ADRENOCHROME UPTAKE AND SUBCELLULAR DISTRIBUTION IN THE ISOLATED RAT HEART

A Thesis

Presented to the

University of Manitoba

In Partial Fulfilment of the

Requirements for the degree

MASTER OF SCIENCE

IN

PHYSIOLOGY

by

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April, 1980

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Acknowledgements

I would like to thank all those people who made possible and helped me with the completion of this thesis. This includes my parents, Maureen Otten, Micheal Daley, Dr. V. Panagia and all the others who helped. I would especially like to thank Dr. Satoshi Takeo for his kind help and I would like to thank Dr. Dhalla for his help and the opportunity given to me to do my work.

This thesis is dedicated to my mother and father, with great love and appreciation.

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I Abstract

Adrenochrome, an oxidation product of epinephrine, has been implicated in the production of myocardial cell damage in catecholamine-induced cardiac necrosis. This study was undertaken to investigate the uptake and subcellular distribution of [C^{14}]adrenochrome in the isolated rat heart. The results revealed that adrenochrome was taken up readily by a low affinity high capacity system somewhat similar to that for extraneuronal catecholamine uptake. The uptake was concentration and time dependent, and obeyed Michaelis-Menton kinetics with a Km of 258 x 10^{-6} M and a Vmax of 54.6 ug/min/gm. Adrenochrome bound strongly to the myocardium and a study on subcellular distribution showed that the highest specific and total activity was found in the sarcolemmal fraction. Adrenochrome caused decreases in contractile force and resting tension of perfused hearts and the uptake of adrenochrome could be dissociated from these effects on heart contractile force and resting tension suggesting the biochemical and structural changes have an important role in these alterations. Adrenochrome uptake was inhibited somewhat by both neuronal and extraneuronal catecholamine uptake inhibitors. Propranolol and iproniazid, which decrease the cardiotoxicity of adrenochrome, reduced a large proportion of adrenochrome uptake. Corticosterone and 17-beta-oestradiol also strongly augmented adrenochrome induced increases in resting tension. The results suggest no simple relationship between adrenochrome uptake and its cardiotoxic

effects, but rather a more complex one involving the binding to various fractions of the heart and affecting their function.

II Introduction and Statement of the Problem

Evidence has been presented that suggests that excessive amounts of catecholamines play a role in heart disease in man (1-3) and catecholamine-induced cardiac necrosis has long been used as a model of catecholamine induced heart failure. Injections of relatively large amounts of epinephrine, norepinephrine or the synthetic catecholameine isoproterenol cause focal necrosis in the heart, along with a variety of ultrastructual, biochemical and functional damage (3-9). These effects have been observed in vivo (3-9), in isolated hearts (10-14), in cultured heart cells (15) and in humans when norepinephrine was used to maintain blood pressure (16,17).

The deleterious actions of exogenously administered catecholamines are also often compared to those of stress (3, 18-30). Various types of stress, either psychological or physiological, can result in increased output of and/or increased sensitivity to catecholamines (28, 30) and thereby produce pathological changes in the myocardium. The increased sensitivity to catecholamines is thought to be related to increased secretion of corticosteroids which is believed to be due to stress (19-21, 29). The various types of stresses can themselves result in pathological changes in the myocardium (19-21) and stress and corticosteroids in combination are particularly detrimental (19-21, 30).

Recently it has been suggested that catecholamines themselves do not induce cardiac necrosis, but rather their

oxidation products are involved (14, 32-39). This view is based on observations that showed that oxidized isoproterenol produces cardiac necrosis in the isolated perfused heart, while fresh isoproterenol does not (14, 33 39). The oxidized isoproterenol was found to have an absorbtion spectrum similar to that of adrenochrome. Subsequently adrenochrome was tested and found to produce myocardial cell damage and contractile failure while other metabolites of epinephrine did not (33, 39). In addition adrenochrome itself is known to have a variety of cellular actions which may be considered detrimental to the heart. These include increased oxygen consumption (40, 40), uncoupling of mitochondrial oxidative phosphorylation (42-44), vasoconstrictor properties (45, 46), inhibition of glycolysis (47-49), inhibition of myosin ATPase activity (50, 51) and still other effects (52-57).

Adrenochrome has also been demonstrated to exert a variety of subcellular effects which might also interfere with the normal functioning of the heart cell. Adrenochrome was found to inhibit sarcolemmal Na^+-K^+ ATPase <u>in vitro</u> or in the perfused heart (38). Adrenochrome decreases both mitochondrial and microsomal calcium uptake and binding (37) <u>in vitro</u> though little is known about whether plasma adrenochrome can enter the heart cell or not. It is therefore the purpose of this study to investigate whether adrenochrome is taken up by the cardiac cell by using the isolated perfused heart, the model most often used to study catecholamine uptake. The subcellular distribution of

adrenochrome will also be looked at by isolating various subcellular fractions, and looking at their adrenochrome content. Since propranolol and iproniazid both been reported to reduce adrenochrome induced cardiotoxicity (31), the effect of these agents on adrenochrome uptake will also be studied

It is well known that catecholamines are selectively removed from the blood stream by the heart and are very concentrated by the myocardium and its adrenergic nerve endings (58, 59). Also catecholamines released from nerve terminals are returned to the neuron by neuronal uptake mechanisms or may be taken up extraneuronally by the heart muscle cells themselves (60). To help understand the nature of adrenochrome uptake the Km and Vmax of adrenochrome uptake will be determined. Also the effects of specific inhibitors of neuronal and extraneuronal catecholamine uptake will be looked at to further compare the adrenochrome uptake to that of these catecholamines. Changes in heart contractile force and resting tension will be monitered and compared to the adrenochrome uptake, to look for any relationship between adrenochrome uptake and adrenochrome effects on contractile force and resting tension. The reversibility of adrenochrome uptake and the revesibility of its effects on contractile force and resting tension will be looked at in order to study the association between adrenochrome content and these two parameters and to investigate how strongly adrenochrome binds to the myocardium.

III Review of the literature

A. Catecholamine Induced Cardiac Necrosis

Injections of high doses of catecholamines have long been known to produce myocardial cell damage (3-9). With injections of epinephrine and norepinephrine, early workers showed that rabbits and dogs developed subendocardial and endocardial hemorrhages, focal lesions, edema, degeneration of myofibrils, arrhythmias and other charachteristic changes (17, 61-63). Such experimental results shed light on a clinical problem involving the use of norepinephrine to maintain blood pressure. Patients were receiving norepinephrine for long periods of time at relatively high concentrations (17) and it was found that the detrimental effects of catecholamines reported in animals were present in humans. The use of norepinephrine was correlated with a nonspecific myocarditis found in hospital patients. Similarly other work showed that norepinephrine therapy decreased the survival rate of dogs in hemorrhagic shock (64). Subsequently both epinephrine and norepinephrine were found to cause lesions in the isolated perfused heart (65).

Since the earlier observations of the deleterious effects of catecholamines isoproterenol, a synthetic catecholamine, has been used for inducing myocardial damage. The initial work was carried out mainly by a group led by G. Rona (6, 66-68). Dose dependent lesions were made using isoproterenol and it was postulated that a "relative myocardial ischemia" was the pathological mechanism with the heart using more energy than it could supply itself with

from the surrounding medium (68).

A number of investigators have now characterized the typical damage produced, though there are some variations depending on the doses and animals used. The first morphological changes appear in as little as two min with large doses of isoproterenol used subcutaneously (69), or in up to 30 min with smaller doses of natural catecholamines (70). Briefly these include focal necrosis of specific areas which involves: a striking hypercontraction and dissarrayment of the myofibrils called myocytolysis (10, 13, 69-76), contracture of sarcomeres (13, 69, 74), swelling of the mitochondria with disruption of the christae and deposition of electron dense bodies which may be calcium salts (13, 69-71, 74, 76, 77) swelling of the sarcoplasmic reticulum (SR) and t-tubles (70, 71, 76), increased number of ribosomes, depletion of glycogen granules and hypertrophy of the golgi apparatus (70). The sarcolemma appears superfically normal but in the early stages there is a dramatic increase in sarcolemmal permeability (76, 78) and tissue enzymes are reported to be released (79).

Various biochemical and metabolic changes have been reported to occur during catecholamine-induced myocardial necrosis. Oxidative phosphorylation is uncoupled, creatine phosphate and ATP stores decrease with an increase in phosphate occuring (80, 81), glycogen stores are depleted (70) and oxygen uptake increases (82). The uncoupling of oxidative phosphorylation is thought to be one of the more important changes and it may be

related to the reported increases in calcium uptake and content (69, 80, 81, 83-89). Magnesium and potassium in contrast, have been reported to decline (75, 87, 88).

The mechanisms by which catcholamines cause myocardial necrosis have been intensively investigated. Aside from the involvement of oxidation products, several different theories have been presented. These can roughly be divided into several groups; one is a relative ischemia or hypoxia theory and a closely related metabolic theory involving energy production, another is a hemodynamic theory implying interference with coronary circulation, a different theory implies electrolyte derrangements as the cause of cell necrosis, while depletion of endogenous norepinephrine stores is cited by some as being a key factor. Several other now less plausible theories also exist such as those involving thrombus formation, congestive heart failure and increases in plasma free fatty acids.

Among the earliest explanations for the cardiotoxicity of catecholamines was that by Raab and coworkers (3, 18). These workers thought that catecholamines, administered exogenously or released endogenously due to stress, caused a relative hypoxia. Catecholamines are well known to greatly increase the work, and hence the energy demand of the heart, but it was believed that the coronary circulation was not able to compensate for the increased oxygen demand. Thus there was a disproportion between supply and demand, which led to the necrosis. Much of Rona's (6, 7, 81) early work supported this idea. Their finding of the

greater myocardial damage due to isoproterenol in comparison to natural catecholamines, was explained in terms of its greater positive chronotropic and inotropic effects. This would greatly increase the cardiac need for oxygen and in addition, isoproterenol in contrast to other catecholamines, caused a decrease in blood pressure which would lead to decreased coronary perfusion and further aggravate the situation. Some authors support this idea (90, 92-94) citing the fact that high energy phosphate stores become depleted and that the increased heart rate decreases the duration of diastole and therefore decreases coronary flow. Furthermore, ischemia is believed to develop in the least perfused areas of the myocardium (90).

Handforth has pointed out that these are mainly unsubstantiated theories, with the actual supply of oxygen to the hearts and the demand not measured (95, 96). Handforth's work supports the idea that local coronary constrictions and dilation of precapillary shunts cause local ischemias and focal necrosis. These experiments involved injection of India ink retrogradely into hearts of animals treated with isoproterenol. The injections were at very early and later stages after isoproterenol administration and revealed that in the treated animals the ink did not perfuse well into all vessels and these correlated with the areas of necrosis. Also some vessels appeared to be constricted after the isoproterenol injections (95, 96). But several workers have directly contrasted these results. Ostadal *et al.* (97) looked at the turtle heart, an unusal but very useful

model for studying isoproterenol induced cardiac necrosis. In this heart, the spongy inner layer is supplied by diffusion from the ventricular lumen. This layer was greatly affected in isoproterenol induced necrosis in these hearts though it should be unaffected if changes in coronary circulation are the cause of the necrosis. Also, Belov and Khastova (10) found an equal distribution of foci of injury throughout the heart, in all cases the foci showed no tendancy whatsoever to correlate with the topography of the coronary vessels. Waldenstrom et al. (13) also found the cappilleries always appeared normally open and had a normal ultrastructure in necrosis induced by norepinephrine administration.

The metabolic theory, involving interference with energy prodcution, is related to both the relative ischemia and electrolytes theory. Interference with energy production in the cell can, especially when coupled with increased demand, seriously deplete ATP and creatinne phosphate stores. If not enough high energy phosphate stores are left for normal metabolic maintenance, serious damage can take place in the cell (83, 84). The relative ischemia theory states that energy supply cannot keep up with demand even at maximal production while others have suggested here that a defect occurs in the production of energy stores which is subsequently subnormal (80, 81, 85). As evidence, mitochondria from catecholamine treated hearts are shown to be uncoupled and high energy phosphate stores to be decreased (80, 81, 85). The causes though are in dispute, some suggest the

mitochondria are affected by catecholamines through electrolyte derrangements (81, 83, 84, 92) while others suggest an important and somewhat different role of endogenous norepinephrine (80, 85).

The theories on electrolyte involvement are fairly well developed. Several authors have proposed that increased calcium uptake by the myocardium leads to decreased energy production and increased usage (69, 80, 81, 84-86). The cause of the intracellular calcium overload may be excessive beta adrenergic stimulation, which results in excessive cAMP levels and is postulated to lead to excessively high calcium entry through the slow inward channels (86, 87, 92, 93). This view is supported by the fact that the more beta receptor stimulating an agent is, the greater is its ability to produce cellular necrosis (98) and that beta blockers prevent catecholamine induced cardiac necrosis (69, 74). Other reports suggest the increased calcium is due to a more general defect somehow induced in the sarcolemma. Soon after isoproterenol administration it was found that the sarcolemma became generally more permeable to the fine sturctural tracer horseradish peroxidase (76, 78).

Regardless of the mechanism, the increased intracellular calcium is believed to cause the necrosis through a variety of mechanisms. Many enzymes, which use or handle calcium, become activated thus using up high energy phosphate stores (86, 87). Also, mitochondria will maintain intracellular calcium homeostasis by sequestering excessive amounts of calcium. This

process requires energy and may occur in preference to ATP formation (99, 100). Such mitochondrial calcium overloading has been shown to impair high energy phosphate production, ultimately leading to irreversible damage to the mitochondria (81, 101). In support of this theory it has been shown that various calcium antagonists reduce the detrimental effects of catecholamine administration (86, 87) and calcium is necessary for the formation of the electron dense deposits found in the mitochondria during the development of the necrosis (77). Some inconsistancies are known in this theory. For example, increasing the dose of isoproterenol consistantly increased cell damage in some experiments, but did not continue increasing calcium content past a certain point. It was suggested that alterations in the subcellualr distribution of calcium were responsible for some of the necrosis (69). Propranolol also, completely prevented the increases in calcium but only partially prevented the necrosis (69). Also, some though not all, of the reports have measured calcium content of the myocardium by looking at content of tissue homogenates. This does not take into account changes in extracellular space and edema, which are known to occur in this type of necrosis (79). Still this theory on calcium involvement remains a strong one, though work is needed on the causes of calcium overload.

Magnesium and potassium are also reported to change in catecholamine induced cardiac necrosis (86-88). Both decrease in contrast to calcium. However it is not yet certain weather they

play a causative role or are secondary changes (86-88). Though potassium and magnesium salts can prevent or reduce the myocardial lesions and high potassium diet can reduce the lesions, these alterations may act through their antagonsim of calcium (86, 87).

Another type of mechanism sometimes suggested and one related to both calcium overload and relative ischemia is that involving endogenous norepinephrine stores. Several authors have stated that the endogenous norepinephrine stores of the heart may be causing the necrosis, with exogenous catcholamines simply causing release of these stores. This results in an upset in the normal cell metabolism (13, 80, 85). Several lines of evidence support this mechanism. Zavodskayan et al. (80) compared the effects of excessive electrical stimulation, which depletes endogenous norepinephrine, to those of exogenous catecholamine administration. The effects were the same with both treatments causing typical necrosis, uncoupled mitochondria and decreased high energy phosphate stores. Waldenstrom et al. (13) used tyramine on isolated hearts to deplete endogenous norepinephrine and found similar results, which could also be prevented by beta blockers. Further work is needed to clarify this role of endogenous norepinephrine.

Several other theories have been proposed to explain catecholamine induced cardiac necrosis but are now less accepted. Thrombus formation was once thought possible but direct evidence in favor of this is lacking and some authors note a lack of

thrombi formation (95, 96). Increases in plasma free fatty acids could be a cause but the production of the same lesions in isolated hearts goes against this theory. There is also no measurable increase in fatty acid content of mitochondria from treated animals and the mitochondria from these animals did not respond to the addition of albumin, which would nullify the effect of free fatty acids (85). Congestive heart failure is also not a factor since this is not shown in treated animals (85), and necrosis is known to occur in the absence of myocardial hypertrophy (102). One report suggested damage to the mitral valve as a factor but others have never substantiated this (94). Another report suggests an involvement of lysosomes, which are made fragile by isoproterenol, but questions arise as to weather these changes are of secondary nature (102).

It is worthwhile also to briefly discuss the physiological signifigance of the cardiac impairment produced by catecholamines and to compare it to other forms of heart diesease. As mentioned, the doses of catecholamines used to induce necrosis are high in comparison to plasma levels, but early workers found that lower physiological doses over prolonged time had the same effects (3, 17, 103). Waldenstrom et al. (13) used catecholamine concentrations of 10^{-6} to 10^{-4} molar in the perfusion medium of their isolated hearts. These workers have stated that these are much higher than the plasma concentrations of 10^{-6} to 10^{-8} molar but the concentrations of catecholamines are 10^{-6} or higher locally when myocardial stores are rapidly depleted under

conditions such as anoxia or ischemia (13, 104).

Catecholamine induced myocardial necrosis has both similarities and dissimilarities to many types of heart failure. Some authors (70, 74, 102) report that at the periphery of experimentally induced myocardial infarcts the same type of contracture bands and degeneration are found. But ischemia produces whole necrotic zones, especially when made by coronary artery occlusion, in contrast to the focal necrosis produced by catecholamine administration (70, 80). The centre of the ischemic zone also differs in that relaxation and not contraction, is shown. Regan et al. (75) noted signifigant differences from ischemia and epinephrine induced myocardial necrosis with regard to potassium ions and triglyceride content, though these might be due to differences in degrees of progression of the two damages. In angina, contracture bands are found in patients which are similar to those produced by norepinephrine in rats (70) and similar myofibrilar degeneration is also shown in stone heart and in patients after heart surgery (76). The first few hours after neurogenic degeneration produces uncoupling of oxidative phosphorylation and decreases in high energy phosphates similar to those caused by norepinephrine injections (80). Overall, it appears that several aspects of catecholamine induced cardiac necrosis are similar to some aspects of a variety of pathological states of the myocardium.