

Clarification of the terminology used for description of calcium transport in different cell types

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Some basic scientific terms in the field of general physiology that studies intracellular calcium transport have a multitude of definitions in the scientific literature. In this article we analyze these definitional ambiguities and try to clarify some basic terms used for the description of calcium transport in cells. The use of ambiguous scientific terminology and conflicting definitions may be a source of misunderstanding among scientists.

Key words: Ca²⁺ transport; flux; common pool models; all-or-none Ca²⁺ release; definitional ambiguity.

INTRODUCTION

Ca²⁺ transport by the endoplasmic reticulum (ER) plays a crucial role in the regulation of intracellular Ca²⁺ signals. Some of the terms which are used for the description of Ca²⁺ transport in different cell types are defined ambiguously in the scientific literature. The increased use of physical terms in biology and their replacement by colloquial terms often results in multiple meanings and inconsistencies in definitions of such basic terms as flux, all-or-none and regenerative Ca²⁺ release, common pool models, etc. The definitional ambiguities make research within the field of general physiology and biophysics difficult to reconcile. Clarifying the meaning of scientific terms is thus a pressing need.

Ambiguous term “flux” in biological literature

Flux is a basic concept for the study of transport phenomena in physics and biology. The change in the Ca²⁺ concentration in the cytosol and ER, which is called sarcoplasmic reticulum (SR) in muscle cells, occurs due to Ca²⁺ fluxes through the membranes surrounding the cellular compartments, i.e., the cytosol, ER, and

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mitochondria, and Ca²⁺ buffering. In physics, transport flux is defined as the rate of flow of some quantity per unit area [1] that in the case of mass transfer is expressed in mol m⁻²s⁻¹. The surface integral of the flux represents the quantity, which passes through the surface per unit time. Flux can also be defined, e.g. electric and magnetic flux in electromagnetism, as the surface integral of a vector field [2]. Due to the conflicting definitions, this term sometimes is used ambiguously, especially in the biophysical and physiological literature, where the term “flux” may be defined as the rate of quantity that passes through a fixed boundary and expressed in mol s⁻¹, e.g., in [3–5], or as the rate of change of calcium concentration and expressed in mol l⁻¹s⁻¹, e.g., in [6–9]. The latter definition may include Ca²⁺ binding with buffers, e.g., [10–12], or may refer only to Ca²⁺ transport between intracellular compartments, e.g., [13]. Sometimes both definitions are used in the same paper [14, 15]. Surprisingly, the strictly physical definition of flux in the case of mass transfer also can be found in the biological literature [16]. In the latter paper a partial derivative of the concentration of a molecular species is equal to the divergence (or the surface integral divided by

the volume) of the flux. To avoid a significant terminological ambiguity, we suggest using the definition that describes the derivatives of Ca^{2+} concentrations as “the rate of change in calcium concentration” instead of “fluxes”. It seems to be a good practice to designate these derivatives by some other symbol than J , which is traditionally used to designate the term “flux” despite of its meaning. For example, in [17] the symbol R was used. As regards the term “flux”, it may be acceptable to define it as the surface integral of the rate of transport of some quantity. In this sense, the term “net flux” that is frequently used in the biological literature means the difference between the two unidirectional fluxes, influx and efflux. Net Ca^{2+} uptake means the difference between uptake and release fluxes, and net Ca^{2+} release means the difference between release and uptake fluxes [6].

In any case, a great care is needed when the term “flux” is used to define some quantities in equations, and papers should always be careful to give the units of these quantities explicitly. This problem becomes especially pronounced when the intercellular movement of Ca^{2+} between interconnected cells is modelled.

Clarification of the term “ Ca^{2+} -induced calcium release”, the modes of Ca^{2+} -induced calcium release and its attributes

Ca^{2+} release from the ER/SR is executed by two families of calcium-release channels, the ryanodine receptors (RyRs), the Ca^{2+} -gated Ca^{2+} channels, and the inositol 1,4,5-trisphosphate (IP_3) receptors. Ca^{2+} -induced calcium release (CICR) usually is defined as Ca^{2+} release from intracellular stores activated by calcium alone, i.e. via RyRs, and Ca^{2+} release via IP_3 receptors is termed IP_3 -induced Ca^{2+} release (e.g., [18, 19]). However, in some papers, CICR was defined as Ca^{2+} release via both RyRs and IP_3 receptors (e.g., [20]). The term CICR is so widely used that the authors usually do not give its definition, and it is not clear without an additional context, which exactly processes they mean. The definition of CICR was discussed in the review of M.

Endo [21]. The author outlines that in the case of the IP_3 receptor, Ca^{2+} can cause Ca^{2+} release only in the presence of IP_3 . Ca^{2+} release at a constant IP_3 concentration can be considered CICR, but if Ca^{2+} is not, by itself, sufficient to evoke Ca^{2+} release, such Ca^{2+} release cannot be considered as CICR. Therefore, although both types of receptors exhibit positive feedback where Ca^{2+} potentiates its own release, CICR should be considered as an exclusive property of RyRs.

To characterize Ca^{2+} release from the endoplasmic reticulum (ER) in nerve cells, the term “CICR modes” was introduced [6, 13] and three modes of CICR, attenuated net uptake, graded net release and regenerative net release were characterized. These modes were simulated for a fixed ER Ca^{2+} concentration. In the first mode, Ca^{2+} uptake into the ER by sarco(endo)plasmic reticulum Ca^{2+} ATPases is faster than Ca^{2+} release from the ER and is attenuated by Ca^{2+} release. Since “attenuated net Ca^{2+} uptake” as a model of “release” [6] sounds a little confusing, a more rigorous term that has a clear meaning could be “the modes of net ER Ca^{2+} transport” instead of “CICR modes”. The modes of net ER Ca^{2+} transport can be characterized by several attributes, such as the direction of net ER Ca^{2+} flux across the ER membrane, the regenerative or non-regenerative behavior, gradation by Ca^{2+} influx, and CICR gain.

The direction of the net ER Ca^{2+} flux determines if the ER acts as a sink or a source [22]. CICR and IP_3 -induced calcium release are both intrinsically self-reinforcing processes since the release of Ca^{2+} leads to regenerative RyR and IP_3 receptor activation. However, net CICR and IP_3 -induced calcium release do not have regenerative character when ER releases Ca^{2+} at a rate that is slower than Ca^{2+} clearance by other pathways. In this case, positive feedback is terminated during the stimulation or at the end of the stimulation (the second mode by Albrecht et al. [6]). In contrary, regenerative net CICR and IP_3 calcium release occur when the ER releases Ca^{2+} at a rate that is faster than

Ca²⁺ clearance by other pathways including slow buffers (the third mode by Albrecht et al. [6]). Net Ca²⁺ release from the ER may lose its regenerative character when the rate of Ca²⁺ release is equilibrated with the rate of Ca²⁺ clearance from the cytosol due to counteracting termination mechanisms. The terms “regenerative CICR” and “all-or-none Ca²⁺ release” are often used in the scientific literature as synonyms [23–25]. Meanwhile, simulations have revealed that the regenerative character of net CICR indeed does not preclude the release of Ca²⁺ in a graded manner with increasing stimulus strength due to the counteracting termination mechanisms [26].

The term “gradation of CICR” can be defined as proportionality of Ca²⁺ release flux to the Ca²⁺ influx through plasma membrane Ca²⁺ channels, which can be linear (“smooth”) or non-linear [27]. All-or-none CICR is characterized by a maximal [Ca²⁺]_i response, which is the same at any strength of a stimulus above the threshold [24]. This happens when the sum of all Ca²⁺ fluxes into the cytosol cannot be compensated by counteracting termination mechanisms, all effluxes and Ca²⁺ binding with slow buffers. Whether regenerative net Ca²⁺ flux can be a nonlinearly graded function or behave in an all-or-none manner depends on the gain amplification of the Ca²⁺ transient triggered by Ca²⁺ current, and the terms “graded” and “regenerative” refer to the distinct attributes of the mode.

There are similarities between Ca²⁺ dynamics and membrane potential dynamics in excitable cells because they both are described by the same mathematical formalism of nonlinear dynamics [15, 24]. Action potentials (APs), similar to all-or-none Ca²⁺ release events, are considered as one instance of a broad class of regenerative events caused by intrinsic positive feedback [28]. Graded regenerative potentials are one of the types of such events [29, 30]. Recently large analog fluctuations in membrane potential were discovered in the dendrites of the neocortex in freely behaving rats [31]. Some

authors incorrectly termed regenerative events as APs [32]. The amplitude and waveform of APs are invariant with respect to the amplitude, duration, and waveform of the stimulus that evoked it. Unlike the APs, the amplitude and waveform of graded regenerative potentials are highly sensitive to the characteristics of the stimulus. It should be noted that in contrast to membrane potentials, the overwhelming majority of Ca²⁺ release events are more or less graded and are not similar to APs.

The term “gain of Ca²⁺ release” is also confusing. Most authors use this term according to the definition suggested by Michael Stern [27] for cardiomyocytes: gain is the ratio of the amount of Ca²⁺ released from the ER to that amount of Ca²⁺ which entered into the cells through plasma membrane Ca²⁺ channels. This definition is applicable to many excitable cells. CICR gain is considered to be low when it is smaller than unity, in which case it provides a robust and graded amplification of the Ca²⁺ signal in the absence of a counteracting termination mechanism [27, 33]. Alternatively, the term “low gain mode” was used as a synonym of net Ca²⁺ uptake [6, 13].

Common pool models and the models of local control

Common pool models were defined by M. Stern in 1992 [27] as those models in which the trigger Ca²⁺ and released Ca²⁺ pass through a common cytosolic pool, and in which all RyRs are controlled by the whole-cell trigger Ca²⁺ current rather than by local openings of single Ca²⁺ channels. These models simulate a spatially homogeneous (global) Ca²⁺ concentration, which is described by only one variable in the whole cell or in each cell compartment [34]. For example, in cardiac cells this occurs when all nanodomains in the junctional, or dyadic, clefts between the sarcolemma and SR coalesce into a single compartment with volume equal to that of all dyads within the cell. Using linear stability theory, M. Stern [27] demonstrated that common pool models cannot achieve both high

gain and smoothly graded Ca^{2+} release, which was observed experimentally in cardiomyocytes. To explain this gradation, M. Stern proposed models of local control of CICR in ventricular myocytes that suggest that voltage-dependent Ca^{2+} channels on the sarcolemma and RyRs on the SR interact via local high Ca^{2+} elevations within the dyadic cleft. Graded release arises as the result of statistical recruitment of spatially uncoupled Ca^{2+} release units (CRUs). CRUs were also defined ambiguously as discrete clusters of RyRs [33], or as the set of release channels together with associated voltage-dependent Ca^{2+} channels [35]. Numerous models of Ca^{2+} sparks (e.g., [5, 10]) are models of local control where voltage-dependent and release Ca^{2+} channels communicate through changes in Ca^{2+} concentration in a restricted subsarcolemmal space, i.e. the trigger Ca^{2+} and released Ca^{2+} pass through a common pool.

The clarity of terms characteristic for ventricular myocytes worsens in publications concerning atrial myocytes. In contrast to ventricular myocytes that have a well-developed system of deep sarcolemma invaginations (t-tubules) where L-type Ca^{2+} channels are localized in the immediate proximity of RyR clusters, only some populations of atrial myocytes have a developed system of t-tubules. Recently a model for a subpopulation of right mouse atrial myocytes with developed transverse-axial tubule system was published [36]. This model is based on the common-pool model in ventricular myocytes with a common dyadic cleft, but the authors claim that their model includes local control of CICR. To model atrial myocytes that do not have a transverse axial tubule system and whose Ca^{2+} release relies on Ca^{2+} diffusion from the submembrane regions, spatial models of atrial myocytes were developed. The first models were one-dimensional models where space was divided into several compartments with homogeneous Ca^{2+} concentrations. The Ca^{2+} transients are large in the periphery of the cell and small in the cell center. The voltage-dependent Ca^{2+} current enters into the peripheral subspace

compartment only. Into other compartments Ca^{2+} enters due to diffusion. In the review of Heijman et al. [37], all spatial models are considered as the opposite of common pool models. The authors propose that, similarly to atrial models, ventricular models can be divided into common-pool and spatial local control models [37]. But the same increase in the number of compartments in the models where the trigger calcium and released calcium pass through common cytosolic pool in each compartment and where only macroscopic SR Ca^{2+} release is described does not make these models different from any other common pool models. The authors of one of such model [38] write that their model shares the general limitations of common pool models such as an approximate description of macroscopic SR Ca^{2+} release. The same concerns spatial neuronal models, where the space is divided by shell compartments.

A more difficult case is presented by 3-dimensional models. In a recent model of local control in ventricular myocytes [9] with realistic reconstruction of intracellular structures, the dyads and junctional SR were treated as single voxels in the spatial geometries. But in some models of atrial myocytes, where spatial grids were modeled as two-dimensional domains [39, 40], the spatial information necessary to model separate dyadic volumes and so Ca^{2+} concentration that locally controls Ca^{2+} release into the dyadic space was not provided [39]. In some other models of atrial myocytes, the dynamics of Ca^{2+} release units was studied in detail at high spatial resolution [40]. Thus, we can see that the terms “common pool models” and sometimes “local control models” do not have accurate definitions and are sufficiently ambiguous to allow for several conflicting interpretations. More rigorous could be the classification of models as spatially homogeneous models (in the whole cell or within each cell compartment) with macroscopic SR/ER Ca^{2+} release versus microdomain models of Ca^{2+} dynamics and elementary Ca^{2+} release units.

CONCLUSIONS

In order to avoid miscommunication of information and to compare more efficiently the results from different publications, ambiguous scientific terms should be avoided or at least defined explicitly. Moving toward a common terminology would benefit future research.

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ПОЯСНЕННЯ ТЕРМІНОЛОГІЇ, ЩО ВИКОРИСТОВУЮТЬСЯ ДЛЯ ОПИСУ ТРАНСПОРТУ КАЛЬЦІУ В РІЗНИХ ТИПАХ КЛІТИН

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Деякі основні наукові терміни у галузі загальної фізіології, що вивчає внутріклітинний транспорт кальцію, мають велику кількість визначень в науковій літературі. В цій статті ми аналізуємо неоднозначності визначень і намагаємося з'ясувати деякі основні терміни, що використовуються для опису транспорту кальцію в клітинах. Використання неоднозначної наукової термінології і суперечних визначень може стати джерелом непорозуміння серед вчених. Ключові слова: транспорт іонів кальцію; потік; моделі загального пулу; вивільнення Ca^{2+} за принципом «все або нічого»; невизначеність визначень.

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ПОЯСНЕНИЕ ТЕРМИНОЛОГИИ, КОТО- РАЯ ИСПОЛЬЗУЮТСЯ ДЛЯ ОПИСАНИЯ ТРАНСПОРТА В РАЗНЫХ ТИПАХ КЛЕТОК

Некоторые основные научные термины в области общей физиологии, которая изучает внутриклеточный транспорт кальция, имеют множественные определения в научной литературе. В этой статье мы анализируем эти неоднозначные определения и пытаемся прояснить некоторые основные термины, которые используются для описания транспорта кальция в клетках. Использование неоднозначной научной терминологии и конфликтующих определений может служить источником непонимания среди ученых.

Ключевые слова: транспорт ионов кальция; поток; модели общего пула; высвобождение Ca^{2+} по принципу «все или ничего»; неоднозначность определений.

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