4:30 p.m.

828-5

Gender Specific Effects of Calcitonin Gene-Related Peptide and Substance P Gene Knockout Mice on Coronary Blood Flow Rates

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Background: The sensory neuropeptides, calcitonin gene-related peptide (CGRP) and substance P (SP) are potent vasodilators. Evidence indicates that CGRP is critical in regulation of basal coronary flow rates. To determine if SP has a similar role and if there are any gender differences between CGRP and SP, we investigated coronary flow rates among wild type (WT) and knockout (KO) mice.
Methods: 109 WT, α-CGRP-KO and SP receptor (NK-1 receptor) KO mice were studied in a Langendorff preparation at various perfusion pressures (50, 40, 30, 20 mmHg). The aorta was cannulated and coronary flow rates measured by pressure differences on both sides of a glass capillary in the perfusion line.
Results: In males, flow rates were decreased 14% and 5% in α-CGRP-KO and NK-1-KO mice respectively, compared to WT controls ($p<0.01$, 50 mmHg). In females, flow rates in α-CGRP-KO mice were decreased 16% compared to WT controls. In contrast, flow rates in NK-1-KO females were increased 19% compared to WT controls ($p<0.01$, 50 mmHg).
Conclusions: In males, both CGRP and SP were significant vasodilators. In females, CGRP was similar to males, however SP appears to be a vasoconstrictor. Thus, there appears to be significant gender specific differences between CGRP and SP in the regulation of the coronary circulation.

	Coronary Flow Rates (ml/min/g)					
Pressure (mmHg)	*Control Female*	*α-CGRP-KO Female*	*NK-1-KO Female*	*Control Male*	*α-CGRP-KO Male*	*NK-1-KO Male*
	n=24	*n=13*	*n=11*	*n= 15*	*n=27*	*n=19*
50	11.45±1.44	9.61± 2.17	13.57±2.11	12.23 ±0.93	10.54±0.93	11.59±1.64
40	9.17±1.08	7.61±1.69	10.57±1.70	10.09±0.75	8.27±0.75	8.89±1.27
30	6.31± 0.80	5.27±1.19	6.97± 1.14	6.98±0.74	5.58± 0.74	5.99±0.90
20	3.89± 0.54	3.11±0.83	3.89± 0.68	5.99±0.54	2.98± 0.57	3.10± 0.55

4:45 p.m.

828-6

Transgenic Inhibition of Cardiac NF-kB Activation Protects Against TNF-a-induced Left Ventricular Dysfunction

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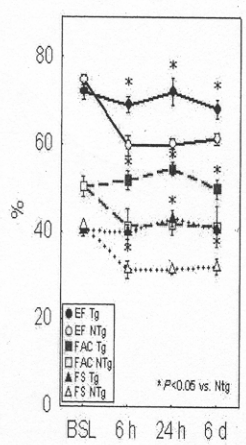
Background: Tumor Necrosis Factor (TNF)-α, a pleiotropic cytokine with diverse biological actions, has been implicated in the development of congestive heart failure. Although TNF-α is known to activate nuclear factor-kappaB (NF-κB), the association between NF-κB activation and LV systolic dysfunction has thus far relied upon pharmacologic inhibition of NF-κB activation.
Methods: We used a transgenic mouse line that overexpresses a dominant negative mutant of IκBα in a cardiac-specific manner. In these mice, NF-κB cannot be activated in cardiomyocytes in response to various stimuli. Age-matched male transgenic (Tg) and nontransgenic (NTg) littermates received TNF-α (120 μg/kg i.p.) and cardiac function was assessed by echocardiography at 6 h, 24 h, and 6 d after TNF-α administration.
Results: In NTg mice, TNF-α administration was associated with a sustained decrement in LV fractional shortening, fractional area change, and ejection fraction that failed to recover at 6 d. In contrast, TNF-α administration did not cause any significant change in LV systolic function in Tg mice. These data constitute the first conclusive genetic evidence that NF-κB activation in cardiomyocytes is crucial for the induction of negative inotropic effects by TNF-α.



Conclusion: We conclude that NF-κB activation in cardiomyocytes is critical for the TNF-α-induced LV systolic dysfunction.

5:00 p.m.

828-7

$ß_2$ Adrenergic Receptor Coupled Phosphoinositide-3 Kinase Constrains cAMP/Protein Kinase A Dependent Increases in Cardiac Inotropy Through cAMP Dependent Phosphodiesterase Activation

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Background: Emerging evidence suggests that Phosphoinositide-3 Kinase (PI3K) may modulate cardiac inotropy, however the mechanism(s) underlying this phenomenon is not understood. We hypothesize that $ß_2$ adrenergic receptor coupled PI3K constrains cAMP/PKA dependent increases in cardiac inotropy through cAMP dependent, PDE Activation.
Methods: We tested the effect of PI3K inhibition on myocardial contractility by measuring simultaneous sarcomere shortening (SS) and intracellular Ca^{2+} transients in fura-2 loaded isolated mouse myocytes at 37°C using a spectrofluorimeter/ videodimension analyzer (Ionoptix Corp).
Results: PI3K inhibition with the specific reversible inhibitor LY294002 ([LY]=10μM) resulted in a marked increase in SS (2.21±0.12 fold increase over baseline, n=14, p<.0001) and Ca^{2+} transient (1.68±0.10 fold increase, n=12, p<.0001). This response could be washed out confirming the reversibility of the inhibition. Since we hypothesized that PI3K modulates the catalytic activity of certain PDE isoforms and therefore the breakdown of adrenergic cAMP, we used the PDE3,4 inhibitor milrinone ([mil]=10μM) alone, and in combination with LY (5μM). Mil alone increased basal SS (1.35±0.08, n=13), however mil+LY showed no added or synergistic effect over LY alone (2.25±0.18 vs. 2.27±0.25, milLY vs. LY, n=13, ns). This supports the hypothesis that mil and LY act through the same pathway. To elucidate the roles of an adrenergic receptor in this basal regulation of PDE activity, we used the $ß_2$ inverse agonist, ICI 118,551 (ICI) (100nM), to suppress the basal agonist independent coupling of this receptor to its G_{sa} and G_{ia} machinery. The increase in contractility induced by PI3K inhibition ([LY]=10μM) was markedly suppressed in a time dependent manner, with the myocyte SS returning to baseline (2.07±.23 vs. 1.06±0.11, LY SS vs. LY-ICI SS over baseline, n=9, p<.001) after 5 min incubation.
Conclusion: These findings demonstrate that PI3K plays a pivotal role in the G_{ia} coupled, basally active, regulation of sympathetically mediated myocardial contractility, through regulation of the catalytic activity of certain PDE isoforms, responsible for the breakdown of adrenergic cAMP.

5:15 p.m.

828-8

Generation of Cyclosporine A-Resistant Anticytomegalovirus-Specific Cytotoxic T Lymphocytes for Transplant Arteriopathy by Dual Gene Delivery Into T cells and Dendritic Cells by Adeno-Associated Virus Vectors

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Background: Transplant patients (and other immunosuppressed persons) are at a very high risk for a variety of opportunistic infections, and most importantly fulminant transplant-associated atherosclerosis. In normal persons cytotoxic T lymphocytes (CTL) clear viral infections by killing virally infected cells. Unfortunately these T cells are specifically affected by the immuno-suppressive drugs such as cyclosporin A (CsA) commonly used to prevent transplant rejection, and have reduced expression of IL-2 and IFN , and limited ability to kill antigen-positive, HLA Class I-restricted target cells.
Methods and Results: Our novel approach is directed at the generation of CsA-resistant, anti-CMV CTL. This *can be done* as CsA-resistant CTL are known to naturally arise and reject transplants. Here we show development of CsA-resistant, anti-CMV CTL by *dual gene therapy* of dendritic cells (DC) and T cells. We had hypothesized that a combination of: 1) transferring the CSA-resistance and Th 1 response-promoting IL-2 cytokine gene into T cells by AAV vector and 2) transferring the CMV pp85 antigen gene into DC by AAV vector will result in a CSA-resistant anti-CMV CTL. Our preliminary data strongly shows that AAV/pp89 gene delivery into dendritic cells did allow these DC to stimulate anti-pp89/antiCMV CTL, which were able to kill 38% of pp89+ target cells (human fibroblasts made positive by AAV/pp89 infection) after only one stimulation. Killing was specific as non-pp89/CMV-positive cells were not killed (eg. normal fibroblasts, lung, breast, and liver cancer cells [5-8% killing]). Intracellular staining showed that 90% of the DC were positive for pp89. Furthermore AAV/IL-2 gene delivery into the pp89 stimulated CTL allowed these T cells to proliferate unimpeded in the presence of 1 μg/ml CsA as measured by 3H incorporation.
Conclusion: These data suggest that this dual antigen/IL-2 gene delivery strategy will be successful. Anti-CMV CTL can easily be generated and be made CsA resistant by the transduction of the IL-2 gene. The approach of dual antigen and cytokine gene delivery offers many possibilities as a novel approach for addressing transplant arteriopathy.