Hypothesis

Role of inositol 1,4,5-trisphosphate in excitation-contraction coupling in skeletal muscle

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The sarcoplasmic reticulum (SR) of skeletal muscle is an intracellular membranous network that controls the myoplasmic Ca^{2+} concentration and the contraction-relaxation cycle. Ca^{2+} release from the terminal cisternae (TC) region of the SR evokes contraction. How electrical depolarization of the transverse tubule is linked to Ca^{2+} release from the junctionally associated TC is still largely unknown. Independent evidence has been recently obtained indicating that either inositol trisphosphate (IP_3) or (and) Ca^{2+} is (are) the chemical transmitter(s) of excitation-contraction coupling. Here we outline the experimental data in support of each transmitter and discuss possible interactive roles of Ca^{2+} and IP_3.

Skeletal muscle *Excitation-contraction coupling* *Ca^{2+} release* *Ca^{2+}* *Inositol 1,4,5-trisphosphate*

1. INTRODUCTION

Following an action potential propagated along the transverse tubule (TT), Ca^{2+} is released from specialized regions of the SR, the terminal cisternae (TC), and muscle contraction ensues [1]. Signal transduction for muscle activation occurs at the triad where TC and TT are junctionally associated via bridging structures called feet [2]. Juxtaposed TC and TT membranes are 120–150 Å apart [2]. Three different hypotheses have been proposed to explain excitation-contraction (EC) coupling during the twitch; however, no conclusive evidence in favour or against any of these hypotheses has been provided [3].

(i) The *mechanical* hypothesis [4] postulates that charge movements at the TT membrane level control Ca^{2+} channels in the junctional SR by altering long-connecting molecules in the feet; such a mechanical linkage might open one SR channel per charge site.

(ii) The *electrical* hypothesis envisions a transient electrical pathway that allows a small current flow across the triadic junction [5]. It seems clear, however, that TT action potential does not propagate along the SR [6].

(iii) The *chemical* hypothesis states that a specific chemical transmitter, e.g., Ca^{2+} or IP_3, is released within the triadic junction in response to an action potential. Simple diffusion across the 120–150 Å junctional space requires less than 1 μs, whereas the latency between the upswing of the TT action potential and the rise of myoplasmic free Ca^{2+} is about 2.5 ms [7]. Thus, EC coupling is not too fast for chemical transmission.

In this article we will focus on the chemical hypothesis for EC coupling, outlining the ex-

Abbreviations: 1,4,5-IP_3, inositol trisphosphate exogenously added; IP_3, inositol trisphosphate generated in stimulated cells, which is likely to be a mixture of the two 1,4,5 and 1,3,4 isomers

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perimental data and their implications, stressing, at the same time, pitfalls and ambiguities of such an hypothesis.

2. IS Ca^{2+} THE CHEMICAL TRANSMITTER?

The Ca^{2+} dependence of Ca^{2+} release from skeletal muscle SR is now well estabilished: (i) Isolated SR vesicles, mainly derived from TC, display Ca^{2+}-induced Ca^{2+} release at micromolar free Ca^{2+} [8–10] with rate constants as high as $100\ s^{-1}$ [11]; (ii) SR of skinned fibres shows Ca^{2+}-dependent Ca^{2+} release [12–14]. Release rates are compatible with those in vivo when the bathing solution contains physiological free Mg^{2+} and 3 μM free Ca^{2+} ([13] and A. Fabiato, personal communication); (iii) Release of Ca^{2+} from the SR of mechanically skinned fibres evoked by depolarization of sealed off TT is Ca^{2+}-dependent [15].

If there is a step of EC coupling which is Ca^{2+}-dependent (fig.1) the unavoidable question is: where is the messenger Ca^{2+} coming from? Two putative sources have been listed:

(i) Ca^{2+} originates from the extracellular space, as in mammalian cardiac muscle [16]. However, in skeletal muscle, external EGTA does not prevent contractile activation [17] and Ca^{2+} channel blockers fully inhibit trans-sarcolemmal Ca^{2+} influx without affecting EC coupling in intact single fibres [18]. Only a late, slow phase of tension development is correlated with the inward Ca^{2+} influx via voltage-sensitive Ca^{2+} channels [19].

(ii) Ca^{2+} is bound to the internal leaflet of the TT membrane (phospholipids?) and is displaced by the incoming action potential [20]. This possibility is weakly supported by the finding that the twitch of intact fibres can be reduced in size and in some cases eliminated without reducing the extracellular free Ca^{2+} to 0 [21].

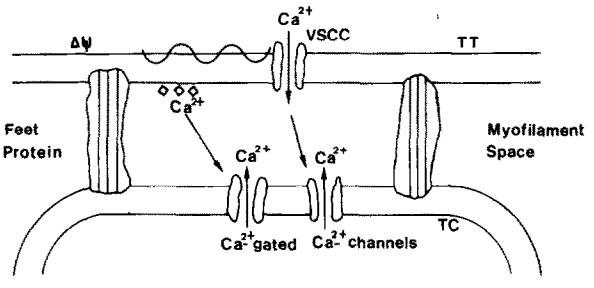

Fig.1. Scheme depicting Ca^{2+} as the messenger for EC coupling. VSCC, voltage-sensitive Ca^{2+} channels; $\Delta\psi$, TT action potential.

Therefore, the fundamental question concerning the occurrence and origin of messenger Ca^{2+} remains the main objection to such an hypothesis.

3. IS IP_3 THE CHEMICAL TRANSMITTER?

IP_3 has been proposed as the messenger coupling extracellular stimuli to Ca^{2+} release from intracellular stores in a variety of cell types [22], including smooth muscle [23–25]. The general scheme outlined by Berridge and Irvine (see fig.1 in [22]) dictates that the appropriate extracellular stimulis triggers the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), located in the inner leaflet of the plasma membrane, into diacylglycerol and 1,4,5-IP_3, the latter compound being a water-soluble second messenger [22]. 1,4,5-IP_3 is hydrolysed by specific phosphatases to inositol 1,4-bisphosphate (IP_2) and inositol 1-phosphate (IP_1).

As far as skeletal muscle is concerned, it has been found that: (i) 1,4,5-IP_3 induces Ca^{2+} release from both isolated TC fractions which are enriched in junctional SR membranes [26] and the SR of skinned fibres ([26,27]; rabbit and frog, respectively). The effect of 1,4,5-IP_3 is antagonized by ruthenium red, a blocker of TC Ca^{2+} channels [26]; (ii) Direct electrical stimulation of intact frog muscles, e.g. a tetanus lasting more than 3 s, increases 2–4-fold the level of IP_1, IP_2 and IP_3 above control [27]; (iii) Prolonged K^+ depolarization increases ^{32}P labelling of phosphatidylinositol in frog muscles [28]; (iv) Stimulation of the nicotinic acetylcholine receptors in chick embryo myotubes leads to accumulation of water-soluble inositol phosphates and increased phosphatidylinositol turnover [29].

A simplified model involving IP_3 in EC coupling, is depicted in fig.2. TT action potential evoked IP_3 production at the level of TT membranes via a PIP_2 phosphodiesterase. IP_3 released within the triadic junction opens IP_3-sensitive Ca^{2+} channels localized in TC (see fig.1 in [26]),

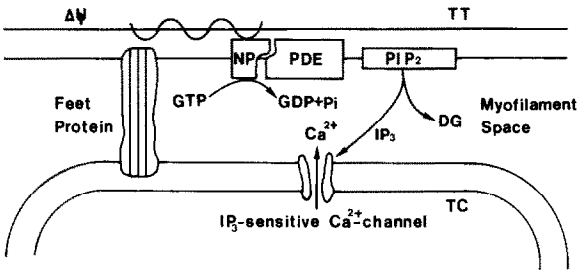

Fig.2. Scheme depicting IP_3 as the messenger for EC coupling. $\Delta\psi$, TT action potential; Np, GTP-binding protein; PDE, PIP_2 phosphodiesterase; DG, diacylglycerol. Other abbreviations are mentioned in the text.

and myoplasmic free Ca^{2+} rises. Several crucial questions remain to be answered.

(i) Are PIP_2, the substrate from which IP_3 derives, and the specific PIP_2 phosphodiesterase present at the TT level? Mitchell, Lindemaien and Jones (personal communication) found PIP_2 in highly purified junctional TT, obtained by French press treatment of isolated triads [30], after labelling with [^{32}P]ATP.

(ii) Does PIP_2 hydrolysis take place during electrical activation of the muscle and *before* contractile activation? Is the rate of phosphoinositide breakdown fast enough (millisecond range) to be causally related to a single twitch? In chick embryo myotubes acetylcholine induces phosphatidylinositol breakdown with a time course compatible with that of depolarization triggered by similar concentrations of acetylcholine [29]. This observation, however, does not necessarily mean that such events occur in adult (mature) muscle fibres. In whole muscle bundles, generation of IP_3 as a result of a direct tetanus [27] and increased ^{32}P labelling of phosphatidylinositol after K^+ depolarization [28] do not cogently prove that IP_3 is produced in a few milliseconds and is causally related to a single twitch. To show unambiguously that IP_3 has a primary role in EC coupling, IP_3 production must be measured after a single twitch given via the motor nerve to muscles which are then completely frozen within 3–4 ms [31]. This experiment should also clarify whether or not the rate of phosphoinositide breakdown is compatible with EC coupling time scale. It is worth mentioning that a lower limit for IP_3-mediated events is currently set at 200 ms [33] in the case of phototransduction [32].

(iii) How is TT depolarization linked to PIP_2 hydrolysis? In other cell systems, GTP-binding proteins have been involved in coupling extracellular stimuli to PIP_2 phosphodiesterase activation [34]. We have recently obtained evidence that a GTP-binding protein plays some role in EC coupling in skeletal muscle [35]: (i) GTPγS, a non-hydrolysable analogue of GTP, causes tension development in skinned fibres; (ii) GTPγS does not act *directly* on the SR, as indicated by lack of effect on Ca^{2+} fluxes in isolated SR fractions. GTPγS, most likely, evokes Ca^{2+} release from the SR by activating PIP_2 phosphodiesterase (see fig.2); (iii) The GTPγS effect occurs at physiological free Mg^{2+} and is inhibited by ruthenium red; (iv) The GTPγS effect is partially blocked by pertussis toxin (IAP), which is believed to inactivate stimulatory GTP-binding protein(s). In neutrophils and platelets, it has been shown that the toxin prevents intracellular Ca^{2+} rises and phosphoinositide breakdown induced by receptor-agonist interaction [34,36,37].

Although a number of critical questions await experimental appraisal, and a negative report on the effect of IP_3 in a crude SR fraction has appeared [38], we think that there is as much evidence for a role of IP_3 in EC coupling as for other cell systems where IP_3 is accepted as the messenger for agonist-induced Ca^{2+} release from intracellular stores [39].

4. ARE Ca^{2+} AND IP_3 INTERACTING IN EC COUPLING?

As a matter of speculation, we will briefly outline two models in which Ca^{2+} and IP_3 are not mutually exclusive transmitters.

If one assumes that Ca^{2+} is the first messenger for EC coupling, IP_3 may be released secondarily following activation of a Ca^{2+}-dependent PIP_2 phosphodiesterase. A late rise in IP_3 may be important in tuning the amount of Ca^{2+} released from TC or in amplifying the response to Ca^{2+}.

If one assumes, instead, that IP_3 is the first messenger, the opening of junctional IP_3-sensitive

Ca^{2+} channels may bring about a small Ca^{2+} efflux which, in turn, leads to massive Ca^{2+} release via Ca^{2+}-gated Ca^{2+} channels. Fast kinetics techniques are needed to discriminate further between these two possibilities.

In conclusion, the chemical hypothesis for EC coupling, though far from being proved, is getting closer than any other hypothesis to explain the nature of EC coupling in skeletal muscle.

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