평활근에서의 칼슘대사

황 광 서(이화여대)

CALCIUM METABOLISM IN VASCULAR SMOOTH MUSCLE CELLS

Kwang Seo Hwang
Ehwa Womens University, College of Pharmacy, Seoul, Korea

INTRODUCTION

Ringer (1883) recognized that calcium was a crucial in contraction of the heart muscle. As the techniques became available for measuring cytosolic free calcium (Ca^{2+}) (Blinks et al., 1976; Grynkiewiez et al., 1985), investigators tried to correlate the cytosolic free Ca^{2+} with muscle contraction and other cellular functions. Now it is well accepted that cytosolic free Ca^{2+} plays a major role in muscle contraction including smooth muscle and also other cellular functions: e.g., secretion, fertilization, proliferation, gene expression and phagocytosis (Tsien et al., 1982; Somlyo and Somlyo, 1986; Lee et al., 1994).

Cytosolic free Ca^{2+} in many types of cells has been estimated to fluctuate between (0.1 uM at rest to 1 uM during activation). Upon stimulation, activating Ca^{2+} proceeds through the channels on plasmalemmal (PL) or sarcolemmal (SL) membrane down the steep gradient of Ca^{2+} raising cytosolic free Ca^{2+}. In order to restore the resting cytosolic free Ca^{2+} level calcium pumps and other systems on PL (or SL) and subcellular organelle(s) remove Ca^{2+} from the cytosol.

THE ROLE OF THE SARCOPLASMIC RETICULUM IN SIGNAL TRANSDUCTION

Subcellular organelle(s) (e.g., endoplasmic (ER) or sarcoplasmic reticulum (SR)) release Ca^{2+} for activation and sequester Ca^{2+} for relaxation. The degree of contribution from these systems on cytosolic free Ca^{2+} varies considerably depending on the types of the cells and the stimuli. In the absence of extracellular Ca^{2+}, appropriate agonist-induced contractions diminish rapidly in cardiac and smooth muscle, while they last for a relatively long period in skeletal muscle. In smooth muscle, the SR accumulates Ca^{2+} during the depolarization-induced Ca^{2+} entry across the surface membrane. However, the contribution of Ca^{2+} accumulation by the SR during the depolarization-induced activation has not been studied.

BAY K8644 STIMULATED CALCIUM ENTRY AND TENSION DEVELOPMENT: CALCIUM AGONIST

In rabbit aorta, physiological salt solution (PSS) containing K^+ upto 15 mM did not produce tension in the absence of Bay K8644 (Hwang and van Breemen, 1985). PSS containing 30 mM K^+ produced maximal tonic tension however, at a slower rate than 80 mM K^+. In the presence of 1 uM Bay K8644 (Schramm et al., 1983), PSS containing 0 and 5 mM K^+ produced 18% of maximal tension (Fig. 1), in contrast to the priming effect of Bay K8644 as reported. However, this was consistent with the hypothesis of Ca^{2+} entry stimulator (Ca^{2+} agonist) as proposed. The tonic tension produced in PSS containing 15 mM K^+ was maximal. Raising the K^+ concentration in PSS, in the presence and absence of 1 uM Bay K8644, stimulated unidirectional ^{45}Ca influx in a graded manner: 12 umol/kg/min at rest to 24 umol/kg/min.

THE SR FUNCTIONS AS A BUFFER BARRIER

It was very interesting to note that in the absence of tension development significant amount of Ca^{2+} entered in PSS containing 5 mM K^+ (12 umol/kg/min, basal ^{45}Ca influx) and this could be raised upto 15 umol/kg/min (threshold ^{45}Ca influx) in PSS containing 15 mM K^+ without further tension development. Beyond this value, tension development was a very steep function of ^{45}Ca influx saturating at 24 umol/kg/min. The Ca^{2+} entry at the level of the threshold would be sufficient to fully activate the myofilaments within minutes if it were not removed by transport mechanism from the cytosol. Such a mechanism may consist of Ca^{2+} extrusion pumps on SL, Ca^{2+} sequestration by the SR, or the combination of these two. The net Ca^{2+} content was also measured under corresponding conditions, showing 200 umol/kg at rest, 270 umol/kg at threshold and 410 umol/kg at maximal. At threshold, the large amount of net cellular Ca^{2+} gain was released by the addition of 50 mM caffeine, which has been shown to share the norepiephrine-sensitive Ca^{2+} store (Bond et al., 1984; Leijten and van Breemen, 1984). This observation that caffeine-induced Ca^{2+} loss into the external medium suggests that the SL has active Ca^{2+} extrusion system. Thus, Ca^{2+} accumulation by the SR was involved in buffering the Ca^{2+} entry since Bay K8644 at normal (5 mM) K^+ concentration stimulated a large net Ca^{2+} gain, which was discharged by 50 mM caffeine.

In summary, the SR in vascular smooth muscle appear to play a crucial role in modulation of Ca^{2+} entry. The superficial SR, for which morphological evidence exist (Bond et al., 1985), may take up Ca^{2+} during its entry followed by vectorial discharge into the subplasmalemmal region, eventually into the extracellular space. This superficial SR functioning as superficial buffer barrier may create a steep Ca^{2+} gradient in the cytosol beneath the surface membrane (Fig 2).

MODULATION BY CYCLIC NUCLEOTIDES

Cyclic AMP, which can be increased by stimulation of beta adrenergic receptors, has been reported to reduce tension in smooth muscle mediated through the reduction of the sensitivity of myosin light chain kinase (MLCK) mediated by the phosphorylation of MLCK by cAMP dependent protein kinase (Adelstein and Eisenberg, 1980). Others showed the evidence that cAMP can affect a number of mechanisms (Cauvin et al., 1984) which lower the cytosolic free Ca^{2+}, including stimulated accumulation of Ca^{2+} in the SR (Mueller and van Breemen, 1979; Saida and van Breemen, 1984).

The presence of either 10 uM forskolin (Seamon and Daly, 1986) or 1 mM dibutyril cAMP (dB cAMP) in PSS containing graded K^+ did not increase the net cellular Ca^{2+} significantly. The net cellular Ca^{2+} increase stimulated by cAMP was about 50 umol/kg for 1 g tension suggesting that the majority of the intracellular Ca^{2+} was sequestered. Both agents reduced Ca^{2+} influx, however, this effect was far less prominent than the reduction in tension. Forskolin raised the threshold of Ca^{2+} influx for tension development. On the other hand, norepinephrin, which has been known to release Ca^{2+} from the SR lowered the threshold. Observations with cyclic GMP support that at least part of its relaxant effect is due to the sequestration of Ca^{2+} by the

smooth muscle SR during activation.

CONCLUSION

The SR of vascular smooth muscle cells clearly modulates Ca^{2+} entry during depolarization-induced Ca^{2+} entry in addition to the well known observations that the SR releases Ca^{2+} for activation and sequesters Ca^{2+} for relaxation. Reduction in tension by cyclic AMP and GMP appear to arise from the enhancement of the SR function as a part of superficial buffer barrier, in addition the reduced sensitivity of MLCK to Ca^{2+} by cAMP. In vascular smooth muscle, the SR constitutes an important regulated buffer barrier to Ca^{2+} entry into the smooth muscle cell.

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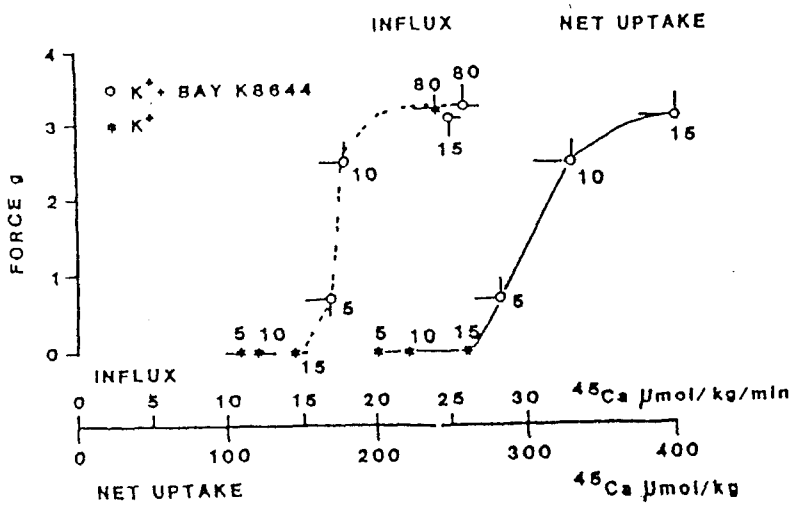
Fig. 1. Correlation between tonic tension developed and Ca^{2+} influx (--- shown on left with calibration on top of abscissa) or net cellular Ca^{2+} content (___ shown on right with calibration on bottom of abscissa) in the presence (o---o) or absence (*---*) of 1 uM Bay K8644. Subthreshold Ca^{2+} influx increased net cellular Ca^{2+} content.

Fig. 2. This model shows the Ca^{2+} gradient formed by the dynamic balance of Ca^{2+} entry and removal during its entry into the vascular smooth muscle cells: superficial buffer barrier.



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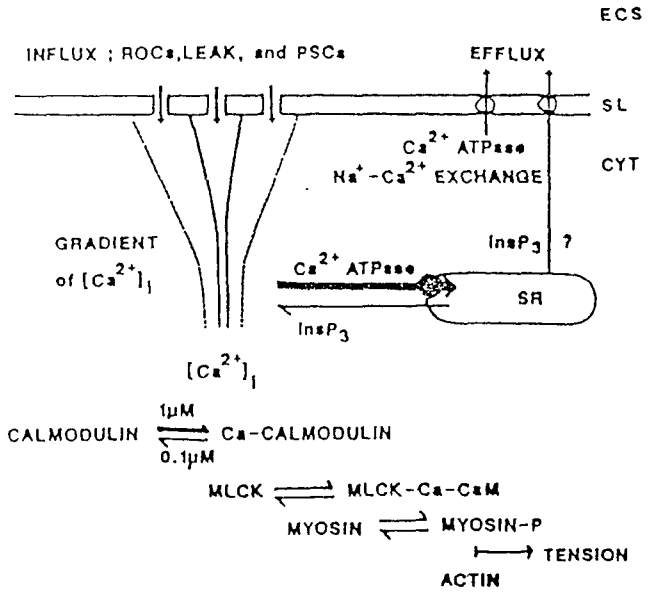

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