# PATHOPHYSIOLOGY AND PHARMACOLOGY OF HEART DISEASE

edited by

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Proceedings of the symposium held by the Indian section of the International Society for Heart Research, Chandigarh, India, February 1988

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**Kluwer Academic Publishers**
Boston/Dordrecht/London

**Distributors for North America:**
Kluwer Academic Publishers
101 Philip Drive
Assinippi Park
Norwell, Massachusetts 02061 USA

**Distributors for all other countries:**
Kluwer Academic Publishers Group
Distribution Centre
Post Office Box 322
3300 AH Dordrecht, THE NETHERLANDS

**Library of Congress Cataloging-in-Publication Data**

Pathophysiology and pharmacology of heart disease : proceedings of a symposium held during the Annual Meeting of the Indian Section of the International Society for Heart Research, 25-28 February 1988, Chandigarh / edited by Inder S. Anand, Purshotam L. Wahi, Naranjan S. Dhalla.
p. cm. — (Developments in cardiovascular medicine ; DICM 102)
"This symposium was sponsored by the Council of Cardiac Metabolism of the International Society and Federation of Cardiology"—Half -title p.
Includes index.
ISBN 0-7923-0367-9
1. Heart—Diseases—Pathophysiology--Congresses. 2. Heart—-Diseases—Chemotherapy—Congresses. I. Anand, Inder S. II. Wahi, Purshotam L. III. Dhalla, Naranjan S. IV. International Society for Heart Research. Indian Section. Meeting (1988 : Chandigarh, India) V. Council on Cardiac Metabolism. VI. Series: Developments in cardiovascular medicine : v. 102.
[DNLM: 1. Heart Diseases—physiopathology—congresses. W1 DE997VME v. 102 / WG 200 P2926 1988]
RC681.A2P37 1989
616.1'207—dc20
DNLM/DLC
for Library of Congress 89-15373
CIP



Printed in the United States of America

To
Professor Santokh Singh Anand
founder-director of the
Postgraduate Institute of Medical Education & Research
Chandigarh, India

# Preface

Research at the molecular and the cellular level has greatly enhanced our understanding of the pathogenesis and management of heart disease. Valuable contributions, towards this end, have been made by scientists from different disciplines including biochemistry, physiology, pathology, molecular biology and biophysics. We felt that it would be of interest and value to bring together experts from diverse specialities to present their work and to discuss the common problems encountered in their endeavours. In accordance, a symposium was organised in February 1988 at the Postgraduate Institute of Medical Education & Research, Chandigarh. It was held during the annual meeting of the Indian section of the International Society for Heart Research. This book is a compilation of some of the papers presented at the symposium.

The symposium was sponsored by the Council on Cardiac Metabolism of the International Society and Federation of Cardiology. A number of Indian organisations gave generous financial help. These included the National Academy of Medical Sciences, Indian Council of Medical Research, Council of Scientific and Industrial Research and Department of Science and Technology.

Desktop publishing was used to prepare this volume. In doing so we came to appreciate the remarkable qualities, skills and help rendered by Professor Dharam Vir. For typing the manuscripts and for other secretarial assistance we gratefully acknowledge the help of Ravinder and Sawtantar.

# 1

# THE NEWBORN PIG HEART, A SUPERIOR ANIMAL MODEL OF CARDIAC HYPERTROPHY

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## Introduction

Animal models of cardiac hypertrophy have been devised that result in rapid cardiac growth. The need for rapid growth in these models results from the goal of determining the extent to which protein synthesis and protein degradation contribute to accumulation of cardiac mass and the precision with which these rates can be measured. If one assumes that these rates can be determined with a standard error of 5%, the rates of synthesis or degradation need to change by at least 15% to reach statistical significance. These changes are huge when considered in relation to the gradual development of naturally occurring hypertrophy. For example, a rat heart weighing 1 g needs to have 1% per day faster rate of protein synthesis than protein degradation to achieve a 35% increase in mass after 30 days.

## Commonly used models of cardiac hypertrophy

The models that have been used include pressure overload, volume overload, chronic exercise, injection of isoproterenol, and injection of thyroid hormone (for review[1]). Pressure overload has been induced by constriction of the ascending aorta proximal to the first branch, the abdominal aorta proximal to the renal arteries, or the pulmonary artery with a haemoclip, suture, or inflatable cuff. These models are defective in regard to mechanical performance, cellular energetics or tissue integrity.[1] In most studies of pressure overload hypertrophy, mechanical performance is depressed.[2-5] The velocity of fibre shortening in papillary muscles correlates with the activity of the calcium/actin-activated myosin ATPase (for review[6]). These same models of pressure-induced hypertrophy also commonly show disturbed energy metabolism as evidenced by decreased contents of high energy phosphates.[1] Reduced energy availability is of particular concern because both protein synthesis and degradation are energy-requiring processes.[7] In regard to morphology, hearts from cats and rabbits

subjected to abrupt banding of the aorta or pulmonary artery may develop areas of focal necrosis and fibrosis.[8] Pressure overload may be produced experimentally without inducing tissue necrosis if the overload is applied gradually,[3] but in this case the rate of growth may be too slow for mechanistic studies of the role of protein synthesis and degradation. Tissue necrosis may provide an explanation for the rapid rates of protein degradation seen in isolated rat hearts from animals subjected to aortic banding.[1] As a result of the deficiencies noted above, hypertrophy induced by acute pressure overload is a poor model for studies of the mechanism of cardiac growth.

Volume overload is usually produced by induction of aortic regurgitation,[9] atrial septal defects,[10,11] or arteriovenous shunts.[9] The abnormalities in mechanical performance, energy metabolism and tissue integrity that are seen with pressure overload do not occur to a significant extent, but the pace of hypertrophy is too slow in volume overload for studies of the relative contributions of synthesis and degradation of protein to growth.

Daily swimming for 6 hours/day[12] has been observed to result in a significant increase in heart weight after 2 days and a 30% increase in heart weight after 14 days. Hearts from rats exercised by swimming demonstrate increased mechanical performance[13] and myosin ATPase activity.[14] The exercise model of hypertrophy has not been used for a rigorous study of effects on protein synthesis and degradation, but this model may be useful for examining the mechanisms of physiological hypertrophy.[15]

Single (25 mg/kg) or multiple injections of isoproterenol (0.1 to 5 mg/kg) result in cardiac hypertrophy (for review[1]). Injections of 5 mg/kg decrease blood pressure and increase the heart rate.[16] Such changes might account for appearance of areas of focal necrosis that have been observed. This same dose (5 mg/kg) of isoproterenol also results in decreased ATP content (16%), as does a single dose of 25 mg isoproterenol/kg (30%).[17,18] Overall, the existence of areas of necrosis along with energy depletion limits the usefulness of isoproterenol-induced hypertrophy for mechanistic studies of cardiac growth.

Thyroxine-induced cardiac hypertrophy results in improved cardiac performance when assessed in isolated hearts or papillary muscles (for review[19]). The enhanced mechanical performance is accompanied by shift of myosin isozymes to the V1 form and increased myosin ATPase activity. Thyroxine administration also increases whole body oxygen consumption, heart rate, systolic pressure and cardiac output.[20] Recent studies by Klein and Hong[21] indicate that thyroxine-induced hypertrophy is mediated indirectly by these changes in cardiac work, but the increased expression of the V1 isozyme is due to a direct ef-

fect on the heart. These studies have also compared changes in the *in situ* working heart to those in a heterotopically transplanted heart of rats injected with 10 μg thyroxine/day. The major defect in this model is the large dose of thyroid hormone that is administered and the multiple effects that occur. Earlier studies from my laboratory[22] demonstrated that rapid cardiac growth could be induced by physiological doses of thyroxine (5 μg/day) if administered to hypophysectomized rats. Both of these models of thyroid hormone induced growth merit further studies at the mechanistic level.

Overall, increased growth induced by exercise and thyroid hormone administration are the only traditional models of hypertrophy that are free of decreased mechanical function, energy depletion and focal necrosis. We have sought a new model of hypertrophy in which rapid physiological growth occurs and the defects of earlier models are absent.

**Rapid growth of newborn pig heart**

In the neonatal period, the left ventricle grows much more rapidly than the right ventricle. Preferential growth of the left heart is due to a change in the afterload imposed on this chamber due to closure of the ductus arteriosus, inflation of the lungs, and the requirement for the left ventricle to pump blood throughout the systemic circulation.[23] These circulatory changes appear to be the stimulus for more rapid growth of the developing left ventricle.[24,25] A possible mechanism for faster growth of the left ventricle is stretch of the ventricular wall which has been shown to accelerate total protein synthesis and ribosome formation in the isolated perfused heart of adult rats.[26]

Accumulations of protein, RNA, and DNA in the right ventricular free wall (RVFW) and left ventricular free wall (LVFW) of hearts of pigs of 1, 5 and 10 days of age are shown in Table 1.[27] The rate of total protein accumulation was 3.5x faster over this period in the LVFW than RVFW. Total RNA accumulated at a similar pace as protein, 3.3 fold greater in the LVFW than RVFW. These findings indicated that the more rapid protein accumulation in the LVFW was due to more ribosomes as indicated by total RNA content. DNA content increased 78% in the RVFW and 124% in the RVFW between 1 and 9 days of age. The greater DNA content in the LVFW probably reflected its larger size and consequently more non-muscle cells. If the protein/DNA ratio is taken as a reflection of cellular hypertrophy, muscle cell size was stable in the RVFW, but increased by 56% in the LVFW during 9 days of postnatal life. These estimates of cellular hypertrophy are minimal values because of the appearance of binucleated muscle cells and hyperplasia of non-muscle cells during this period. Morphometric studies of the neonatal pig heart are needed to assess the extent of hypertrophy

of the muscle cells, particularly because non-muscle cells are more numerous and their numbers have a large effect on protein/DNA ratios.

The neonatal pig heart offers a further advantage in that it can be perfused *in vitro* under conditions that allow rates of protein synthesis to be calculated from rates of incorporation of phenylalanine, an amino acid that otherwise is not metabolised by heart,[28] and the specific radioactivity of the immediate precursor for protein synthesis, phenylalanyl-tRNA.[27] After 1 hour of perfusion, the specific radioactivity of tRNA-bound phenylalanine was 92% of phenylalanine in the perfusate.[27] This finding justified the routine use of specific radioactivities of perfusate phenylalanine for calculations of protein synthetic rates. During 1 hour of perfusion of hearts of 5-day old pigs with Krebs-Henseleit bicarbonate buffer containing 5 mM glucose, 5 mM lactate, normal plasma concentrations of 19 amino acids and 0.57 mM [$^{14}$C] phenylalanine, the rate of protein synthesis in the LVFW was double that in the RVFW (Table 2). This increase was accounted for by a 35% greater RNA content (capacity for protein synthesis) and a 52% higher efficiency of synthesis (nmol phenylalanine incorporated.mg RNA$^{-1}$.h$^{-1}$). In earlier experiments from this laboratory,[39] cardiac work increased the efficiency of protein synthesis. Recently, Nagal et al.[30] reported that increased efficien-

**Table 1**

*Effect of age on protein, RNA, and DNA contents of newborn pig hearts.*

| | Days postpartum: | 1 | 5 | 10 |
|---|---|---|---|---|
| Total protein, mg per heart portion | RVFW | 100 ± 11 | 150 ± 14 | 184 ± 5 |
| | LVFW | 116 ± 6 | 279 ± 28* | 407 ± 8* |
| Total RNA, mg per heart portion | RVFW | 100 ± 11 | 141 ± 13 | 184 ± 8 |
| | LVFW | 150 ± 9* | 327 ± 34* | 442 ± 9* |
| Total DNA, mg per heart portion | RVFW | 100 ± 8 | 149 ± 15 | 178 ± 14 |
| | LVFW | 148 ± 6* | 275 ± 31* | 333 ± 17* |
| Total protein/ total DNA | RVFW | 100 ± 4 | 101 ± 3 | 107 ± 7 |
| | LVFW | 79 ± 3* | 103 ± 4 | 124 ± 7 |

Means ± SE of 5 hearts at each age are given.

Values are expressed as a percentage of the quantity of protein, RNA, or DNA in the RVFW of 1-day old hearts. Protein/total DNA ratios are expressed in a similar manner. These RFVW base values were 234 mg protein, 3.21 mg RNA, 3.45 mg DNA, and the protein/total DNA ratio was 67.3.

* $p < 0.05$ versus RVFW of same age.

**Table 2**

*Efficiency and capacity of protein synthesis in isolated perfused hearts of 5-day old pigs.*

| | | Protein synthesis | RNA | Efficiency |
|---|---|---|---|---|
| Control | RVFW | 100 ± 9 | 100 ± 3 | 100 ±10 |
| | LVFW | 205 ± 11* | 135 ± 6* | 152 ± 7* |
| +Insulin | RVFW | 141 ± 7# | 95 ± 3 | 148 ±10# |
| | LVFW | 219 ± 8* | 136 ± 5* | 160 ± 7 |

Means ± SE of 7 or 8 hearts are given.

Values are expressed as a percentage of the rate of protein synthesis, RNA content, or efficiency of synthesis observed during 1 h of perfusion in the RVFW of control hearts. These RVFW control values were 946 ± 86 nmol phenylalanine.g dry heart$^{-1}$, 11.5 ± 0.4 mg RNA.g dry heart$^{-1}$, and 83 nmol phenylalanine.mg RNA$^{-1}$.h$^{-1}$.

* $p < 0.05$ versus RVFW of control hearts.
# $p < 0.05$ versus same portion of control hearts.

cy of synthesis was a major factor leading to right ventricular hypertrophy following pulmonary artery constriction. When 5-day old pig hearts were perfused with the same buffer containing 1.7 x $10^{-7}$ M insulin, a pharmacological concentration, the rate of protein synthesis in the RVFW increased 40% due to greater efficiency of synthesis. The rate of protein synthesis in the LVFW did not increase with the addition of insulin and, in the presence of the hormone, efficiencies of synthesis were the same in RVFW and LVFW. In the presence of insulin, faster protein synthesis in the LVFW was due entirely to greater capacity for synthesis, as reflected in increased RNA content.

## Conclusions

Studies of the mechanisms of cardiac hypertrophy must employ experimental models that induce rapid growth of either the left or the right heart. These models involve either induction of a pathological overload to the ventricles or a se[illegible] physiological stimulus, such as exhaustive exercise of thyrotoxicosis. The LVFW of the newborn pig heart grows 3x faster than the RVFW without any pathological or supraphysiological stimulus. From this point of view, the newborn pig heart is not a model of hypertrophy, but rather a totally normal situation in which rapid cardiac growth occurs. The newborn heart does not suffer from energy depletion which distorts measurements of protein and RNA synthesis and degradation. Focal necrosis does not develop, and growth can be accounted for by faster rates of protein synthesis.[27] Both increased capacity and efficiency for protein synthesis may contribute to the faster growth, but

greater capacity as reflected in RNA content plays the predominate role. In other experiments (Camacho, Peterson, White and Morgan, *unpublished*), a 50% faster rate of ribosome formation was observed in the LVFW than RVFW of 2-day old pigs. These findings suggest that increased ribosome content is based on accelerated rates of synthesis of ribosomal proteins and transcription of rDNA. Further studies of the newborn heart should provide information regarding the physiological control of cardiac growth.

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# 2

# SUBCELLULAR ALTERATIONS IN HYPERTROPHIED PIG HEART DUE TO PRESSURE OVERLOAD

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It is well known that $Ca^{2+}$ plays a crucial role in determining the heart function and cellular integrity.[1-5] While the interaction of $Ca^{2+}$ with myofibrillar proteins is of prime importance in eliciting the contractile events, different membrane systems such as sarcolemma, sarcoplasmic reticulum and mitochondria are considered to be involved in the delivery as well as removal of $Ca^{2+}$ from the contractile apparatus. The superficial store of $Ca^{2+}$, which is represented as ATP-independent $Ca^{2+}$-binding with the sarcolemmal membrane, is believed to be a buffer site between extracellular and intracellular compartments of $Ca^{2+}$ and serves as an immediate source of $Ca^{2+}$ for contraction. Although most of the $Ca^{2+}$ enters the myocardial cell through $Ca^{2+}$-channels located in the sarcolemmal membrane, the biochemical basis for opening $Ca^{2+}$-channels is far from clear. Recently, the involvement of a sarcolemmal $Ca^{2+}/Mg^{2+}$ ATPase, which is activated by millimolar concentrations of $Ca^{2+}$, has been suggested in a gating mechanism for the influx of $Ca^{2+}$ into the cardiac cell.[1,3,6,7] While $Ca^{2+}$-stimulated ATPase (activated by micromolar concentration of $Ca^{2+}$ in the presence of $Mg^{2+}$ and ATP-dependent $Ca^{2+}$-binding in the sarcolemmal membrane are believed to serve as a $Ca^{2+}$-pump mechanism for the efflux of $Ca^{2+}$, the sarcolemmal $Na^{+}$-$Ca^{2+}$ exchange mechanism has been implicated both in entry and removal of $Ca^{2+}$ from the cell.[1,3,8-10] The $Ca^{2+}$-pump mechanism ($Ca^{2+}$-stimulated ATPase and ATP-dependent $Ca^{2+}$-uptake) present in the sarcotubular system is involved in the sequestration of $Ca^{2+}$ from the cytoplasm and has been claimed to be intimately associated with the relaxation of the cardiac muscle; a major portion of $Ca^{2+}$ required for cardiac muscle contraction is also considered to be released from this intracellular store upon excitation of the cell.[1,3] Although mitochondria are also known to accumulate $Ca^{2+}$ by energy-dependent mechanisms, their role

in the regulation of intracellular $Ca^{2+}$ in the heart has been suggested under pathological rather than physiological conditions.[2,3]

From a physiological viewpoint, cardiac hypertrophy is considered to be an adaptive process in response to increased work load.[11] It is by now clear that adaptation of the hypertrophied heart is not only associated with an increase in muscle mass where new contractile units are added but is also accompanied by changes in function of sarcolemma, sarcoplasmic reticulum and mitochondria with respect to their $Ca^{2+}$ translocation abilities.[11,12] On the basis of the work from several laboratories[13-28] it has been suggested that changes in subcellular organelles depend upon the type and stage of cardiac hypertrophy.[11] In this report we describe changes in sarcolemmal, sarcoplasmic reticular, mitochondrial and myofibrillar activities in the hypertrophied heart due to pressure overload induced by banding supravalvular aorta in pigs for 12 weeks. This experimental model has been employed in our laboratory for defining functional and subcellular alterations in the myocardium both at 4 weeks and 8 weeks after inducing a pressure overload.[29-34]

## Materials and methods

Cardiac hypertrophy in pigs was induced by banding the supravalvular aorta for 12 weeks as described earlier.[29] Sham operated animals without aortic banding were used as controls. The animals were sacrificed by a stunning blow on the forehead and the left ventricular tissue was dissected out. The left ventricular weight/body weight ratio was increased by about 30% of the control value indicating the presence of cardiac hypertrophy. It should be pointed out that none of the animals used showed any clinical signs of heart failure. Sarcolemmal fraction was isolated by the hypotonic shock-LiBr treatment method.[36] Fragments of sarcoplasmic reticulum were isolated and purified by the method described previously.[37] Mitochondria were isolated by the method of Sordahl and Schwartz.[18] Myofibrils and myosin were isolated and purified according to techniques described elsewhere.[38,39] It should be pointed out that marker enzyme activities, as monitored by methods employed previously,[36,37] revealed minimal but an equal extent of cross contamination in both control and experimental membrane preparations. $Ca^{2+}$-binding, $Ca^{2+}$-uptake and different ATPase activities in the membrane fractions as well as ATPase activities of myofibrils and myosin were studied under assay conditions employed previously.[31-33,35]

## Results

The data shown in Table 1 indicate that ATP-independent $CA^{2+}$ binding was significantly increased in the sarcolemmal preparation from 12-week hyper-