Contribution of Sarcoplasmic Reticulum Ca^{2+} Release and Ca^{2+} Transporters on Sarcolemmal Channels to Ca^{2+} Transient in Fetal Mouse Heart

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ABSTRACT: Sarcoplasmic reticulum (SR) Ca^{2+} release has been shown not to be the predominant mechanism responsible for excitation-contraction (E-C) coupling in fetal myocytes. However, most of the studies have been conducted either on primary cultures or acutely isolated cells, in which an apparent reduction of ryanodine receptor density have been reported. We aimed to elucidate the contribution of SR Ca^{2+} release and Ca^{2+} transporters on sarcolemmal channels to Ca^{2+} transients in fetal mouse whole hearts. On embryonic day 13.5, ryanodine significantly reduced the amplitude of the Ca^{2+} transient to $27.2 \pm 4.4\%$ of the control, and both nickel and SEA0400 significantly prolonged the time to peak from 84 ± 2 ms to 140 ± 5 ms and 129 ± 6 ms, respectively, whereas nifedipine did not alter it. Therefore, at early fetal stages, SR Ca^{2+} release should be an important component of E-C coupling, and T-type Ca^{2+} channel and reverse mode sodium-calcium exchanger (NCX)-mediated SR Ca^{2+} release could be the predominant contributors. Using embryonic mouse cultured cardiomyocytes, we showed that both nifedipine and nickel inhibited the ability of NCX to extrude Ca^{2+} from the cytosol. From these results, we propose a novel idea concerning E-C coupling in immature heart. (*Pediatr Res* 69: 306–311, 2011)

Myocardial remodeling plays important roles in the development of heart failure and arrhythmogenesis after myocardial infarction. During cardiac remodeling, the expressions of fetal genes such as those for the T-type Ca^{2+} channel (TCC) (1,2) and sodium-calcium exchanger (NCX) (3–5) are up-regulated, and the resultant T-type Ca^{2+} currents are thought to be involved in the pathological process that leads to systolic dysfunction and arrhythmogenesis (6,7). In the fetal heart, however, the roles played by Ca^{2+} transporters remain unclear.

In adult hearts, excitation-contraction (E-C) coupling is initiated by a rapid increase in the myoplasmic Ca^{2+} concentration, generated mainly by Ca^{2+} release from the sarcoplasmic reticulum (SR). This SR Ca^{2+} flux is mediated by the opening of ryanodine receptors (RyRs) in response to a small trigger Ca^{2+} stimulus [Ca^{2+} -induced Ca^{2+} release; calcium-induced calcium release (CICR)], which is provided by the

voltage-dependent Ca^{2+} channels [L-type Ca^{2+} channels (LCCs)] located in the sarcolemma (8).

In isolated ventricular myocytes obtained from fetuses or newborns, RyR-mediated CICR has been reported to provide only a small amount of Ca^{2+} influx (9–12). According to a previous study on neonatal whole hearts, a sarcolemmal Ca^{2+} influx *via* TCCs and reverse mode NCXs is predominantly responsible for the rapid increase in the cytosolic Ca^{2+} concentration (13–15).

However, most of the studies have been conducted either on primary cultures or acutely isolated cells and an apparent reduction of RyR density (16) and alterations in cell shape (13) have been reported. Previously, we used fetal mouse whole heart models to examine the effect of a SR Ca^{2+} ATPase inhibitor on this Ca^{2+} transient and indicated that Ca^{2+} reuptake from the SR might be functional even on embryonic day (ED) 12.5 (17). We hypothesized that Ca^{2+} release from the SR might be responsible for the increase in intracellular Ca^{2+} concentration even in fetal hearts and that Ca^{2+} transporters such as TCCs and NCXs might be involved in cardiac contraction in such hearts. In this study, we concluded that during the developmental changes 1) RyR plays an important role in CICR, 2) TCC and reverse mode NCX are more effective than LCC as a trigger for CICR, and 3) nifedipine and nickel may not only inhibit Ca^{2+} channels but also inhibit NCX.

METHODS

The study was performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (National Institutes of Health Publication No. 85-23, revised 1996), and the protocols were approved by the Institute Animal Research Committee of the National Defense Medical College.

Experiment 1

Whole heart preparation and fluorescence measurements. Fetal mouse whole hearts at ED 10.5, 13.5, and 16.5 were obtained as previously reported (17). Briefly, the hearts were stained by immersion in a $20 \mu\text{M}$ solution of the Ca^{2+} -sensitive fluorescent indicator Fluo-3 AM (Molecular Probes, Eugene, OR) for 15 min at 37°C . After loading, the heart was transferred to a chamber on a microscope (OLYMPUS IX-70) and then

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Abbreviations: AP, action potential, CICR, calcium-induced calcium release, E-C, excitation-contraction, ED, embryonic day, LCC, L-type Ca^{2+} channel, NCX, sodium-calcium exchanger, RyR, ryanodine receptor, SR, sarcoplasmic reticulum, TCC, T-type Ca^{2+} channel, TTP, time to peak, T_{50} , time to 50% relaxation

washed out at 37°C with control solution (18) [NaCl 137 mM, KCl 5.4 mM, MgCl₂ (6H₂O) 1.0 mM, CaCl₂ 1.8 mM, glucose 5.5 mM, and HEPES 5 mM; pH 7.4]. Fluorescence signals in beating hearts stimulated electrically at 0.5 Hz were recorded using a photomultiplier (CAIM-50Z; Image works, Inc.).

Ca²⁺-transient analysis. The amplitude, time to peak (TTP), and time to 50% relaxation (*T*₅₀) values of the Ca²⁺ transient were measured in fetal mouse whole hearts before and after application of the agents listed below. The effects of Ca²⁺ blockers on its amplitude, TTP, and *T*₅₀ values were analyzed. The agents used were 100 μM ryanodine (SR Ca²⁺-release blocker), 5 μM nifedipine (LCC blocker), 100 μM (low dosage) nickel (TCC blocker) (19–22), and 1 μM SEA0400 (23) (blocker of the reverse mode Na⁺-Ca²⁺ exchanger), whereas 2–5 mM nickel has often been used to inhibit NCX activity. Inhibition ratio (%) values were calculated using the equation: 100 – [(amplitude of the Ca²⁺ transient at 15 min after application)/(amplitude of the Ca²⁺ transient before application) × 100]. Prolongation ratio (%) values for the TTP study were calculated using the equation: [(TTP at 15 min after application) – (TTP before application)]/(TTP before application) × 100.

Figure 1. Caffeine-induced Ca²⁺ transients in fetal mouse cultured cardiomyocytes. Caffeine-induced Ca²⁺ transients were recorded in control solution, in zero Ca²⁺ (for 3 min), in zero Ca²⁺ + zero Na⁺ (for 3 min), and finally in zero Ca²⁺ + caffeine. Both the third and the final solution varied among the protocols. In this experiment, we analyzed the *T*₅₀ value of the caffeine-induced Ca²⁺ transient (see Fig. 2).

Experiment 2

Ventricular myocytes: isolation and cultures. Ventricular myocytes were enzymatically isolated from fetal mouse hearts (24,25). Fetal mice at ED 14.5 were taken out, and the hearts were quickly removed. Only the ventricles were minced into 0.5–1.0 mm³ pieces. There were added to 0.03% trypsin + 0.03% collagenase in saline (total 10 mL), put into a trypsinizing flask, stirred as a trypsin step, and the top layer transferred to another tube with culture medium. After repeating the trypsin step four times, the myocytes were spun in a centrifugal separator at 200 × g for 10 min. Sedimented myocytes were scattered by pipetting in Ham F-12 (Life Technologies Japan, Tokyo) + Medium 199 (Life Technologies Japan, Tokyo) + penicillin-streptomycin (total 10 mL) containing 10% FCS, passed through a filter, and put on eight 25-mm diameter glass coverstrips in a 100-mm diameter dish. After addition of adequate medium, they were cultured in a CO₂ incubator at 37°C for 2 d.

Caffeine-induced Ca²⁺ transients. Caffeine-induced Ca²⁺ transients (26,27) in fetal self-beating ventricular myocytes were recorded using the photomultiplier described above. Caffeine releases Ca²⁺ from the SR (28) and elimination of intracellular Ca²⁺ is attributed to NCX. The analysis of the time course of the decay of the caffeine-induced Ca²⁺ transient allows evaluation of the functions of forward-mode NCX and sarcolemmal calcium pump, when nifedipine or nickel is present. We used four protocols in which solutions were applied sequentially as follows (Fig. 1).

Protocol 1: control solution – zero Ca²⁺ (3 min) – zero Ca²⁺, zero Na⁺ (3 min) – zero Ca²⁺, zero Na⁺, caffeine (20 mM).

Protocol 2: control solution – zero Ca²⁺ (3 min) – zero Ca²⁺, zero Na⁺ (3 min) – zero Ca²⁺, caffeine.

Protocol 3-1: control solution – zero Ca²⁺ (3 min) – zero Ca²⁺, zero Na⁺, plus nifedipine (3 min) – zero Ca²⁺, caffeine, plus nifedipine (5 μM).

Protocol 3-2: control solution – zero Ca²⁺ (3 min) – zero Ca²⁺, zero Na⁺, plus nickel (3 min) – zero Ca²⁺, caffeine, plus nickel (100 μM).

Protocol 4: control solution – zero Ca²⁺ (3 min) – zero Ca²⁺, zero Na⁺, plus either nifedipine or nickel (3 min) – zero Ca²⁺, zero Na⁺, caffeine, plus either nifedipine or nickel.

In protocol 1, myocytes were incubated in zero Ca²⁺ solution with intact NCX function to stop contraction and extract cytosolic Ca²⁺ and then incubated in a zero Ca²⁺ and zero Na⁺ solution to block NCX function. Finally, caffeine was added to release Ca²⁺ from the SR. Thereafter, intracellular Ca²⁺ is slowly extruded *via* sarcolemmal Ca²⁺ pump (Fig. 2A). In protocol 2, after incubation in a solution containing caffeine and normal Na⁺, intracellular Ca²⁺ is rapidly extruded mainly *via* NCX (Fig. 2B). In protocol 3, myocytes were incubated in a solution containing zero Ca²⁺ and zero Na⁺ plus either nifedipine or nickel, followed by a solution containing caffeine, normal Na⁺, and either nifedipine or nickel (Fig. 2C and D). This allowed us to examine the effects of these Ca²⁺-channel blockers on NCX. In protocol 4, a solution

Figure 2. Effects of Ca²⁺-channel blockers on the *T*₅₀ value of the caffeine-induced Ca²⁺ transient. The final solution contained (in addition to caffeine) zero Na⁺ in protocol 1 (A) *n* = 5, normal Na⁺ in protocol 2 (B) *n* = 9, normal Na⁺ + nifedipine in protocol 3-1 (C) *n* = 3, or normal Na⁺ + nickel in protocol 3-2 (D) *n* = 10. *T*₅₀ values for the caffeine-induced Ca²⁺ transients were compared among each protocol (E). **p* < 0.05, ***p* < 0.01.

Figure 3. Effects of Ca^{2+} -channel blockers on the amplitude of the Ca^{2+} transient in ED 13.5 mouse hearts. The reagents are nifedipine (A) $n = 14$, nickel (B) $n = 8$, SEA0400 (C) $n = 6$, ryanodine (D) $n = 4$. Each panel shows the change in the amplitude of the Ca^{2+} transient at 15 min after Ca^{2+} -channel blocker application. The amplitude of the Ca^{2+} transient measured before application of the blocker was expressed as 100%. $*p < 0.05$ and $**p < 0.01$ vs. before the application. Inhibition ratio were calculated using the equation: $100 - [\text{amplitude of the } \text{Ca}^{2+} \text{ transient at 15 min after application}] / [\text{amplitude of the } \text{Ca}^{2+} \text{ transient before application}] \times 100$, and were compared upon each reagent (E). $**p < 0.01$.

containing caffeine, zero Na^+ , and either nifedipine or nickel was used. This allowed us to examine the effects of these blockers on the sarcolemmal Ca^{2+} pump. In these protocols, we measured the T_{50} value of each caffeine-induced Ca^{2+} transient.

Statistical analysis. Data are presented as means $\pm$ SE. Statistical significance was determined using two-sample t tests as follows: paired t test (t test) for the amplitude of the Ca^{2+} transient, paired t test (Welch's test) for the prolongation ratio (%), and unpaired t test for the TTP and T_{50} of the Ca^{2+} transient. Turkey's multiple-comparison test was used for comparisons among three or more groups, and $p < 0.05$ was taken to indicate significance.

RESULTS

Experiment 1

Effects of Ca^{2+} -channel blockers on the amplitude of the Ca^{2+} transient in fetal mouse whole hearts. In ED 13.5 mouse whole hearts, the amplitude of the Ca^{2+} transient was reduced by $72.8 \pm 4.2\%$, $34.4 \pm 4.2\%$, $26.5 \pm 4.2\%$, and $16.7 \pm 6.6\%$ on ryanodine, nifedipine, nickel, and SEA0400 application, respectively ($p < 0.01$, $p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively; Fig. 3). The inhibition ratio was significantly greater upon ryanodine application than upon nifedipine, nickel, or SEA0400 application ($p < 0.01$; Fig. 3E). In both of ED 10.5 and 16.5 mouse whole hearts, the inhibition ratio upon ryanodine application was also significantly greater than that upon the other sarcolemmal Ca^{2+} channels blocker application (data not shown).

Effects of Ca^{2+} -channel blockers on TTP and T_{50} values for Ca^{2+} transients in fetal mouse whole hearts. There was no significant difference of TTP between before and after application of nifedipine, while there was a significant prolongation with both nickel and SEA0400 at all embryonic stages ($p < 0.01$; Table 1). At ED 10.5, the prolongation of TTP was significantly greater with nickel than with SEA0400 ($p < 0.05$). The prolongation ratio of the TTP upon nickel application was significantly greater on ED 10.5 than on ED 16.5 ($p < 0.05$; Fig. 4A), but no such difference was detected for SEA0400 (Fig. 4B).

Table 1. Effects of Ca^{2+} -channel blockers on TTP and T_{50} of Ca^{2+} transient in fetal mouse whole hearts

	ED 10.5	ED 13.5	ED 16.5
TTP (msec)			
Control	77 ± 2 ($n = 48$)	84 ± 2 ($n = 28$)	90 ± 3 ($n = 27$)
Nifedipine	81 ± 3 ($n = 23$)	77 ± 4 ($n = 14$)	97 ± 3 ($n = 11$)
Nickel	$142 \pm 4^\dagger$ ($n = 14$)	$140 \pm 5^\dagger$ ($n = 8$)	$144 \pm 7^\dagger$ ($n = 7$)
SEA0400	$122 \pm 6^\dagger$ ($n = 11$)	$129 \pm 6^\dagger$ ($n = 6$)	$127 \pm 7^\dagger$ ($n = 9$)
T_{50} (msec)			
Control	104 ± 1 ($n = 48$)	116 ± 3 ($n = 28$)	110 ± 3 ($n = 27$)
Nifedipine	$129 \pm 4^\dagger$ ($n = 23$)	$138 \pm 7^\dagger$ ($n = 14$)	$148 \pm 7^\dagger$ ($n = 11$)
Nickel	$163 \pm 7^\dagger$ ($n = 14$)	$150 \pm 8^{* \dagger}$ ($n = 8$)	$174 \pm 8^\dagger$ ($n = 7$)
SEA0400	$152 \pm 7^\dagger$ ($n = 11$)	$168 \pm 9^\dagger$ ($n = 6$)	$150 \pm 8^\dagger$ ($n = 9$)

Values are presented as means $\pm$ SE; n represents number of whole hearts. $*p < 0.05$ vs control. $^\dagger p < 0.01$ vs control.

Figure 4. Prolongation ratio (%) values for the TTP of the Ca^{2+} transient after application of nickel [A: $n = 14$ (ED 10.5) and $n = 7$ (ED 16.5)] or SEA0400 [B: $n = 11$ (ED 10.5) and $n = 9$ (ED 16.5)]. Prolongation ratio (%) values were calculated using the equation: $[(\text{TTP of the } \text{Ca}^{2+} \text{ transient after application}) - (\text{TTP of the } \text{Ca}^{2+} \text{ transient before application})] / (\text{TTP of the } \text{Ca}^{2+} \text{ transient before application}) \times 100$. $*p < 0.05$.

Unexpectedly, the T_{50} value of the Ca^{2+} transient value was significantly greater after application of nifedipine, nickel, and SEA0400 than in control at all embryonic stages [$p < 0.01$, except for nifedipine on ED 13.5 ($p < 0.05$); Table 1].

Experiment 2

Effects of Ca²⁺-channel blockers on caffeine-induced Ca²⁺ transient in fetal mouse cultured myocytes. To elucidate the effects of nifedipine and nickel, which has been often used as LCC and TCC blockers, respectively, on the sarcolemmal Ca²⁺ pump and NCX, we measured the T_{50} value of the caffeine-induced Ca²⁺ transient using fetal mouse cultured myocytes (Figs. 1 and 2). NCX function kept intact in the final solution containing Na⁺ on the protocol 2, 3-1, and 3-2. As a result, the amplitude of caffeine-induced Ca²⁺ transients on these protocols were smaller than that on protocol 1, because Ca²⁺ was continuously extruded *via* NCX as SR released Ca²⁺ after caffeine application. There was no significant difference of the amplitude of caffeine-induced Ca²⁺ transients between protocol 2 and 3 (data not shown). Therefore, to investigate the effect of nifedipine and nickel on NCX function, we evaluated T_{50} of caffeine transients of protocol 3, compared with protocol 2.

The T_{50} value of the caffeine-induced Ca²⁺ transient in zero-Na⁺ solution was 5.24 ± 0.37 s ($n = 5$), whereas in normal Na⁺ solution, it was 1.08 ± 0.15 s ($n = 9$; Fig. 2A, B, and E). The T_{50} values of the caffeine-induced Ca²⁺ transient in solutions containing normal Na⁺ + nifedipine and normal Na⁺ + nickel were 2.38 ± 0.10 s ($n = 3$) and 3.86 ± 0.17 s ($n = 10$), respectively (Fig. 2C, D, and E). T_{50} values on protocol 3-1 and 3-2 were significantly larger than that on protocol 2. In protocol 4, the T_{50} values were not significantly different *versus* protocol 1 (data not shown), indicating that neither nifedipine nor nickel had any influence on the sarcolemmal Ca²⁺ pump.

DISCUSSION

In this article, three new contributions are presented. First, during the developmental changes, RyR plays an important role in CICR even at the early embryonic stage. Second, TCC and reverse-mode NCX were each more effective than LCC as a trigger for CICR at the sarcolemmal Ca²⁺-channel level in fetal cardiomyocytes. Third, nifedipine and nickel may not only inhibit Ca²⁺ channels but also NCX on the basis of data obtained in mouse cultured cardiomyocytes.

Contribution of SR Ca²⁺ release, LCC, TCC, and reverse mode NCX to the Ca²⁺ transient. The reduction in the amplitude of the Ca²⁺ transient upon application of ryanodine to the fetal mouse heart at ED 13.5 was about 73%, whereas the reductions seen with blockers of LCC, TCC, and reverse mode NCX were only 34, 27, and 17%, respectively, indicating that SR Ca²⁺ release may be functional and be the major contributor to E-C coupling even at early fetal stages. This is the first study to demonstrate the importance of SR Ca²⁺ release in mouse whole hearts at early fetal stages. Our data are similar to those obtained in two previous studies. Using fetal rat isolated cardiomyocytes, Seki *et al.* (29) reported an 84% reduction in the amplitude of the Ca²⁺ transient upon application of ryanodine. Using d 1 rat whole hearts, Ostálová *et al.* (30) found a >50% reduction in contraction upon application of ryanodine. In contrast, Escobar *et al.* (13) reported that ryanodine reduced the amplitude of the Ca²⁺

transient only 10% on d 1 and of 32% on d 5, using rat neonatal whole hearts stimulated at 2 Hz. The discrepancy may derive from the use of different stimulation frequencies (0.5 Hz *versus* 2 Hz) (31,32), a species difference, or an age difference.

It is well known that the SR is both at lower density and immature at fetal and neonatal stages (33). However, a recent report demonstrated that RyR densities were reduced in cell culture, indicating that SR function might be underestimated in culture (16). Moreover, intrinsic cardiac adrenergic (ICA) cells play an important role in fetal heart development (34). These cells, which exist before sympathetic innervation, are capable of catecholamine synthesis, release, uptake, and generate spontaneous Ca²⁺-transient activity. In studies on isolated single myocytes, however, ICA cells might be lost during the physical and chemical manipulations, leading to underestimation of the Ca²⁺-transient activity.

Roles of TCC and reverse mode NCX as triggers of CICR.

In a preliminary study, we found that upon high-dose nickel (500 μ M) application, the amplitude of the Ca²⁺ transient was greatly reduced, and its TTP was greatly prolonged. These results are consistent with both TCC and reverse-mode NCX being inhibited by high-dose nickel, as indicated by a previous study (13). Accordingly, we used low-dose (100 μ M) nickel to make separate evaluations of TCC from NCX.

In this study on fetal mouse whole hearts, the TTP of the Ca²⁺ transient became significantly longer after application of nickel or SEA0400, indicating that TCC and reverse-mode NCX possibly contribute to sarcolemmal calcium entry as triggers of CICR. This speculation may be supported by the up-regulations of TCC and reverse mode NCX mRNAs reported in fetal and neonatal cardiomyocytes (22,35–37), and by the opening of TCC at more negative membrane potentials than LCC. Furthermore, the prolongation of TTP at ED 10.5 was significantly greater with nickel than with SEA0400. TCC expression was reported to be greatest in chick ventricles at early embryonic stages (ED 5) (20). It has been reported that TCC blockers prolonged the TTP of the Ca²⁺ transient more effectively than LCC blockers in rat neonatal whole hearts (13). Although a previous report claimed that the distance between the sarcolemmal LCCs and SR Ca²⁺-release channels needed to be short (38), our results may raise the possibility that colocalization with RyR is tighter for TCCs than for LCCs at early fetal stages. To resolve this issue, further investigations concerning TCC function as a trigger of CICR will be necessary.

Effects of Ca²⁺-channel blockers on cardiac diastolic phase in fetal mouse cultured myocytes. Using the fetal mouse cultured myocytes, we found that 1) application of nifedipine or nickel partially inhibited the extrusion of Ca²⁺ from the cytosol *via* NCX and 2) the NCX-inhibitory effect of nickel was greater than that of nifedipine. That is, at fetal stages, nifedipine and nickel have influences over NCX function in the diastolic phase of the Ca²⁺ transients, while nifedipine has been widely used as LCC blocker and nickel as NCX and/or TCC blocker. To the best of our knowledge, this is the first report that nifedipine has an inhibitory effect on forward-mode NCX rates. Because the mRNA levels for the

sarcolemmal NCX reach peak near birth in developing hearts (37), it would be important to study the pharmacological effects on NCX in developing hearts furthermore.

There are several limitations of this study. First, we could not investigate the effects of action potential (AP) configuration in our stimulated whole hearts, although the amplitude of Ca^{2+} transient has been shown to be exquisitely sensitive to changes in AP configuration in immature myocardium (39,40). It has been reported that the shortened AP result in less time for NCX-mediated Ca^{2+} influx and thereby reduce the amplitude of Ca^{2+} transient (40). As the major source of Ca^{2+} influx in neonatal myocyte is through the NCX (41,42), Ca^{2+} homeostasis in immature hearts should be largely affected by AP configuration. Nevertheless, the lack of these considerations should not change our conclusions that were reached by relative comparisons in whole hearts stimulated electrically by same frequency and pulse duration.

Second, the agents used to dissect the various calcium transport pathways were not entirely selective. There is a possibility that they might have some effects on other transporters, because we did not demonstrate the presence of each Ca^{2+} channel current. However, low concentration nickel has been reported to useful to investigate TCC function in previous articles (20–22), although higher concentration nickel has often be used to inhibit NCX activity (19). SEA0400 also has been reported to be more selective inhibitor of the reverse mode NCX than the previously used agents like KB-R7943 (23). Therefore, we believe that the lack of current data would not make our conclusions feeble.

Third, it has recently been reported that there is an important role for IP_3R -gated calcium stores in contributing to calcium transients and contraction in neonatal rat myocytes. Primary subsarcolemmal IP_3 -mediated Ca^{2+} release was shown to be capable of activating local RyRs to produce a secondary CICR and was suppressed in the presence of ryanodine (43). Therefore, the lack of the selective inhibition to IP_3 -mediated Ca^{2+} release in our study would not change our conclusions for the important role of RyR in CICR, although we should investigate the role of IP_3R on CICR in fetal hearts in future.

In conclusion, on the basis of our results, we propose the following a novel ideas: 1) SR Ca^{2+} release may be the major contributor to E-C coupling even in the fetal mouse heart, 2) TCC and reverse-mode NCX functions may contribute more than LCCs to CICR in the fetal mouse heart, and 3) nifedipine and nickel inhibit not only Ca^{2+} entry through Ca^{2+} channels but also Ca^{2+} extrusion through NCX in the fetal mouse heart. We believe that the present results may not only contribute to the medication rationale for fetal and neonatal heart failure but also be a useful contribution to the discussion of the medication target for adult heart failure, because the shifts in sarcolemmal Ca^{2+} channels occurring during early embryonic stages can be regarded as a reflection of the changes that take place in adult heart failure.

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