Evidence For Enhanced Ischemic Preconditioning Through Combination δPKC-Inhibition and εPKC-Activation

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Introduction

Ischemic pre-conditioning, which is defined as the resistance of heart tissue to prolonged ischemia after an initial period of transient ischemia, is the second most significant form of myocardial protection, after reperfusion therapy known to date.1,2 Six protein kinase C (PKC) enzymes have been shown to translocate to the membrane upon activation in cardiac myocytes, and among these δPKC and εPKC have been linked to ischemia preconditioning.3 Earlier work in the Dorn lab has demonstrated that the use of a rationally-designed εPKC agonist, pseudo-εRACK (ψεRACK), can induce εPKC activation, translocation, and sustained ischemic preconditioning.4 Activation of δPKC, on the other hand, has been shown to increase ischemic damage in the cardiac myocyte.5 Recent work in the lab has focused on studying the effect of inhibiting δPKC activation and translocation with a peptide inhibitor, δV1. Inhibition of δPKC with δV1 is indeed cardioprotective and effective at ischemic preconditioning.6 Additionally, inhibition of δPKC or activation of εPKC alone both confer a reduction in ischemic damage greater than 50%.5

Hypothesis

Simultaneous activation of εPKC and inhibition of δPKC is more cardioprotective than either treatment alone.

Methods

Experimental mice were anesthetized with avertin i.p. (per protocol) and their hearts were rapidly removed to reduce ischemia and cannulated via the aorta in a Krebs-Henseleit buffer on a Langendorff *ex vivo* perfusion apparatus. Left ventricular pressure and real time derivative (dP/dt) was measured via a catheter in the apex of the ventricle. The heart was perfused for 20 minutes to allow equilibration. Simulated ischemia was induced by interruption of the perfusate for 40 minutes, followed by 30 minutes of reperfusion. Measurements were made at 1 minute intervals throughout the reperfusion period. Cardiac damage was assessed using the diagnostic marker creatine kinase (CK) via an *in vitro* assay kit (Sigma) performed on the post-ischemic perfusate. Samples were rapidly frozen to preserve CK activity and allowed to thaw completely at room temperature before being assayed.

Results

Mice that simultaneously expressed the δV1 and ψεRACK peptides had a more rapid and greater return to baseline hemodynamic function as measured by left ventricular pressure and real-time derivative (~2900 mmHg/sec) as compared to ψεRACK (~2600 mmHg/sec) and

NTG (~2000 mmHg/sec) alone. Creatine kinase release values were comparable in crossed δV1 and ψεRACK mice (168.50 ± 38.65 IU) to those in ψεRACK (115.00 ± 33.32) mice and non-transgenic (575.00 ± 298.00) mice.

Conclusions

The data suggest that simultaneous expression of δV1 and ψεRACK peptides in mice has a greater cardioprotective effect than expression of either peptide alone. CK activity of the crossed mice do not show a significant decrease in ischemic injury over either peptide alone. The reduction in CK activity is significant when compared to non-transgenic mice and comparable to either of the CK levels in the mice who expressed the proteins separately. More work needs to be done to further define the roles of δPKC and εPKC in ischemic pre-conditioning. Future work will also aim at exploiting the potential therapeutic applications of δPKC inhibitor and εPKC activator proteins in inducing ischemic pre-conditioning and mitigating cardioprotection.

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