MINIREVIEW

Synchronization of Ca$^{2+}$ oscillations: a capacitative (AC) electrical coupling model in neuroepithelium

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Structural organization of intracellular Ca$^{2+}$ stores and coupling modes of Ca$^{2+}$ increase

Chemical coupling and DC electrical coupling

The lumen of the endoplasmic reticulum (ER) is continuous with a space between the outer nuclear membrane (ONM) and inner nuclear membrane (INM) [1–3]. Intracellular Ca$^{2+}$ stores are formed within the ER lumen and the space between the ONM and the INM [1,2]. In cells with a centralized nucleus surrounded by the ER (Fig. 1A), intercellular communication may be mediated by the release of a transmit-

Abbreviations

AC, alternating current; BK channel, big K$^+$ channel; CNS, central nervous system; DC, direct current; DiOC$_{3}$ (3), 3,3'-dipentylxocarbocyanine iodide; ER, endoplasmic reticulum; I$_{c}$, capacitative current; INM, inner nuclear membrane; Ins(1,4,5)P$_{3}$, inositol 1,4,5-trisphosphate; mAChR, muscarinic acetylcholine receptor; ONM, outer nuclear membrane; Pyk2, proline-rich tyrosine kinase 2; RGC, retinal ganglion cell.
ter (e.g. ATP) and its receptors, which stimulate the release of Ca\textsuperscript{2+} from intracellular Ca\textsuperscript{2+} stores (Fig. 1B and Koizumi in this minireview series). This mode of coupling is referred to as chemical coupling. When gap junctions are present between adjacent cells, electrical coupling through gap junction channels may synchronize plasma membrane potentials, and Ca\textsuperscript{2+} influx through voltage-activated Ca\textsuperscript{2+} channels should lead to a synchronous increase in intracellular [Ca\textsuperscript{2+}] (Fig. 1B and Imtiaz et al. in this minireview series). This coupling mode is mediated by direct currents (DCs) through gap junction channels, and may be called DC electrical coupling.

Alternatively, a second messenger molecule such as inositol 1,4,5-trisphosphate [Ins(1,4,5)P\textsubscript{3}] and/or Ca\textsuperscript{2+} ions may pass gap junction channels, and such passive diffusion might lead to a synchronous increase in intracellular [Ca\textsuperscript{2+}]. However, the results of our studies on the retinal neuroepithelium contradict this diffusion model and provide evidence for an alternative model. We have found that Ins(1,4,5)P\textsubscript{3}-mediated robust Ca\textsuperscript{2+} increases induced by a supramaximal amount of an agonist do not synchronize, despite strong gap junctional coupling in the retinal neuroepithelium [4,5]. It has also been shown that synchronous Ca\textsuperscript{2+} oscillations occur in newborn retinal ganglion cells (RGCs), which lose gap junctions [5]. On the basis of these findings, an alternative model to the passive diffusion of Ins(1,4,5)P\textsubscript{3} or Ca\textsuperscript{2+} through gap junction channels is provided to explain the synchronization of Ca\textsuperscript{2+} oscillation between these cells.

Fig. 1. Structure of intracellular Ca\textsuperscript{2+} stores and coupling modes of intracellular [Ca\textsuperscript{2+}] increase. (A) Cells in which the nucleus is located in the center of the cell and is surrounded by ER. Modified from Fig. 1 in [1] with permission. (B) Chemical coupling and DC electrical coupling. Stored Ca\textsuperscript{2+} ions are released by the activation of receptors by a transmitter, such as ATP (chemical coupling). Depolarization \(\Delta V\) synchronized by gap junctional coupling activates voltage-dependent Ca\textsuperscript{2+} channels to cause synchronous Ca\textsuperscript{2+} influx (DC electrical coupling). The Ca\textsuperscript{2+} influx may cause Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release to amplify the [Ca\textsuperscript{2+}] increase. (C) Neuroepithelial cells in which the ONM is closely apposed to the plasma membrane (PM) and the cells are tightly packed in the basal layer. Modified from Fig. 2 in [6]. (D) Capacitative (AC) electrical coupling. Efflux of Ca\textsuperscript{2+} from Ca\textsuperscript{2+} stores and counterinflux of K\textsuperscript{+} cause fluctuations in the membrane potential of the Ca\textsuperscript{2+} store, inducing ACs, which can pass the membranes as capacitative currents (I\textsubscript{C}). The current loop is closed via cytoplasm and the PM or gap junction (GJ), and also via the extracellular space, even in the absence of GJs. NPC, nuclear pore complex; Nu, nucleoplasm.
Capacitative alternating current (AC) electrical coupling

A novel mechanism of coupling between cells that does not depend on gap junctions or transmitters has been proposed, on the basis of the observation that the membrane potential of Ca²⁺ stores oscillates synchronously between cells in the retinal neuroepithelium [4,6]. The voltage change exhibited a bistable alteration of fast rising and fast falling, which oscillated at the same frequency as the Ca²⁺ oscillations [4]. The voltage change was recorded using an organelle-specific, voltage-sensitive fluorescent dye, 3,3′-dipentyloxacarbocyanine iodide [DiOC₅(3)], and a highly sensitive video camera, which was connected to a high-speed confocal scanner (Nipkow disk type) [4]. When the voltage change was recorded using a photomultiplier, it was found, surprisingly, that the bistable voltage alteration consisted of periodic repeats of a burst of high frequency (> 200 Hz) voltage fluctuations [5]. The low time resolution of the video camera (15 images per second) did cover the high-frequency voltage fluctuation.

To explain the synchronization of the store potential, a capacitative (AC) electrical coupling model has been proposed, because the fast voltage change across the store membrane produces ACs, which can pass the plasma membrane capacitatively when the store membrane is in close proximity to the plasma membrane. The neuroepithelium consists of bipolar cells, in which the nuclei are positioned at different levels (pseudostratified columnar epithelium). In the retinal neuroepithelium, the ONM is closely apposed to the plasma membrane. The Closing of the store BK channels increases the time constant for the store membrane to dampen the high-frequency voltage fluctuations. In fact, an increase in intracellular [Ca²⁺] coincides with an increase in DiOC₅(3) fluorescence, which is caused by the burst of high-frequency voltage fluctuations [5].

It has been shown that voltage- and Ca²⁺-activated K⁺ channels [big K⁺ channels (BK channels)] are present in the membrane of the Ca²⁺ store or the ONM [4,7]. The store BK channels are activated by a positive voltage change on the luminal side and by an increase in the luminal [Ca²⁺] [4,7]. Because the closing of the store BK channels attenuates Ca²⁺ release [4], the Ca²⁺ efflux will decrease when the luminal Ca²⁺ levels decrease to the point at which the store BK channels close. The decrease in the luminal [Ca²⁺] should also decrease the driving force for Ca²⁺ efflux. The closing of the store BK channels increases the time constant for the store membrane to dampen the high-frequency voltage fluctuation of the Ca²⁺ store, which will inhibit the synchronous burst of the voltage fluctuations of the Ca²⁺ store [5]. When the Ca²⁺ store is replenished with Ca²⁺ ions by Ca²⁺ pumps in the store membrane, and the store BK channels are reactivated, the voltage fluctuations of the Ca²⁺ store will resume.

Synchronous intracellular Ca²⁺ increase in central nervous system (CNS) development

Figure 2 illustrates the development of neural activities relative to the cellular events that occur during the course of CNS development. Neurons are born from neuroepithelial cells after they have exited the cell cycle. It has been shown that the Ca²⁺ mobilization (Ca²⁺ release from Ca²⁺ stores) and the synchronous Ca²⁺ oscillations are essential for neuroepithelial cell proliferation, for ventricular cell proliferation, and for cell cycle progression [8–17]. Thus, the synchronous Ca²⁺ oscillations continue during neurogenesis. Cell death occurs naturally, leading to a reduction in the
number of neurons by approximately one-half. The surviving neurons begin to generate action potentials. At this stage, the surviving neurons exhibit a characteristic synchronous burst spiking, which leads to transient, synchronous increases in intracellular \([\text{Ca}^{2+}]\) between the cells [18,19]. Although transmitters may play a role in modulating the bursting activity, chemical transmission is unlikely to mediate the synchronization of spikes between the cells [18] (discussed later). It has been proposed that the synchronous increase in intracellular \([\text{Ca}^{2+}]\) is essential for the fine-tuning of synaptic connections [18–20]. Glial cells are born following neurogenesis [21]. The glial cells provide electrical insulation to neurons, thereby making it possible for individual neurons to generate action potentials asynchronously, depending on the synaptic inputs that they receive. Thus, neural circuits are precisely formed, and each neuron can respond to appropriate natural stimuli.

**Biological significance of \([\text{Ca}^{2+}]\) synchronicity**

The above overview of the steps of CNS development raises questions regarding the molecular events that accompany the synchronous increase in intracellular \([\text{Ca}^{2+}]\). The following sections describe a new model and provide possible explanations regarding the biological significance of the synchronous increases in intracellular \([\text{Ca}^{2+}]\) between cells.

**Cell cycle-dependent \([\text{Ca}^{2+}]\) mobilization and cell–cell adhesion in the neuroepithelium**

Neuroepithelial cells undergo interkinetic nuclear migration along the apicobasal axis during cell cycle progression [21,22]. Stimulation of G-protein-coupled receptors causes the robust release of \([\text{Ca}^{2+}]\) from intracellular \([\text{Ca}^{2+}]\) stores in S-phase cells in the basal layer, whereas the ER and the nuclear envelope are broken down and the \([\text{Ca}^{2+}]\) mobilization declines in M-phase cells in the apical layer [12]. Spontaneous, synchronous \([\text{Ca}^{2+}]\) oscillations occur between S-phase neuroepithelial cells and newborn RGCs [4,5].

The interkinetic cell shows a polarized bipolar structure, whereas the M-phase cell is round. Fujita and Yasuda [23] have suggested that this morphological difference is due to a change in cell–cell adhesion that is mediated by cadherin–catenin complexes within each cell and by cadherin–cadherin interactions between the two cells. The interkinetic cells adhere to each other via cadherin–catenin complexes, and these complexes are anchored to F-actin (Fig. 3A). During M-phase, the cadherin–catenin complex dissociates, thereby disrupting cell–cell adhesion [23]. As a result, M-phase cells are round (Fig. 3B). These morphological and molecular changes point to a relationship between cell–cell adhesion and the synchronous \([\text{Ca}^{2+}]\) oscillations, and suggest that cadherin–catenin complexes connect interkinetic cells with each other. Synchronous \([\text{Ca}^{2+}]\) oscillations occur in S-phase cells and newborn RGCs. In contrast, in M-phase cells, the \([\text{Ca}^{2+}]\) mobilization system, including the ER and the nuclear envelope, disappears and cadherin–catenin complexes are disassembled. It is proposed that cell–cell adhesion may be regulated by the synchronous increases in intracellular \([\text{Ca}^{2+}]\), as described below.

**Synchronous increases in intracellular \([\text{Ca}^{2+}]\) and disassembly of cadherin–catenin complexes**

The cytoplasmic domain of cadherin interacts with F-actin via \(\beta\)-catenin and \(\varepsilon\)-catenin; \(\beta\)-catenin binds to cadherin and \(\varepsilon\)-catenin, which in turn interacts with F-actin (Fig. 3A) [24]. Thus \(\beta\)-catenin plays a pivotal role in the regulation of cell–cell adhesion. The interaction of \(\beta\)-catenin with cadherin is regulated by tyrosine phosphorylation of \(\beta\)-catenin [25,26], which leads to disassembly of the cadherin–catenin complex. \(\beta\)-Catenin is directly tyrosine-phosphorylated by the nonreceptor protein tyrosine kinase proline-rich tyrosine kinase 2 (Pyk2) [26,27], or is indirectly tyrosine-phosphorylated by Src family kinase, which can be activated by Pyk2 [28]. It is likely that tyrosine phosphorylation of \(\beta\)-catenin is triggered by \([\text{Ca}^{2+}]\) ions, because Pyk2 is activated by an increase in intracellular \([\text{Ca}^{2+}]\) [29,30].

If Pyk2 is transiently activated by an increase in intracellular \([\text{Ca}^{2+}]\) to phosphorylate \(\beta\)-catenin in two
adherent cells, the synchronous increase in \([\text{Ca}^{2+}]\) between the cells could lead to a significant change in the homophilic binding of cadherins. The coactivation of Pyk2/ Src kinase between the cells would result in the dissociation of cadherin–catenin complexes (Fig. 3A). However, if intracellular \([\text{Ca}^{2+}]\) were increased in only one of the two adherent cells, the transient activation of Pyk2 would only occur in that cell. In this case, the \(\beta\)-catenin would be rapidly dephosphorylated by a phosphatase in that cell without disrupting the homophilic binding of cadherins (Fig. 3C).

If the synchronous increase in intracellular \([\text{Ca}^{2+}]\) were responsible for the disruption of cell–cell adhesion, it would seem paradoxical that the synchronous \(\text{Ca}^{2+}\) oscillations would occur in S-phase cells, but not in M-phase cells. S-phase cells, however, may gradually disconnect themselves from the surrounding cells before M-phase, at which point almost all cadherin–catenin complexes are disassembled. After mitosis, the cells are reattached by cadherins, and the ER and the nuclear envelope are reorganized before S-phase. Newborn RGCs are also free from surface adhesion as they extend dendrites (Fig. 3B).

In summary, a new model is put forward in which synchronous, transient increases in intracellular \([\text{Ca}^{2+}]\) between cells can facilitate the disruption of cell–cell adhesion to destabilize cell surface contact. A reduction in the stability of cell–cell adhesion may be an output of a coincidence detector of cellular activities. This decrease in cell-cell contact, in other words, the increase in freedom of cell surface, may play an essential role in the regulation of mitosis, dendrite extension, and synaptic plasticity.

### Capacitative (AC) electrical coupling in cortical development

Synchronous \(\text{Ca}^{2+}\) oscillations occur in the developing cortex even before synapse formation [8,31,32]. \(\text{Ca}^{2+}\) oscillations in the retinal ventricular zone are driven by a muscarinic acetylcholine receptors (mAChRs), which cause the release of \(\text{Ca}^{2+}\) from intracellular \(\text{Ca}^{2+}\) stores [13,14,33]. The activation of mAChRs also induces strongly synchronized electrical activities in the subplate of the cortex of newborn mice [32]. The mAChR-driven electrical activity is blocked by tetrodotoxin, suggesting that the activation of mAChRs results in the generation of action potentials [32]. However, it remains unknown how the activation of mAChRs induces the synchronous firing activity.

The capacitative (AC) coupling model may account for the generation of synchronous bursts of spikes. The AC currents caused by the voltage fluctuations of the \(\text{Ca}^{2+}\) store may pass the plasma membrane capacitatively (Fig. 4A). This current may function as a noisy stimulus current to evoke action potentials (Fig. 4B).
If the voltage fluctuations of the Ca\textsuperscript{2+} store are synchronous between the cells, synchronous bursts of spikes could be generated. Such capacitative coupling may be the underlying mechanism that mediates the synchronization of spikes during the early stages of neurodevelopment.

References

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Capacitative electrical coupling of Ca\(^{2+}\) release


