SUBCELLULAR BASIS OF THE CARDIOTOXIC EFFECTS OF ADRENOCHROME

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BY

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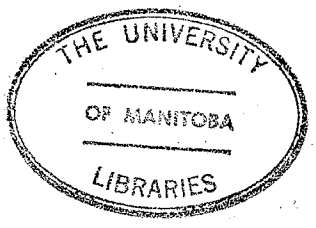

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SUBCELLULAR BASIS OF THE CARDIOTOXIC

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ABSTRACT

It has been reported by various investigators that catecholamines in high concentrations can produce myocardial cell damage. Recently it has been shown that this cardiotoxic effect of catecholamines was due to the formation of adrenochrome, an oxidation product of catecholamines, rather than catecholamine per se. However, information in regard to the possible mechanisms involved in the cardiotoxicity of adrenochrome was lacking and it is for this purpose the present study was undertaken.

The effects of adrenochrome on the various activities associated with rat heart sarcolemma, fragments of sarcoplasmic reticulum (microsomes) and mitochondria were examined by using in vitro as well as isolated heart preparations. Furthermore, the adenine nucleotide levels in hearts perfused with adrenochrome were measured. Amongst all the sarcolemmal enzymes investigated, $Na^+ - K^+$ ATPase was the only enzyme affected significantly by adrenochrome (10 - 100 µg/ml) and the inhibitory effect of adrenochrome on this enzyme was found to be not only irreversible but also independent of pH (6.6 - 7.8). Furthermore, $Na^+ - K^+$ ATPase was the only enzyme inhibited in hearts perfused with adrenochrome.

The microsomal ATPase as well as Ca^{2+} binding and uptake activities in vitro were significantly decreased by varying concentrations of adrenochrome. The Ca^{2+} uptake was most sensitive to the depressant effect of adrenochrome (1 µg/ml) and this effect of adrenochrome on

microsomal Ca^{2+} uptake was independent of pH (6.0 - 8.0) and calcium concentrations (10 - 200 μM). The results of kinetic study showed that the inhibitory effect of adrenochrome on Ca^{2+} uptake was of a mixed type. In hearts perfused with adrenochrome, it was found that contractile failure was both time (5 - 30 min) and dose (5 - 50 μg/ml) dependent, however, depression of microsomal Ca^{2+} uptake and binding as well as ATPase activities were relatively independent of time and dose. Furthermore, the depressant effect of adrenochrome was irreversible in both in vitro and isolated heart preparations.

Adrenochrome essentially had no effect on the ability of mitochondria to hydrolyse ATP in vitro, however, mitochondrial Ca^{2+} binding and uptake were significantly decreased by adrenochrome and Ca^{2+} uptake was more time - dependent than Ca^{2+} binding. It was found that the inhibitory effect of adrenochrome on mitochondrial Ca^{2+} uptake was irreversible and was independent of pH (6.0 - 8.0), however, it was partially antagonized by high concentrations of calcium. Furthermore, results from kinetic study indicated that the inhibitory effect of adrenochrome on mitochondrial Ca^{2+} uptake was of a mixed type. In hearts perfused with adrenochrome, the mitochondrial ATPase activity was not altered regardless of the dose of adrenochrome (5 - 50 μg/ml) or the length of perfusion (5 - 30 min), however, Ca^{2+} uptake was significantly decreased and this depressant effect of adrenochrome on mitochondrial Ca^{2+} uptake was independent of time or dose.

Since mitochondrial oxidative phosphorylation was not affected in hearts perfused with adrenochrome and ATP levels were decreased in these hearts, the direct effect of adrenochrome on mitochondrial oxidative phosphorylation was investigated in vitro. It was found that adreno-

chrome as well as calcium were able to uncouple mitochondrial oxidative phosphorylation and they potentiated each others effect when present together.

The results clearly indicate that adrenochrome is capable of altering the functional activities of sarcolemma, sarcoplasmic reticulum and mitochondria. In view of the fact that these membrane systems play an important role in the regulation of calcium movements, alterations in their functional integrity will undoubtedly produce disturbances in intracellular calcium concentrations, and thus resulting in intracellular Ca^{2+} - overload. A sequence of events, such as reduction of myocardial ATP content secondary to overstimulation of myofibrillar ATPase and uncoupling of mitochondrial oxidative phosphorylation, will occur as consequences of intracellular Ca^{2+} - overload. Therefore it is possible that the formation of adrenochrome and the occurrence of intracellular Ca^{2+} - overload are the major factors responsible for the cardiotoxic effects of catecholamines.

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I. INTRODUCTION AND STATE OF THE PROBLEM

It has been repeatedly demonstrated that prolonged infusion of catecholamines as therapeutic agents in the treatment of shock or excessive endogenous secretion of catecholamines as in the case of pheochromocytoma produce infarct-like lesions in the myocardium. Different hypotheses have been formulated in an attempt to explain the possible mechanisms by which catecholamines produce myocardial damage. Hemodynamic alterations as well as changes in substrate availability and utilization have been considered to be the major factors responsible. Ionic disturbances in general received a lot of emphasis and particular attention has been focused on the phenomenon of intracellular Ca^{2+}-overload as the significant factor in the catecholamine - induced myocardial damage. Recently it has been reported that adrenochrome, an oxidation product of catecholamines, rather than catecholamine per se was responsible for the myocardial necrosis and contractile failure. However, no information was available in the literature concerning the possible mechanisms by which adrenochrome produce myocardial cell damage. This study was therefore undertaken to provide some information on this point.

It is known that various membrane systems, such as sarcolemma, sarcoplasmic reticulum and mitochondria, play an important part in the regulation of myocardial function and metabolism. Various enzyme systems such as Na^{+} - K^{+} adenosine triphosphatase (ATPase), adenylate cyclase, Ca^{2+} - ATPase and Mg^{2+} ATPase, have been shown to be associated with sarcolemma, and to play an important role in the regulation of ionic movements across the cell membrane. Sarcoplasmic reticulum and mitochondria were also shown to have the ability to accumulate as well as to release calcium, and decrease in calcium accumulating ability by these

organelles have been observed in different types of heart failure. In addition to serve as an intracellular calcium regulator, mitochondria also responsible for replenishing cellular ATP stores; "the power house of the cell". Therefore it is conceivable that alterations in these membrane systems would inevitably affect myocardial function and possibly produce cell damage. Therefore the present study was designed to investigate the effect of adrenochrome on these membrane systems by using in vitro as well as isolated heart preparations. The reasons for using isolated heart preparations in this study rather than intact animals were several: 1) the contractile force generation can be monitored during adrenochrome perfusion and the degree of contractile failure can be recorded; 2) the exact concentrations of adrenochrome can be controlled and adjusted and 3) hemodynamic, metabolic as well as other possible systemic effects of adrenochrome can be eliminated and the effects of adrenochrome on the heart will not be complicated by these changes. It is hoped that the results of this study will contribute to the further understanding of the possible mechanisms by which catecholamines exert cardiotoxicity.

II. REVIEW OF LITERATURE

Catecholamines are considered to play a role in various human diseases (1). According to Raab (2), catecholamines are involved in some of the most common and fatal cardiovascular diseases such as angina pectoris, myocardial infarction, post-infarction syndrome and congestive heart failure. All these conditions were shown to be associated with an increase in plasma catecholamine levels (4). Myocardial damage was evident either histologically or was suggested by electrocardiographic changes in these situations. Similar lesions were found in patients with pheochromocytoma (5, 6), in patients receiving therapeutic dose of catecholamines in the treatment of shock (7, 8), and in animals injected with large doses of catecholamines (9, 10). Furthermore, it was reported that compounds which act by releasing heart catecholamines also produced cardiac lesions in experimental animals (11). Various mechanisms including reduction in myocardial oxygen availabiiity, increase in free fatty acids and derangement in electrolyte distributions, have been proposed for the cardiotoxic effects of catecholamines, however no single factor has been agreed upon.

A. Reduction in Myocardial Oxygen Availability:

Infusion with catecholamines such as epinephrine, norepinephrine and isoproterenol influence the hemodynamic states of the cardiovascular system. Blacket et al. (12) found that in rabbits, with continued infusion, the pressor effect of norepinephrine could not be maintained even with increasing dose level and a substantial fall in blood pressure was noted when the infusion was stopped. Szakacs and Cannon (13) reported similar findings in human subjects. Since isoproterenol dilates

On the other hand, cardiac lesions can be produced by other sympathomimetic amines such as methoxamine, which has no positive inotropic effect (22). In a subsequent study (23), the same author compared the cardiovascular effects of different sympathomimetic amines at pharmacological and lesion producing doses. They postulated three mechanisms: myogenic, physical and metabolic, which are responsible for the production of cardiac lesions by catecholamines. The myogenic mechanism deals with a direct depressant effect on the myocardium to decrease cardiac pumping action. The physical mechanism concerns a reduction in aortic flow by means of aortic constriction. The metabolic mechanism represents a shift of myocardial metabolism from aerobic to anaerobic in the presence of a normal arterial oxygen content and in so doing alters substrate utilization.

Challoner and Steinberg (26) have reported that the increased myocardial oxygen consumption induced by catecholamines was not entirely due to enhanced myocardial mechanical activity, since epinephrine was able to increase the myocardial oxygen consumption by 50% in potassium-arrested hearts. The increased oxygen consumption may be due to stimulation of the non-phosphorylating respiration by the epinephrine (27), or to stimulation of ATP-wasting metabolic cycle as suggested by Hauge and Øye (28). The oxygen wasting effect of catecholamines has been observed by several investigators (29, 30). Since oligomycin did not further stimulate epinephrine increased oxygen consumption, Challoner (31) concluded that the observed effect of epinephrine on oxygen consumption was due to non-phosphorylating pathways rather than secondary to an increase in ATP-wasting cycle.

B. Increase in Free Fatty Acids:

Increased myocardial free fatty acids have been found in the presence of epinephrine (32, 33). Elevated plasma free fatty acids concentrations have been reported in patients with pheochromocytoma and it was suggested that free fatty acids may be the mediators of increased oxygen consumption induced by catecholamines (34). In addition, infusion of triglycerides have been shown to cause an increase in heat production by the heart (35), and the synthetic beta adrenergic blocker, butidine, was able to decrease the myocardial oxygen consumption and free fatty acid uptake (36). Furthermore, increases in myocardial free fatty acids have also been shown in norepinephrine - induced cardiac necrosis and it was suggested that excessive mobilization of free fatty acids may be responsible for the development of heart lesions (37).

The possibility that free fatty acids play a role in mediating the increased myocardial oxygen consumption induced by catecholamines was substantiated by Mjøs (38). He demonstrated that inhibition of lipolysis by nicotinic acid, beta pyridyl carbinol or high plasma glucose concentration can substantially reduce the isoproterenol - induced rise in oxygen consumption without affecting the action of isoproterenol on myocardial performance. By comparing isoproterenol, glucagon and calcium, Mjøs (39) further demonstrated that these three agents produced a similar increase in $dP/_{dt}$ but the oxygen consumption was less for glucagon and calcium than for isoproterenol, even though the increase in heart rate was more with glucagon than with isoproterenol. Calcium produced the smallest rise in heart rate and the oxygen consumption per beat was also less with calcium than with isoproterenol. He concluded

that the increased oxygen consumption was mediated by free fatty acids since the myocardial free fatty acid uptake was increased three fold by isoproterenol, whereas calcium and glucagon had no effect on this parameter.

The direct effects of free fatty acids on myocardial function have been studied by several investigators. Kurien and Oliver (4)) postulated that intracellular accumulation of free fatty acids may have a detergent effect on the cell membrane thus enhancing the efflux of potassium ions. Opie (41) stressed the possibility that free fatty acids may alter mitochondrial metabolism resulting in uncoupled respiration whereas Henderson et al. (42) proposed that accumulation of free fatty acids intracellularly may cause a decrease in free calcium concentration thus affecting myocardial function. However, more recent data provided evidence against the involvement of free fatty acids as a causative factor in increasing myocardial oxygen consumption (43). Young et al. (44) found that infusion of free fatty acids into dog increased the heart rate to the same extent as that of catecholamine. However, ST segment alterations suggesting myocardial ischemia were produced only by infusion of catecholamines. They suggested that the cardiotoxic effect of catecholamines was not mediated by free fatty acids. Furthermore, impaired mitochondrial oxidative phosphorylation in catedholamine treated rats has been reported by Sobel et al. (45), although they did not find any associated increase in myocardial free fatty acid levels nor was addition of albumin able to reverse mitochondrial impairment. In view of these descrepancies, Opie et al. (46) suggested that the cardiotoxic actions of free fatty acids may be due to prior sensitization by the catecholamines.

C. Derangement in Electrolyte Distributions:

The heart contains high concentrations of catecholamines, second only to adrenal glands (47). When circulating catecholamines are increased, heart muscle and other vascular tissues have the ability to selectively accumulate them (48, 49). It was suggested that tissue binding of catecholamines is responsible for this accumulation (50) and tissue bound catecholamines play a more significant part in the pathogenesis of myocardial lesions than circulating catecholamines (8). There has been evidence indicating that catecholamines cause an net efflux of potassium from the myocardial cells (51) and dietary potassium deficiency and loss of body potassium have been shown to cause cardiac lesions (52, 53). Selye and Bajusz (54) have reported that potassium depletion enhanced the development of myocardial necrosis induced by stress, which is known to be associated with elevated levels of plasma catecholamines. Chappel et al. (55) also reported that pre-treatment with "electrolyte steroids" such as deoxycorticosterone acetate or fluorocortisol potentiated the cardiotoxic response of isoproterenol and this effect was abolished by a diet supplemented with KCl. Furthermore, the isoproterenol induced necrosis was more severe in rats on a potassium deficient diet than on a normal diet, and a diet deficient in sodium produced an amelioration of the isoproterenol induced cardiac lesions (56). Therefore, it appears that the interrelationship between these electrolytes plays an important role in the development of myocardial lesions by catecholamines. Histochemical studies have demonstrated that cellular depletion of potassium and gain of sodium preceeded the catecholamine induced necrotic lesions (57). Dusek et al.

(58) reported that potassium depletion or sodium excess were important factors in isoproterenol induced atrial necrosis. Due to their structural and functional characteristics, atria are normally more resistant to the damaging effects of catecholamines. These authors suggested that potassium depletion, and sodium and water excess will result in mitochondrial swelling as well as producing some membrane alterations. Increased membrane permeability would allow large amounts of calcium to enter the mitochondria, thus resulting in uncoupled oxidative phosphorylation and possibly irreversible structural damage. In addition to potassium, magnesium is another ion found to play a part in the genesis of catecholamine-induced cardiac necrosis (59). Lehr et al. (60) demonstrated that myocardial injury produced by sympathomimetic amines was preceeded by a decrease of not only potassium but also magnesium and a concomitant increase in sodium and calcium contents. Heggtveit et al. and Heggtveit (61, 62) reported that magnesium deficiency was also able to produce cardiac lesions. It was suggested that loss of cellular magnesium was associated with uncoupling of oxidative phosphorylation and disruption of Mg^{2+} - dependent intra-mitochondrial enzyme systems which resulted in myocardial necrosis (63). Earlier observations have shown that magnesium salt protects the myocardium against necrosis induced by various methods (64, 65). More recent data have demonstrated that magnesium also prevented catecholamine-induced metabolic changes and necrosis in the heart (66, 67). The protective effect of Mg^{2+} or K^{+} was thought to be due to their physiological Ca^{2+}-antagonistic properties (68). These ions act either by limiting Ca^{2+} uptake into the myocardium (69), by preventing Ca^{2+} from reaching con-

tractile elements or by competing with Ca^{2+} ions at a site on myofibrillar adenosine triphosphatase (ATPase) (70). According to Sordahl and Silver (71) the protective effect of Mg^{2+} is on the phosphorylating mechanism of the mitochondria; when Mg^{2+} is present, the heart mitochondria retain the ability to phosphorylate ADP even with increased Ca^{2+} uptake.

Previously it has been shown that there is an association between increased serum or myocardial calcium levels and cardiac necrosis. Agents, such as dihydrotachysterol, Vit D_3 or NaH_2PO_4, which are known to have either direct or indirect influence on calcium metabolism, produced cardiac lesions (72, 73, 74). It has been repeatedly demonstrated that catecholamines cause a marked increase in myocardial calcium content (68, 75). According to Fleckenstein et al. (76) intracellular Ca^{2+} - overload excessively activates Ca^{2+} - dependent ATPases and impairs the phosphorylating capacity of the mitochondria thus resulting in high-energy phosphate exhaustion. Myocardial necrosis will occur when more than 50% of the myocardial ATP has been broken down to useless products. By using radioactive calcium, they further demonstrated that six hours after isoproterenol injection, the calcium taken up by the myocardium was 180% greater than the plasma level, and pretreatment with 9 α - fluorocortisol acetate, dihydrotachysterol or NaH_2PO_4 greatly potentiated the myocardial calcium uptake induced by isoproterenol. The myocardial ATP and creatine phosphate (CP) levels, on the other hand, showed maximum depression two hours following isoproterenol treatment. Subsequently, CP levels began to fluctuate toward normal while ATP levels remained depressed. A greater decrease in ATP was