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Features

Open Question

Does muscle activation occur by direct mechanical coupling of transverse tubules to sarcoplasmic reticulum?

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Our knowledge of the physiological and biochemical constituents of skeletal muscle excitation has increased greatly during the last few years but this has not led to a consensus of the physiological mode of muscle activation. Three hypotheses of transmission, involving either transmitter–receptor interaction or direct mechanical coupling, are still under active consideration. The hypothesis of direct mechanical coupling currently being evaluated proposes that the dihydropyridine receptor in the transverse tubules serves as a voltage sensor that communicates directly with the junctional foot protein/\(\text{Ca}^{2+}\) channel of sarcoplasmic reticulum to initiate opening of the channel.

During the last 20 years three basic hypotheses of the mechanism of excitation–contraction coupling in skeletal muscle have been proposed. These have found favor and disfavor in a cyclical fashion much as the waves of the sea. Our understanding of the physiological and biochemical processes of muscle activation has increased vastly during this period but, we cannot as yet delineate unequivocally between the theories.

The three basic hypotheses of muscle excitation expressed in general terms are:

1. Direct or capacitive electrical communication between the transverse tubule (T-tubule) and the terminal cisternae causes the passage of a wave of potential change that is responsible for the opening of the \(\text{Ca}^{2+}\) channel in the sarcoplasmic reticulum (SR).
2. Depolarization of the T-tubule causes the release of a transmitter into the junctional space which binds to a receptor and causes \(\text{Ca}^{2+}\) channel opening in the SR.
3. Direct mechanical communication occurs between the T-tubule and the terminal cisternae of the SR in which depolarization of the T-tubule causes a conformational alteration of the spanning structure, thus opening the \(\text{Ca}^{2+}\) channel of SR.

At present, the first of these hypotheses has little experimental support. It is hard to define within the context of the known ionic gradients across the SR membrane and the transverse tubular membrane. The other two hypotheses have been involved in a long running dogfight for supremacy. This review questions whether direct mechanical communication can account for muscle activation. The answer should be viewed in the context of alternative modes of excitation–contraction coupling.

The transmitter–receptor hypothesis may be expressed more specifically in terms of two potential transmitters for muscle activation: (1) \(\text{Ca}^{2+}\); and (2) inositol 1,4,5-trisphosphate (IP3). These two hypotheses reduced to their minimal constituents are illustrated in Fig. 1. Variations of each hypothesis are possible but have as yet little experimental support. For example, \(\text{Ca}^{2+}\) may be released from intracellular storage sites on muscle depolarization rather than being transported across the membrane.

**Trigger \(\text{Ca}^{2+}\)-induced \(\text{Ca}^{2+}\) release**

A remarkable feature of skeletal muscle is the high content of dihydropyridine receptor in the transverse tubules. In other cellular systems, this receptor has been associated with \(\text{Ca}^{2+}\) channel activity. Experimental data in skeletal muscle support the view that drugs which bind to and inhibit this receptor also inhibit the slow \(\text{Ca}^{2+}\) channel. Estimates of dihydropyridine receptor content in T-tubules vary from 20 to 200 pmol per mg protein. Assuming each receptor is a channel, this content would probably not be quite adequate to supply the \(\text{Ca}^{2+}\) necessary for contraction. This number of channels, however, could supply a source of trigger \(\text{Ca}^{2+}\) which, in its turn, stimulates opening of the \(\text{Ca}^{2+}\) channel of SR. In a similar context, experiments with skinned muscle fibers (in which the plasma membrane has been mechanically removed or chemically rendered leaky), as well as experiments in isolated SR demonstrate that \(\text{Ca}^{2+}\) release from SR may be activated in the presence of micromolar concentrations of \(\text{Ca}^{2+}\) and inhibited in very low \(\text{Ca}^{2+}\) concentrations. The rate of \(\text{Ca}^{2+}\) release from the SR initiated by this process although under the non-physiological conditions of low \(\text{Mg}^{2+}\), is adequate to account for the rapidity of muscle contraction.

Why, then, is this hypothesis not generally accepted as the mode of muscle activation? The initial evidence against this hypothesis was presented by Armstrong et al. who showed that muscle could continue to contract when immersed in a solution containing the \(\text{Ca}^{2+}\) chelator EGTA. Subsequent experiments have supported this observation but more sophisticated experimental approaches have demonstrated a reduction in \(\text{Ca}^{2+}\) release associated with EGTA treatment. In addition, Baylor and Hollingworth have shown that muscle contraction is not significantly affected by the inclusion in the cytoplasm of high concentrations of the \(\text{Ca}^{2+}\)-chelating agent, FURA-2. Thus, in skeletal muscle the curious situation
the Ca\(^{2+}\) release initiated in these other cells is hormonally activated and takes place on a relatively slow time scale. In contrast, Ca\(^{2+}\) release from skeletal muscle is activated by depolarization. It begins and is terminated within a few milliseconds of depolarization\(^{11}\). The evidence in favor of IP\(_3\)-induced Ca\(^{2+}\) release is that injection of this compound into skinned muscle fibers induces contraction of the muscle\(^{12,13}\). However, these observations are still fraught with experimental inconsistencies among different investigators. Critical issues such as the concentration of IP\(_3\) required to initiate release have not been unequivocally answered. The most efficacious response reported indicates that a concentration of \(~0.5 \mu M\) is adequate to activate Ca\(^{2+}\) release\(^{14}\). The kinetics have not been studied directly using IP\(_3\) since diffusion time limits the response to the treatment. Walker et al.\(^{15}\) have administered an inactive precursor (caged IP\(_3\)) to skeletal muscle and converted it by a photoreaction into IP\(_3\). They observed a very slow onset of contraction. Similarly, in cardiac muscle Fabiato\(^{16}\) has shown which is held in apposition to the transverse tubule, although PI kinase acts predominantly with the terminal cisternae portion of the SR. It is this domain which is held in apposition to the transverse tubule. Thus, direct mechanical coupling between the T-tubule and the SR could occur through the junctional structures which, in their turn, may interact with or be a part of the Ca\(^{2+}\) channel of the SR.

The original hypothesis of direct mechanical communication arose through the observation that a non-linear capacitive current was observable when the membrane potential of the muscle was altered. The size of this charge movement was half-maximal at a level of depolarization which gave half-maximal activation of muscle contraction. The charge movement was originally modeled as a plunger which could occur through the junctional structures which, in their turn, may interact with or be a part of the Ca\(^{2+}\) channel of the SR.

**Direct mechanical coupling hypothesis**

An alternative scheme of contraction by direct coupling between T-tubules and SR has been proposed to bypass the limitations of transmitter–receptor interaction. Among the attractions of this hypothesis, originally proposed by Schneider and Chandler\(^{17,18}\), is the favorable morphology of the junction. It has been known for a considerable period that the T-tubule is held in immediate juxtaposition to the SR by electron-dense protrusions called junctional feet. It has also become apparent largely through the work of Meissner et al.\(^{20}\), that Ca\(^{2+}\) release is associated predominantly with the terminal cisternae portion of the SR. It is this domain which is held in apposition to the transverse tubule. Thus, direct mechanical coupling between the T-tubule and the SR could occur through the junctional structures which, in their turn, may interact with or be a part of the Ca\(^{2+}\) channel of the SR.
this crude but simple model has withstood the test of time. Most particularly, charge movement is very closely connected to muscle activation not only as a function of membrane potential but also in the response to pharmacological manipulation. The limitation of the model was that it did not lead to the defining of a specific moiety through which the message of transmission occurred. The details of this model, as well as the alternative protocols, has been fleshed out through a growing knowledge of the molecular constituents of the triad junction.

The Ca$^{2+}$-release site

Our ability to obtain molecular insight into the triad junction has arisen through fractionation procedures at the level of the vesicular components for the junction and, subsequently, at the level of the protein constituents of these vesicles. Sarcoplasmic reticulum has been physically separated into longitudinal reticulum and terminal cisternae. The terminal cisternae fraction has been shown to contain T-tubules associated in the form of a triadic junction, and the triadic junction has itself been separated into its component organelles. The T-tubules have been shown to be an extremely rich source of dihydropyridine receptor, the putative Ca$^{2+}$ channel. The identification of the junctional constituent has been achieved through two complementary approaches. Cadwell and Caswell identified a high molecular weight protein (confined to the terminal cisterna) that exhibited expected properties of the junctional feet. This protein was subsequently extracted and isolated.

At the same time, several investigators have followed the pharmacological approach of identifying a drug which binds and reacts specifically with the Ca$^{2+}$ release channel of sarcoplasmic reticulum. One drug (ryanodine) has now been radiolabeled as a means to the isolation of the channel. The recent isolation of the channel has shown that it is identical to the protein previously identified as the junctional feet. This now allows us to see the model of direct mechanical coupling a more specific context: The feet processes themselves contain the Ca$^{2+}$ channel and this channel is in immediate juxtaposition to the T-tubule. Therefore, the logic of direct mechanical coupling becomes more compelling, but the caveat remains that the understanding of muscle contraction has been deviled by compelling logic which has not translated into direct demonstration.

The voltage sensor

A further insight into the mechanism of muscle contraction has arisen through the development of our understanding of the role of the dihydropyridine receptor, the putative Ca$^{2+}$ channel of T-tubules. The existence of this channel was originally invoked to support the concept of Ca$^{2+}$-induced Ca$^{2+}$ release, but it soon became apparent that the mode of action of this protein is more complicated. The early experiments on drugs which inhibited this channel failed to elicit a significant inhibition of muscle contraction. In addition, the Ca$^{2+}$ current associated with the action potential was switched on by muscle depolarization significantly after the contractile event had begun. Furthermore a long-standing discrepancy existed between the biochemical data in which dihydropyridine drugs, such as nitrdenpine, gave a $K_d$ for the receptor in the nanomolar range while inhibition of the slow Ca$^{2+}$ current requires much higher concentration.

Hui et al. demonstrated that the Ca$^{2+}$-blocking drug, D600, was indeed able to inhibit contraction although this inhibition occurred only after the muscle had been subjected to K$^+$ contracture and cooled. Rios and Brum subsequently reported that inhibition of contraction could be elicited with nitrenpine. These authors added an interesting corollary, postulating that the dihydropyridine receptor might be acting not in the capacity of a Ca$^{2+}$ channel but in the role of a voltage sensor. The earlier electrophysiological experiments demonstrated that the opening of the Ca$^{2+}$ channel occurred on depolarization of the fiber. Rios and Brum have argued that the dihydropyridine receptor can have a dual role.

In skeletal muscle the main role of this channel is to sense the electrical potential of the T-tubule and to transmit, via a conformational change, the information to the SR without necessarily initiating Ca$^{2+}$ influx.

The physiological evidence germaine to this hypothesis rests largely on a requirement for the following correlations:

1. (1) activation or inhibition of the dihydropyridine receptor is associated with concomitant activation or inhibition of charge movement (Qβ) and the two are temporally linked;
2. (2) activation or inhibition of charge movement is associated with concomitant activation or inhibition of Ca$^{2+}$ release from SR and contraction;
3. (3) activation or inhibition of Ca$^{2+}$ movement into the cell is not associated with concomitant activation or inhibition of charge movement and contraction.

Most of the data currently available support these conclusions although with some reservation. Two classes of agents which are known to bind to and inhibit the dihydropyridine receptor’s action as a Ca$^{2+}$ channel are also known to inhibit charge movement and contraction. The conditions in which D600 blocks contraction require that the muscle first be depolarized and cooled and the inhibition can be reversed by hyperpolarization. A possible explanation for these requirements is that D600 only binds the receptor when it is in an inactive state after depolarization. The blockage of charge movement and of contraction are both complete. In the presence of dihydropyridine drugs blockage of charge movement and Ca$^{2+}$ release from the SR appears to be partial and critically dependent on the condition of the fiber. When the holding potential of the muscle fiber is hyperpolarized (−100 mV) the blockage by dihydropyridine is quite limited, but when the holding potential is at −70 mV a blockage by about 70% is observed. Rios and Brum have shown that this is associated with a concomitant inhibition of the rate and extent of Ca$^{2+}$ release from the SR. The concentrations required to produce even a partial blockade are considerably in excess of the $K_d$ estimated from isolated T-tubules. Lamb has found that increasing dihydropyridine to extremely high concentration does not create more than partial blockade. In neonatal muscle there is evidence that the half-maximal blocking concentration effected by the dihydropyridine, PN200-110, is considerably decreased by depolarization of the fiber. If blockade by PN200-110 of charge movement is similarly voltage dependent this may explain the discrepancy in the biochemical $K_d$. The physiological half-maximal concentration for the dihydropyridine effect on charge movement may be high because the muscle is polarized, while in biochemical experiments the drug binds with higher affinity to its receptor because it is in its inactive (depolarized) state. What is not yet clear is whether that portion of
the charge movement that is refractory to
dihydropyridines represents a separ-
ate component of charge movement or
whether it simply represents the fact
that under the experimental conditions
employed, only a portion of the dihy-
dropyridine receptor has been placed
in an inactive state capable of binding
the drug. In addition, Lamb et al. has
found, in rabbit muscle fibers, that
although charge movement is consis-
tently blocked (to approximately 50%)
by nifedipine, the effect on contraction
is quite variable, some fibers being
completely refractory to the effects of
the drug while others are completely
paralysed. This, in its turn, raises the
issue as to whether the charge move-
ment invariably reflects the activation
of the muscle.

Evidence of a temporal link between
activation or inactivation of the dihy-
dropyridine receptor and muscle activa-
tion is, as yet, unavailable. The
dihydropyridine-sensitive Ca\(^{2+}\) chan-
nel is switched on with a slow time
course. It could be argued, however,
that this represents a multistage pro-
cess in which a rapid conformational
response to a voltage change may pre-
cede the slow gating of the channel.
When the receptor is operating as a
voltage sensor for muscle activation,
only the conformational change may be
required. It is also unclear whether
blockage of the receptor by drugs gives
time-correlated blockade of acti-
vation. It is possible that the inhibition
of muscle activity may be consequent
not to direct inhibition of the receptor
but to the modified Ca\(^{2+}\) homeostasis
following channel inhibition. T-tubules
contain an active Ca\(^{2+}\)-extrusion pump
which may deplete cytoplasmic stores
directly and SR stores indirectly if
Ca\(^{2+}\) entry is blocked.

The third requirement for the hy-
pothesis that the dihydropyridine
receptor directly mediates the message
of excitation to the SR is that there
should be a dissociation between Ca\(^{2+}\)
influx and activation. There is no doubt
that under normal conditions a consid-
erable influx of Ca\(^{2+}\) is elicited by an
action potential but this Ca\(^{2+}\) current is
slow in onset. A second component of
much more rapid activity has also been
discerned in neonatal muscle. This
fast Ca\(^{2+}\) current is uninfluenced by
Ca\(^{2+}\)-blocking agents and, therefore,
cannot play a role in dihydropyridine
blockage of contraction. The slow Ca\(^{2+}\)
current is too slow to account for the
rapidity of Ca\(^{2+}\) release from the SR.
In addition, Baylor and Hollingworth
have observed that Ca\(^{2+}\) release from
the SR may take place in a stimulated
muscle even when the cytoplasmic
environment is bathed with a rapidly
binding Ca\(^{2+}\)-chelating agent, further
suggesting that any Ca\(^{2+}\) influx which
may occur is not directly responsible
for Ca\(^{2+}\) release.

**A biochemical model?**

If this hypothesis of direct mechani-
cal coupling expressed in its present
form is to fulfill the role of excitation-
contraction coupling then the physio-
logical data must be supported by bio-
chemical evidence that direct mechan-
ical communication does occur. In
many ways the hypothesis is rendered
more attractive by the observation that
Ca\(^{2+}\) release is elicited by the junc-
tional foot protein. For this now
requires of the mechanical coupling
hypothesis only that there be communi-
cation between this protein and the
voltage sensor in the T-tubules as illus-
trated in Fig. 1c. This could be fulfilled
if the junctional foot protein is physi-
cally attached to the dihydropyridine
receptor. One would further expect
that the attachment should be specific
in the sense that all junctional foot
particles would have an associated
dihydropyridine receptor or receptor
cluster.

Block et al. have observed tetrads
of large intercalated particles in freeze-
fracture replicas of skeletal muscle
T-tubules. The large size of the inter-
calated particle is consistent with the
view that this particle may be the dihy-
dropyridine receptor whose total mol-
ecular weight must be in the range of
400 000. An unexpected feature of the
observation of these authors is that
there is one tetrad per two feet indicat-
ing that only half the feet are asso-
ciated with these particles.

The biochemical evidence to support
direct connection between the dihydro-
pyridine receptor and the junctional
foot protein is weak. We have em-
ployed three techniques to determine
which proteins in the T-tubule bind
to the foot protein. These are:

1. Affinity chromatography, employ-
ing the isolated protein and dissolv-
ing T-tubular vesicles;
3. Crosslinking using hetero bifunc-
tional crosslinking agents.

In each case we have clear evidence for
an association between the glycolytic
enzymes, glyceraldehyde phosphate
dehydrogenase and aldolase, with the
junctional foot protein. We have not
been able to observe any direct associ-
ation between the junctional foot pro-
tein and the dihydropyridine receptor
or, for that matter, any intrinsic
T-tubular protein. Chadwick et al.,
using a hetero bifunctional agent, have
described an association between the
junctional foot protein and a protein
of molecular weight 72 000 in the
T-tubule. This is not a subunit of the
dihydropyridine receptor and is, in
any event, an extrinsic protein. Using
gel overlay procedures, we have observed
an association between glyceraldehyde
phosphate dehydrogenase and the a-
subunit of the dihydropyridine recep-
tor; this, however, is not specific in the
sense that the glycolytic enzyme binds
to a number of other T-tubular consti-
ituents. Thus, there remains the possi-
bility that the junctional foot protein
binds indirectly through the glycolytic
enzymes to the DHP receptor but it is
doubtful that this could serve for direct
mechanical coupling.

It needs to be emphasized that the
hypothesis that the dihydropyridine
receptor is the voltage sensor for
muscle excitation does not, in itself,
require that the receptor binds directly
to the junctional feet. Communication
could take place through indirect
means; we are, therefore, currently in
the ambiguous situation that our knowl-
edge on the processes of muscle excita-
tion has increased vastly but we still are
limited by our inability to produce a
theory that is fully consistent with the
existing data, although much of our
current knowledge is in accord with
the hypothesis of direct mechanical cou-
lping. The molecular mechanism of
direct mechanical coupling has not
been explained in skeletal muscle but
could be analogous to the sliding of
subunits which has been described in
gap junctions.

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Talking Point

How ‘hidden’ reading frames are expressed

Roberto Cattaneo

Secondary reading frames, ‘hidden’ under other reading frames, are used for coordinated expression of proteins in several eukaryotic viruses. In some genes, ribosomal frameshifting and initiation or reinitiation of protein synthesis on internal AUG codons is translational mechanisms allowing access to such ‘hidden’ reading frames. In others, secondary reading frames are translated from alternatively spliced or edited mRNAs.

Eukaryotic mRNAs are generally monocistronic, but for several viral transcripts as well as for one cellular transcript, it has been shown that proteins encoded in different reading frames are expressed. Since eukaryotic ribosomes typically start protein synthesis at the first AUG codon only, it is not immediately evident how secondary reading frames overlapping with or following the first reading frame can be expressed.

Ribosomal frameshifting

A situation present in many viral eukaryotic transcripts is illustrated in Fig. 1: the first open reading frame (top, stippled box) overlaps with a second one (top, black box). Thus, if translation begins at the first AUG codon, only one protein will be expressed (bottom left, stippled box). Ribosomal frameshifting, subsequent to initiation on the first AUG codon, may enable expression of the overlapping reading frame, creating a fusion protein (bottom right, fused stippled and black boxes). Since only a fraction of the ribosomes change frame at a frameshift signal, fusion proteins are produced in addition to, rather than instead of, the normal protein.

Frameshifting in the -1 frame is used by most retroviruses to access the reverse transcriptase reading frame, hidden in the gag mRNA, and by coronaviruses to express their RNA replicase encoded by two different reading frames. Until now, no clear-cut case of ribosomal frameshifting has been observed in cellular genes of higher eukaryotes, and it seems that cellular eukaryotic genes avoid sequences on which frameshifting could occur. In contrast, the yeast retroviral-like element Ty shifts to the +1 frame to express its reverse transcriptase, and the ribosomes of E. coli can slip, shift, step backward and hop forward (−2, −1, +1, +2, +5 and +6 frameshifts).

Internal initiation

The use of an internal AUG codon for initiation of translation is an alternative to ribosomal frameshifting, and does not result in production of fusion proteins (Fig. 1, bottom center, black box). Several conditions may allow the use of an internal AUG codon. (1) An internal AUG is sometimes used in cases where the first AUG occurs in an unfavourable context for translation initiation. (2) Termination of protein synthesis at a stop codon may lead to reinitiation at a nearby AUG in another frame. (3) A ‘ribosome landing pad’ may direct the ribosome to an internal position in the mRNA, as described for the uncapped genomic RNA of picornaviruses. (4) In a capped mRNA of a paramyxovirus, some ribosomes pass from the cap directly to an initiation codon far downstream.

It is interesting to note that the cousins of retroviruses and the yeast Ty elements, the hepatitis B-like viruses (HBVs), also have the reverse transcriptase reading frame hidden under an overlapping reading frame initiating upstream. However, in contrast to the retroviruses, HBVs avoid ribosomal frameshifting and instead use an internal AUG codon to express reverse