Potentiation of Contraction of Glycerol-Extracted Muscle by Deoxycholate (35213)

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Deoxycholate, a bile salt, is known to affect contractile systems. Several years ago, Dr. R. S. Filo, in the course of some preliminary investigations in this laboratory, found that this detergent potentiates the ATP-induced contraction of glycerol-extracted arterial muscle. Hellam and Podolsky (1) have observed a similar effect of deoxycholate on the ATP-induced contraction of skinned skeletal muscle fibers. In the present study, it was demonstrated that deoxycholate potentiates the rate and extent of ATP-induced contractions of both glycerol-extracted arterial muscle and skeletal muscle fiber bundles, and the mechanism of the potentiation was explored.

This potentiation of the ATP-induced contractile response of these muscle models could result from direct action of deoxycholate on the contractile protein. Alternatively, it could be an indirect result brought about through alteration of the concentration of ATP at the active site. This latter hypothesis was tested by examining the relative degree of potentiation of thick and thin fiber bundles, where different diffusion conditions would exist. The former hypothesis was tested by studying the effect of deoxycholate on the ATPase activity of isolated actomyosin. Evidence is presented that the potentiating action of deoxycholate is related to its ability to enhance the diffusion of ATP, increasing its availability to the contractile protein.

Materials and Methods. Contraction of glycerol-extracted fiber bundles. Arterial fiber bundles were prepared from the media uncoiled from the wall of the hog carotid, using the technique described by Bohr *et al.* (2). Skeletal muscle fiber bundles were taken from rabbit psoas. Both were extracted in 50% glycerol–water solution (v/v) containing 8.75 m*M* imidazole buffer (pH 7.0,) at 4° for 24 hr, and stored at −18° for 4–60 days before use. For extractions, arterial fiber bundles were affixed to glass rods at a stretch about 10% beyond their unloaded equilibrium length. Skeletal muscle fiber bundles were tied onto rods at resting length.

Arterial fiber bundles were separated into two groups, control and experimental. Each fiber bundle in the experimental group was paired with a bundle in the control group which had approximately the same cross-sectional geometry and which came from an adjoining portion of the same artery. Skeletal muscle fiber bundles were similarly paired. Only fibers which were of uniform geometry along their length were used. Thickness measurement is reported as the shortest distance through the center of the fiber bundle. This measurement is more relevant for the study of diffusion phenomena than measurement of cross-sectional area since diffusion may take place more rapidly through a flat fiber bundle than through a thick one even though both have the same cross-sectional area. Fibers were mounted in a bath and stretched by a small force, 60 mg for arterial and 5 mg for skeletal muscle fiber bundles. Force developed was recorded using a Grass FT .03 transducer and polygraph.

The standard medium for obtaining "control" contractions contained 150 m*M* KCl, 5 m*M* $MgCl_2$ and 17.5 m*M* morpholinopropane sulfonic acid (MOPS) (pH 7.0 at 25°). From this standard solution the other solutions used were prepared by various additions, as follows: (i) 1 m*M* Ethyleneglycol-bis-(β-aminoethyl ether)N,N′-tetraacetic acid, for a Ca-free "rinse" solution; (ii) 1 m*M* EGTA and 7.5 m*M* Na deoxycholate, for the

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"treatment" solution; (iii) 1 m*M* CaEGTA + 5 m*M* ATP for the "contracting solution"; (iv) 1 m*M* EGTA and 5 m*M* ATP for an "equilibrating solution."

One fiber of a pair was put in a Ca-free rinse solution, the other in deoxycholate treatment solution, for 15 min, during which time the solutions were gently agitated by aeration. Both fibers were then put in agitated Ca-free rinse solution for 7 min, to remove the deoxycholate from the treated fiber. The fibers were then put in contracting solution, and the contractions were recorded. Responses of the thick and thin arterial and skeletal muscle fiber bundle to the contracting solution were recorded. Thick arterial fiber bundles were also equilibrated in 5 m*M* MgATP in the absence of Ca ions to permit the diffusion of ATP into the fiber bundle before addition of Ca ions to cause contraction.

Actomyosin ATPase activity. Actomyosin was prepared from skeletal muscle according to the method described by Murphy *et al.* (3). ATPase activity was determined at 25° using 0.4 mg of actomyosin/ml, 17.5 m*M* MOPS buffer (pH 7.0 at 25°), 5 m*M* MgATP, KCl to give an ionic strength of 0.1, and 1 m*M* CaEGTA. Enzyme reactions were stopped by the addition of trichloroacetic acid. ATPase activity was calculated on the basis of inorganic phosphate liberation, measured by the method of Rockstein and Herron (4).

Deoxycholate was added to suspensions of actomyosin to give concentrations of 2.5 and 7.5 m*M*. After 15 min of gentle stirring, the deoxycholate was removed by centrifuging the suspension twice for 15 min at 16,000*g*. After each centrifugation, the supernatant was discarded and the actomyosin was resuspended in a solution containing 100 m*M* KCl, 4.4 m*M* MOPS (pH 7.0 at 0°) buffer, 5 m*M* NaN_3 and 0.5 m*M* dithiothreitol. Control actomyosin suspensions were treated similarly but without the addition of deoxycholate. Protein concentrations were determined by the biuret method (5).

Results. Both the extent and the rate of force development by thick fiber bundles from arterial smooth muscle are increased by deoxycholate treatment (Fig. 1). In thick

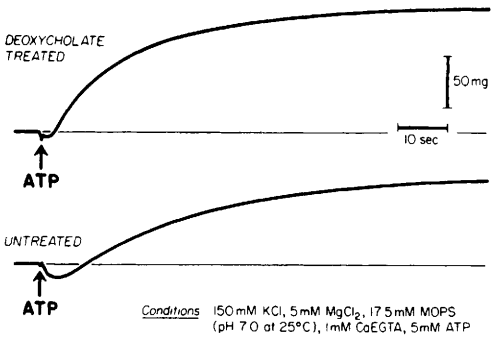

FIG. 1. Potentiating effect of deoxycholate on rate and magnitude of ATP-induced contraction of thick (450μ) arterial muscle fiber bundles.

fiber bundles from skeletal muscle the rate but not the extent of force development is increased. However, thin bundles from either muscle were not affected by the treatment. The results of these experiments are summarized in Table I.

Thick arterial fiber bundles equilibrated in 5 m*M* MgATP in the absence of Ca showed no effect from deoxycholate pretreatment when Ca was added to the medium. These results suggest that slow diffusion of the bulky ATP molecule into the bundle might be limiting the contraction of the fibers untreated with deoxycholate.

The activity of actomyosin, on the other hand, was not potentiated by deoxycholate treatment. In fact, the ATPase activity of skeletal muscle actomyosin, as measured by amount of inorganic phosphate liberated, was inhibited by both 2.5 and 7.5 m*M* deoxycholate, as shown in Table II.

Discussion. These observations do not support the possibility that deoxycholate potentiates the contraction of glycerol-extracted fibers by directly affecting the ATPase activity of the contractile proteins. Briggs and Fuchs (6) observed that 0.5, 1.0, or 5.0 m*M* deoxycholate had little effect on myofibrillar ATPase activity, whereas 10 m*M* deoxycholate caused a 50% inhibition of this activity. The results of the present study are consistent with these findings. Any effect on contraction that this detergent might have through its action on the contractile proteins would be inhibitory rather than potentiating.

Our evidence favors the hypothesis that deoxycholate reduces diffusion barriers in the

TABLE I. Effect of Deoxycholate on the ATP-Induced Contraction of Glycerol-Extracted Fiber Bundles.

Fiber bundle	Av thickness (μ)	Maximum force developed (mg ± SEM)			Maximum rate of force development (mg/sec ± SEM)		
		Control	Deoxycholate pretreated	Potentiation (%)	Control	Deoxycholate pretreated	Potentiation (%)
Arterial							
Thick	483 ± 33.1	82.7 ± 5.2 $n=27$	136.1 ± 2.2 $n=27$	65[a]	1.00 ± 0.09 $n=20$	2.56 ± 0.38 $n=18$	156[a]
Thin	178 ± 23.4	13.9 ± 4.1 $n=7$	18.1 ± 4.1 $n=7$	30 NS[b]	0.06 ± 0.01 $n=7$	0.07 ± 0.01 $n=7$	18 NS
Thick bundles equilibrated with ATP	485 ± 30.7	159.2 ± 24.5 $n=11$	154.4 ± 16.3 $n=13$	−3 NS	0.71 ± 0.01 $n=10$	0.68 ± 0.01 $n=10$	−4 NS
Skeletal							
Thick	700 ±119.6	490 ± 89.3 $n=7$	585 ± 90.3 $n=7$	19 NS	38.3 ± 13.4 $n=7$	246.6 ± 42.6 $n=7$	544[a]
Thin	135 ± 8.3	229.5 ± 13.0 $n=11$	171.8 ± 17.3 $n=11$	−25 NS	82.6 ± 17.2 $n=10$	97.3 ± 18.5 $n=10$	18 NS

[a] Significant at 0.001 level.
[b] NS = not significant.

TABLE II. Deoxycholate Inhibition of Skeletal Muscle Actomyosin ATPase Activity.

Deoxycholate (m*M*)	Inhibition (% ± SEM)	
	0–2 min	4–8 min
2.5	16.0 ± 2.2 $n=7$	16.0 ± 2.1 $n=8$
7.5	22.8 ± 5.8 $n=10$	19.9 ± 6.1 $n=10$

fiber bundle. These barriers may be due to the presence of remaining structures (membranes) which hinder the diffusion of ATP to the contractile proteins. Briggs and Fuchs (6) and Martonosi *et al.* (7, 8) have provided evidence that deoxycholate can destroy such membrane systems. The effects of the impaired diffusion may be magnified by peripheral hydrolysis of ATP around the fiber core, which would maintain an ATP concentration gradient and prevent this energy source from reaching the core cells in an effective concentration.

Assuming that thick fiber bundles have an inner core of cells which do not receive ATP and are thus not able to contract, removal of diffusion barriers by deoxycholate would enable ATP to reach the core cells which could then contract, and more force would be developed. Increased maximum rates of force development can also be accounted for by the removal of diffusion barriers, allowing ATP to reach all of the cells faster. The maximum rate of force development would thereby be increased. Small fiber bundles do not show the potentiating effect of deoxycholate, presumably because they do not have long diffusion pathways and therefore all cells have a ready supply of ATP. The results given for skeletal muscle fiber bundles are consistent with those of Bowen and Martin (9) who, using a similar preparation, found that bundles up to 110 μ thick could be used without serious diffusion problems, but with larger fibers there were ATP deficient cores.

Chaplain and Abbott (10) found that the detergent Tween 80 reduces diffusion barriers in glycerol-extracted fiber bundles. They found that glycerol-extracted insect muscle fiber bundles will perform oscillatory work in 2 m*M* ATP after treatment with the detergent Tween 80, but not before. Enhancement of diffusion by Tween 80 is further evidenced by the fact that after this treatment the ATPase activity of fiber bundles more closely approaches the rate found in homogenates. These observations suggest that access of ATP to the myofibrillar ATPase sites of the core of fibers is impeded in the untreated glycerol-extracted fiber bundles.

The lack of an effect on arterial muscle fiber bundles due to deoxycholate pretreatment after equilibration in 5 m*M* MgATP, supports the idea that deoxycholate enhances the diffusion of ATP through the bundle. It also indicates that any change in the diffusion of Ca ion produced by deoxycholate is not great enough to be measured as a change in rate or magnitude of contraction. Since CaEGTA is used in the contracting solution to maintain the Ca-ion concentration at a supraoptimal level around the contractile element (11), any mechanism of the deoxycholate potentiation which involves increased activation by the addition of Ca ions is unlikely. Hellam and Podolsky (1) observed that more force was developed by skinned skeletal muscle fibers only at Ca-ion concentrations which are partially activating. They attributed the potentiation to a direct effect on the contractile proteins, which increased the affinity of myofilaments for Ca ions. This direct effect would not have been seen in the present study since the Ca-ion concentration was maintained at a supraoptimal level by CaEGTA.

This study suggests that the diffusion barriers of arterial muscle fiber bundles are somewhat different from those of skeletal muscle fiber bundles. After treatment with deoxycholate, arterial muscle fiber bundles show a potentiation of both maximum force developed and the rate of force development, whereas skeletal muscle fiber bundles show a much greater rate potentiation, but little change in the maximum force developed. This may mean that skeletal muscle fiber bundles have a moderate diffusion barrier. Treatment with deoxycholate allows rapid attainment of maximum force in the presence of an optimal MgATP concentration. On the other hand, arterial fiber bundles apparently

have a more severe diffusion barrier which prevents maximum force development without deoxycholate treatment. Deoxycholate reduces the diffusion barriers enabling core cells, which previously did not receive ATP, to contract. Murphy *et al.* (3) have found that a high concentration of ATP will inhibit the ATPase activity of arterial actomyosin. Such an overoptimal average concentration of ATP may account for the fact that the rate increase in contraction of arterial muscle fiber bundles is not as dramatic as that of skeletal muscle glycerol-extracted fiber bundles. An overoptimal concentration of ATP may also account for the low rate of contraction of the thick arterial bundles equilibrated with ATP.

Treatment with the detergent deoxycholate subsequent to glycerol extraction provides better material for studying the contractile system, by minimizing the ATP concentration gradient due to diffusion barriers and peripheral ATP hydrolysis. The use of deoxycholate makes possible the use of larger arterial fiber bundles with a minimal diffusion problem; maximum force developed and maximum rate of force development by these fibers are more nearly attained.

Summary. Treatment with the detergent deoxycholate potentiates the rate and extent of the ATP-induced contraction of both thick (485 μ) glycerol-extracted arterial muscle and thick (700 μ) skeletal muscle fiber bundles. Deoxycholate does not affect the ATP-induced contraction of thin glycerol-extracted fiber bundles (180 and 135 μ for arterial muscle and skeletal muscle fiber bundles, respectively). It does not affect the Ca-induced contraction of arterial muscle fiber bundles pre-equilibrated with 5 m*M* MgATP, and inhibits the ATPase activity of skeletal muscle actomyosin. It thus appears that the potentiating action of deoxycholate on the contraction of thick glycerol-extracted fiber bundles is due to its ability to enhance the diffusion of ATP through the fiber bundle.

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