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Review

Personal recollections on the discovery of the ryanodine receptors of muscle

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Abstract

The intracellular Ca^{2+} release channels are indispensable molecular machinery in practically all eukaryotic cells of multicellular animals. They serve a key role in cell signaling by way of Ca^{2+} as a second messenger. In response to a signaling event, the channels release Ca^{2+} from intracellular stores. The resulting rise in cytoplasmic Ca^{2+} concentration triggers the cell to carry out its specialized role, after which the intracellular Ca^{2+} concentration must be reduced so that the signaling event can again be repeated. There are two types of intracellular Ca^{2+} release channels, i.e., the ryanodine receptors and the inositol triphosphate receptors. My focus in this minireview is to present a personal account, from the vantage point our laboratory, of the discovery, isolation, and characterization of the ryanodine receptors from mammalian muscle. There are three isoforms: ryanodine receptor 1 (RyR1), first isolated from rabbit fast twitch skeletal muscle; ryanodine receptor 2 (RyR2), first isolated from dog heart; and ryanodine receptor 3 (RyR3), first isolated from bovine diaphragm muscle. The ryanodine receptors are the largest channel structures known. The RyR isoforms are very similar albeit with important differences. Natural mutations in humans in these receptors have already been associated with a number of muscle diseases.

Keywords: Ryanodine receptor; Calcium release channel; Skeletal muscle; Heart muscle; Sarcoplasmic reticulum; Terminal cisternae; Junctional face membrane; Excitation–contraction coupling; Muscle; Triads; Smooth muscle

By the passing of Professor Setsuro Ebashi, we lost one of the towering figures in muscle research. Dr. Ebashi was responsible for a number of seminal concepts that we now take for granted. I will cite two of these.

One major discovery was elucidating the role of sarcoplasmic reticulum in Ca^{2+} uptake, responsible for muscle relaxation. He described an isolated skeletal muscle fraction, ''relaxing factor'', referable to a membrane fraction (fragmented sarcoplasmic reticulum) capable of ATP-driven uptake of Ca^{2+}. This uptake causes the muscle to relax. He also demonstrated that the energized sequestering of the calcium ions was by means of a Mg^{2+}- activated ATPase in the sarcoplasmic reticulum membrane. Since a rise in Ca^{2+} concentration had already been known to be responsible for muscle to contract, lowering the Ca^{2+} concentration enables muscle relaxation.

A second major discovery was the description of troponin/tropomyosin as the switch within the thin filaments of the sarcomere, which responds to Ca^{2+} causing muscle to contract.

Prof. Ebashi was chairman of the prestigious Department of Pharmacology at the University of Tokyo for nearly a quarter of a century (1959–1983) and thereby was responsible for training a number of outstanding leaders in the field of muscle research. His intellect was widely felt through his leadership in learned societies. He served as President of the International Union of Pure and Applied Biophysics (1978–1981), and as President of the International Union of Basic and Clinical Pharmacology (1981). He organized the Eighth World Congress of Pharmacology held in Tokyo in 1981. Prof. Ebashi was the recipient of numerous awards in recognition of his profound scientific achievements.

Abbreviations: RyR, ryanodine receptor; CRC, calcium release channel; SR, sarcoplasmic reticulum; LT, longitudinal tubules; TT, transverse tubules; TC, terminal cisternae; JFM, junctional face membrane; JTC, junctional terminal cisternae; FKBP, FK506 Binding Protein; FS, foot structures; CPM, calcium pump membrane.

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My own personal remembrance of Tetsuro was that he was brilliant and creative, albeit modest in nature. He was reserved, but could be surprising in a delightful way. For example, after an international conference on muscle in Poland, we sat around chatting after dinner when Tetsuro burst into song. A fond personal memory that I will treasure is the presentation to me of the Medallion of the Red Gate of the University of Tokyo by Prof. Ebashi, on behalf of the Tokyo Society of Medical Sciences and the Faculty of Medicine of the University of Tokyo. The last Christmas card that I received from him was in 2005. His cards were copies of lovely Japanese woodblock prints, which I happen to be fond of. Tetsuro wrote me that there would be a symposium celebrating the 40th anniversary of his finding of troponin in Okazaki, Japan. He had suffered a stroke five years earlier and was still suffering from its complications. He had hoped to be well enough to attend this symposium, but sadly passed away before the symposium was held in 2006. We shall miss our dear friend and colleague.

Personal recollections of the discovery and characterization of the calcium release channels (ryanodine receptors) of sarcoplasmic reticulum

In the early 1980s very little was known about the Ca^{2+} release machinery at the molecular level. My focus in this mini-review is to present a personal account of the discovery of the intracellular Ca^{2+} release channels, i.e., how we went about to isolate and characterize the Ca^{2+} release machinery, which mediates Ca^{2+} release from intracellular stores. My aim is not to cover the literature, but to provide a personal account.

My approach to identify the Ca^{2+} release machinery on a molecular level was to first isolate highly purified membrane fractions of rabbit fast twitch skeletal muscle [1–3]. With such membrane fractions in hand, we would aim to deduce where the molecular machinery for Ca^{2+} release is localized. We would then have to develop an in vitro assay to measure calcium release on a subcellular level which can be modulated by ligands. Modulation by high affinity ligands could then be used to follow the purification. The isolation of the molecular machinery would then be feasible. After some success with skeletal muscle, similar studies were carried out on heart muscle.

Background information

The mammalian skeletal muscle fiber (myofiber) is a huge multinucleate cell resulting from the fusion of many myoblasts [4]. The dimensions of the mammalian myofiber can be 100 μm in diameter and many centimeters long. In one myofiber there can be hundreds of myofibrils extending the full length of the myofiber. Myofibrils consist of linear arrays of sarcomeres, the fundamental units of muscle contraction. The sliding of the thick myosin filaments between the thin filaments, consisting mainly of actin and a number of regulatory components including troponin/tropomyosin, results in the shortening of the length of the sarcomere. This shortened length of the sarcomere is amplified many-fold to macroscopic dimensions in the length of the myofiber, since the myofibrils consist of linear arrays of hundreds of sarcomeres. Despite the large size of the myofiber, the response time is rapid, in the millisecond time-scale. This is because the myofiber is electrically excitable. The depolarization of the sarcolemma of the myofiber is rapidly transferred longitudinally along the full length of the fiber and transversely to within the fiber by way of the transverse tubules (TT), invaginations from the plasma membrane. The transverse tubules are associated with the sarcoplasmic reticulum (SR) to form the sarcotubular network system. The macroscopic phenomenology of muscle contraction in skeletal muscle is referred to as "depolarization induced Ca^{2+} release". That is to say, excitation at the plasma membrane results in contraction of the muscle fiber, by way of release of Ca^{2+} release from the sarcoplasmic reticulum compartment into the myoplasm. The rise in myoplasmic Ca^{2+} concentration causes muscle to contract. No external Ca^{2+} is necessary. The reuptake of Ca^{2+} by the ATP-energized calcium pump protein (Ca^{2+} stimulated ATPase) of the calcium pump membrane of SR enables muscle to relax again. Each and every sarcomere of the myofibril is surrounded by the sarcotubular membrane system which surrounds the myofibrils like a tight sleeve (Fig. 1). Ca^{2+} uptake and release by the sarcotubular mem-

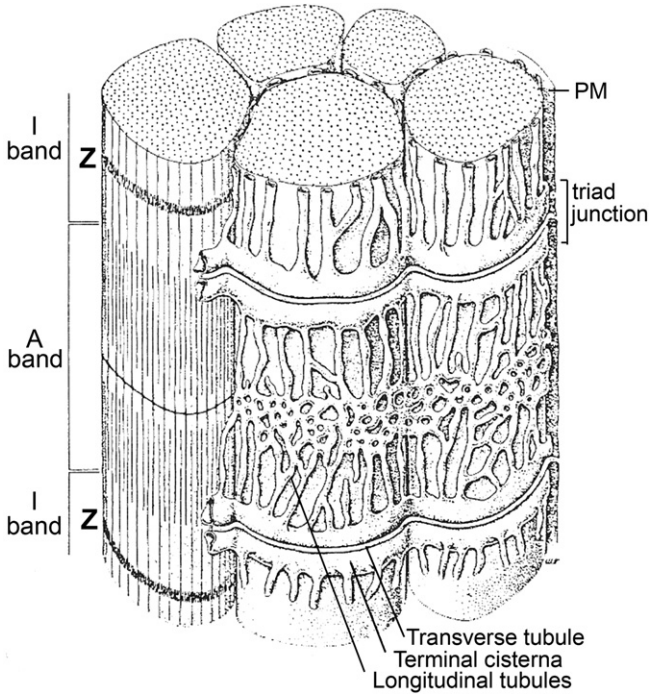

Fig. 1. Diagram of a portion of a mammalian striated muscle fiber. Two transverse tubules innervate one sarcomere. The transverse tubules are invaginations of the sarcolemma, close to the line where the A and I bands meet. Two terminal cisternae of SR are junctionally associated with one transverse tubule and connect with the longitudinal sarcotubules of SR located around the A band. The tripartite structure, seen in cross-section of two terminal cisternae (one from each adjacent sarcomere) flanking the transverse tubule, constitutes a triad (see Fig. 3). The sarcotubular network surrounds each sarcomere like a tight sleeve.