Supplemental Material and Methods

Blunting of cardioprotective actions of estrogen in female rodent heart linked to altered expression of cardiac tissue chymase and ACE2

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Real-time PCR

Real-time PCR was used to quantify mRNA expression for specific genes in LV tissue that are listed below. Total RNA was extracted from frozen, pulverized LV tissue from each group using TRIzol reagent, and processed according to the manufacturer's recommendations. The quality and quantity of RNA samples were determined by spectrometry and agarose gel electrophoresis. Complementary first-strand DNA was synthesized from oligo (dT)-primed total RNA, using the Omniscript RT kit (Qiagen Inc, Valencia, CA). Relative quantification of mRNA levels by real-time PCR was performed using a SYBR Green PCR kit (Qiagen Inc). Amplification and detection were performed with the ABI7500 Sequence Detection System (Applied Biosystems). Only one peak from the dissociation curve was found from each pair of oligonucleotide primers tested. Real-time PCR was carried out in duplicate; a no-template control was included in each run to check for contamination. It was also confirmed that no amplification occurred when samples were not subjected to reverse transcription. Sequence-specific oligonucleotide primers were designed according to published GenBank sequences (www-ncbi-nlm-nih-gov.go.libproxy.wakehealth.edu/Genbank) and confirmed with OligoAnalyzer 3.0. The relative target mRNA levels in each sample were normalized to S16 ribosomal RNA. Expression levels are reported relative to the geometric mean of the control group.

Table 1. Primer Sequences for Real-time PCR

Gene name	Sequence	Product Size	Accession Number
ACE	TTGACGTGAGCAACTTCCAG TGTCAGATCAGGCTCCAGTG	194	AF539425
Chymase	TCTGGAGGACCTCTCCTGTG TGCATTCGGATGTACGTAGG	66	NM_013092
Angiotensinogen	AATTCGGGGATCCTACAACC CTCAGCACCCAAAAGGGTAG	70	XM_008772597
AT1aR	TCCTGCCACATTCCCTGAGTT CGAAATCCACTTGACCTGGTG	116	M74054
ACE2	CGCTGTCACCAGACAAGAA GCCATTATTTCGTCCAATCC	139	NM_001012006
MAS	ACTGTCGGGCGGTCATCATC GGTGGAGAAAAGCAAGGAGA	263	XM_006227868
ANF	AGGGCTTCTTCCTCTTCCTG CCAGGTGGTCTAGCAGGTTC	117	NM_012612
BNP	GCCAGTCTCCAGAACAATCC CCTTGGTCCTTTGAGAGCTG	97	NM_031545
NOX2	CGAAAACTTCTTGGGTCAGC AGGAGATTCCGACACACTGG	101	AF298656
SERCA2	ACCTCATCTCCTCCAACGTG GGGGGTTTGTTCATGATGTC	175	NR_027839
Phospholamban	TGACGATCACAGAAGCCAAG GCCGAGCGAGTAAGGTATTG	160	NM_022707
ERbeta	AGCCTTAGTTCCCCTGCTTC TGCTGCCGGGAACACTGTAG	160	XM_006240221.2
GPR30	TCTACCTAGGTCCCGTGTGG AGGCAGGAGAGGAAGAGAGC	151	NM_133573.1
GAPDH	CTCCCATTCTTCCACCTTTG AGGGCCTCTCTCTTGCTCTC	157	NG_028301