Inhibition by KB-R7943 of the Reverse Mode of the Na+/Ca2+ Exchanger Reduces Ca2+ Overload in Ischemic-Reperfused Rat Hearts

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Ca2+ influx via the Na+/Ca2+ exchanger (NCX) may lead to Ca2+ overload and myocardial injury in ischemia–reperfusion. Direct evidence that increased cytoplasmic Ca2+ concentration ([Ca2+]i) is mediated by the reverse mode of the NCX is limited, so in the present study the [Ca2+]i dynamics and left ventricular pressure were monitored in perfused beating hearts. The effects of KB-R7943 (KBR), a selective inhibitor of the NCX in the reverse mode, were analyzed during low-Na+ exposure and ischemia–reperfusion. Hearts from Sprague-Dawley rats were retrogradely perfused and loaded with 4 μmol/L fura-2 to measure the fluorescence ratio as an index of [Ca2+]i. To evaluate KBR effects on the reverse mode exchanger, the increase in [Ca2+]i induced by low-Na+ exposure (Na+: 30 mmol/L; KCl: 10 mmol/L; caffeine pre-treatment) was measured with and without 10 μmol/L KBR (n=5). In another series, the hearts were subjected to 10 min of low-flow ischemia with pacing, followed by reperfusion in the absence (n=6) or in the presence of 10 μmol/L KBR (n=6). Background autofluorescence was subtracted to estimate the ratio in the ischemia–reperfusion protocol. KBR significantly suppressed the increase in [Ca2+]i induced by low-Na+ (40.2±11.2% of control condition, p=0.014), as well as on increase in diastolic [Ca2+]i during ischemia (% increase from pre-ischemia in [Ca2+]i at 10 min: KBR, 17.9±6.4%; no KBR, 44.4±7.7%; p=0.024). After reperfusion, diastolic [Ca2+]i normalized more rapidly in KBR-treated hearts (% increase at 1 min: KBR, 4.5±7.0%; no KBR, 39.8±12.2%; p=0.03). Treatment with KBR also accelerated recovery of the rate–pressure product on reperfusion (1 min: KBR, 8,944±1,554 min–1·mmHg; no KBR, 4,970±1,325; p<0.05). Thus, inhibition of the reverse mode exchanger by KBR reduced ischemic Ca2+ overload and possibly improved functional myocardial recovery during reperfusion in a whole heart model. (Circ J 2002; 66: 390–396)

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glucose; gassed with 100% O2, pH 7.4, maintained at 37°C) was used as the perfusate and the perfusion flow rate was maintained at 13–14 ml/min with a peristaltic pump (505S, Watson Marlow, Cornwall, UK). The pulmonary vein was cannulated for measurement of left ventricular pressure (LVP) by insertion of an 18G catheter into the left ventricle (LV) with a pressure transducer and polygraph system (SEN3101, AP641G, Nihon Kohden, Tokyo, Japan). The electrocardiogram was also monitored via carbon electrodes attached to the epicardium of the LV. Pacing leads were attached to the cannulas placed in the aorta and pulmonary vein.

Fluorescence Measurements
Cytoplasmic Ca2+ concentrations were measured using a cell-permeable, Ca2+-sensitive fluorescent dye, the acetoxymethyl ester of fura-2 (fura-2; Dojindo Laboratories, Kumamoto, Japan) and an intracellular ion analyzer (CAF-110, CA-200DP; Japan Spectroscopic Co, Tokyo, Japan) as described previously. Briefly, the fluorescent dye was initially dissolved in dimethyl sulfoxide containing Cremophor EL (Sigma, St Louis, MO, USA; 25% w/v). The heart was loaded by re-circulating the Langendorff perfusion with Tyrode solution containing 4 mmol/L fura-2 for 30 min followed by washout with standard Tyrode solution for 20 min. Excitation light of an ultraviolet (UV) frequency was transferred fiberoptically from a Xe lamp to the epicardial surface of the LV. Fluorescent light was collected through another fiber and conducted to the photomultiplier. The ratio of 500 nm fluorescence intensity for the differences in fura-2 loading conditions in each heart:17,18

\[ \Delta \text{diastolic } [\text{Ca}^{2+}] \text{i} (\%) = \frac{(R - R_{\text{pre}}/A_{\text{pre}}) \times 100}{R_{\text{pre}}} \]

where R is the diastolic ratio, R_pre is the pre-ischemic diastolic ratio, and A_pre is the amplitude of the pre-ischemic ratio. Each ratio was calculated from the fluorescence intensity at 340 nm divided by the intensity at 380 nm and was expressed in arbitrary units. We chose the diastolic concentration because of its minimal variation during the cardiac cycle. Calibration of fura-2 was not performed. Throughout the experiment, we simultaneously recorded fura-2 fluorescence, LVP, and heart rate. Original tracings for fura-2 fluorescence (F340, F380), ratio (F340/F380), and LVP in an untreated heart are shown in Fig 1. The tracing representing transported Ca2+ transients (presented as the ratio) showed phasic changes, whereas the systolic and diastolic values of the fluorescence ratio remained constant.

Experimental Protocol
Series A: Low-Na+ Exposure Fig 2 is the original tracings of recordings of [Ca2+]i as the fura-2 fluorescence intensity, ratio (F340/F380) and LVP, and heart rate. Original tracings for fura-2 fluorescence (F340, F380), ratio (F340/F380), and LVP in an untreated heart are shown in Fig 1. The tracing representing transported Ca2+ transients (presented as the ratio) showed phasic changes, whereas the systolic and diastolic values of the fluorescence ratio remained constant.

Series B: Ischemia–Reperfusion Protocol In the untreated group (n=6), hearts were perfused aerobically before they were exposed to low-flow ischemia for 10 min and then reperfused. Low-flow ischemia was induced by reducing the perfusion flow rate to 10% of the pre-ischemic value, while electrical pacing (140 beats/min, 0.5–1.0 V output, 2 ms pulse) was conducted. Pacing was discontinued at the end of ischemia. We had previously confirmed metabolic changes, such as an increase in lactate and a decrease in pH levels in the coronary effluent, during low-flow ischemia. In the KBR-treated group (n=6), treatment with 10 μmol/L KBR was initiated at 5 min prior to induction of ischemia and discontinued after 1 min of reperfusion.

Statistics
Data are expressed as the means±SEM, sometimes after normalization relative to the baseline. Student’s t-test was used to evaluate data, considering p<0.05 to indicate a statistically significant difference.

Results
Changes in [Ca2+]i and LVP During Low-Na+ Exposure
This report is the first to demonstrate inhibition of the reverse mode of the NCX by KBR by studying [Ca2+]i dynamics in the perfused whole heart; previous experiments have only clearly demonstrated the inhibitory effect of KBR in isolated cardiac myocytes. With normal oxygenation, pre-treatment with 10 mmol/L caffeine markedly reduced the amplitude of Ca2+ transients and LVP, while increasing the diastolic [Ca2+]i: this reflected SR Ca2+ release and Ca2+ depletion (Fig 2). A small decline in [Ca2+]i during the initial phase of exposure to caffeine represented Ca2+ efflux via the forward mode of the NCX. In this heart model, the lower trace showing that...
LVP further decreased to zero mmHg and gradually recovered during the exposure indicated an occasional phenomenon. Subsequent low-Na⁺ exposure induced a rapid, marked rise in \([\text{Ca}^{2+}]_i\) associated with abolition of LV developed pressure and an increase in resting pressure, which restoration of normal-Na⁺ conditions reversed. This rise in \([\text{Ca}^{2+}]_i\) resulted mainly from \(\text{Ca}^{2+}\) influx via the reverse NCX, because prior SR \(\text{Ca}^{2+}\) depletion by caffeine ruled out the SR as a source. Removal of caffeine resulted in a recovery of both the amplitude of \(\text{Ca}^{2+}\) transients and LVP. We then repeated the low-Na⁺ exposure to compare the increases in diastolic \([\text{Ca}^{2+}]_i\) in untreated and KBR-treated hearts. In untreated hearts, this second low-Na⁺ exposure provided a result similar to the first (Fig 3A). Treatment with 10 mmol/L KBR for the second exposure.

Because of the difference in \([\text{Ca}^{2+}]_i\) dynamics between untreated and KBR-treated hearts, we further investigated the role of SR \(\text{Ca}^{2+}\) stores in diastolic \([\text{Ca}^{2+}]_i\) dynamics, using 10 mmol/L KBR as a selective inhibitor of \(\text{Ca}^{2+}\)-ATPase activity. Table 1 shows the hemodynamic parameters in untreated and KBR-treated hearts. The heart was exposed to 10 mmol/L caffeine for 5 min and then to low-Na⁺ (30 mmol/L) solution for 30 s, followed by perfusion with Tyrode’s solution containing caffeine and then washout of the caffeine.
KBR before and during the second low-Na+ exposure did not affect the increase in \([Ca^{2+}]\); (data not shown). In contrast, treatment with 10 \(\mu\)mol/L KBR suppressed the increase in \([Ca^{2+}]\) during the second low-Na+ exposure (Fig 3B). In 5 untreated hearts (Fig 4, left), the increase in \([Ca^{2+}]\) during the second low-Na+ exposure was 84.1±3.6% of that during the first exposure (first, 0.39±0.07 arbitrary units; second, 0.33±0.06 arbitrary units; NS). In 5 hearts treated with 10 \(\mu\)mol/L KBR (Fig 4, right), the second, treated increase in \([Ca^{2+}]\) was significantly suppressed to 40.2±11.2% of the first, untreated increase in \([Ca^{2+}]\); (first, 0.37±0.07 arbitrary units; second, 0.16±0.05 arbitrary units; p=0.014).

Changes in \([Ca^{2+}]\) and LVP During Ischemia and Reperfusion

Fig 5 shows the original tracings obtained from individual hearts throughout the ischemia-reperfusion experiment, without subtraction of background autofluorescence. In both untreated hearts and those treated with 10 \(\mu\)mol/L KBR, diastolic \([Ca^{2+}]\) rose during ischemia and returned to the pre-ischemic value after reperfusion, in agreement with previous studies monitoring \([Ca^{2+}]\) in the low-flow ischemic rat heart using fura-217,18 or indo-114. The immediate increase in the diastolic ratio might be caused in part by a change in autofluorescence associated with induction of ischemia18 because myocardial autofluorescence is known to increase during ischemia, representing increased mitochondrial derived NADH fluorescence14. In a previous study of myocardial autofluorescence in ischemia-reperfusion,13 we found the ischemia-induced increase in total fluorescence to be less in the unloaded heart than in the fura-2 loaded heart. After subtraction of the autofluorescence value excited at 340 nm and 380 nm, an ischemia-associated increase in the ratio was still observed in the fura-2 loaded heart,13 so the present increase reflected the rise in \([Ca^{2+}]\); in addition to the change in autofluorescence. During ischemia, the diastolic \([Ca^{2+}]\) in the untreated heart increased to exceed the pre-ischemic systolic \([Ca^{2+}]\); (Fig 5A), although this change in \([Ca^{2+}]\) was attenuated in hearts treated with 10 \(\mu\)mol/L KBR (Fig 5B), in agreement with previous experiments using cardiac myocytes.7 LVP rapidly decreased after induction of ischemia, but partially recovered after reperfusion. In the untreated heart (Fig 5A), LVP transiently recovered and gradually decreased during ischemia. This phenomenon was occasionally observed with and without treatment. Only at 1 min of ischemia, LVP was greater in untreated hearts than in KBR-treated hearts (Table 1) but no significant difference was observed between the 2 groups in the %LVP at baseline (46.1±8.4% vs 30.7±3.4%). The amplitude of Ca2+ transients decreased slightly during ischemia and partially recovered after reperfusion (Fig 5). Similar results were obtained from our previous studies using perfused hearts and isolated cells.18,25 The decrease in amplitude of Ca2+ transients might be explained mainly by reduction of Ca2+ release from the SR or depletion of Ca2+ stores in the SR. No significant difference in the amplitude of transients was noted between the 2 groups. Pre-ischemic, end-ischemic and reperfused values of the amplitude were 0.16±0.02, 0.12±0.02, 0.13±0.01 (arbitrary units) in untreated hearts and 0.17±0.01, 0.13±0.01, 0.15±0.01 in KBR-treated hearts.

Changes in Diastolic \([Ca^{2+}]\) During Ischemia and Reperfusion

Before subtraction of autofluorescence, pre-ischemic diastolic \([Ca^{2+}]\) was similar (fluorescence ratio; 0.79±0.03 vs 0.80±0.01, NS) in untreated and KBR-treated hearts. Fig 6 shows the mean values for changes in diastolic \([Ca^{2+}]\) during ischemia-reperfusion. The ischemic increase in diastolic \([Ca^{2+}]\) was attenuated by KBR treatment and this effect of KBR on \([Ca^{2+}]\) was confirmed by subtraction of autofluorescence (Fig 7). Pre-ischemic diastolic \([Ca^{2+}]\) was also similar (fluorescence ratio; 0.66±0.05 vs 0.67±0.01, NS) in untreated and KBR-treated hearts. In the untreated
hearts, diastolic [Ca²⁺]: increased by 43.1±6.0% and 44.4±7.7% of the pre-ischemic value at 5 min and 10 min, respectively, of ischemia. An increase by 39.8±12.2% was still evident after 1 min of reperfusion. Three of 6 hearts developed VF associated with elevation of diastolic [Ca²⁺]. When the hearts were treated with 10 μmol/L KBR, diastolic [Ca²⁺] increased by only 17.9±6.4% at the end of ischemia. After reperfusion, diastolic [Ca²⁺] promptly declined to essentially pre-ischemic values, with an increase of only 4.5±7.0% after 1 min of reperfusion.

Changes in Hemodynamics

Hemodynamic parameters during the pre-ischemic, ischemic and reperfusion conditions are summarized in Table 1. Treatment with 10 μmol/L KBR did not influence heart rate or LVP in the pre-ischemic state. Although the reverse mode of the NCX has been shown to contribute to contraction, blockade of the NCX did not affect the hemodynamic in nonischemic hearts. Treatment of guinea-pig papillary muscle with 10 μmol/L KBR did not significantly affect the action potential parameters.

After reperfusion, the LVP tended to be greater in KBR-treated hearts than in untreated hearts (LVP at 1 min of reperfusion: see Table 1, p=0.18; %LVP of baseline at 1 min: 84.5±7.2 vs 64.3±15.8, p=0.22). Moreover, KBR-accelerated recovery of the rate–pressure product (Fig 8).

Discussion

We investigated the effects of KBR, a selective inhibitor of the reverse mode of the NCX, on Ca²⁺ transients and LVP during low-Na⁺ exposure and ischemia–reperfusion in the beating intact heart. Treatment with KBR suppressed the increase in [Ca²⁺], resulting from Ca²⁺ influx via reverse mode NCX induced by low-Na⁺ exposure, and also reduced the ischemia-induced rise in diastolic [Ca²⁺]. After reperfusion, KBR improved cardiac function in terms of the rate–pressure product.

Effect of KBR on the NCX

Although KBR predominantly inhibits the reverse mode of the NCX in isolated myocytes, only a few studies have addressed the intracellular ionic dynamics of the isolated perfused heart because of the difficulties in assessing ion transporter function. Baartscheer et al estimated that the relative contribution of Ca²⁺ influx via reverse mode NCX to the increase in [Ca²⁺], induced by zero-Na⁺ exposure in isolated rat ventricular cardiac myocytes was approximately 45% of the total increase in [Ca²⁺], and ryanodine-sensitive Ca²⁺ release from SR similarly accounted for approximately 45%; Ca²⁺ influx via verapamil-sensitive Ca²⁺ channels was negligible. In addition, reduction of extracellular Na⁺ from 156 to 29 mmol/L sufficiently reversed the driving force, the free energy of NCX in myocytes pretreated with ryanodine. If SR function is inhibited, the functional activity of the reverse mode of NCX can be evaluated essentially in isolation by observing the increase in [Ca²⁺]. The caffeine-induced slight increase in diastolic [Ca²⁺] is unlikely to offset the reversal of the NCX. Caffeine also has a phosphodiesterase (PDE) inhibitory action and may increase cAMP levels. These effects could be related to positive inotropism and arrhythmogenesis. However, the use of caffeine is well-established as a tool for assessing cellular Ca²⁺ handling and a high concentration of caffeine depletes the SR Ca²⁺. Thus, these additional effects of caffeine would have had a minimal influence on the current results.

In isolated myocytes, treatment with KBR inhibited the increase in [Ca²⁺] induced by exposure to a zero-Na⁺ solution in a concentration-dependent manner, with complete abolition at 10 μmol/L. However, reduction of the [Ca²⁺] increase by 10 μmol/L KBR was only approximately 60% in this study. The lesser effectiveness of KBR on the NCX in the intact heart might be explained by differences in the extracellular environment: isolated myocytes may be more directly exposed to the drugs. Another explanation for discrepancy is the difference in workload or metabolic consequences.

Effects of the NCX Inhibitor on [Ca²⁺]: During Ischemia and Reperfusion

Several investigations using whole-heart preparations12-14,17,18 as well as isolated cell models6-8,25 have demonstrated alterations in Ca²⁺-dependent fluorescence transients during ischemia and reperfusion. No-flow ischemia arrested the heart beat, resulting in abolition of Ca²⁺ transients. In the present study we used a model of low-flow ischemia in which we could evaluate pharmacologic inhibition of Ca²⁺ overload during ischemia in the beating heart. A similar slight decrease in the amplitude of the Ca²⁺ transient associated with a decrease in LVP occurred during ischemia in both untreated and KBR-treated hearts. Assessment of intracellular Ca²⁺ kinetics using rapid caffeine application indicated that reduction of SR Ca²⁺ stores during anoxia25 and an ischemia-induced reduction of SR Ca²⁺ uptake was demonstrated using a biochemical assay. It is unlikely that KBR modified SR dysfunction during ischemia.

In an investigation of the correlation between diastolic [Ca²⁺] and ventricular contractility after reperfusion, hearts exposed to ischemia for a longer period (30 min) exhibited significantly slower contractile recovery (LVP) and higher diastolic [Ca²⁺] in early reperfusion than hearts subjected to a shorter period of ischemia (10 min). Therefore, elevation of diastolic [Ca²⁺] might be an important determinant of contractile dysfunction in ischemia–reperfusion. Another study also showed a significant role of the degree of the end-ischemic Ca²⁺ overload in reoxygenation-induced myocardocyte hypercontracture. In the present study, treatment with KBR significantly suppressed the ischemic increase in diastolic [Ca²⁺], and accelerated the reperfusion-induced decline of diastolic [Ca²⁺], which is consistent with results obtained in isolated cells again suggesting that the cardio-protective effect of KBR results in part from reduction of [Ca²⁺] overload by inhibiting the reverse mode of the NCX.

Our finding of the ischemic increase in diastolic [Ca²⁺], was consistent with reported results in intact hearts12-14,17,18 Nuclear magnetic resonance detected an increase in [Ca²⁺] after 15–20 min of ischemia and the onset of increase in [Ca²⁺] might depend on the method of investigation. In our previous studies21,25 hypoxia caused inhibition of the exchanger in a few minutes. Therefore, the rise in diastolic [Ca²⁺] during the early phase of ischemia might result partly from functional suppression of the forward mode of the NCX. Inhibition of the NCX may be explained by a decrease in intracellular pH9,30 extracellular pH31 and/or ATP. We have demonstrated intracellular acidosis in intact hearts during global ischemia using a fluorescent intracellular pH indicator32.

Taking these findings into consideration, how did KBR,
a blocker of the NCX, provide a cardioprotective reduction of \([\text{Ca}^{2+}]\) overload during ischemia? Previous studies have suggested that \([\text{Ca}^{2+}]\) overload during or after ischemia or hypoxia occurs principally through the reverse mode of the NCX.\(^{6-8}\) The NCX normally functions as one of the major efflux pathways for cytoplasmic \([\text{Ca}^{2+}]\) during diastole. Ischemia-induced accumulation of \([\text{Na}^+]\) may drive the NCX in the reverse mode, which results in \([\text{Ca}^{2+}]\) influx. The increase in \([\text{Na}^+]\) during ischemia is thought to occur via activation of the Na\(^+\)/H\(^+\) exchanger by intracellular acidosis. In the early phase of ischemia, diastolic \([\text{Ca}^{2+}]\) may begin to rise because of inhibition of the forward mode of the NCX.\(^{23}\) In the present study, a suppressive effect of KBR on increase in diastolic \([\text{Ca}^{2+}]\) was clearly observed at the end of 10 min of ischemia. At that time, the reverse mode of the NCX may predominate, leading to a \([\text{Ca}^{2+}]\) influx that can be prevented by KBR. A previous study demonstrated that Na\(^+\) withdrawal activated the reverse mode of the NCX, even in extracellular acidosis (pH 6.4), and that KBR suppressed the NCX. Stimulation of the reverse mode of NCX could overcome its acidosis-induced inhibition.

Whether \([\text{Na}^+]\) increases in the early phase of ischemia is not yet clear. Interestingly, KBR also attenuated the anoxic increase in \([\text{Na}^+]\) in cardiac myocytes,\(^{7}\) and this effect might inhibit functional activity of the reverse mode of the NCX.

Despite earlier suggestions that myocardial \([\text{Ca}^{2+}]\) content might increase during reperfusion,\(^{48}\) most studies using fluorescent dye, such as in the present study, have shown a decline in diastolic \([\text{Ca}^{2+}]\) during reperfusion following ischemia.\(^{12-14}\) In a less injured (reversible damaged) heart exposed to brief ischemia, removal of cytoplasmic \([\text{Ca}^{2+}]\) by SR might overcome the influx of \([\text{Ca}^{2+}]\) via the reverse mode of the NCX after reperfusion. The contribution of the SR to \([\text{Ca}^{2+}]\) extrusion is greater in the rat heart than in rabbit or guinea-pig hearts.\(^{35}\) Recovery of the amplitude of \([\text{Ca}^{2+}]\) transients was also mainly mediated by restoration of SR \([\text{Ca}^{2+}]\) uptake and \([\text{Ca}^{2+}]\) stores.\(^{25,26}\) In the present study, untreated hearts still exhibited a higher diastolic \([\text{Ca}^{2+}]\); early reperfusion. More rapid decline (normalization) of diastolic \([\text{Ca}^{2+}]\) in the KBR-treated heart might also be a consequence of the inhibitory effect of this agent on the NCX. Hearts from mice overexpressing the NCX may be more susceptible to ischemia–reperfusion injury.\(^{36}\) On the other hand, artificially enhanced gene expression for the NCX has been found to preserve \([\text{Ca}^{2+}]\) homeostasis during ischemia and hypoxia in failing mouse hearts.\(^{37}\) The role of the NCX in abnormal \([\text{Ca}^{2+}]\) handling during the late phase of ischemia and in reperfusion remains a matter of debate.

**Study Limitations**

It has been reported that KBR is not a purely selective inhibitor of NCX. In guinea-pig ventricular myocytes, the IC50 was 0.32 \(\mu\text{mol/L}\) for inhibition of the reverse mode of the NCX, but was 17 \(\mu\text{mol/L}\) for the forward mode. KBR also inhibited the voltage-gated \([\text{Na}^+]\) current, \([\text{Ca}^{2+}]\) current and the inward \([\text{K}^+]\) current, but with a higher IC50s of 14, 8 and 7 \(\mu\text{mol/L}\), respectively.\(^{38}\) Although we could not completely exclude the effect of KBR on these ion transporters, a previous study reported that 10 \(\mu\text{mol/L}\) KBR did not affect the Na\(^+\)/H\(^+\) exchanger, \([\text{Ca}^{2+}]\) channel, SR \([\text{Ca}^{2+}]\)-ATPase or \([\text{Na}^+]\)/K\(^+\)-ATPase in rat smooth muscle cells and cardiac myocytes.\(^{10}\)

We should consider certain problems arising from using a fluorescent dye to measure \([\text{Ca}^{2+}]\) in the isolated perfused heart. Changes involving a fraction of fura-2 fluorescence derived from nonmyocyte sources, particularly endothelial cells, have been suggested\(^{14,15,18,39}\) although the fraction derived from endothelial cells is unlikely to change remarkably during the cardiac cycle. Thus, meaningful differences in \([\text{Ca}^{2+}]\); dynamics could be found between the untreated heart and the KBR-treated heart. Miyata et al showed that the intensity of indo-1 fluorescence in mitochondria also changed during anoxia, whereas the characteristics of \([\text{Ca}^{2+}]\) transients were stable in normoxic myocytes.\(^{30}\) Assessment of ischemic change in mitochondrial fura-2 fluorescence remains to be established in the whole-heart model. We observed fura-2 fluorescence mainly from the epicardium. A previous study evaluated the relationship between indo-1 fluorescence intensity and distance from the sampling probe in the isolated rabbit heart and found that 90% of fluorescence arises from within 850 \(\mu\text{m}\) of the surface (ie, subepicardium). Although the fluorescence seems to vary synchronously with contraction, the change in \([\text{Ca}^{2+}]\) was in part concealed in the mid- and endomyocardium.\(^{31}\) We did not calibrate fura-2 fluorescence, which will be necessary for comparison of \([\text{Ca}^{2+}]\) in the intact heart with that in models representing diseased hearts. Despite these problems, the characteristics of fura-2 fluorescence \([\text{Ca}^{2+}]\) transients) obtained from isolated perfused rat hearts were appropriate for assessment of intracellular \([\text{Ca}^{2+}]\) handling. The advantages of fluorescence indicators include the ability to monitor real-time changes in \([\text{Ca}^{2+}]\); and LVP simultaneously, and to assess the pharmacologic effect on \([\text{Ca}^{2+}]\); dynamics during ischemia–reperfusion in the intact heart.

In conclusion, inhibition of the reverse mode of the NCX by KBR, a selective inhibitor, resulted in reduction of intracellular \([\text{Ca}^{2+}]\) overload and acceleration of recovery of myocardial contractility during ischemia and reperfusion in the intact heart model.

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**References**


