Relationship between Cellular Calcium and Catecholamine on Myocardial Contractility*

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During the past few years an interest in the excitation-contraction coupling of calcium ion has been focused on their influence on myocardial contractility. On the other hand, recent investigations have shown that the sympathetic nervous system mediated the increase in the frequency of contraction and the augmentation of the inotropic properties of the myocardium. Especially, beta adrenergic stimulation as a result of the release of norepinephrine from sympathetic nerve endings, lead to enhancement of the contractile properties of the cardiac chambers. Therefore, it is an interesting subject to investigate the role of calcium ion in contractile force of myocardium induced by catecholamine. The purpose of this paper is to investigate the changes of distribution of calcium ion in cardiovascular response to catecholamine.

METHOD

Rabbits (about 2kg) were anesthetized with Thiopental soda (1.0 to 2.0 mg/kg iv). Their hearts were quickly excised, set on Langendorff apparatus and infused by modified Ringer solution (37°C), the composition of which has been described by Koch-Weser. Oxygenation and control of pH was achieved bubbling with a mixture of 95%O₂—5%CO₂ gas in the Ringer solution in the reservoir bottles.

Statam-transducer connected to apex of the heart was used for the continuous measurement of the maximum active isometric tension that is produced by increase of resting tension. And the leak of Ringer's solution from infused heart was measured consistently as the indication of coronary flow.

In these apparatus the heart was infused by Ringer solution containing 2.3mMol calcium for 30 minutes initially, and secondary infused by Ringer solution containing various doses of calcium for 30 minutes.

Norepinephrine was added to infused solution at final 5 minutes. These hearts were transferred quickly to a sucrose solution at 0°C, then weighed after separate the ventricles. And these was homogenized with 20 times volume of sucrose solution containing EDTA., and then separated in subcellular fractions with ultracentrifuge. The measurement of calcium and protein in each subcellular fractions were employed respectively.

RESULTS AND DISCUSSION

1. Influence of various concentration of calcium on myocardial contractility, cardiac rate and coronary flow.

Myocardial contractile force was increased.

Key Words:

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Fig. 1. Changes of cardiac performance (contractile force, cardiac rate and coronary flow) infused by solution containing various concentration of calcium. The left panel was recorded by infusion with high calcium concentration, while right was low calcium.

transiently by infusion containing 4.5 or 9.0 mMol calcium, and gradually followed by bradycardia. By infusion of low calcium concentration (0.6 & 1.2 mMol), myocardial contractile force is markedly decreased. (Fig. 1)

2. Subcellular calcium concentration of myocardium infused by the solution with various calcium concentration.

Although, total calcium content of myocardium and calcium concentration of mitochondrial fraction were influenced markedly by the changes of calcium concentration in infused solution, calcium concentration of heavy microsome fraction that is occupied by sarcoplasmic reticulum was changed slightly. (Fig. 2)

3. Effects of norepinephrine on myocardial contractility, heart rate and coronary flow under the infusion of low calcium containing solution. By administration of norepinephrine 0.5 μg/min, myocardial contractile force and coronary flow were increased about

Fig. 2. Calcium concentration in subcellular fraction of myocardium infused by solution containing various concentration of calcium.

Ca_T: Calcium concentration in whole heart.
Ca_Mit: Calcium concentration in mitochondrial fraction.
Ca_MS: Calcium concentration in heavy microsome fraction.

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40% and 20% respectively, otherwise heart rate were hardly changed. (Fig. 3)

When 1.0 μg/min of norepinephrine was infused, the increment of myocardial contractile force and coronary flow became progressively, and 20% increase of cardiac rate was observed (Fig. 4). Furthermore, by administration of excessive dose (2.0 μg/min) of norepinephrine, myocardial contractile force and coronary flow had reached to the maximum level; that is 70% and 50% respectively, while increasing cardiac rate is 60% in these experiments. (Fig. 5)

Calcium concentration in whole heart and each subcellular fractions had increased by increasing dose of norepinephrine. Accordingly, the increment of calcium concentration in whole heart and mitochondrial fraction were proportionally with norepinephrine dose. However, the calcium concentration in heavy

Fig. 3. Records of contractile force, cardiac rate and coronary flow following the norepinephrine administration of 0.5μg/min in rabbit heart.

Fig. 4. Records of contractile force, cardiac rate and coronary flow following the norepinephrine administration of 1.0μg/min in rabbit heart.

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Fig. 5. Records of contractile force, cardiac rate and coronary flow following the norepinephrine administration of 2.0 μg/min in rabbit heart.

Fig. 6. Calcium concentration in subcellular fraction of myocardium infused by solution containing 0.6mMol calcium and various dose of norepinephrine.

Fig. 7. Calcium concentration in subcellular fraction of myocardium infused by solution containing various concentration of calcium and norepinephrine (1.0μg/min)

microsome fraction was increased markedly until 1.0 μg/min of norepinephrine and became stable by more increment of norepinephrine. (Fig. 6)

4. Influence of norepinephrine on the intracellular distribution of calcium in myocardium infused by solution containing various concentration of calcium.

Although, the increment of subcellular calcium concentration in myocardium infused by solution containing 0.6 um calcium and 1.0
μg/min of norepinephrine was significant, especially in heavy microsome, but when infused by solution containing 2.3 mMol calcium and 1.0 μg/min of norepinephrine, no changes of subcellular calcium concentration were observed. The almost same value of calcium concentration in heavy microsome after administration of norepinephrine were observed regardless the changes of calcium concentration in infused solution. Furthermore, in these experiment, the absolute value of myocardial contractile force after the addition of norepinephrine were within same range. Thus, the close relationship was observed between calcium concentration in heavy microsome fraction and contractile force in cardiac performance. (Fig. 7)

For this reason, it is of interest to investigate the influence of increasing cardiac rate which is observed with increasing contractile force concomitantly on cardiac calcium concentration. Therefore, the cardiac rate was increased to 40% compared to control levels by electrical stimulation, however, no changes of calcium content in heavy microsome, myocardial contractility and coronary flow were observed. (Fig. 8)

Effects of norepinephrine on subcellular calcium content and cardiac dynamics were abolished by pretreatment of propranolol. (Fig. 9)

![Fig. 8. Effects of norepinephrine and increasing cardiac rate on calcium concentration in subcellular fraction of myocardium infused by solution containing 0.6mMol calcium.](image)

![Fig. 9. Influence of Propranolol on calcium concentration in subcellular fraction infused by solution containing 0.6mMol calcium with or without norepinephrine.](image)

**Summary**

Positive inotropic action of norepinephrine were demonstrated in rabbit heart that was perfused by solution containing various calcium concentration.

In these experiments, the changes of calcium content in subcellular fractions of myocardium were observed.

1) The calcium concentration in each subcellular fraction of myocardium was increased in the state of increasing contractile force
by norepinephrine. Especially, there are similar pattern of changes between calcium concentration in heavy microsome fraction and contractile force of myocardium.

2) Increase of cardiac rate had no effects on the changes of calcium concentration in myocardium. These phenomena induced by norepinephrine were inhibited by pretreatment of propranolol.

Thus, it should be emphasized that calcium ion in cardiac sarcoplasmic reticulum plays an important role in increasing contractile force by norepinephrine.

REFERENCE