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PROGRESS REPORT (final)
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BIOCHEMICAL ASSESSMENT OF STRESS IN CARDIAC TISSUE IN RESPONSE TO WEIGHTLESS SPACE TRAVEL

PERSONNEL

Name	UCSD Title	Role in Grant
Laurence L. Brunton, Ph. D.	Professor of Pharmacology and Medicine, UCSD	Principal Investigator
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HYPOTHESIS

The absence of unit gravity may cause physiological changes in the cardiovascular system. For instance, in the absence of Earth's gravity, venous return to the heart may increase due, in part, to decreased pooling of the blood in the extremities. We hypothesize that this would produce an increase in the heart's work load, ultimately resulting in hypertrophy.

ORIGINAL PROPOSAL AND ITS REVISION

Originally, we proposed a two project for approximately $50,000 per year. NASA awarded $20,000 for one year. This required scaling back the aims. The original included a menu from which items to be measured would be chosen based on the most current knowledge. Original proposed measurements centered on transmembrane signaling (cyclic AMP system) and on inducible genes (fos, jun, HSP70 and others mentioned below). In scaling back to met the exigencies of the budget, we chose to focus on mRNA expression, since multiple probings could be

made from a single mRNA gel blot, and since a careful examination of the recent literature convinced us that certain genes were very good markers of stress and hypertrophy in cardiac tissue.

TESTING THE HYPOTHESIS

As a test of the hypothesis, we have analyzed the mRNA levels of ANP in cardiac tissue from rats subjected to weightless space travel.

ANP is normally synthesized and secreted by atrial myocytes, however its expression can be induced in the ventricles during hypertrophy and is widely accepted as a molecular marker of myocardial growth.

RESULTS

In this study, Sprague-Dawley rats were divided into ground control and flight specimens, with the latter being subjected to two weeks of space travel. Upon their return to Earth, both flight specimens and ground controls were sacrificed and atria and ventricles were isolated. We received the frozen heart tissue from NASA.

We have powdered each tissue, then extracted mRNA from each sample and analyzed it by Northern analysis using a cDNA probe specific for ANP. The techniques applied are all standard, although some minor adapting was required for the tissue and for the qualities of the specific mRNA and cDNA.

ANP mRNA levels were elevated in the flight specimens, with the most dramatic increase occurring in the ventricular tissues. Densitometric assessments of autoradiographs demonstrated a 30% increase of ANP message in atrial tissue and a 230% increase of ANP message in the ventricular tissue of rats subjected to weightlessness. Ground controls showed no change in expression of ANP in either ventricular or atrial tissues. The increase of ANP message supports our hypothesis that prolonged space travel leads to cardiac hypertrophy.

Recently, we have been constructing cDNA probes for HSP70 and SERCA2, as described below.

CONTINUATION OF THIS WORK

One of the interesting bonuses of assessing expression of mRNAs by Northern analysis is that the blots may be probed, stripped and re-probed multiple times. In addition, we have tissue and mRNA for multiple gels/blots. Thus, we can continue to study this set of tissues. We are currently using additional cDNA probes and Northern analysis to assess the effects of weightless flight on other markers of growth and stress in cardiac tissue, including SERCA2 and HSP70.

SERCA2 is the protein that regulates the accumulation of Ca^{++} back into the sarcoplasmic reticulum to permit (or cause) relaxation of the heart. We have evidence that the SERCA2 gene is regulated by cAMP and the PI/PKC pathways and that the mRNA for SERCA2 is down-regulated by the hormone endothelin, which is also a cardiac growth factor and positive inotropic agent. Down regulation of the sarcoplasmic reticulum calcium ATPase (SERCA2) is associated with cardiac dysfunction in the failing heart. Plasma levels of the growth peptide endothelin (ET-1) levels are elevated in cardiac hypertrophy and failure. Investigating the capacity of ET-1 to regulate SERCA2 gene expression in cardiac myocytes, we used Northern analysis studies of mRNA of both adult and neonatal cardiac myocytes. The data indicate that after a 24 hour treatment with ET-1, SERCA2 expression is decreased by 30% and 50%, respectively. In transfection experiments using the SERCA2 promoter region linked to CAT activity, ET inhibits basal SERCA2 promoter activity as well as thyroid hormone stimulated SERCA2 promoter activity. Preliminary data indicate that the signaling pathways by which ET down-regulates SERCA2 expression require ET-1 activation of both G_q and G_i linked pathways and the lowering of intracellular cAMP levels (1).

Based on this knowledge, we believe that probing the cardiac tissue of the NASA "astronaut rats" will be exciting. We have produced a cDNA probe for SERCA2 and will shortly begin working out conditions for probing the mRNA blots for the message for SERCA2.

We are also trying to set up a collaboration with colleagues at UCSD who study the capacity of cardiac fibroblasts to "re-model" the extracellular matrix and who study angiogenesis. Our thought here is that alteration of the extracellular matrix could be a prelude to hypertrophic responses in the heart and that expression of different

collagens may alter the mechanical properties of the ventricles. Similarly, expression of VEGF (Vascular Endothelial Growth Factor) is an early marker for an angiogenic response that is often observed in stressed cardiac tissue when O_2 demand exceeds O_2 supply. Whether this gene is activated by weightless space flight is not known but readily determined and we are preparing to obtain the cDNA probe.

CAVEAT

A complete analysis cannot be made until NASA sends us the full key to the sample code. Much of the system of labeling is apparent but we need to be certain that we have the key and that we interpret our results correctly according to the protocol NASA used. We have requested the key and are currently filing a second request.

PUBLICATIONS SUPPORTED BY THIS GRANT

1. Lio FM, Meszaros JG, Brunton LL. (1997). Induction of mRNA for Atrial Natriuretic Peptide (ANP) in Hearts of Rats Subjected to Weightless Space Travel. Abstract for National Minority Biomedical Research Symposium, New Orleans, October, 1997.

2. A manuscript based on the above work is being prepared but will not be completed until NASA sends the full key to the labeling of the samples.

3. Meszaros JG, Brunton LL and Bloor CM. "Animal Models of Angiogenesis in Cardiovascular Tissues" in, Angiogenesis in Cardiovascular Disease, ed., JA Ware and M Simons, Oxford University Press, for late 1997/early 1998.

REFERENCES

1. Hilal-Dandan R, Brunton LL and Dillmann W (1996). Endothelin Regulates Sarcoplasmic Reticular Calcium ATPase (SERCA2) Gene Expression in Rat Cardiac Myocytes. Circulation (abstracts for AHA Meetings, Fall, 1996)

2. Martin J L, Mestril R, Hilal-Dandan R, Brunton LL and Dillmann WH (1997). Small heat shock proteins and protection against ischemic injury in cardiomyocytes. Circulation, in press.