na^+ accumulation increases Ca^{++} overload and impairs function in anoxic rat heart. J. Mol. Cell. Cardiol. 22, 57–72.

Xue, Y., Aye, N., Hashimoto, K., 1996. Antiarrhythmic effects of HOE 642, a novel Na^+–H^+ exchange inhibitor, on ventricular arrhythmias in animal hearts. Eur. J. Pharmacol. 317, 316–319.

Yasutake, M., Ibuki, C., Hearse, D., Avkiran, M., 1994. Na^+/H^+ exchange and reperfusion arrhythmias: protection by intracoronary infusion of a novel inhibitor. Am. J. Physiol. 267, H2430–H2440.