ENZYMATIC ADAPTATION: MOLECULAR BASIS FOR CARDIAC GLYCOSIDE ACTION?

1. Increase in rat heart actomyosin and mitochondrial ATPase specific activities following digoxin injection.

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In spite of a vast knowledge of the effects of cardiac glycosides, the fundamental causes of these effects remain largely unknown. These drugs are known to strengthen the contractile force of failing hearts and also to have a profound influence on the metabolism of such organs (Kako & Bing, 1958; Hadju & Leonard, 1959; Lee, 1960; Rebar, Rebar, & Omachi, 1957). In the present series of investigations we are posing the question, "Can enzymatic adaptation account for some or all of the physiological effects of cardiac glycosides?" The term adaptation is used in its broadest sense to mean an alteration in the amount of active enzyme in an organism.

The pharmacology of a number of drugs has been shown to include enzymatic adaptation, for example, the work of Mayer and Morgan (1960) in which activation of myocardial glycogen phosphorylase by epinephrine, norepinephrine, isoproterenol, and ephedrine was correlated with cardiac contractile force. One of the prime considerations which prompted our investigation was the chemical similarity between the cardiac glycosides and the steroid hormones which are known to exert their effects primarily by enzymatic adaptation.

In this paper evidence is presented that the specific activity of actomyosin and mitochondrial ATPase is increased in rat hearts following injection of digoxin. In addition the effect of digoxin on these enzyme levels will be shown to be diminished but not abolished by actinomycin D.

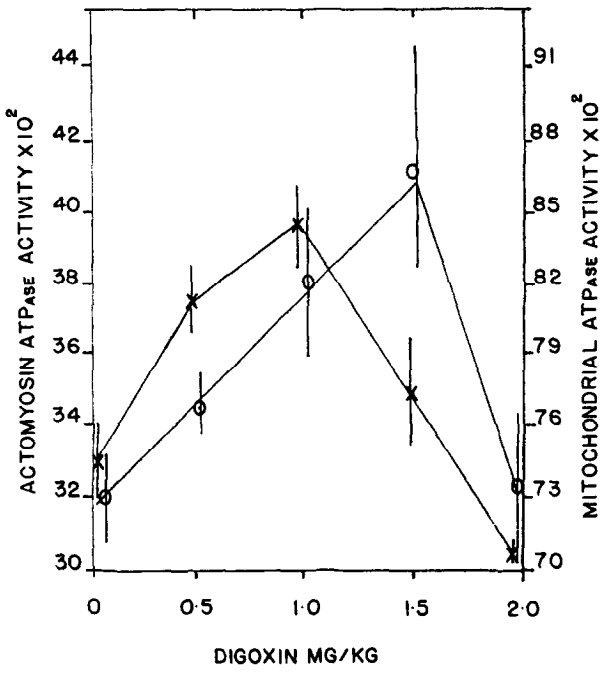

Fig. 1. Dose-response curve. actomyosin ATPase (X) specific activities determined 4 hours after administration of indicated digoxin dose. Mitochondrial ATPase (O) specific activities determined 3 hours after digoxin injection. Each point represents the average value obtained from 6 rats. Vertical lines represent the range of values determined by statistical analysis. The change in actomyosin and mitochondrial ATPase activities is highly significant.

METHODS

Male, fed, 175 to 250 gram Sprague-Dawley rats were injected intraperitoneally with 0.5 to 2.0 mg/kg of digoxin. The rats were killed and their hearts removed at intervals from zero to six hours after injection. After removal the hearts were frozen at -12°C and then homogenized in 0.25 M sucrose and 0.03 M Tris-Cl^- buffer, pH 7.4, to form a 10% homogenate. The homogenate was dialyzed overnight in the same buffer and then centrifuged at 600 xg for 15 minutes to remove nuclei, membranes, and myosin. The supernatant was then centrifuged at 27,000 xg for 15 minutes to sediment the mitochondria. The supernatant from this centrifugation contained

actomyosin and other soluble proteins. The mitochondrial and nuclear fractions were resuspended in the tris buffer and tested for ATPase activity along with the soluble fraction. All centrifugations were performed at 4°C in a Sorvall RC-2 refrigerated centrifuge.

ATPase activity was measured by the enzymatic method previously described (Fritz & Hamrick 1965). Protein was estimated by the biuret method using bovine plasma albumin as a standard. Specific activity is expressed as micromoles of ATP hydrolyzed per minute per mg of protein.

RESULTS

Increased actomyosin and mitochondrial ATPase activity was observed in the presence of increasing concentrations of digoxin (Fig. 1). The optimum dose for actomyosin ATPase was 1.0 mg/kg whereas that for mitochondrial ATPase was 1.5 mg/kg. The highest dose tested was 2.0 mg/kg which had no noticeable effect on actomyosin ATPase activity, but caused the specific activity of mitochondrial ATPase to be diminished to a level below that of the control. The actomyosin ATPase data presented in figure 1 were obtained 4 hours after injection of digoxin, whereas the mitochondrial ATPase data represent 3 hour time points. These were the times of maximum response for the respective enzymes as seen in figure 2. The data presented in figure 2 were obtained using a dose of 1 mg/kg which was sub-optimum for the mitochondrial enzyme.

Several experiments were performed to verify the fact that the increases in actomyosin and mitochondrial ATPase activities were caused by digoxin. Vehicle injected and non-injected controls showed no change in the enzyme activities tested. Adrenalectomized rats responded to the drug in precisely the same way as the normal animals. The pellet obtained after low speed centrifugation of the homogenate was resuspended and assayed for ATPase activity; the small amount of activity found, presumably due to membrane ATPase, did not change after digoxin injection. Furthermore no change in lactate dehydrogenase activity was observed which was

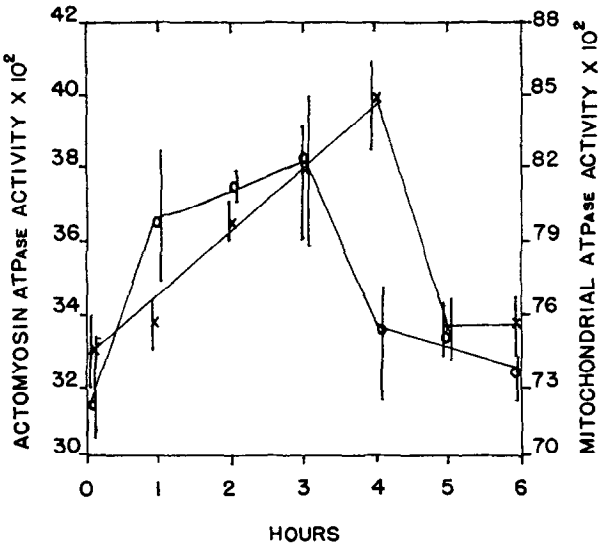

Fig. 2. Time-response curve. Actomyosin ATPase (X) and mitochondrial ATPase (O) activities determined at times indicated after administration of 1 mg per Kg of digoxin. Each point represents the average value obtained from 6 rats. Vertical lines represent the range of values determined by statistical analysis. The change in actomyosin and mitochondrial ATPase activities is highly significant.

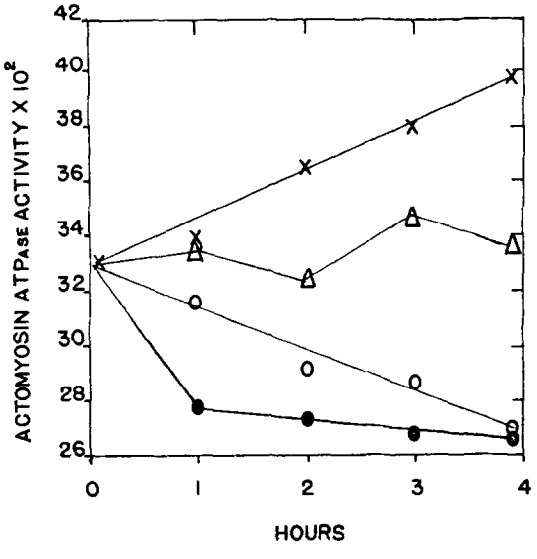

Fig. 3. Effect of actinomycin D on actomyosin ATPase specific activity. X - Digoxin, 1 mg/Kg; -Actinomycin D, 0.7 mg/Kg + digoxin, 1 mg/Kg; O-Actinomycin D, 1.0 mg/Kg + digoxin, 1 mg/Kg; O - Actinomycin D, 1 mg/Kg. Actinomycin D was injected intraperitoneally 30 minutes before digoxin in rats that received both drugs. Enzyme values were obtained at the indicated times after digoxin injection.

interpreted to mean that no myocardial damage had occurred as a result of the digoxin injections.

Figures 3 and 4 reveal the effect of actinomycin D on actomyosin and mitochondrial ATPase activities respectively. The results were essentially the same with both enzymes, i.e., in the absence of digoxin the antibiotic caused a marked decrease in their activitieswhich could be partially restored when digoxin was administered along with the actinomycin D.

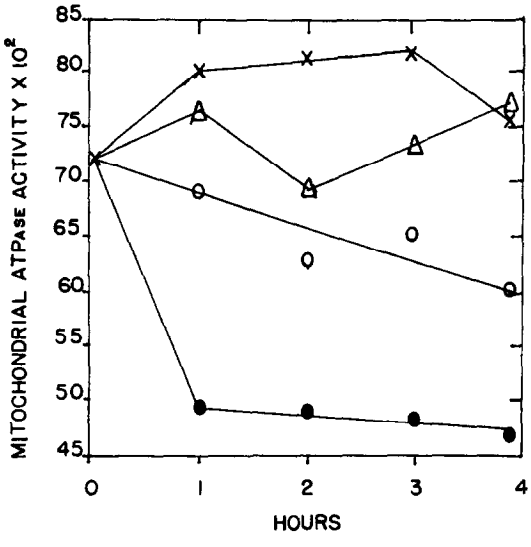

Fig. 4. Effect of actinomycin D on mitochondrial ATPase specific activity. See legend to fig. 3 for meaning of symbols and protocol.

DISCUSSION

The results of Takahashi et. al. (1965) as well as those of Blum and Morales (1953) indicate that there is a direct correlation between contraction of myofibrils and actomyosin ATPase activity. From this it follows that an increase in actomyosin ATPase, as herein reported, would be accompanied by or followed by an increase in myofibrillar contractile strength. Actomyosin bands are known to have diminished contractile

strength in failing human hearts (Kako & Bing, 1958; Olson, 1961); and furthermore Alpert and Gordon (1962) have reported abnormally low myofibrillar ATPase activity in myocardial failure. In addition Meerson (1960) has pointed out that decreased protein synthesis may be responsible for the alteration of contractile proteins in cardiac decompensation. These considerations suggest the possibility that the cardiac glycosides may exert their inotropic effect by causing an increase in the intracellular level of active actomyosin ATPase.

The role of actinomycin D in inhibiting protein synthesis has been extensively investigated and it seems clear that one of its major effects is upon RNA metabolism (Hurwitz & August, 1963). The fact that digoxin can overcome the inhibitory effect of actinomycin D on actomyosin and mitochondrial ATPase may be interpreted in a number of ways but the most general conclusion is that the glycoside is functioning by affecting protein synthesis. It is interesting to note that Sonnenblick et. al. (1964) have observed that 90% of injected tritiated digoxin was found in the sarcoplasmic reticulum of cat myocardium. It is well known that this is the intracellular site of protein biosynthesis.

A number of investigations have revealed that cardiac glycosides are effective in altering myocardial metabolism. For example Lee et. al. (1960) found a decrease in the concentration of ATP as well as phosphocreatine accompanied by an increase in oxygen consumption and contractile strength following incubation of isolated cat papillary muscle in a ouabain solution. These changes can be rationalized in terms of an increased mitochondrial ATPase activity leading to a more rapid breakdown of ATP and thus an alteration in the intracellular concentrations of ATP, ADP, & AMP as well as inorganic phosphate. Atkinson (1965) in referring to the effect of these adenylates on phosphofructokinase, isocitrate dehydrogenase, and citrate synthase has suggested that they may be primary factors in the control of fermentative as well as respiratory energy metabolism.

In summary, our studies support the hypothesis that the molecular basis for digoxin action involves the enzymatic adaptation of actomyosin and mitochondrial ATPase.

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