EFFECT OF NITROGLYCERIN ON Ca EFFLUX IN THE CORONARY ARTERY

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Abstract—Effects of nitroglycerin on the $^{45}$Ca efflux were studied on the isolated coronary artery preparation of the dog. After loading with $^{45}$Ca for 3 hours and rinsing with cold physiological salt solution (PSS), the preparation was transferred to a series of vials containing oxygenated PSS at 10 min intervals. After 100 min, Ca efflux was induced from within the cell by metabolic inhibition with dinitrophenol plus monooiodoacetate following the method of Van Breemen et al. (1975). Nitroglycerin did not produce any effect on the Ca efflux. Although not complete, lanthanum (1 mM) produced an inhibition of the Ca efflux, which was not reversed by nitroglycerin. It is concluded that our previous findings of relaxation of the lanthanum contracture of the coronary artery by nitroglycerin was not brought about by an increase in Ca efflux from the coronary artery.

The contractile status of the vascular smooth muscle, which plays an important role in the pathogenesis of the cardiovascular disease, is determined by intracellular free Ca concentration (1, 2). A part of the activator Ca enters a smooth muscle cell through the plasma membrane and a part may be released from intracellular stores.

Reduction of this activator Ca concentration in the cytoplasm, which results in relaxation of the smooth muscle, can occur through three mechanisms: (1) a decrease of Ca influx, (2) an increase of Ca extrusion (Ca efflux), and (3) an accumulation in the intracellular stores. Vasoactive agents may affect these three mechanisms.

Nifedipine, a Ca antagonist, produces a relaxation of the vascular smooth muscle through inhibition of transmembrane Ca influx (3), while the inhibition of the transmembrane Ca influx is not conceivable with respect to the smooth muscle relaxant effect of nitroglycerin, a representative antianginal drug, for as we previously reported (4, 5), contracture produced in the intestinal smooth muscle by high-potassium medium cannot be reversed by nitroglycerin. K-contracture of the intestinal smooth muscle is effectively inhibited by nifedipine, and is generally related to the transmembrane Ca influx. Conversely, lanthanum-contracture in the coronary strip is effectively inhibited by nitroglycerin, but not by nifedipine (5).

In this paper, the effects of nitroglycerin on Ca efflux were studied. In addition the effects of lanthanum on Ca efflux were studied. Since an inhibition of Ca efflux by lanthanum was observed, further experiments were done to observe whether or not the inhibition would be reversed by nitroglycerin.

MATERIALS AND METHODS
Mongrel dogs weighing 15–25 kg were
anesthetized with thiopental sodium (30 mg/kg i.v.) and bleed from the carotid artery. The heart was rapidly excised, and transferred into a dissection bath containing chilled normal physiological salt solution (normal PSS, see below) bubbled with 100% oxygen. The circumflex branch of the left coronary artery (LCA) was isolated and cleaned of adherent fat and connective tissues. The LCA was then prepared into 2–3 mm cross-sectional rings. Three or five such cross-sectional rings weighing 10–20 mg were fixed to a stainless steel rod, and preincubated in normal PSS for 30 min at 37°C.

The physiological salt solutions (PSS) used contained (mM): 1) normal PSS, NaCl 125; KCl 2.7; MgCl₂ 1.2; CaCl₂ 2.0; tris (hydroxymethyl) aminomethane (E. Merk) 23.8, glucose 11.0. 2) “lanthanum method” PSS, NaCl 113; KCl 2.7; MgCl₂ 1.2; tris (hydroxymethyl)aminomethane 23.8, glucose 11.0; LaCl₃ 10.0. 3) “efflux” PSS, NaCl 125.5; KCl 2.7; MgCl₂ 1.2; CaCl₂ 1.5; tris (hydroxymethyl)aminomethane 23.8; glucose 11.0. The pH of these solutions was adjusted to 7.4 with conc. HCl and the preparations were bubbled with 100% oxygen.

Time course for cellular uptake of ⁴⁵Ca was determined using the lanthanum method (6). The tissue was loaded for several hours in 10 ml normal PSS containing 20 µCi of ⁴⁵Ca (New England Nuclear ⁴⁵CaCl₂ in water), after which it was transferred to “lanthanum method” PSS and incubated for 1 hr. After rinsing for 5 sec in 50 ml of normal PSS, the tissue was digested in 3 ml of Soluene-350 (Packard Instrument Company) for 2 hr at 37°C. The amount of cellular ⁴⁵Ca was determined by liquid scintillation counting using a scintillation cocktail (Aquazol-2, New England Nuclear) with scintillation spectrometer (Packard model 3320).

⁴⁵Ca efflux measurement was performed as follows: After loading of the preparation, the tissue was rinsed for 5 sec in 50 ml of normal PSS to remove the adherent solution. Thereafter the preparations were transferred at 10 min intervals through a series of glass scintillation vials containing the oxygenated “efflux PSS”. After 100 min, the transmembrane ⁴⁵Ca efflux was induced with 10⁻⁴ M of 2,4-dinitrophenol (DNP) plus 10⁻³ M of moniodoacetic acid (IAA) (7) and the amount of ⁴⁵Ca appearing in the “efflux” medium was determined with scintillation spectrometer.

In order to examine the effects of lanthanum on ⁴⁵Ca efflux, 1 mM lanthanum was added 30 min prior to metabolic inhibition with DNP plus IAA. In nitroglycerin experiments 10⁻⁶ g/ml of nitroglycerin, which was found to produce an almost complete inhibition of lanthanum-contraction in our previous study (5), was added 30 min prior to metabolic inhibition. In experiments in which the effects of nitroglycerin on the lanthanum-induced inhibition of ⁴⁵Ca efflux were studied, 1 mM lanthanum was added simultaneously with 10⁻⁵ g/ml of nitroglycerin 30 min prior to metabolic inhibition.

Statistical analyses were conducted by Student’s t-test (two-tailed). A value of p<0.05 was considered to be significant.

RESULTS

1) Setting of loading time for ⁴⁵Ca experiment: Figure 1 illustrates the rate of intracellular ⁴⁵Ca accumulation in the coronary artery measured using the “Lanthanum method”. The amount of intracellular ⁴⁵Ca reached a plateau after incubation in ⁴⁵Ca medium for 3 to 4 hours. Thus, loading time of 3 hours was adopted for ⁴⁵Ca efflux experiment.

2) Effect of lanthanum on ⁴⁵Ca efflux induced with DNP plus IAA: Figure 2 shows the rate of ⁴⁵Ca washout in “efflux PSS”. The cellular ⁴⁵Ca efflux was induced with DNP plus IAA as described in the “Method”. The amount of ⁴⁵Ca effluxed from the intra-
cellular space, estimated by measuring the area under the efflux curves was $8.10\pm1.98$ μmole/kg LCA (mean±S.E., n=7), while in the lanthanum-treated group, this value was $2.66\pm0.89$ μmole/kg LCA (Mean±S.E., n=6), indicating that $^{45}$Ca efflux induced with DNP plus IAA was significantly inhibited by 1 mM lanthanum ($p<0.05$).

3) Effect of nitroglycerin on the inhibition of $^{45}$Ca efflux produced by lanthanum: The inhibition by lanthanum of $^{45}$Ca efflux induced with DNP plus IAA was not reversed by $10^{-6}$ g/ml of nitroglycerin, as shown in Fig. 3. The amount of $^{45}$Ca effluxed from the intracellular space in nitroglycerin-treated group was $2.55\pm0.37$ μmole/kg LCA (mean±S.E., n=4).

4) Effect of nitroglycerin on $^{45}$Ca efflux induced with DNP plus IAA: As shown in Fig. 4, $^{45}$Ca efflux induced with DNP plus IAA was not significantly affected by $10^{-6}$ g/ml of nitroglycerin. The amount of $^{45}$Ca effluxed from the intracellular space in nitroglycerin-treated group was $4.66\pm0.61$ μmole/kg LCA (mean±S.E., n=4).
Discussion

In the present study, the time required for the equilibrium of cellular $^{45}$Ca was 3 to 4 hours. Thus, we adopted the 3 hours’ loading time of $^{45}$Ca in order to maximally label the cellular Ca compartments. This loading time was similar to the findings of other investigators (7, 8).

We carried out experiments on $^{45}$Ca efflux in solutions containing 1.5 mM Ca (7). An augmentation of $^{45}$Ca efflux was observed after DNP plus IAA in agreement with observations by Van Breemen et al. (7, 9) in other tissues. This extra efflux was used as a measure of the $^{45}$Ca effluxed from within the cell. The reason why we chose such a procedure is that the large portion of $^{45}$Ca washout curve of the smooth muscle represented the washout of extracellular $^{45}$Ca rather than that from within the cell, as described previously by Van Breemen et al. (7). Accordingly, we could not differentiate the cellular efflux from that emerging from the extracellular space without using the special procedure.

Lanthanum blocked $^{45}$Ca efflux initiated by metabolic inhibition suggesting that one of the possible mechanisms for lanthanum-contraction reported by Fermum et al. (10) and Imai and Kitagawa (5) is the inhibition of Ca efflux. Accordingly, it became important to rule out the possibility that the reversal by nitroglycerin of lanthanum-contraction is brought about by removal of lanthanum-induced blockade of Ca efflux. However, nitroglycerin, in a dose which was effective in relaxing the lanthanum-contraction of the coronary artery strip had no effect on the inhibition of $^{45}$Ca efflux produced by lanthanum. Moreover, nitroglycerin did not affect $^{45}$Ca efflux induced by DNP plus IAA.

These results indicate that the enhancement of calcium efflux from within the cell is not involved in the relaxation by nitroglycerin of the contracture of the coronary artery, as induced with lanthanum.

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