Biochemical Pharmacology, Vol. 21, pp. 601–605. Pergamon Press. 1972. Printed in Great Britain

INOTROPIC EFFECT OF CARDIAC PHOSPHOLIPIDS ON THE CONTRACTION OF RABBIT HEART PERFUSED WITH A CALCIUM DEFICIENT MEDIUM

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(*Received* 21 *June* 1971; *accepted* 8 *October* 1971)

Abstract—The contractile strength of isolated rabbit heart is dependent on the Ca^{2+} concentration in the perfusion medium. At suboptimal Ca^{2+} concentration, the addition of exogenous phospholipids extracted from beef heart improves myocardial contractility. Evidence is presented that phospholipids significantly potentiate the inotropic effect of digitoxin. The possible mechanism by which phospholipids facilitate Ca^{2+} transport is discussed.

SEVERAL enzymes have a phospholipid requirement for maximal activity.[1] Moreover, it has been shown that in mitochondrial membranes, phospholipids play an important role in regulating properties of semipermeability and in transporting cations.[1] In particular, the integrity of the phospholipid structure of the inner mitochondrial membrane is required for translocating Ca^{2+} across the membrane.[2] Patriarca and Carafoli[3] have shown recently that in the intact rat, intraperitoneal injection of radioactive calcium leads to more extensive labeling of the mitochondria than any other cellular component of the heart.

On the other hand, Ca^{2+} has been shown to be the agent which initiates muscle contraction[4] and relaxation occurs when the calcium concentration falls below a certain level.[5] In the myocardium, diffusion of calcium from the extracellular spaces to the contractile proteins is considered the process that triggers muscle shortening.[4,6]

In the present paper it is shown that cardiac phospholipids added to Ca^{2+} deficient perfusion medium significantly improve myocardial contractility and specifically potentiate the "digitalis effect".

MATERIALS AND METHODS

Male "Fulvi di Borgogna" rabbits weighing 3000–3500 g were maintained on stock diet *ad lib.* prior to an 18- to 24-hr period of fasting. The animals were killed by decapitation, the hearts were quickly removed, washed in a Tyrode solution and isolated by the use of a modified Langendorf preparation.[7]

The concentrations of the solutes in the Tyrode solutions were as follows: 140 mM NaCl; 2·5 mM KCl; 0·5 mM $MgCl_2.6H_2O$; 0·35 mM $NaH_2PO_4.H_2O$; 5·5 mM glucose; 12 mM $NaHCO_3$; 1·80 mM $CaCl_2$. The final pH was 7·3.

The venous effluent, collected from the coronary sinus in a graduated beaker, represented the coronary flow. The solution was oxygenated for at least 30 min before

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washing and perfusion and was maintained at 38° in a thermostatically controlled water bath. The amplitude of contraction was measured from the apex of the left ventricle after recording on a kymograph.

We used "Merck" digitoxin. Phospholipids were extracted from beef hearts and purified as described by Bruni *et al.*[8] Before using they were suspended in water and sonicated at 20,000 kcycles for 30 min. The chromatographic analysis[9] revealed the presence of phosphatidyl-choline, lysophosphatidyl-choline, cephaline, cholamin-cephaline, sphingomyeline, monophosphoinositol, phosphatidyl-serine, cardiolipins, and traces of cholesterol, triglycerides, diglycerides and fatty acids.

RESULTS

Figure 1 shows that the contractile strength of the perfused heart was dependent on the Ca^{2+} concentration in the perfusion medium. At the suboptimal concentration of 0·9 mM, upon addition of beef heart phospholipids the impaired contractile amplitude recovered proportionally to the phospholipid concentration (Fig. 2). The amount of added phospholipids required to counteract the effect of Ca^{2+} deficiency was proportional to the extent of the Ca^{2+} deficiency itself. However, when Ca^{2+} concentration was reduced to, or below, 0·36 mM, the addition of phospholipids did not determine any detectable effect.

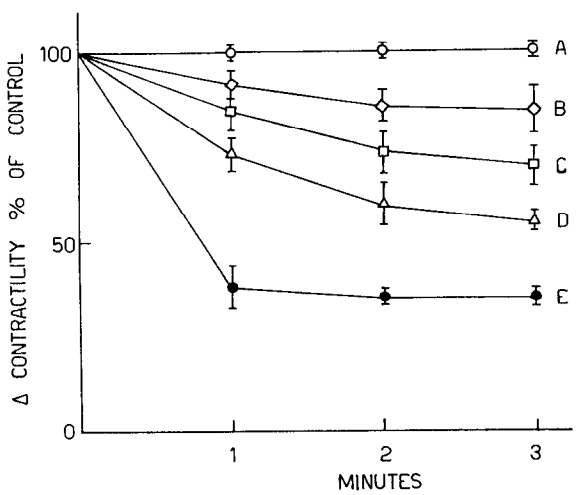

FIG. 1. Effect of varying the concentration of Ca^{2+} in the perfusion medium on the extent of the contractility of isolated perfused rabbit heart. A: 1·80 mM Ca^{2+}; B: 1·44 mM Ca^{2+}; C: 1·08 mM Ca^{2+}; D: 0·72 mM Ca^{2+}; E: 0·36 mM Ca^{2+}. Each curve represents the mean of the values obtained from nine different preparations. Standard deviations indicated by the bars.

At this critical concentration of 0·36 mM Ca^{2+}, the addition of digitoxin caused only a slight increase in the contraction amplitude. However, upon subsequent addition of phospholipids, a remarkable increase in contraction amplitude was observed (Fig. 3). This synergism between digitoxin and phospholipids is also seen in Fig. 4, in which the amplitude of contraction determined by the digitoxin concentration in the perfusion medium is significantly improved by the addition of phospholipids.

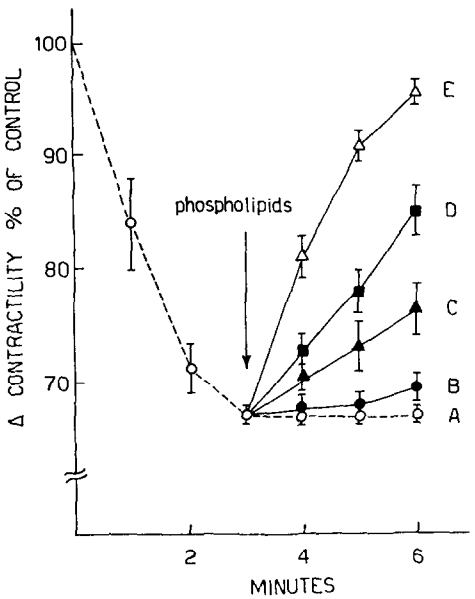

FIG. 2. Effect of added phospholipids on the contractility of isolated rabbit heart perfused with suboptimal Ca^{2+} concentration (0·90 mM Ca^{2+}) of the perfusion medium. A: No phospholipids; B: 10 μg/ml; C: 50 μg/ml; D: 100 μg/ml; E: 200 μg/ml. Each curve represents the mean of the values obtained from eight different preparations. Standard deviations indicated by the bars.

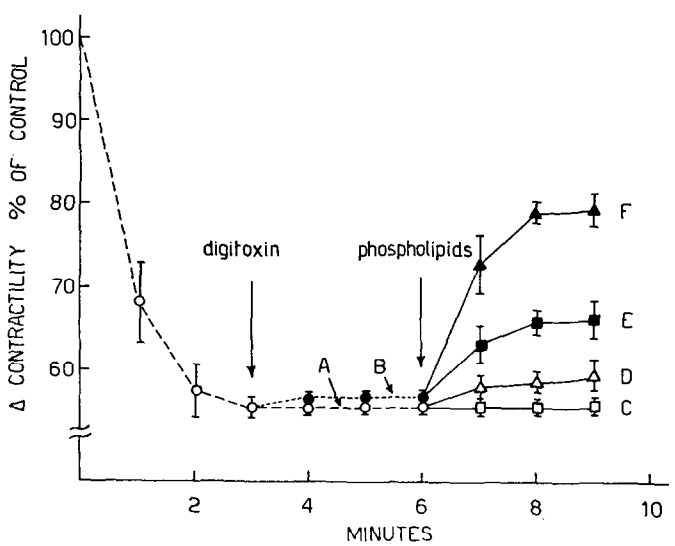

FIG. 3. Effect of successive additions of digitoxin and phospholipids on the recovery of the contractility of isolated rabbit heart. The perfusion medium contains 0·36 mM Ca^{2+}. A: control without digitoxin; B: treated for 3 min with 1 μg/ml digitoxin; C and E: 25 μg/ml phospholipids, D and F: 50 μg/ml phospholipids. Each curve represents the mean of the values obtained from eight different preparations. Standard deviations indicated by the bars.

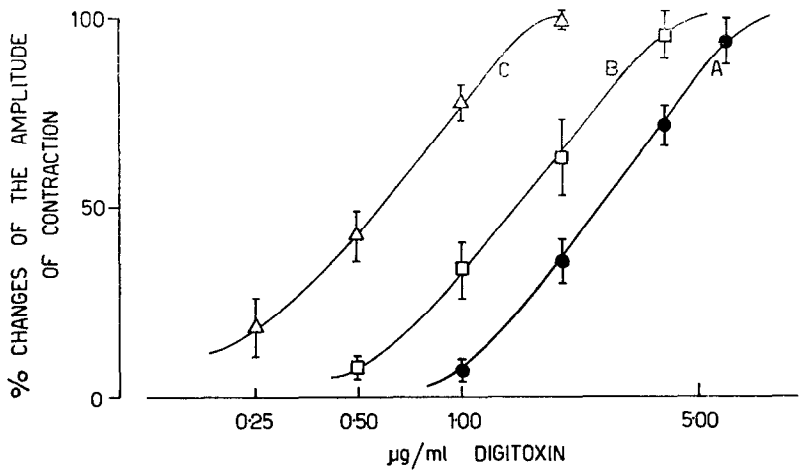

FIG. 4. Rabbit heart perfused in 0·36 mM Ca^{2+} at 37°. Log concentration–response curves for digitoxin in the presence of various concentrations of phospholipids in the perfusion medium. A: Digitoxin; B: digitoxin + 40 μg/ml phospholipids; C: digitoxin + 200 μg/ml phospholipids. The drugs were applied concomitantly to the reduction of Ca^{2+} concentration in the perfusion medium. The effects on contractility have been calculated after 3 min. The data are expressed as the percentage of change in the amplitude of contraction. It has been assumed that the maximum recovery of the contractility (100 per cent) corresponds to the value obtained in the presence of 1·80 mM Ca^{2+}. On the contrary, the minimum recovery of contractility (0 per cent) corresponds to the value obtained in the presence of 0·36 mM Ca^{2+}. Each point represents the mean of at least five different preparations. Standard deviations indicated by the bars.

DISCUSSION

The results reported indicate that exogenous phospholipids exert a positive inotropic and a potentiating effect on digitalis action on isolated heart perfused with a medium containing a suboptimal concentration of Ca^{2+}. Since both these effects were detected only when the perfusion medium contained suboptimal concentrations of calcium ions, the assumption that phospholipids facilitate calcium translocation across the cellular and intracellular membranes seems reasonable. The validity of the concept that endogenous phospholipids, as polar heads of membranes, contain the ion-exchange sites controlling cation permeability has been repeatedly demonstrated.[1,10,16]

Carpenedo *et al.* have observed that quercitin, a flavonoid, at a low calcium concentration exerts an effect very similar to that described here. Since quercitin is able to chelate divalent ions to form loosely dissociable complexes,[12] it was suggested that quercitin could make calcium readily available to a contractile system. The described phospholipids effect may be interpreted similarly.

Moreover, since it is well known that in the presence of a calcium-free perfusate, depolarization fails to evoke mechanical activity,[13] it seems reasonable to conclude that the critical intracellular concentration of free-ionized calcium required for mechanical activity must be transferred across the membranes from an extracellular environment or from a surface-bound calcium complex.[14] It is very likely that added phospholipids bind calcium and bring it either across the membranes or to some

membrane site suitable for the successive release of free Ca^{2+} to the Ca^{2+} -requiring intracellular structures.

The observed potentiation of digitalis action by phospholipids is not easily explained. It has been suggested that drugs, such as ouabain, digitoxin and lanatoside c, which increase the efficiency with which the heart performs mechanical work probably do so via a calcium-regulated pathway.[15] In the light of this possible mechanism, one can assume that phospholipids facilitate calcium availability to the cellular structure which is susceptible to digitalis action.

Acknowledgements—We are very grateful to Ing. W. Wolff and to Mr. G. Guagliumi, the General Director of "Aesculapius".

The careful technical assistance of Mrs. M. Follo, Mrs. M. L. Costa and Mr. T. Bussei is gratefully acknowledged.

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