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ABNORMAL CALCIUM HANDLING BY VENTRICULAR MYOCYTES OF HYPERTENSIVE, CALCIUM-DEFICIENT CHICK EMBRYOS

Takashi Miyahara, and Rocky S. Tuan. Thomas Jefferson University, Philadelphia, PA

Hypertension and tachycardia develop in Ca^{2+}-deficient, shell-less (SL) chick embryos cultured ex ovo, accompanied by elevated circulating catecholamines and higher α-adrenergic sensitivity of cardiovascular functions. We report here that Ca^{2+} handling is altered in the heart cells of SL embryos. For this study, isolated and cultured ventricular myocytes of SL and normal (NL) embryos were loaded with Fura-2 via transient membrane damage with glass beads. Compared to Fura-2/AM, bead loading yielded similar $[Ca^{2+}]_i$ values and kinetic profile and did not affect cell viability and beating activity. The procedure should be generally applicable for Fura-2 loading, particularly for cells with low esterase activity. The Fura-2 loaded cells were washed in Ca^{2+}-free buffer and then analyzed for kinetic changes in $[Ca^{2+}]_i$ as a function of $[Ca^{2+}]_o$ and adrenergic modifiers by fluorescence imaging microscopy. At 0.5 and 1.0 mM $[Ca^{2+}]_o$, SL cells showed higher $[Ca^{2+}]_i$ (R values: SL, 0.67 & 0.93; NL, 0.50 & 0.74, respectively; $p<0.05$) and faster rate of increase in $[Ca^{2+}]_i$ compared to NL cells. At higher $[Ca^{2+}]_o$ (3-5 mM), there was no significant difference in $[Ca^{2+}]_i$ between NL and SL cells. Treatment of NL or SL cells with norepinephrine (NE; 0.01-1 μM) at 1mM $[Ca^{2+}]_o$ substantially increased $[Ca^{2+}]_i$. In NL cells, the NE effect was completely inhibited by ß-blockade (1μM propranolol), which was only partially effective in SL cells. On the other hand, α-blockade (1μM prazosin) partially inhibited $[Ca^{2+}]_i$ increase by NE in SL cells, but not in NL cells. The altered Ca^{2+} handling and adrenergic regulation of the heart cells may contribute to the Ca^{2+} deficiency related development of impaired cardiovascular functions in the chick embryos.

HEMODYNAMIC AND VASCULAR CHARACTERISTICS OF DUP 753: A SPECIFIC ANGIOTENSIN II ANTAGONIST, IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR).

Robert J. Cody, Garrie J. Haas, Philip F. Binkley, Douglas M. Brown. The Ohio State University, Columbus, OH

DUP 753, an angiotensin II antagonist, produces a dose dependent antihypertensive effect. The present study characterized the hemodynamic profile of IV bolus DUP 753 in the SHR, in preparation for studies of orally administered DUP 753. IV DUP 753 as 1 or 3 mg/kg had minimal to-modest effect, but 10 mg/kg produced marked pressure reduction. In 9 anesthetized, instrumented, open-chest SHR (age 14±1.2 mo, wgt 413± 13 grams) DUP753(10mg/kg) lowered mean aortic pressure (MAP) from 151±18 to 112±22 mmHg ($p<0.01$), persisting from 15 to 60 minutes. MAP reduction was mediated by systemic arterial resistance reduction from 1.97±.43 to 1.41± .43 units ($p<0.01$). While heart rate and cardiac output(ml/min) were unchanged,peak flow (234±55 to 283±59 ml/min $p<.01$) and pulse pressure (41±12 to 49±8 mmHg $p<.04$) increased. Characteristic aortic impedance (10758±3823 to 9419± 2377 dx5/cm^5 $p<.05$) and wave reflectance index (15401±6744 to 10790±3298 dx5/cm^5 $p<.05$) also decreased. Thus, the effect of DUP753 is mediated by vasodilation without adverse cardiac effects. These studies document reversal of an adverse effect of angiotensin II on the conduit function of the aorta, as well as favorable vasodilation of resistance size arteries.

INDUCTION OF THE EARLY GROWTH RESPONSE GENE-1, C-FOS, AND PROTEIN SYNTHESIS IN ADULT CARDIOMYOCYTES BY ANGIOTENSIN II AND ENDOTHELIN

Ludwig Neyses, Janis Nouskas, Joachim Luyken, Stefan Fronhoffs, R. Sanders Williams, Vikas P. Sukhatme, Hans Vetter, Medical Policlinic, University of Bonn, Federal Republic of Germany

We assessed the pressure-independent role of angiotensin II (AII) and endothelin-1 (E) in the development of myocardial hypertrophy using isolated adult rat cardiomyocytes.
The transcriptional factors "early growth response gene-1" (Egr-1) and c-fos were investigated by Northern blotting using a 2.1 kb fragment of murine Egr-1 and v-fos (1 kb) as probes. At 10^{-7}M, AII and E induced c-fos approximately 10 and 25-fold, respectively (maximum after 15 min, return to basal levels at 60 min). There was a dose-response relationship with induction in the physiological concentration range of AII and E. Egr-1 was maximally induced at 30 min by AII and E with a similar dose-response relationship as c-fos. The action of AII, not E, was potentiated 3-fold by fetal calf serum. AII stimulated protein synthesis (measured using cycloheximide-inhibitable ^{3}H-Phenylalanin incorporation) by 110+/-14% (n=6, $p<0.05$, AII vs control). E increased protein synthesis by 223+/-18% ($p<0.05$).
Conclusion: These results indicate that AII and E are able to induce probable early (growth-related transcriptional factors) and late (protein synthesis) steps in the chain of events leading to myocardial hypertrophy and/or remodeling.

CALCIUM VOLTAGE CHANNEL ANTAGONISM PREVENTS ENDOTHELIN-INDUCED HYPERTENSION.

Robert J. Cody, Garrie J. Haas, Douglas M. Brown. The Ohio State University, Columbus, OH

We have previously reported that endothelin (ET3) administration to normal rats causes brief hypotension followed by sustained hypertension, mediated by concordant changes in systemic arterial resistance (SAR,mmHg/CO per kg). In vitro vascular studies suggest ET3 vasoconstriction may be mediated by increased vascular smooth muscle cytosolic calcium. This hypothesis remains controversial and has not been fully tested in intact animals. We gave ET3 (1 nanomole) intravenously to 6 anesthetized Sprague-Dawley rats (age 15±0.5 mo; weight 525±64 grams), during hemodynamic instrumentation. Compared to baseline, and following the initial brief hypotensive phase, ET3 increased mean pressure (MAP,mmHg) from 110±12 to 143±19($p<0.01$), mediated by an increase of SAR (1.17±0.7 to 2.30±0.9;$p<0.01$). At peak vasoconstriction, infusion of a calcium channel antagonist (nicardipine, 3.1±1.2mcg/kg/ min), reversed MAP from 143±10 to 106±13 ($p<0.01$), and decreased SAR from 2.30±0.9 to 1.40±0.6 ($p<0.01$). In 4 additional rats prior administration of 1.7 mcg/kg/min nicardipine produced minimal MAP reduction, but completely prevented subsequent ET3 hypertension. These in vivo data suggest that the hypertensive response to ET3 can be reversed and prevented by antagonizing voltage channel calcium influx in vascular smooth muscle.