Calcineurin Inhibition Does Not Prevent Left Ventricular Hypertrophy but Does Suppress Its Molecular Markers in a Rat Model of Low-Renin, Mild Pressure Overload

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Background: The role of the Ca^2+ dependent calcineurin (Cn) signalling pathway in the pathogenesis of pressure overload induced left ventricular hypertrophy (LVH) is controversial. The aim of this study was to investigate the relationship between the Cn pathway and the development of LVH in a rat model of low-renin, mild pressure overload.

Methods: Male Wistar rats were fed for 4 weeks a high salt and low renin diet (8% NaCl diet and 1% NaCl drinking water) or a low salt diet (0.3% NaCl diet and distilled water). Blood pressure was measured 3 times per week. After 4 weeks, rats were randomized to receive either 1 mg/kg FK506 daily IP (FK) or an equivalent volume of saline, IP (S). After 3d, half of each group was then subjected to banding of the aortic arch (B). The remaining animals were sham-operated controls (C). LV body weight ratio (LV/BW) and molecular markers of hypertrophy were measured 1 week after surgery. Results: Typical transient hypertrophic response (normalization of LV hypertrophy) was found in the control (SC) group of ANP, α-skeletal actin and BNP in LV tissue samples was assessed by RT-PCR.

Results: At 7d, there was no increase in LV / BW in SB animals compared with SC. Consistent with this finding, there was no change in the expression of the molecular markers examined. In contrast, after 21d an increase in LV / BW of 13±8% (p<0.005) was found in SB; but there was no difference between SB and FK in the degree of LVH induced (0.002±3±0.002 vs 0.003±0.003; n.s.). Peak acute pressure gradients were similar in SB and FK at 21d (30±15, n=10 vs 35±16, mmHg, n=9 respectively) and banding per se did not cause an increase in plasma renin activity. The RT-PCR data (n=5-6 per group) at 21d indicated a 4-fold increase in ANP expression (p=0.03), a 10-fold increase in α-skeletal actin expression (p<0.04) and a 9-fold increase in BNP expression (p<0.06; n.s.) in B compared with SC. No change in the expression levels of these markers was observed in FKB animals.

Conclusions: 1. FK506 does not prevent pressure overload hypertrophy in this model, suggesting that the Cn pathway per se is not critical for the development of LVH when renin is not elevated. 2. The expression of molecular markers associated with LVH without the suppression of LVH in FKB indicates that these proteins are causally related to the Cn cascade but not necessary for the genesis of LVH. 3. Collectively, these data suggest that alternative intracellular signalling pathways are responsible for LVH in this model.

Stimulation of Arachidonic Acid Release From Mas-Transfected COS Cells Is Not Restricted to Angiotensin-(1-7) and Does Not Involve AT1 and AT2 Receptors

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Background: Besides angiotensin (Ang) II, other Ang peptides, as Ang III (Ang-(2-8)), Ang IV (Ang-(3-8)), and Ang-(1-7) have also biological activities. Especially Ang-(1-7) has become an angiotensin of interest, since its vascular and baroreflex actions counteract those of Ang II. Recently, it could be demonstrated that Ang-(1-7) is a functional ligand of the G protein-coupled receptor Mas. We wanted to examine whether other Ang receptors are involved in the ligand/receptor interaction and if other Ang metabolites also affect the signalling pathway of Mas.

Methods: COS cells were transfected with a pcDNA-Mas construct (Mas-transfected cells). COS cells, transfected only with the plasmid pcDNA, served as control cells. Cells were incubated with (3H)arachidonic acid (AA) for 18h. 15 min after adding the angiotensin, Ang-(1-7), Ang IV, Ang-(2-8), Ang III, Ang II, Ang-(3-8), Ang I1 showed no changes. The (3H)AA release induced by Ang-(1-7) was totally increased (p<0.06; n,s.) in SB compared with SC animals; no change in the expression levels of these markers was observed in FKB animals.

Conclusions: 1. FK506 does not prevent pressure overload hypertrophy in this model, suggesting that the Cn pathway per se is not critical for the development of LVH when renin is not elevated. 2. The expression of molecular markers associated with LVH without the suppression of LVH in FKB indicates that these proteins are causally related to the Cn cascade but not necessary for the genesis of LVH. 3. Collectively, these data suggest that alternative intracellular signalling pathways are responsible for LVH in this model.

Characterization of a Novel Insolito 1,4,5-Trisphosphate Receptor/Calcineurin Signaling Pathway in Cardiac Hypertrophy: Effects of Heparin

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Background: Cardiac hypertrophy may be regulated primarily by hypertrophy-stimulating factors. However, little is known about the cardiac growth-inhibitory factors that negatively regulate the formation of cardiac hypertrophy. We postulated that activation of InsP3R which is an intracellular calcium release channel. We postulated that activation of InsP3R mediated CN / NFTAD / GATA4 pathway have a potential role in regulation of cardiac hypertrophy and heparin may antagonize this signaling pathway. Methods and Results: primary neonatal rat cardiomyocytes were cultured using established techniques. Fluorescence-spectrophotometric measurements using fura2 showed that InsP3 caused a dose-dependent increase of cytosolic free calcium concentration in cardiomyocytes (IP 3 100 nM: 138±7 vs 223±8 nM, p<0.01). InsP3 increased 3H-thymidine (IP 3 100 nM: 186±17±17 vs 186±100±0±44± cm2/pmol, p<0.01) and 3H-nucleosine (IP 100 nM: 88±19±153±14±11±cm2/pmol, p<0.01) incorporation in a time- and dose-dependent manner. Immunoblotting showed that InsP3 significantly enhanced the expression of c-fos, c-myc, alpha-actin and beta-MHC in cardiomyocytes. Administration of InsP3 time-dependently increased the expression of CN, NFAT3 and GATA4. The InsP3-induced CN expression was blocked by heparin administration. Conclusions: The study indicates that the InsP3 sensitive calcium pool is related to cardiac hypertrophic gene regulatory pathways. Antagonizing the InsP3R/CN/NFAT3/GATA4 signaling pathway may be a new therapeutic target for cardiac hypertrophy. (Supported by grant from NSFC No. 39725013.)