Supplementary Material

The Alpha1 Isoform of Soluble Guanylate Cyclase Regulates Cardiac Contractility but is not Required for Ischemic Preconditioning

Basic Research in Cardiology

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	WT		**sGCα1KO**		**P**
	Control	**IPC**	**Control**	**IPC**	
N	18	19	12	13	
Flow Rate ml/min	1.4 ± 0.7	1.4 ± 0.6	1.4 ± 0.3	1.6 ± 0.3	0.3673
LVDevP mmHg	16 ± 16	24 ± 19	20 ± 16	33 ± 24	0.0385
dP/dt_{max} mmHg/s	549 ± 545	855 ± 743	602 ± 563	1150 ± 858	0.0195
dP/dt_{min} mmHg/s	-388 ± 415	-583 ± 498	-481 ± 464	-863 ± 698	0.0359

Supplementary Table 1: Functional parameters in WT and sGCα1KO hearts after 20 min of reperfusion

Hearts were paced at 7 Hz during the measurements. N: number of mice per group; LVDevP: left ventricular (LV) developed pressure; dP/dt_{max}: maximum rate of developed LV pressure; dP/dt_{min}: minimum rate of developed LV pressure; P: P-value for the effect of IPC vs control in all groups after two-way ANOVA analysis. No significant differences were found after two-way ANOVA with Bonferroni post-hoc tests to compare between control and IPC values for each genotype

Supplementary Discussion

We observed a significant effect for the treatment factor (IPC versus control ischemia-reperfusion) in all groups when using two-way ANOVA to analyze LVDevP, dP/dt_{max}, and dP/dt_{min} (supplemental table 1), indicating that IPC increased post-ischemic cardiac function. However, we did not detect a statistically significant IPC-induced improvement in functional recovery in WT or sGCα1KO hearts using Bonferroni post-tests. This was due to the large variation that we observed in cardiac function during the reperfusion phase. Nevertheless, the reduction in infarct size that we observed was very robust, showing a highly significant decrease of 35 - 40% after IPC versus control ischemia-reperfusion in both genotypes (Figure 3). This decrease in infarct size is considered the most important endpoint for measuring the effects of IPC, having more relevance than the degree of post-ischemic functional recovery to gauge the efficacy of cardioprotection by IPC [2, 4]. Indeed, the extent of immediate postischemic contractile recovery is largely a measure of the severity of myocardial stunning, an independent transient phenomenon that is caused by free radical damage [1], and which is not affected by IPC [3].

Supplementary Method

cGMP measurements

Cardiac effluents were collected during Langendorff perfusion of hearts before and after administration of 1 μmol/L DEA/NO, and immediately frozen in liquid nitrogen. Prior to the cGMP determination, samples were purified by ice-cold ethanol precipitation to remove proteins. Subsequently, the samples were dried by vacuum centrifugation and resuspended in assay buffer. cGMP concentrations in the samples were then determined using an enzyme immunoassay (Biomedical Technologies, Stoughton, MA) according to the manufacturer's recommendations. cGMP concentrations in the samples were then multiplied by the flow rate in the Langendorff system at the time of collection to obtain the amount of cGMP produced per minute.

Supplementary Legend

Supplementary Fig. 1 cGMP release in the cardiac effluent of isolated hearts before and after treatment with DEA/NO (1 µmol/l). N=4-9 per group; ***: P<0.001 vs baseline sample by Bonferroni's post hoc test after repeated measures two-way ANOVA

Supplementary References

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Supplementary Figure 1