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Ca^{2+} in biological systems

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1) Introduction

Ca^{2+} is now accepted as the most fundamental regulatory factor for various kinds of intracellular processes. The most crucial step to the discovery of the roles of Ca^{2+} was made by muscle scientists who showed the indispensable nature of Ca^{2+} for the interaction of thick and thin filaments in skeletal muscle fibers[5].

Professor Edith Bülbring has explored a new front in physiology, pioneering an electrophysiological approach to smooth muscle function. She and her colleagues have clearly indicated[1,12] that the inward current of the action potential in smooth muscle is entirely different from that of nerve or skeletal muscle. This led to the conclusion that the inward current of vertebrate smooth muscle is carried by Ca^{2+} [17,21].

This was an important milestone in the history of muscle physiology in general. Before this discovery, most scientists were of the opinion, consciously or unconsciously, that smooth muscle would be, in a sense, a dull skeletal muscle. Hence, it might not be necessary to pursue smooth muscle penetratingly, and the only thing that would need to be done with smooth muscle would be to make clear how this muscle differed quantitatively from skeletal muscle. However, smooth muscle is quite distinct from skeletal muscle in many respects, not only quantitatively but also qualitatively, and exhibits very complicated properties which could not have been deduced from studies on skeletal muscle[8].

It is needless to say that the inward Ca current of smooth muscle is the basis for the action of Ca entry blockers, the study of which is now established as an important genre of pharmacology, modulating not only physiological processes but also some pathological ones.

With these points in mind, the roles of Ca^{2+} in biological systems will be reviewed with a historical emphasis.

2) Recognition of the essential role of Ca in contractility

In addition to the famous work on frog cardiac muscle in 1883[26], having recognized the essential role of Ca^{2+} in its contractility, Ringer made another important contribution to muscle physiology in 1886[27], i.e. the lack of Ca^{2+} in the bathing medium compelled skeletal muscle fibers to make repetitive twitches, which could be terminated by the addition of Ca^{2+}. These two findings, apparently contradicting each other, already indicated the direct role of Ca^{2+} on cardiac contractile systems. The level of scientific knowledge at that time, however, did not allow physiologists to distinguish between electrical phenomena at the sarcolemma and the contractile processes in the sarcoplasm. Consequently, the events were interpreted on the basis of the ratio between Ca^{2+} and K^+ concentrations, of which the proper value should maintain the physiological state of muscle, viz., the rhythmical contraction of cardiac muscle and the quiescent state of skeletal muscles.

Heilbrunn might have been the first person (1940) to hold the idea that Ca^{2+} could be the very factor that induced contractile processes under physiological conditions[10]. He soaked a frog skeletal muscle bundle, both ends of which had been cut, in isotonic $CaCl_2$. It shortened rather slowly but intensely, becoming one-fifth or less the initial length in about 20 s.

Kamada was deeply interested in this work and further extended it in collaboration with Kinosita in 1943[15]. He achieved beautiful results using microinjection technique to inject $CaCl_2$ solution into sarcoplasm by use of a glass micropipette with a tip diameter of about 2–5 μm. He described his observation in the following way (the italics are from the present author):

'The injection causes around the tip of the micropipette *a localized slow contraction* (a hump-like swelling due to local shortening and thickening of the fiber, associated with a localized movement of protoplasm toward the tip of the micropipette)'.

The very important point of his report was that he noticed the reversible nature of contraction:

'The cross-striations, which have been condensed at about the injected spot, are soon made to scatter again, and the fiber can restore the original state once more so as to *respond to a second injection* as before. Hence the reaction induced by the microinjection may be considered as fundamentally of *a reversible nature.*'

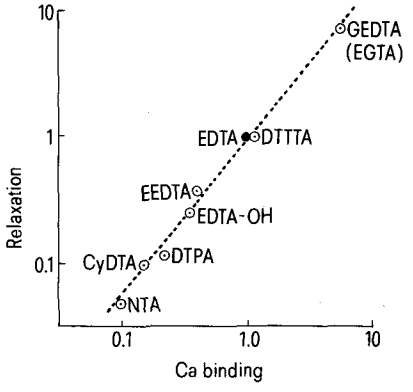

Figure 1. Relationship between Ca binding capacities and relaxing effects on glycerinated muscle fibers of various chelating agents. For details of experimental conditions, see Ebashi et al.[4]. The results in Ebashi[3] are recalculated and plotted in the graph, allowing for experimental conditions, particularly the presence of Mg ion which would interfere with Ca binding of chelating agents. Abbreviations: EDTA, ethylenediaminetetraacetic acid; EEDTA, ethyletherdiaminetetraacetic acid; GEDTA, glycoletherdiaminetetraacetic acid (more commonly abbreviated as EGTA); DTPA, diethylenetriaminepentaacetic acid; EDTA-OH, hydroxyethylethylenediaminetriacetic acid; DTTTA, dimethyltriethylenetetraminetetracetic acid. DTTA was a generous gift of Prof. G. Schwarzenbach (quoted from Ebashi[4]).

Owing to unfavorable circumstances, however, this paper was not internationally recognized. In 1947 Heilbrunn also reported essentially the same experiment in collaboration with Wiercinski[11].

In spite of these remarkable findings, the importance of Ca^{2+} was not recognized by muscle scientists for many years. One of the reasons for this ignorance was the dramatic impact of the actomyosin-ATP system discovered by Albert Szent-Györgyi and his schools around 1941–1942[28]. The research workers pursuing this system could not find any effect of Ca^{2+} on it, because their reaction systems contained sufficient Ca^{2+} which had leaked out from the glassware, which was made of soft glass at that time. In this way, 20 years passed in vain until the role of Ca in the contractile mechanism was confirmed on the molecular level[4].

To reach the final conclusion a pharmacological approach to the mechanism of relaxation was used, which served as a preparatory experiment for later research. The results of this experiment[3,4], carried out with glycerinated muscle fibers (fig. 1), coincided well with those of experiment with purified proteins, thus dispelling the suspicion that the latter was a mere in vitro artifact.

3) A great variety of Ca regulation mechanism

The discovery of troponin might have appeared to have settled the problem as regards the control mechanism of muscle contraction[5]. However, this was only the beginning of the studies on 'Ca regulation'. It is now widely agreed that the mechanism of Ca regulation is characterized by its great variety. We have now three major regulatory systems for muscle contraction, the troponin system, the myosin-linked system and the vertebrate smooth muscle system[8].

In addition to them, reference should be made to the regulatory system in non-muscle tissues. There is no doubt that an actomyosin-ATP system is playing an essential role in cytoplasmic streaming, which is also controlled by Ca^{2+}. It was a general belief that Ca^{2+} should be the activator of all actomyosin-dependent contractile systems, though their fundamental mechanisms might be different from one another (fig. 2). Hence, it was quite difficult to explain the inhibitory effect of Ca^{2+} on cytoplasmic streaming by its activating effect on the actomyosin system. Using carefully prepared actomyosin from slime mold, however, Kohama[16] has shown that the actin-myosin-ATP interaction of slime mold is inhibited by Ca^{2+}. Now we can have a perspective view of the molecular mechanism of cytoplasmic streaming. In this way, the mode of action of Ca^{2+} is quite unexpected, being different from one system to another.

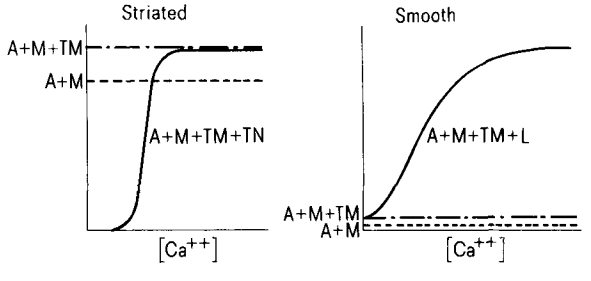

Figure 2. Characteristics of actin-mysoin-ATP interactions of vertebrate striated and smooth muscle. A, actin; M, myosin; TM, tropomyosin; TN, troponin; L, activating factor for smooth muscle contractile system (myosin light chain kinase for one group and leiotonin, an actin-linked factor, for the other group). The inherent nature of skeletal and cardiac actomyosin is to respond to ATP by contraction. Relaxation can be induced only in the presence of troponin and absence of Ca^{2+}; Ca^{2+} removes this inhibitory effect of troponin. Thus the apparently activating effect of Ca^{2+} should be interpreted as a de-repressive type activation. In contrast with this, smooth muscle actomyosin is quiescent even in the presence of ATP; for contraction some factor(s) and Ca ion are necessary. Thus Ca acts as a true activator, demonstrating that smooth muscle is qualitatively different from striated muscle. Most actomyosin systems of myosin-linked regulation behave as a kind of de-repressive type like vertebrate striated muscle, but some of them resemble the smooth muscle type (quoted from Ebashi[8]).

4) Ca regulation in metabolic systems

The Ca concept leaped out from the field of muscle contraction when Ozawa et al.[22] found the activation of phosphorylase *b* kinase by the same range of Ca^{2+} concentration which activated the contractile system. It was then quite natural to suppose that this activation would not be confined to skeletal muscle. Indeed, phosphorylase *b* kinases of cardiac muscle[23] and smooth muscle[25] (fig. 3) were found to behave in the same way.

Furthermore, this kinase in the brain[24] is also activated by Ca in the same concentration range. Thus the problem of Ca regulation of metabolic systems was completely emancipated from muscles, suggesting that the Ca regulation might be a common problem of living organisms[9]. The well-known activation of phosphorylase *b* kinase by cyclic AMP could be seen only in the presence of Ca^{2+}[23], indicating that the Ca regulation should be more fundamental than is the cyclic AMP regulation. It is interesting as well as puzzling that phosphorylase *b* kinase of smooth muscle is not stimulated by cyclic AMP (fig. 3).

5) Calmodulin

All these findings, however, did not provoke the attention of most biochemists at that time to Ca^{2+} except Kakiuchi, who had been working on cyclic AMP metabolism through brain phophodiesterase. He pursued research related to Ca^{2+} and eventually found that this enzyme was also activated by Ca in the same range of concentration as that for muscle contraction, and that

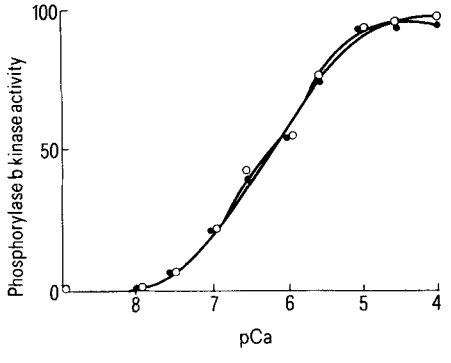

Figure 3. Ca dependence of smooth muscle phosphorylase *b* kinase in the absence and presence of cyclic AMP. ○, with cyclic AMP; ●, without cyclic AMP. For experimental conditions, see Ozawa et al.[23] (quoted from Ozawa[25] but redrawn for this article).

this activation depended on a protein factor, named 'modulator protein' (1970)[13,14], now called calmodulin. It has happened very often in the history of science that a novel substance was found quite independently by several investigators through entirely different approaches. This was the case of calmodulin. Cheung[2] was pursuing the activator of phosphodiesterase in a snake venom without paying attention to Ca and finally isolated the factor, calmodulin, at the same time as did Kakiuchi.

There is no need to refer to the explosive expansion of Ca research which has occurred after the establishment of calmodulin as the key protein in Ca regulation.

6) Calcium era

Ca research is to some extent a matter of Ca binding proteins. Calmodulin certainly represents one such protein; perhaps it is the ancestor protein of many Ca binding proteins. Even so, it is still one of them. An urgent task may be to enumerate Ca binding proteins in tissues as far as possible. In this respect, it is worthy of note that a simple method of detecting Ca binding proteins even from such a crude preparation as tissue homogenate has been developed[18]. It would sometimes be an efficient way to detect a Ca binding protein first, then to isolate it, and finally to investigate its physiological function in detail. It has been known that chemical agents acting on membrane surface receptors, viz., transmitters, hormones and related drugs, activate inosital lipid metabolism[19]. Indebted to the discovery of protein kinase C by Nishizuka, there has appeared a strong possibility that the events induced by such agents may by and large be explained by mechanisms involving Ca^{2+} in a central role[7,20].

Now Ca^{2+} is the most fashionable subject in biology. On the other hand, we are still only at the entrance of the 'Calcium era'; so many important things remain to be revealed*.

7) Concluding remarks

Life was born in the sea, of which the inorganic ion composition is virtually retained in our blood**. One of the most characteristic features of living organism is the biased distribution of inorganic ions outside and inside the cells (fig. 4). The unbalanced distribution of Na and K ions, respectively, is elegantly utilized by nerve and some muscles as the source of action current. A much more sharply one-sided distribution is exhibited by Ca^{2+}. It is quite understandable that the living organisms use this unique property for their own purposes in a very subtle way. Since such a biased distribution can be seen in very primitive living organisms, it must have been acquired at a very early stage of evolution, perhaps at the time when the cell was formed. This point has so far been overlooked in the evolutional research; it must be rightly appreciated in future studies.

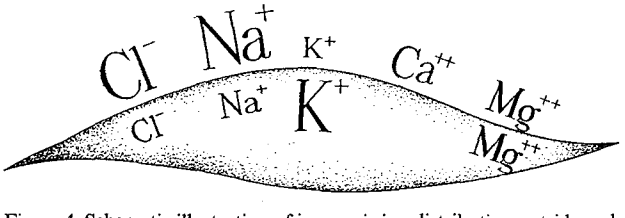

Figure 4. Schematic illustration of inorganic ion distribution outside and inside the cell. It is now agreed that the Ca^{2+} concentration in the cytoplasm of most cells in the resting state is 10^{-7} M or less. Since the Ca^{2+} concentration in mammalian serum is 2.5 mM, the difference across the cell membrane is more than 10,000-fold (quoted from Ebashi[6] but redrawn for this article).

*At present it is not clear whether or not Ca^{2+} is so deeply involved in protein metabolism as is in 'physiological' functions[7]. The situation is much the same for the processes related to developmental aspects, though involvement of Ca^{2+} in some crucial reactions has been confirmed. It is very probable that Ca may also be the most important regulator also in these areas as a whole, but its mode of action is unlikely to be as straightforward as in 'physiological' functions, being even more complicated than the inositol lipid-protein kinase C system mentioned above.

**Since various kinds of intracellular processes depend on Ca and they are related to one another in a complex manner, it is quite possible that a minor shift in the outer milieu from normal Ca concentration could cause an intense deviation of the cell function as a whole. Therefore, it is quite reasonable that the Ca^{2+} concentration in the serum is kept strictly constant as it has been so in the sea water. It might not be so absurd to postulate that the primary role of endocrine organs concerning Ca metabolism is to serve for stabilizing the serum Ca^{2+} concentration.

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Calcium and sodium distribution and movements in smooth muscle

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Summary. Electron probe microanalysis (EPMA) has been used to study the subcellular distribution of Ca, Na, K, Cl, and Mg in smooth muscle. The EPMA results indicate that the sarcoplasmic reticulum (SR) is the major *intracellular* source and sink of activator Ca: norepinephrine decreases the Ca content of the junctional SR in portal vein smooth muscle. Mitochondria do not play a significant role in regulating cytoplasmic free Ca^{2+}, but mitochondrial Ca content can be altered to a degree compatible with suggestions that fluctuations in matrix Ca contribute to the control of mitochondrial metabolism. The rise in *total* cytoplasmic Ca during a maintained, maximal contraction is very much greater than the rise in free Ca^{2+}, and is probably in excess of the known binding sites available on calmodulin and myosin. Cell Ca is not increased in normal cells that are Na-loaded. The non-Donnan distribution of Cl is not due to compartmentalization, but reflects high cytoplasmic Cl. Na-loading of smooth muscle in K-free solutions is temperature dependent, and may exhibit cellular heterogeneity undetected by conventional techniques. The total cell Mg is equivalent to approximately 12 mM, and less than 50% of it can be accounted for by binding to ATP and to actin. Mitochondrial monovalent cations in smooth muscle are relatively rapidly exchangeable.
Key words. Ca, Mg, Na; electron probe analysis; mitochondria; sarcoplasmic reticulum; inositol trisphosphate.

Introduction

The importance of calcium and sodium in smooth muscle function has been overshadowed only by the difficulty of determining, by conventional methods, the distribution and movements of these ions. Large extracellular spaces and binding sites and the uncertainties of identifying the number and anatomical site of kinetic compartments are only some of the problems facing physiologists attempting to measure cellular sodium and to determine the distribution of cellular calcium (for review, see Jones[28] and Brading[6]). Electron optical techniques now permit the localization and quantitation of these elements in ultrathin cryosections of rapidly frozen tissues (for review, see Somlyo[48], Hall[24]). We shall summarize here the results of some studies of Ca, Na and other electrolytes in smooth muscle with these methods and functional implications concerning excitation-contraction coupling.

Methods

The methods used for rapid freezing and cryoultramicrotomy[33,53] and for quantitative electron probe analysis[4,5,34] have been described in detail, as have the physiological experiments showing contraction of smooth muscle in Ca^{2+}-free solutions[4].

Calcium in sarcoplasmic reticulum and Ca release

The rise in cytoplasmic Ca^{2+} that activates smooth muscle contraction[21] (for review, see Hartshorne[25]) can be triggered electrically by action potentials[8] (for review, see Kuriyama[36] and Johansson[27]) and graded depolarization, and/or by a mechanism independent of changes in membrane potential, pharmacomechanical coupling[11,20,26,54]. Some forms of activation are associated with Ca^{2+} influx