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Involvement of mitochondrial permeability transition pore (mPTP) in cardiac arrhythmias: Evidence from cyclophilin D knockout mice

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ABSTRACT

In the present study, we have used a genetic mouse model that lacks cyclophilin D (CypD KO) to assess the cardioprotective effect of mitochondrial permeability transition pore (mPTP) inhibition on Ca^{2+} waves and Ca^{2+} alternans at the single cell level, and cardiac arrhythmias in whole-heart preparations. The protonophore carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone (FCCP) caused mitochondrial membrane potential depolarization to the same extent in cardiomyocytes from both WT and CypD KO mice, however, cardiomyocytes from CypD KO mice exhibited significantly less mPTP opening than cardiomyocytes from WT mice ($p < 0.05$). Consistent with these results, FCCP caused significant increases in CaW rate in WT cardiomyocytes ($p < 0.05$) but not in CypD KO cardiomyocytes. Furthermore, the incidence of Ca^{2+} alternans after treatment with FCCP and programmed stimulation was significantly higher in WT cardiomyocytes (11 of 13), than in WT cardiomyocytes treated with CsA (2 of 8; $p < 0.05$) or CypD KO cardiomyocytes (2 of 10; $p < 0.01$). (Pseudo-)Lead II ECGs were recorded from *ex vivo* hearts. We observed ST-T-wave alternans (a precursor of lethal arrhythmias) in 5 of 7 WT hearts. ST-T-wave alternans was not seen in CypD KO hearts ($n = 5$) and in only 1 of 6 WT hearts treated with CsA. Consistent with these results, WT hearts exhibited a significantly higher average arrhythmia score than CypD KO ($p < 0.01$) hearts subjected to FCCP treatment or chemical ischemia-reperfusion ($p < 0.01$). In conclusion, CypD deficiency-induced mPTP inhibition attenuates CaWs and Ca^{2+} alternans during mitochondrial depolarization, and thereby protects against arrhythmogenesis in the heart.

1. Introductions

Mitochondria have been shown to play a vital role in the regulation of intracellular Ca^{2+} (Ca_i^{2+}) homeostasis in cardiomyocytes [1–3]. Mitochondrial Ca^{2+} influx is primarily mediated by the mitochondrial calcium uniporter (mCU), a low-affinity, high-capacity ion channel [4,5]. Mitochondrial Ca^{2+} efflux is dependent on two channels: the Na^+-Ca^{2+} exchanger (mNCX), the primary channel in physiologic conditions, and the mitochondrial permeability transition pore (mPTP), which opens during times of pathophysiologic stress [6]. These Ca^{2+} channels and transporters derive their driving forces from the Ca^{2+} gradient and mitochondrial membrane potential ($\Delta\Psi_m$) established by a proton gradient generated by the mitochondrial electron transport chain [1,7–9].

Previous studies have shown that mitochondria are physically associated with the sarcoplasmic reticulum (SR) through electron-dense tethering structures opposite the location of ryanodine receptors [10]. Mitofusin 2, a protein necessary for mitochondrial membrane fusion, creates a tether between the mitochondrial outer membrane and the SR [11,12]. This link is approximately 10–50 nm wide, and likely creates a micro-domain in which Ca_i^{2+} levels can be readily manipulated to induce a variety of ionic flux responses by either the mitochondrial or the SR Ca^{2+} handling pathways.

Our previous work has demonstrated that mitochondrial Ca^{2+} fluxes likely alter Ca_i^{2+} levels within this micro-domain, and thus modulate Ca_i^{2+} handling properties, including the behavior of the SR ryanodine receptors (RyR) [13]. We found that mitochondrial uncoupling by the protonophore carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone (FCCP) results in $\Delta\Psi_m$ depolarization, which subsequently causes mitochondrial Ca^{2+} release *via* the mPTP. This mitochondrial Ca^{2+} release promoted

Abbreviations: CaWs, Ca^{2+} waves; FCCP, carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone; CsA, cyclosporin A; CypD KO, CypD knockout mouse model; DHPR, α1 subunit of L-type Ca channel, dihydropyridine receptor; Ca_i^{2+}, intracellular Ca^{2+}; I/R, ischemia/reperfusion; mCU, mitochondrial calcium uniporter; $\Delta\Psi_m$, mitochondrial membrane potential; mPTP, mitochondrial permeability transition pore; mNCX, Na^+-Ca^{2+} exchanger; PCL, pacing cycle length; PLN, phospholamban; RyR, ryanodine receptors; SR, sarcoplasmic reticulum; Tha, thapsigargin.

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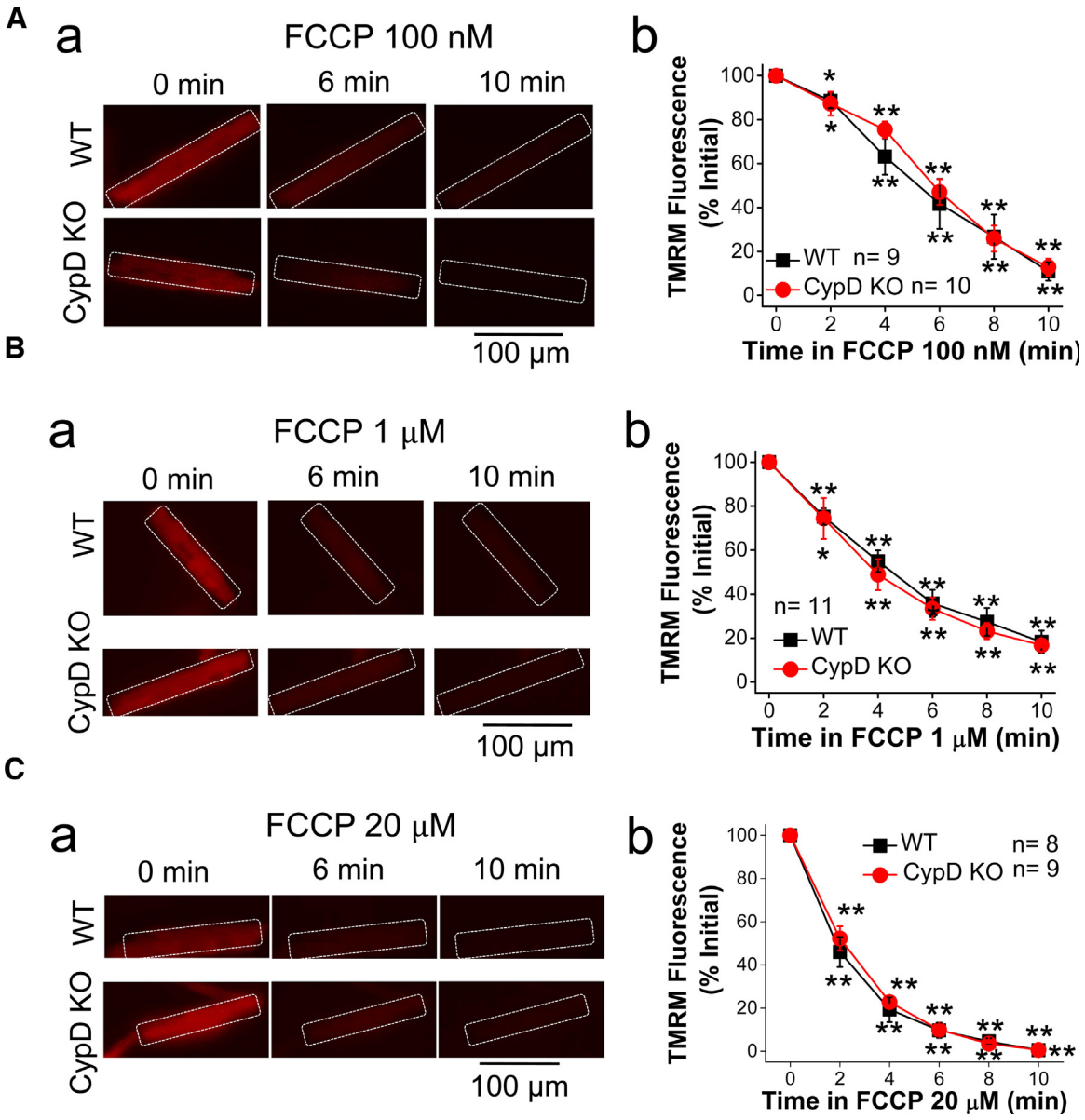

Fig. 1. FCCP Depolarized the $\Delta\Psi_m$ to the Same Extent in WT and CypD KO Myocytes. FCCP (A, 100 nM; B, 1 μM; C, 20 μM) was used to depolarize $\Delta\Psi_m$. The fluorescence in the presence of 30 μM FCCP was set as 100% dissipation (not shown). (A-a, B-a, C-a) Snapshots of TMRM fluorescence at baseline (0), 6, and 10 min in the presence of FCCP are shown. (A-b, B-b, C-b) Summary data showing a decrease in TMRM fluorescence, $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the respective baseline value.

spontaneous Ca^{2+} release from the SR, and exacerbated Ca^{2+} waves (CaWs), which can be potentially arrhythmogenic. However, the following issues remained to be elucidated: 1) the less than ideal selectivity of cyclosporin A (CsA) as a mPTP blocker; 2) the relevance of the mPTP to arrhythmogenesis at the whole heart level.

To further delve into mechanistic research, we have employed the *Ppif*$^{-/-}$, CypD knockout mouse model (CypD KO). CypD has been demonstrated to be a necessary component and regulator of the mPTP. As a result, the mPTP opening should be severely reduced in CypD KO cardiomyocytes. It has been shown that CypD KO mice are generally protected from ischemia/reperfusion (I/R) injury *in vivo*, and mitochondrial swelling, Ca^{2+} overload, and ROS-induced cell death *in vitro* [14,15]. These cardioprotective effects are thought to occur as a result of the inability of the mPTP to open in a high-conductance mode during instances of mitochondrial matrix Ca^{2+} overload or other severe mitochondrial stress, such as membrane potential dissipation *via* superoxide flashes [16]. This ultimately prevents mitochondrial inner membrane permeabilization and apoptosis. On the other hand, CypD KO may also have deleterious consequences under different stress conditions. For example, CypD KO mice demonstrate increased hypertrophy, fibrosis, and accelerated development of congestive heart failure in response to transaortic constriction pressure-overload models [17]. This may be mediated by increased mitochondrial matrix Ca^{2+} overload and/or lack of dynamic cardiac metabolic range. Our previous study has shown that suppression of mPTP by CsA attenuates CaWs, suggesting a potential antiarrhythmic effect. However, it still remains to be elucidated if a selective genetic approach to knock out CypD ameliorates arrhythmogenesis.

In the present study, we have found that inhibition of the mPTP by CypD KO reduces mPTP-mediated pathological mitochondrial Ca^{2+} effluxes, attenuates spontaneous CaWs, and prevents Ca^{2+} alternans development. Furthermore, CypD KO prevents arrhythmogenesis at the whole heart level in an *ex-vivo* model. A preliminary report has been communicated to the American Heart Association annual meeting [18].

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