

## **FKBP12.6 ‘stabilises’ cardiac SR Ca<sup>2+</sup>-release by antagonising high-affinity reversible activation of RyR2 by FKBP12**

FKBP12.6 is thought to play an important cardioprotective role, however, the underlying mechanism is not understood. Since FKBP12 is structurally similar to FKBP12.6 but is found at much higher levels (1-3  $\mu$ M), we investigated the effects of both FKBP12 and FKBP12.6 on RyR2 single-channel function and on SR Ca<sup>2+</sup>-release in rat isolated permeabilised cardiomyocytes. FKBP12 increased RyR2 open probability (Po) in a concentration-dependent, reversible manner (EC<sub>50</sub> 51 nM). Physiological levels of FKBP12 (3  $\mu$ M) increased Po from 0.187 $\pm$ 0.051 to 0.657 $\pm$ 0.111 (SEM; n=14; *P*<0.001). FKBP12.6 (200 nM), itself, did not significantly alter RyR2 Po, but was a very effective antagonist of FKBP12, shifting the FKBP12 EC<sub>50</sub> to 4  $\mu$ M. In permeabilised myocytes perfused with Fluo-5F, spontaneous waves of Ca<sup>2+</sup>-induced Ca<sup>2+</sup>-release were induced by 234 nM Ca<sup>2+</sup> in the mock cytosolic solution. Perfusion with FKBP12 (3  $\mu$ M) increased wave frequency from 0.34 $\pm$ 0.04 Hz to 0.52 $\pm$ 0.07 Hz (SEM; n=14; *p*<0.03). 10 mM caffeine produced a larger Ca<sup>2+</sup>-transient in control (2.21 $\pm$ 0.11; F/Fo) than in FKBP12 (1.47 $\pm$ 0.16; n=6; *p*<0.003) indicating lower SR Ca<sup>2+</sup>-content. Perfusion with FKBP12.6 (200 nM) alone, had no significant effect yet it reduced the ability of FKBP12 to increase wave frequency (49.9 $\pm$ 5.8% increase over control in the absence of FKBP12.6 vs. 16.2 $\pm$ 2.1% in the presence). Our single-channel experiments demonstrate that FKBP12 is a high affinity, potent activator of RyR2. FKBP12.6 acts as an antagonist of FKBP12 at RyR2 but itself possesses minimal efficacy. Our cellular experiments suggest that this is the underlying mechanism by which FKBP12.6 acts to ‘stabilise’ or reduce SR Ca<sup>2+</sup>-release in cardiac cells. Thus, the balance between the opposing actions of FKBP12 and FKBP12.6 on RyR2 gating may be crucial for normal EC-coupling in cardiac cells.

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