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COBRA VENOM CARDIOTOXINS AS PROBES OF ALTERED MEMBRANE STRUCTURE IN DYSTROPHIC SKELETAL MUSCLE

C. J. Parker, Jr. and R. A. Hudson

Department of Biochemistry
Wayne State University School of Medicine
Detroit, Michigan

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SUMMARY: Cardiotoxin from the venom of the Thailand cobra, Naja naja siamensis, causes a rapid influx of calcium into muscle from the normal Syrian hamster. This does not occur, however, in the corresponding muscle from the dystrophic hamster. The toxin may be opening "calcium channels" in normal muscle and not in the dystrophic. In contrast, cardiotoxin has no effect on the rate of efflux of small molecular weight substances from either type of muscle.

Measuring subtle differences which may exist between the membranes from normal and diseased tissue can be difficult. Small changes in the phospholipid or protein compositions may be within the error limits of detection. A chemical probe that can interact with and detect subtle differences in the membranes from normal and diseased tissues may be of great value. Such a probe would detect differences in the molecular or supramolecular assemblies within the region of the membrane to which the probe binds or alternatively detect differences in the functional components of the membrane coupling with the former probe interacting sites. In this communication we report the utility of cardiotoxins as probes of the functional differences between normal and dystrophic muscle membranes.

Cardiotoxins interact with the membranes of virtually all cells (1-5). Although a wide range of effects are produced by these protein toxins according to cell type, most of the reported effects, e.g., hemolysis of erythrocytes (6), depolari-

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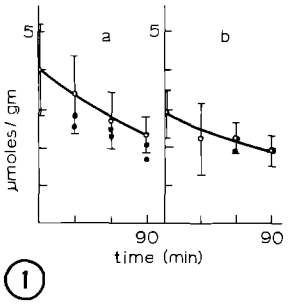
zation of excitable tissue (7), blockade of axonal conduction (8), can be traced to cardiotoxin induced alterations of the regulated cation transport of the affected membrane. Since various muscles are uniquely marked by the presence of small molecules such as carnosine, anserine or taurine (9) which leak out slowly, if at all (10), maintenance or disruption of the plasma membrane barrier can be inferred from the analysis of the muscle content of these substances in the presence of cardiotoxins. In the present case efflux measurements of such small molecules were useful in demonstrating the integrity of the membrane in the presence of cardiotoxin induced alterations in calcium ion transport.

EXPERIMENTAL PROCEDURE

The interior abdominal wall muscle of the normal and dystrophic (11) Syrian hamster was used in experiments reported. The latter were obtained from Trenton Experimental Animal Colony, Bar Harbor, ME. In all experiments cardiotoxin D from the venom of the Thailand cobra, Naja naja siamensis, was used. The toxin was purified by the method of Zusman et al. (12). The efflux of carnosine and taurine was measured by placing muscles (0.5 gm) in a 50 ml bath of Hank's solution with and without cardiotoxin. At times indicated samples were removed, blotted dry, weighed and assayed for the carnosine and taurine remaining in the muscle (13). Calcium influx rates were determined by placing weighed samples of the muscle in 10 ml of Hank's solution (1 mM Ca^{+2}) containing 0.1 μC $^{45}Ca^{+2}$ per ml. After removal from the bath the tissue was extracted in 5 ml water at 100° C for 15 min. Aliquots of the extract were transferred to cellulose filter discs, dried and counted. The results were expressed as dpm per gm wet weight of muscle per dpm per ml of bath. Total calcium in the muscle was determined by the method of Baginski et al. (14) after extracting muscle samples as described above.

RESULTS AND DISCUSSION

The efflux of carnosine from normal and dystrophic muscle is shown in fig 1a and 1b. In both cases there is about 50% release over a 90 min period. The efflux of taurine is shown in fig 2a and 2b. The results are similar to those seen with carnosine. Apparently, cardiotoxin has no effect on either



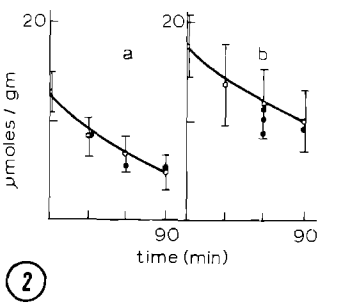
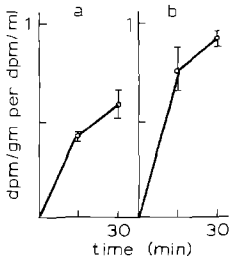


Fig. 1. The effect of cardiotoxin on the efflux of carnosine from normal (a) and dystrophic (b) hamster muscle. Open circles, without cardiotoxin, closed circles, 1.0 μM cardiotoxin.

Fig. 2. The effect of cardiotoxin on the efflux of taurine from normal (a) and dystrophic (b) hamster muscle. Open circles without cardiotoxin, closed circles 1.0 μM cardiotoxin.

taurine or carnosine efflux in normal or dystrophic muscle. Although cardiotoxin at the concentration employed has a marked effect on the integrity of the erythrocyte membrane, it appears to have little or no effect on the muscle membrane with regard to permeability of small molecules. Other reagents such as potassium chloride and EDTA stimulate the efflux of carnosine from rabbit psoas muscle (15).

Cardiotoxin, however, does affect muscle. Over a period of 5 to 10 min there is a marked contracture. The muscle appears to go into rigor and is no longer pliable but hard in texture. This contracture may be due to an influx of calcium from the bath in an amount sufficient to saturate the storage capacity of the sarcoplasmic reticulum resulting in a rise in the concentration of Ca^{+2} in the cell to a level that would prevent relaxation. The effect of cardiotoxin on the influx



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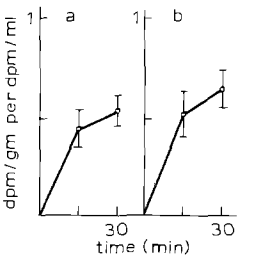

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Fig. 3. The effect of cardiotoxin on the influx of $^{45}Ca^{+2}$ into normal hamster muscle, (a) no cardiotoxin, (b) 1.0 μM cardiotoxin.

Fig. 4. The effect of cardiotoxin on the influx of $^{45}Ca^{+2}$ into dystrophic hamster muscle, (a) no cardiotoxin, (b) 1.0 μM cardiotoxin.

of Ca^{+2} is shown in figs 3 and 4. In normal muscle (fig 3a) there is a rapid rise in muscle radioactivity in the first 15 min to about 40% of the activity of the bath followed by a slow net uptake of calcium by the muscle. These results contrast markedly with what is seen in the presence of cardiotoxin (fig 3b). Over a 30 min period the radioactivity of the muscle and the bath are nearly equal. While it is not possible to distinguish between exchange and net uptake in these experiments, there must be some cardiotoxin stimulated net uptake. Both types of muscles contain about 0.5 μM of Ca^{+2} per gm of wet weight. If the increase in radioactivity were due only to exchange, the ratio of activity inside to outside would never exceed 0.5 to 0.7 depending on the extracellular space. This is clearly not the case.

These results suggest that cardiotoxin may be opening "calcium channels" in the membrane. That cardiotoxin has no

effect on the efflux of carnosine or taurine rule out the opening of "holes" in general in the membrane. Chang (16) suggests that there is a generalized translocation of ions across the membrane occurring in response to the action of cardiotoxin. Lin Shiau et al. (17) has shown that stimulated efflux of Ca^{+2} occurs from chick biventer muscle in response to the action of cardiotoxin. We have shown that cardiotoxin D depolarizes muscle cells from -90 to -15 mv within the same time span during which stimulated Ca^{+2} influx and contracture occurs.

The effect of cardiotoxin on Ca^{+2} influx in dystrophic hamster muscle is shown in figs 4a and 4b. It can be seen in fig 4a that the influx of radioactivity in dystrophic muscle is similar to that seen in normal muscle. In the presence of cardiotoxin, however, there is a marked difference between the two types of muscles. The results show that cardiotoxin has no effect on the influx of Ca^{+2} into dystrophic muscle.

In summary, the results presented indicate that cardiotoxin induced $^{45}Ca^{+2}$ uptake differentiates normal and dystrophic skeletal muscle membranes by reporting differences in the properties of the "calcium channels" in the two muscle membranes. The toxin may affect the membrane in other ways. Since cardiotoxin has no effect on calcium influx in dystrophic muscle, one might expect an absence of rigor. This is not the case. Both types of muscles exhibit cardiotoxin induced contracture, suggesting more than one effect of the toxin on the muscle.

Earlier studies with dystrophic muscle have implicated altered Ca^{+2} permeability (18, 19). Defective Ca^{+2} transport has also been noted for erythrocytes from dystrophic patients (20). Altered fluxes of other important cations have also

been observed. Young et al. (21) showed that both K^+ influx and efflux are altered (the latter more so) in hereditary muscular dystrophy in mice. A number of studies, notably with heart muscle from the dystrophic Syrian hamster, have suggested a Ca^{+2} transport defect (22-24). Calcium accumulation and associated cytotoxin effects appear to be the major pathogenesis in these hereditary cardiomyopathies. Interestingly, studies by Jasmin et al. (25) have shown that drugs which limit the transport of Ca^{+2} into muscle have a therapeutic effect. These workers have suggested that a primary defect in the muscle cell membrane (presumably involving the transport of Ca^{+2}) may be responsible for the disease. Our experiments show no differences between the apparent calcium uptake rates in normal and dystrophic muscles.

The observation of cardiotoxin induced differences in apparent Ca^{+2} uptake rates in normal and dystrophic muscles focus on probable differences in the structures of the membranes themselves. Such differences may eventually be found in either the receptor sites for cardiotoxin binding, the manner in which such sites may be interacting with channels for cations in general or calcium in particular or differences in the number of calcium (cation) channels activatable by cardiotoxin.

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