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Review

Hierarchical organization of calcium signals in hepatocytes: from experiments to models

Geneviève Dupont [a,*], Stéphane Swillens [b], Caroline Clair [c], Thierry Tordjmann [c], Laurent Combettes [c]

[a] *Université Libre de Bruxelles, Faculté des Sciences CP231, Boulevard du Triomphe, B-1050 Brussels, Belgium*
[b] *Université Libre de Bruxelles, IRIBHN, Faculté de Médecine, Campus Erasme, Route de Lennik 808, B-1070 Brussels, Belgium*
[c] *Unité de Recherche U442, Institut National de la Santé et de la Recherche Médicale, Université de ParisSud, bât 443, 91405 Orsay, France*

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Abstract

The proper working of the liver largely depends on the fine tuning of the level of cytosolic Ca^{2+} in hepatocytes. Thanks to the development of imaging techniques, our understanding of the spatio-temporal organization of intracellular Ca^{2+} in this – and other – cell types has much improved. Many of these signals are mediated by a rise in the level of inositol 1,4,5-trisphosphate ($InsP_3$), a second messenger which can activate the release of Ca^{2+} from the endoplasmic reticulum. Besides the now well-known hepatic Ca^{2+} oscillations induced by hormonal stimulation, intra- and intercellular Ca^{2+} waves have also been observed. More recently, subcellular Ca^{2+} increases associated with the coordinated opening of a few Ca^{2+} channels have been reported. Given the complexity of the regulations involved in the generation of such processes and the variety of time and length scales necessary to describe those phenomena, theoretical models have been largely used to gain a precise and quantitative understanding of the dynamics of intracellular Ca^{2+}. Here, we review the various aspects of the spatio-temporal organization of cytosolic Ca^{2+} in hepatocytes from the dual point of view provided by experiments and modeling. We first focus on the description and the mechanism of intracellular Ca^{2+} oscillations and waves. Second, we investigate in which manner these repetitive Ca^{2+} increases are coordinated among a set of hepatocytes coupled by gap junctions, a phenomenon known as 'intercellular Ca^{2+} waves'. Finally, we focus on the so-called elementary Ca^{2+} signals induced by low $InsP_3$ concentrations, leading to Ca^{2+} rises having a spatial extent of a few microns. Although these small-scale events have been mainly studied in other cell types, we theoretically infer general properties of these localized intracellular Ca^{2+} rises that could also apply to hepatocytes.

Keywords: Liver; Local signal; Calcium wave; $InsP_3$ receptor

1. Introduction

The liver is a multifunctional organ, responsible for vital functions such as the intermediary metabolism of the body or the control of the endocrine system. It also plays a key role in the defensive system of the organism and as a store for blood volume. Among this variety of functions of the liver, many of them are controlled by calcium. For example, the production of glucose by the liver is mediated by a hormone-induced Ca^{2+} increase. Many events related to bile secretion are also regulated by Ca^{2+}, such as

* Corresponding author. Fax: +32-2-650-5767; E-mail: gdupont@ulb.ac.be

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vesicular trafficking, canalicular exocytosis, permeability of tight junctions or canalicular contraction. Ca^{2+} is also involved in cell survival by contributing to the regulation of cell growth, cell division, apoptosis and necrosis. It is therefore not surprising that the level of Ca^{2+} is highly regulated in liver cells.

The concentration of free Ca^{2+} in the cytosol ($[Ca^{2+}]_i$) is actively kept much lower (100–200 nM) than extracellular (1–2 mM) and intrareticular (0.5 mM) Ca^{2+} concentrations [1]. The cytosol, with its very low concentration of free calcium, is located at the interface of these two very calcium-rich environments. This results in the cytosol being a site of major, rapid variations in $[Ca^{2+}]_i$ in response to the transfer of small quantities of Ca^{2+} from the extracellular medium or intracellular storage compartments [2]. These variations are induced by hormones and neurotransmitters and are described as 'calcium signals'. It is becoming increasingly evident that such calcium signals are extraordinarily well organized in both space and time, from the subcellular to the whole tissue level [2–4].

The predominant pathway for Ca^{2+} elevation in hormone-stimulated hepatocytes, as in most electrically non-excitable cells, involves the activation of the inositol-phosphate system, which finally leads to the release of Ca^{2+} stored in the endoplasmic reticulum (ER), through the stimulation of $InsP_3$-sensitive Ca^{2+} channels. The highly organized character of these $InsP_3$-induced Ca^{2+} signals stems from various factors among which the most important are the regulation of the $InsP_3$-sensitive Ca^{2+} channels by cytosolic Ca^{2+} itself, the detailed characteristics of diffusion of cytosolic Ca^{2+} as well as the specificity of $InsP_3$ synthesis, metabolism and movement.

In this review, we focus on the spatio-temporal organization of calcium signals in hepatocytes from the subcellular to the multicellular level. The general idea of the present study is to use the synergy provided by an experimental and a theoretical approach to apprehend complex phenomena, such as Ca^{2+} oscillations, waves or gradients from a clear and sound point of view. In a natural manner and following the historical progress in experimental observations, we first consider the calcium dynamics in isolated hepatocytes. It was first shown by Woods et al. [5] that the Ca^{2+} signals in response to hormonal stimuli consist of a series of spikes in $[Ca^{2+}]_i$ (oscillations) with a period of a few seconds to a few minutes. It appeared later that each Ca^{2+} spike is also organized spatially: the Ca^{2+} concentration first increases locally, then the increase propagates in the whole cell as a wave, traveling at a speed of 10–20 μm s^{-1} [6]. In Section 2, we give a rather detailed description of these phenomena in hepatocytes and discuss the most plausible molecular mechanism underlying this intracellular organization.

At the level of the liver, hepatocytes are coupled through gap junctions in a spatially organized manner. Individual cells from different types present in the liver also communicate indirectly via a chemical messenger released into the extracellular medium [7]. As a result, Ca^{2+} waves are seen to propagate intercellularly along the hepatocyte plates. Although this phenomenon of intercellular Ca^{2+} wave propagation has been observed in other tissues, its mechanism could be tissue-specific. Section 3 is devoted to the latter question in hepatocytes by dealing in fact with the mechanism of coordination of Ca^{2+} signals on a simplified system made of a small group of connected hepatocytes known as 'multiplet'.

Oscillations, intra- and intercellular Ca^{2+} waves all tightly depend on the subcellular properties of the Ca^{2+} releasing entities, namely the $InsP_3$-sensitive Ca^{2+} channels. The arrangement of these channels on the surface of the ER appears to considerably affect the resulting Ca^{2+} signal [8,9]. Recent advances in the Ca^{2+} imaging techniques have allowed the visualization of the Ca^{2+} increase caused by a single Ca^{2+} channel (a Ca^{2+} event known as a 'Ca^{2+} blip') or by a small group of channels (known as a 'Ca^{2+} puff'). The detailed observation of these phenomena provide important information about the in vivo regulation of the $InsP_3$ receptors ($InsP_3R$) as well as on the properties of Ca^{2+} diffusion in a real cytoplasmic environment. Given the technical limitation inherent to the analysis of such small-scale events, a theoretical approach of Ca^{2+} blips and puffs is particularly useful. In Section 4, we focus on the microscopic organization of such Ca^{2+} signals. The final aim of this approach would be to understand how these Ca^{2+} increases highly localized in time and space could interact to give a coordinated signal at the cellular level, which signal would in turn organize with respect to other individual hepatocytes to give

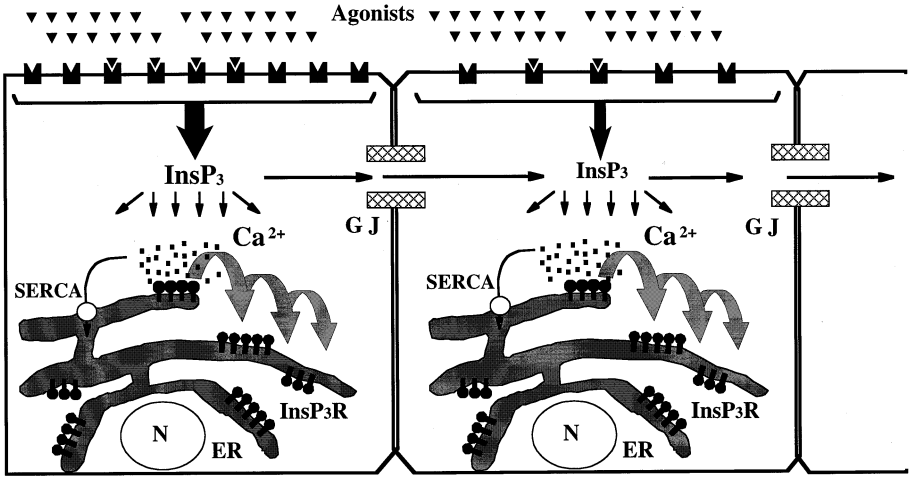

Fig. 1. Schematic representation of the spatio-temporal organization of calcium signals in hepatocyte: from Ca^{2+} blips to intercellular Ca^{2+} waves. Those different levels of organization are discussed throughout the text. ER, endoplasmic reticulum; GJ, gap junction; IP_3R, IP_3 receptor–Ca^{2+} channel; N, nucleus.

a coordinated Ca^{2+} signal at the level of the whole liver (see Fig. 1).

2. Calcium signaling at the cellular level: oscillations and waves

2.1. Ca^{2+} oscillations in isolated hepatocytes

In hepatocytes, as in most electrically non-excitable cells, Ca^{2+} oscillations originate from the periodic opening of Ca^{2+} channels located in the membrane of the ER, following activation of the phosphoinositide cascade. The binding of an agonist to the extracellular side of a membrane-bound receptor activates the Gα-subunit of a G-protein complex coupled to the receptor. This activated G protein in turn stimulates phospholipase C (PLC) activity. The latter enzyme catalyzes the hydrolysis of the membrane-bound phosphatidyl-inositol bisphosphate (PIP_2) into diacyl-glycerol and $InsP_3$. Ca^{2+} release from the internal stores is ensured by the $InsP_3Rs$. The kinetics of these receptors has been studied in great detail (for review, see e.g. [10]). The $InsP_3R$ is an homotetramer that can bind up to 4 $InsP_3$ molecules. Whether the latter process is cooperative or not remains a matter of debate [11–13]. The equilibrium open probability of this Ca^{2+} channel presents a bell-shaped dependence on cytosolic Ca^{2+}. The reversible Ca^{2+}-induced inhibition of Ca^{2+} release observed at high Ca^{2+} levels develops more slowly than the activation by Ca^{2+} [10]. The decrease of $[Ca^{2+}]_i$ in the cytosol is due to the activity of the Ca^{2+} ATPases (SERCA pumps), which actively transport Ca^{2+} from the cytosol into the ER. As will be emphasized in the modeling Section 2.4, Ca^{2+}-regulated $InsP_3Rs$ and Ca^{2+} ATPases are together sufficient to generate Ca^{2+} oscillations.

In most cases, Ca^{2+} oscillations in hepatocytes take the form of repetitive, sharp spikes sometimes preceded by a slower, pacemaker-like elevation in the cytosolic Ca^{2+} concentration (Fig. 2A). These periodic increases in the level of free Ca^{2+} in the cytosol from about 0.1 μM up to 1 μM have been observed in hepatocytes in response to stimulation by a large number of agonists such as noradrenaline, vasopressin, phenylephrine, etc. Depending on the nature and the concentration of the agonists, the period can vary from a few tens of seconds to a few minutes. A general property of these oscillations is that their frequency increases with the level of stimulation. This parallel rise in the concentration of external agonist and the frequency of Ca^{2+} oscillations is known as 'frequency-encoding', since the level of external stimulation is encoded in the frequency of Ca^{2+} oscillations. In some instances, Ca^{2+} oscillations appear as small, symmetrical fluctuations on a raised basal level of Ca^{2+} (Fig. 2B). This type of oscillation, much less frequent, is often referred to as 'sinusoidal oscillations' and is also based on the receptor-activated

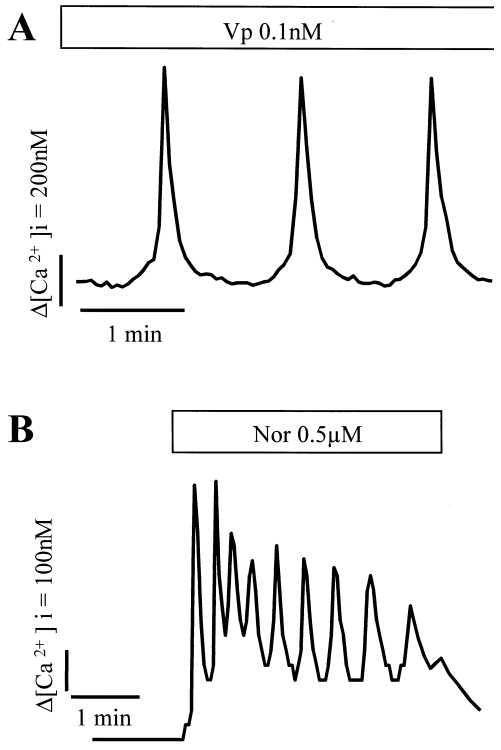

Fig. 2. Calcium oscillations in an isolated hepatocyte. Ca^{2+}-associated fluorescence variations of fura2-loaded hepatocytes were recorded with a frame ratioing of 1 image/3 s as described in Combettes et al. [72]. (A) Addition of low concentration of $InsP_3$-dependent agonists (vasopressin, Vp 0.1 nM, in this case) induces typical slow Ca^{2+} oscillations. Note the slow 'pacemaker' phase preceding the Ca^{2+} burst. (B) Addition of intermediate concentration of $InsP_3$ dependent agonists induces 'sinusoidal' Ca^{2+} oscillations on a sustained Ca^{2+} increase.

$InsP_3$ synthesis and the subsequent Ca^{2+} release from internal stores. The present review is devoted to the study of $InsP_3$-induced repetitive spiking (as in Fig. 2A), which prevails in most types of hormonal stimuli.

Besides the level of external stimulation (and thus the internal concentration of $InsP_3$), other factors regulate these oscillations in cytosolic Ca^{2+}. The shape of the oscillations (and specifically the slope of the decreasing part of the spikes) is clearly agonist-dependent [5]. The level of extracellular Ca^{2+} – and thus the rate of Ca^{2+} influx – affects the frequency of Ca^{2+} oscillations. Moreover, a basal level of external Ca^{2+} is required to avoid a progressive damping of the oscillations. As in many cell types, the decrease of Ca^{2+} concentration in the Ca^{2+} stores appears as the driving force for Ca^{2+} entry from the external medium into the cytosol. The molecular basis of this mechanism, known as 'capacitative Ca^{2+} entry' [14] is very actively investigated. Mitochondria have also been shown to modulate cytosolic Ca^{2+} oscillations and waves in some cell types. In permeabilized hepatocytes, it has been shown that Ca^{2+} uptake by mitochondria, located in close association with the ER, can substantially affect the feedback exerted by Ca^{2+} on the $InsP_3Rs$ [15]. The permeability transition pores could furthermore release Ca^{2+} in an autocatalytic manner, in a process known as 'mitochondrial Ca^{2+}-induced Ca^{2+} release' [16]. However, the most probable hypothesis is that external Ca^{2+} and mitochondria only modulate Ca^{2+} oscillations, while the properties of the channels involved in the release of Ca^{2+} from internal stores play the major role in the generation of sustained Ca^{2+} oscillations.

$InsP_3R$ activity is also regulated by other intracellular messengers such as cAMP and cGMP. Indeed, it has been shown that activation of cAMP- or cGMP-dependent protein kinases, probably via the phosphorylation of the $InsP_3R$, could extend the window of $InsP_3$ concentrations able to elicit Ca^{2+} oscillations [17–21]. It has been also shown that the $InsP_3R$ can undergo time-dependent ligand-induced desensitization [22]; this phenomenon could provide a mechanism for the termination of the Ca^{2+} spikes, alternative to – or superimposed on – the inhibition of $InsP_3R$ activity by high cytosolic Ca^{2+}. Although various other hypotheses have been put forward as, for example, depolarization of the ER membrane [23], the mechanism for spike termination, and more specifically, for keeping the system silent for a duration equal to the observed period of Ca^{2+} oscillations, is one of the major problem that remains to be solved to get a complete understanding of the mechanism of Ca^{2+} oscillations.

2.2. *Intracellular Ca^{2+} waves in isolated hepatocytes*

Each cell is a spatially inhomogeneous medium. Although the ER appears to be quite evenly distributed in many cell types, the $InsP_3Rs$ are not evenly distributed all over the cytoplasm. For example, in hepatocytes, $InsP_3Rs$ are more abundant at the apical region [24]. Basic calculations show that, for a typical cell dimension of 20 μm (corresponding to an

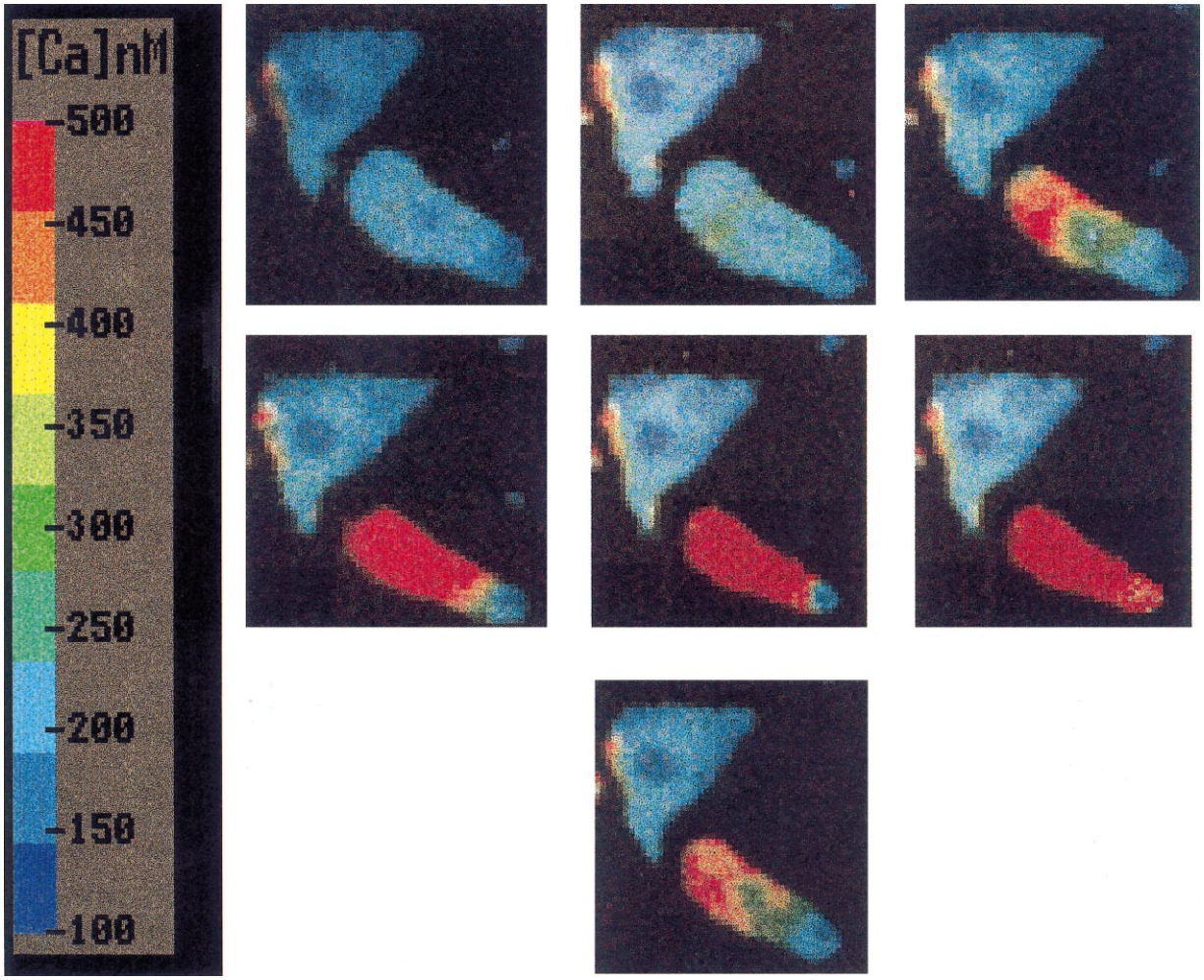

Fig. 3. Intracellular calcium wave in hepatocyte. Primary culture of hepatocytes (24 h) were loaded with fura2 as described in [72]. Images are false color representations of cytosolic free Ca^{2+}. The first image represents cytosolic Ca^{2+} at rest. As shown in the next five images, addition of vasopressin (2 nM) elicited an intracellular Ca^{2+} wave in the lower cell (1 image/800 ms) which originated from a specific locus. The last image shows that subsequent Ca^{2+}increase (about 25 s later) started from the same locus.

hepatocyte), the number of $InsP_3Rs$ that are open simultaneously during the occurrence of a global Ca^{2+} spike is smaller than 100. The coherent behavior of the cell with respect to Ca^{2+} signaling thus comes from the fact that Ca^{2+} and $InsP_3$ both diffuse in the cytoplasm, a phenomenon which allows each Ca^{2+} spike to propagate as a Ca^{2+} wave throughout the cell.

In hormone-stimulated hepatocytes, Ca^{2+} first rises in a restricted region of the cell and then spreads through the whole cell during each Ca^{2+} spike (Fig. 3). The wave front propagates with a fairly constant amplitude and at a relatively constant rate. As the width of the front is of the order of the size of the cell, the wave has the appearance of a Ca^{2+} tide [25]. The propagation velocity is of the order of 20 μm s^{-1} and does not depend on the agonist concentration [4]. Other factors could however influence the rate of propagation. High levels of Ca^{2+} buffering in the cytosol can decrease the rate of propagation [26], while very high concentrations of buffer can even abolish the Ca^{2+} wave as shown in *Xenopus* oocytes [27].

Besides normal hormonal stimulation, Ca^{2+} waves in hepatocytes can also be induced by direct introduction of a non-metabolizable analog of $InsP_3$, which strongly suggests that Ca^{2+}, and not $InsP_3$, plays the major role in the propagation of the wave

[28]. This hypothesis is corroborated by the observation that agents, such as *tert*-butyl hydroperoxide (tBHP), or certain bile acids, which raise Ca^{2+} in the cytosol independently of $InsP_3$, also induces Ca^{2+} waves [6,29].

2.3. Quantal Ca^{2+} release and incremental detection

Another property of $InsP_3$-induced Ca^{2+} release is revealed under direct stimulation of permeabilized cells by step increases in $InsP_3$. In these conditions, it clearly appears that low $InsP_3$ concentrations fail to release the total Ca^{2+} content of the stores. As the first hypothesis proposed to explain this phenomenon relied on an all-or-none discharge of a fraction of the total releasable pool, it was called 'quantal release' [30]. That this phenomenon is also not due to classical desensitization by $InsP_3$ is indicated by the fact that successive, minute increases in $InsP_3$ provoke repetitive Ca^{2+} discharges, whose amplitude directly depends on the quantity of $InsP_3$ added to the system. The latter phenomenon was called incremental detection [31]. Besides intrinsic differences in the sensitivities of the Ca^{2+} pools, incremental detection has often been ascribed to some desensitization processes mediated by a decrease in luminal Ca^{2+}. However, this hypothesis appears quite unrealistic, given the fact that this inactivation of the receptor only occurs when the level of intraluminal Ca^{2+} has drastically dropped [32].

Another plausible explanation for quantal Ca^{2+} release and incremental detection has been formulated in terms of a theoretical model in which the activity of the $InsP_3R$ is regulated by the Ca^{2+} level in an intermediate domain, located at the border between the ER and the cytosol [33]. As we will see later, this hypothesis would only be corroborated by a detailed knowledge of the microscopic organization of the $InsP_3Rs$ and of the associated Ca^{2+} increases, which is the focus of Section 4.

2.4. Model for Ca^{2+} oscillations and waves

Numerous theoretical models for Ca^{2+} oscillations have been proposed (for reviews, see [34–37]). A significant distinction between the various models is that some require the periodic variation of $InsP_3$ to generate Ca^{2+} oscillations [38,39] while other only rely on the regulatory properties of the $InsP_3R$ [40–42]. In the former type of models, Ca^{2+} oscillations rely on the stimulation of $InsP_3$ synthesis through PLC activation by Ca^{2+} [43]. That cytosolic $InsP_3$ concentration oscillates concomitantly with $[Ca^{2+}]_i$ has received recent experimental support by the indirect demonstration of $InsP_3$ oscillations in ATP-stimulated MDCK cells [44]. However, it should be kept in mind that such $InsP_3$ oscillations could simply originate from the Ca^{2+}-enhanced degradation of $InsP_3$ by 3-kinase [45]; in the latter case, $InsP_3$ oscillations would passively follow Ca^{2+} oscillations but would not take part in the central oscillatory mechanism.

That $InsP_3$ primes the cytoplasm of the cell to put it into an excitable state with respect to Ca^{2+} increases is the basic assumption of the second class of models. These models are thus based on the experimentally well-established dual control of the activity of the $InsP_3R$ by Ca^{2+} [46–48]. The first detailed theoretical model for the $InsP_3R$ was proposed by De Young and Keizer [40]. The $InsP_3R$ is there assumed to consist of 3 identical and independent subunits. Each subunit can bind one activating $InsP_3$, one activating Ca^{2+}, and one inhibiting Ca^{2+}. The channel is open when the three subunits have both $InsP_3$ and activating Ca^{2+} bound; provided an adequate choice of parameter values, the model nicely reproduces the bell-shaped dependence of the open probability of the $InsP_3R$ as a function of Ca^{2+}. Given that each subunit can exist in eight different states, the model is relatively heavy and has been simplified in various ways [36,49]. Model simplifications arise from the possible separation of time scales, as activation of the Ca^{2+}-releasing activity of the $InsP_3R$ by Ca^{2+} and $InsP_3$ occurs on a much shorter time scale than inhibition of the receptor by Ca^{2+}.

Another model that takes the same simplifying assumption into account is based on experiments performed with synaptosomes under Ca^{2+} clamp conditions [41]. This rather simple three-variable model reproduces the steady-state open probability of the $InsP_3R$ in the synaptosomes [48] as well as the peak response, time-to-peak and rate of inactivation in response to steps of Ca^{2+} or $InsP_3$ [35]. More recent studies focus on the regulatory differences between the different $InsP_3R$ isoforms (for review, see

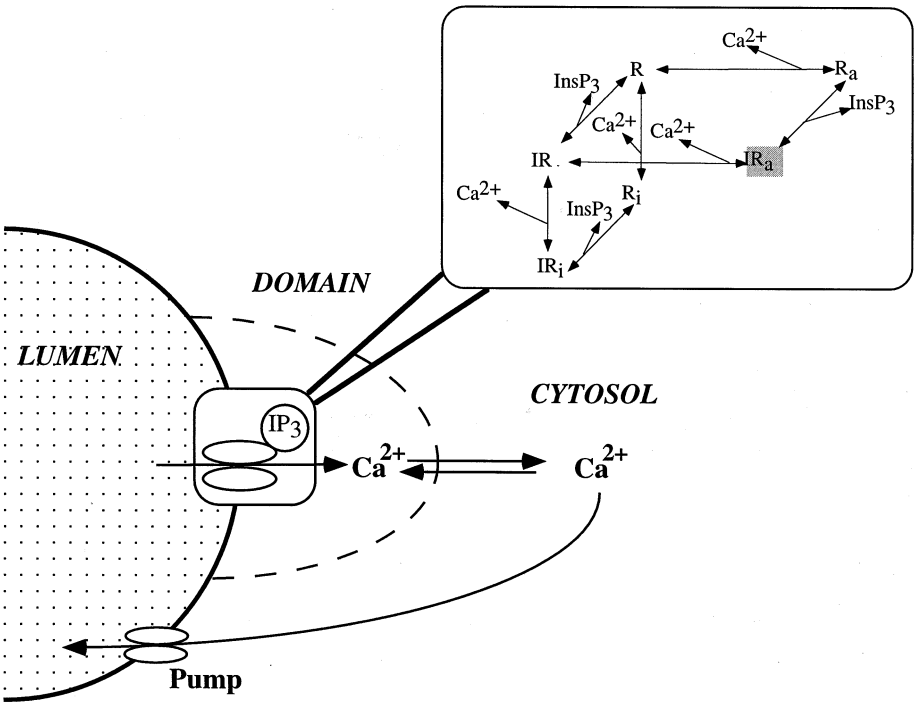

Fig. 4. Schematic representation of a model for intracellular Ca^{2+} oscillations and incremental detection based on the kinetic behavior of the $InsP_3$ receptor [33,42]. The model considers the domain around the channel as a specific compartment, an hypothesis which is necessary to account for incremental detection but not for oscillations. The detailed kinetics of the $InsP_3$ receptor is schematized in the upper right box. The receptor has three binding sites, and only the state with $InsP_3$ and activating Ca^{2+} bound (represented in grey) can release Ca^{2+} from the lumen into the cytosol.

[50]). A model specifically devoted to the study of the type 1 $InsP_3R$ thus accounts for the observation that high $InsP_3$ concentrations are able to overcome Ca^{2+}-dependent inhibition of channel activity [51], a phenomenon that was also proposed by Mak et al. [52].

One of the model for Ca^{2+} oscillations based on the description of the kinetics of the $InsP_3R$ was initially developed to account for quantal Ca^{2+} release (see 2.3; [33]) and later extended to study Ca^{2+} oscillations [42]. This model is schematized in Fig. 4. Intracellular calcium is supposed to be distributed between three homogeneous compartments: the lumen ($InsP_3$-sensitive store), the intermediate domain (downstream from the channel gate), and the cytosol. The physiological significance of such a postulated domain remains to be demonstrated and will be addressed in Section 4. Channel activity (Fig. 4, inset) is stimulated by $InsP_3$ and regulated in a biphasic manner by the level of Ca^{2+} in the domain. Ca^{2+}-induced deactivation develops slowly whereas $InsP_3$- and Ca^{2+}-mediated activations are instantaneous. Ca^{2+} released from the stores passes through the domain before entering the cytosol. Efflux from the stores is regulated by the fraction of non-desensitized receptors with only activating Ca^{2+} and $InsP_3$ bound to their respective sites, and is proportional to the Ca^{2+} gradient between the lumen and the domain; a small $InsP_3$-independent efflux is also considered.

The concentration of Ca^{2+} in the domain that surrounds the mouth of the channel is given by the balance between what comes from the lumen and what goes into the cytosol. The efflux from the lumen depends on both the fraction of $InsP_3Rs$ in the open state and on the concentration difference between the lumen and the domain. Efflux from the domain into the cytosol is linear and proportional to the concentration difference between both compartments. The fact that the various compartments have highly different volumes is also taken into account. Finally, the level of Ca^{2+} in the cytosol increases due to the efflux of Ca^{2+} from the domain and decreases

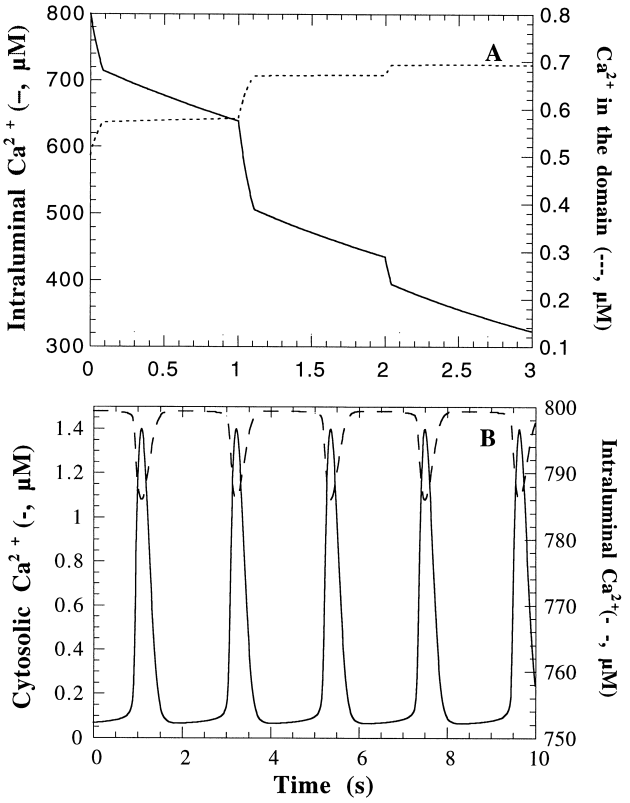

Fig. 5. Incremental detection (A) and Ca^{2+} oscillations (B) in the model based on the kinetic behavior of the $InsP_3$ receptor schematized in Fig. 4. In panel A, the three successive decreases in the level of luminal Ca^{2+} correspond to step increases in the concentrations of $InsP_3$. The model reproduces the typical biphasic Ca^{2+} release, the amplitude of which directly depends on the $InsP_3$ concentration. To simulate incremental detection, the volume of the 'cytosol' is assumed to tend to infinity, an assumption which corresponds to the situation where cells are permeabilized. To simulate the oscillations shown in panel B, the cell is supposed to be intact. Otherwise, all parameter values are similar to those used in panel A. See [42] for equations and parameter values.

because of Ca^{2+} pumping back into the lumen through Ca^{2+}-ATPases. A detailed explanation of the equations of the model can be found in previous publications [33,42].

To simulate quantal release, it is assumed that cytosolic Ca^{2+} is maintained at a constant concentration and that pumping back into the stores is negligible; these constraints reproduce the conditions of stopped-flow experiments performed in permeabilized cells. Fig. 5A shows that, in these conditions, the system defined here above responds to an $InsP_3$ stimulus above a certain threshold by a transient, rapid decrease of Ca^{2+} in the lumen, followed by a much slower diminution. Because the receptors are only partially desensitized, this biphasic release of Ca^{2+} can be reproduced if the system is submitted to successive $InsP_3$ elevations. When, in contrast to the situation of Fig. 5A, the level of Ca^{2+} in the cytosol is allowed to vary – thus restoring the conditions of an intact cell – numerical integration of the model with the same values of parameters as in Fig. 5A generates sustained Ca^{2+} oscillations (Fig. 5B). In agreement with experimental observations, the period of oscillations decreases when increasing the level of $InsP_3$; finally, for supra-maximal levels of stimulation, oscillations disappear and are replaced by a sustained, elevated level of cytosolic Ca^{2+}. Another factor which affects the period of Ca^{2+} oscillations is the rate at which the $InsP_3R$ resensitizes. However, if the rate of Ca^{2+} transfer from the domain into the cytosol is very fast, the period can become much larger than the time needed by the $InsP_3R$ to resensitize; in these conditions indeed, the stimulatory increase of Ca^{2+} around the mouth of the channel is prevented for some time because the Ca^{2+} released by the lumen is rapidly flowing out of the domain into the cytosol [42].

Intracellular Ca^{2+} waves can be simulated by all models for Ca^{2+} oscillations provided that diffusion of cytosolic Ca^{2+} is taken into account. In these conditions, the rate of propagation of the simulated waves depends on various factors among which the most important are the diffusion coefficient for Ca^{2+} and the buffering capacity of the cell [35].

2.5. *Possible physiological significance of Ca^{2+} oscillations in hepatocytes*

Oscillations of cytosolic Ca^{2+} represent one of the most widespread examples of periodic behavior at the cellular level [2]. As Ca^{2+} is involved in a variety of physiological processes, it is interesting to investigate the effect of such Ca^{2+} oscillations on the cellular responses mediated by Ca^{2+}. The sensitivity of the latter responses on the frequency of Ca^{2+} spikes most probably rely on a variety of molecular mechanisms. One of these certainly involves a Ca^{2+}-calmodulin activated protein kinase (CaMKII), which acts as a widespread mediator between the Ca^{2+} spikes and the physiological response [38]. Recent

experiments have shown that CaMKII is sensitive to the temporal pattern of high frequency Ca^{2+} spikes [53]. Such a capability of decoding the frequency of Ca^{2+} oscillations can be ascribed to the complex mode of regulation of CaMKII activity by Ca^{2+}, in the form of autophosphorylation and CaM trapping [54].

The liver provides another very good system to investigate the role of Ca^{2+} as a key-modulator of a physiological response. Upon stimulation, Ca^{2+} indeed acts as an important second messenger controlling the phosphorylation-dephosphorylation cascade that governs the switch between glycogen synthesis and degradation. Glycogenolysis is indeed promoted by hormones, like noradrenaline and vasopressin, which induce repetitive Ca^{2+} spikes. This increase in Ca^{2+} affects the dynamics of phosphorylase kinase, an enzyme activating the glycogen-degradating enzyme, namely glycogen phosphorylase (Fig. 6).

As the phosphorylation-dephosphorylation cascade involved in glycogen metabolism has been well characterized [55], the effect of Ca^{2+} oscillations on cellular regulation in the liver can be approached by a theoretical model [56]. Such an approach – in which a model for Ca^{2+} oscillations is coupled to a model for the control of glycogen metabolism in the liver – predicts that a given level of active phosphorylase kinase can be induced by lower average Ca^{2+} levels when Ca^{2+} oscillates (Fig. 7). This is easily intuitively understood, as during oscillations, Ca^{2+} can sometimes exceed the threshold for kinase activation, even if the average level of Ca^{2+} remains below the latter threshold. Thus, it looks as if Ca^{2+} oscillations could sensitize the phosphorylation-dephosphorylation cascade to low stimulation levels.

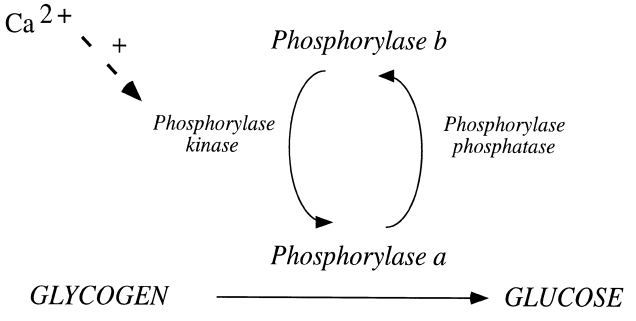

Fig. 6. Schematic representation of the pathway through which an increase in cytosolic Ca^{2+} provokes an enhanced glycogen degradation in hepatocytes.

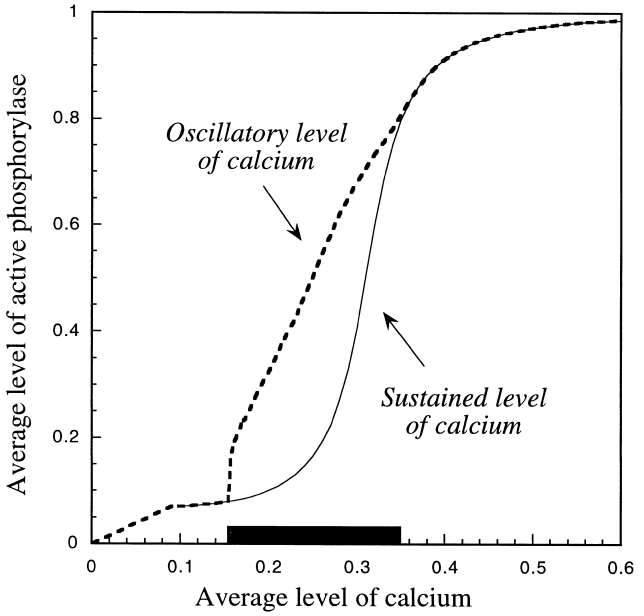

Fig. 7. Potentiation of a hormonal stimulation by Ca^{2+} oscillations, as predicted by a theoretical approach of the role of Ca^{2+} oscillations on glycogen degradation in hepatocytes. The mean level of Ca^{2+} increases with the level of stimulation. For the same average level of Ca^{2+}, the mean fraction of active phosphorylase (corresponding to phosphorylase a in Fig. 6) is higher when the level of Ca^{2+} oscillates (dashed curve) as compared to the situation of a sustained Ca^{2+} increase (plain curve). As the oscillatory domain of Ca^{2+} (indicated by the black bar) is bounded by two critical values of mean Ca^{2+}, the two curves are identical for low and high levels of stimulation. See [56] for equations and parameter values.

This theoretical prediction for glycogen degradation in the liver is in qualitative agreement with the results obtained by Dolmetsch et al. [57] as to the expression of transcription factors in lymphocytes. It would therefore be interesting to use similar experimental techniques to measure the effect of Ca^{2+} oscillations on glycogenolysis. If the theoretical prediction is correct, it could be concluded that, in addition to avoiding potential damage to the cell and increasing the robustness in signal detection at low levels of stimulation [58], Ca^{2+} oscillations in hepatocytes optimize the effect of hormonal stimulation.

The mitochondrial metabolic Ca^{2+} output has also been shown to be optimized by an oscillatory level of Ca^{2+} [59,60]. In hepatocytes, increases in cytosolic Ca^{2+} can indeed be rapidly transported into the mitochondrial matrix. This increase in mitochondrial Ca^{2+} in turn stimulates various mitochondrial dehy-

drogenases. As the uptake mode of the mitochondria is short-lived -probably to avoid mitochondrial Ca^{2+} overload-, a sustained Ca^{2+} increase in the cytosol only induces a transient increase in oxidative metabolism. In contrast, in the presence of cytoplasmic Ca^{2+} oscillations, the resulting mitochondrial Ca^{2+} oscillations are integrated and produce a sustained increase in NADH [59,60].

3. Calcium signaling at the multicellular level: intercellular Ca^{2+} waves

As in many other cell types, intracellular movements of Ca^{2+} in hepatocytes, induced by hormones and neurotransmitters, may be propagated from cell to cell creating an apparent intercellular wave. In a large number of different cultured cell types it is believed that intercellular calcium waves are mediated by the diffusion of a messenger through gap junctions [61]. Indeed, it has been shown in many cell types, among others in hepatocytes [62] and in epithelial cells of the respiratory tract [61,63] that mechanical stimulation of one cell can induce a Ca^{2+} increase in adjacent cells and that the inhibition of junctional coupling reduces or abolishes the propagation of intercellular calcium waves. Such a propagation most probably relies on the progressive diffusion of $InsP_3$ from the mechanically stimulated cell to its neighbors [61,64]. However, in more physiological conditions, in which cells are globally stimulated by Ca^{2+}-mobilizing agonists, the inhibition of junctional coupling results in the desynchronization of the calcium signals of the initially coupled cells [65–68].

3.1. Specificity of intercellular Ca^{2+} waves in hepatocytes

That junctional coupling is required for coordination of the Ca^{2+} responses is particularly true for multicellular rat hepatocyte systems – i.e., for doublets or triplets of hepatocytes connected by gap junctions [65–69] – where glycogenolytic agonists such as vasopressin or noradrenaline, induce tightly coordinated intracellular Ca^{2+} increases (Fig. 8A). Such coordination was also observed for a whole perfused liver [7,70,71]. Increasing the time resolution of the analysis revealed a sequential pattern of Ca^{2+} increases in the successive coupled cells creating the appearance of intercellular Ca^{2+} waves (Fig. 8B). However, a striking feature of these responses is that the order in which the cells respond is always the same for a given agonist, resulting in apparent unidirectional intercellular Ca^{2+} waves. This sequence of cellular responses is maintained when stimulation is repeated and does not depend on agonist concentration [72]. Such coordinated and sequential signals were also observed in the intact perfused liver in which vasopressin elicits waves of $[Ca^{2+}]_i$ increases running along hepatocyte plates across the lobules, at a dose-dependent speed of 20–120 μm s^{-1}. Although these waves propagate towards only one direction, the starting area of vasopressin-induced waves in the liver lobule remains a matter of controversy [70,71,73]. Thus, interhepatocyte Ca^{2+} waves, although elicited by global agonist stimulation, appear to be oriented in a *specific direction* in multiplets or in the perfused intact liver [7,70–73].

So far, unidirectional Ca^{2+} waves have only been observed in excitable cells. The latter cells are most often highly polarized such as neurons or heart cells and it is known that action potentials are propagated in one direction along a specific intercellular circuit. In neurons, it is due to asymmetrical chemical synapses and clustering of neurotransmitter receptors and ion channels. In heart cells, including cardiac pacemaker cells, it is thought to be ensured by the tissue micro-architecture and the distribution of gap junctions and ionic channels. In the liver, unidirectional Ca^{2+} waves could result from a gradually decreasing cellular sensitivity to hormonal stimuli from the first to the last responding cell. Indeed, it is well known that hepatocytes contribute unequally to various liver functions according to their position in the liver cell plate [74]. This hepatocyte heterogeneity is particularly well established for the metabolism of carbohydrates, amino acids and ammonia [75,76].

We have shown that hepatocytes isolated from periportal and perivenous areas, exhibited significantly different cellular sensitivities to the agonists. Also, one cell population could be more sensitive to one agonist but less sensitive to another. Periportal hepatocytes were more sensitive than perivenous hepatocytes to ATP; in contrast, the opposite was true for vasopressin, noradrenaline and angiotensin II

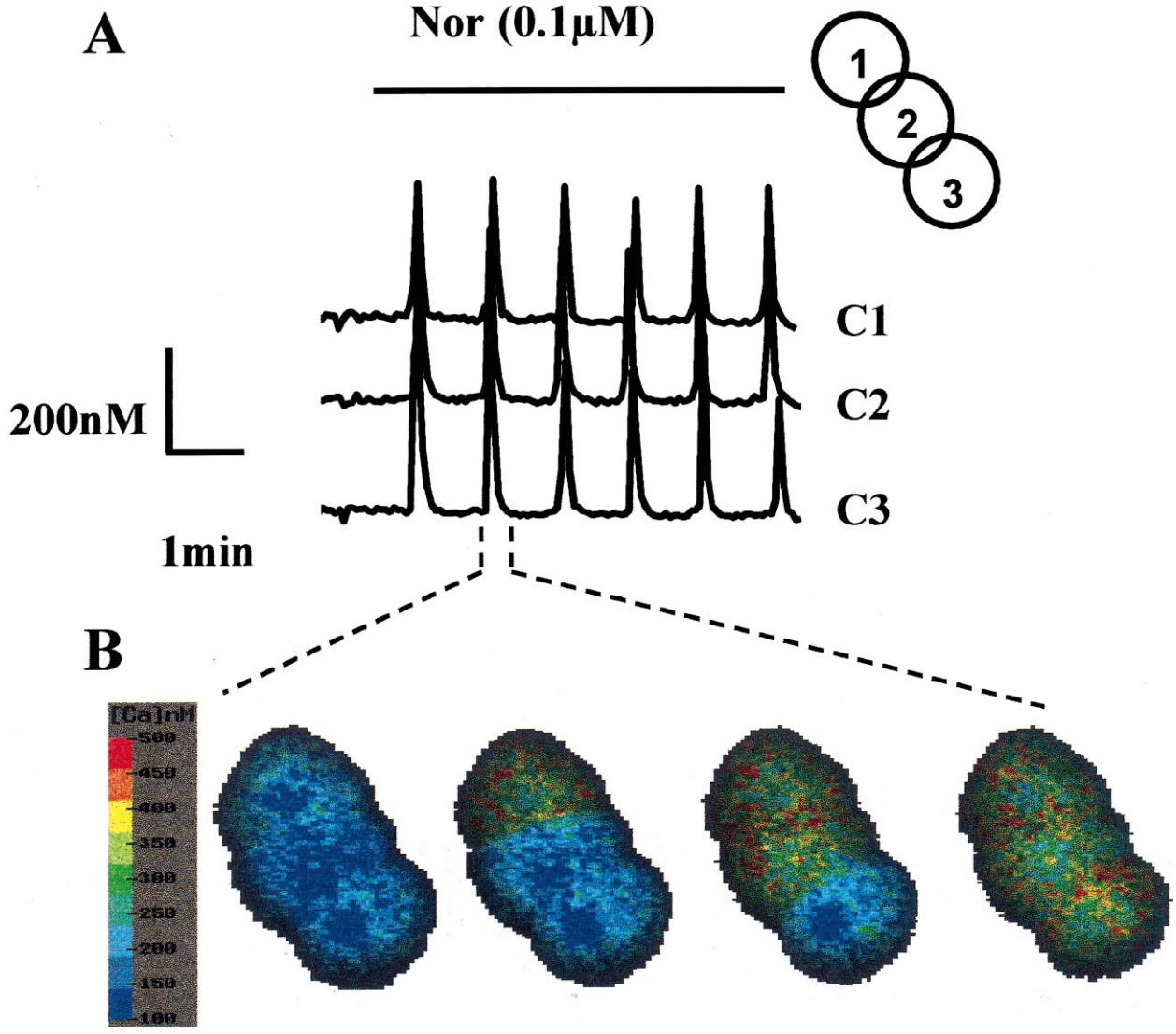

Fig. 8. Tightly coordinated Ca^{2+} oscillations and apparent intercellular calcium wave in a triplet of hepatocytes. Freshly isolated hepatocytes loaded with fura2 were challenged with $InsP_3$-dependent agonists (vasopressin, Vp, 0.1 nM in this case) for the time shown by the horizontal bars, as described in Combettes et al. [72]. (A) Addition of vasopressin to the bath induced tightly coordinated Ca^{2+} oscillations in the three connected cells. (B) Expended time resolution (1 image/700 ms) shows that spikes appear in a sequential manner, giving the appearance of an intercellular Ca^{2+} wave. Modified from [72].

[77,78]. The differences in Ca^{2+} responses to these agonists resulted from differences upstream from the G-protein; Ca^{2+} increases were indeed identical from cell to cell when the transduction pathway was stimulated immediately downstream from the hormonal receptors [78]. Also, the photolytic release of $InsP_3$ from caged $InsP_3$ in connected hepatocytes caused simultaneous Ca^{2+} responses in the connected cells, in contrast to the situation with vasopressin or noradrenaline, which induced a sequential Ca^{2+} increase. These results strongly suggested that heterogeneity in hormonal sensitivity between the periportal and perivenous populations of hepatocytes was due to differences in number or affinity of the hormonal receptors. Finally, binding experiments performed on periportal and perivenous hepatocyte membranes have shown a significantly higher density of vasopressin receptors in the perivenous area [78].

Similar qualitative results were obtained on hepatocyte multiplets. Hormone receptors cannot be

easily quantified for isolated cells. However, successive excisions of hepatocytes within a triplet, an experimental approach similar to that used in studies of pacemaker activity in cardiac sinoatrial cells, resulted in an increase in the time lag of the response to vasopressin or noradrenaline of the remaining cells [72]. Again, these results support the notion that there is a gradient in agonist sensitivity along the liver cell plate. Moreover, as indicated previously, they also suggest that the diffusion of an intercellular messenger may sensitize adjacent cells, accelerating their response.

3.2. Models for intercellular Ca^{2+} wave propagation in hepatocytes

The two most likely candidates that may flow through gap junctions and thereby coordinate Ca^{2+} spiking among adjacent hepatocytes are Ca^{2+} and $InsP_3$. As, at the present time, experiments do not allow to determine the nature of this intercellular messenger, this question has been approached by modeling. Thus, a simple model describing the Ca^{2+} dynamics in a pair of coupled hepatocytes characterized by different frequencies predicts that junctional calcium fluxes are effective in synchronizing calcium oscillations in coupled hepatocytes [79]. In this model, intrinsic frequencies of oscillations are supposed to rely on random variations in structural properties (cell size, shape or ER content) or $InsP_3$ levels. The author proposes that synchronization of Ca^{2+} spiking between adjacent cells can be achieved provided that the permeability of gap junctions is high enough. As intuitively expected, the value of the permeability coefficient needed to synchronize the cells should increase with the intrinsic differences between adjacent cells. It would be interesting to test with this model, and those values for the permeability coefficient, if focal stimulation of one cell of a doublet is unable to induce Ca^{2+} oscillations in the adjacent cells, as observed experimentally [67, 78].

Also, this model appears somewhat oversimplified by the way in which cytosolic Ca^{2+} diffusion and gap junctions are considered. As each cell is considered as an homogeneous system (intracellular Ca^{2+} diffusion is neglected), the amount of Ca^{2+} flowing through gap junctions is proportional to the differences between the average Ca^{2+} levels in both hepatocytes. In reality, this amount only depends on the differences between the Ca^{2+} concentrations at both sides of the plasma membrane [64]. The levels of Ca^{2+} in both modeled cells thus tends to equalize more than in real cells, where such an effect exists only locally. Thus, synchronization of Ca^{2+} spiking is probably artificially favored in such a model in which intracellular Ca^{2+} diffusion is neglected.

Another theoretical model developed to account for intercellular Ca^{2+} waves in connected hepatocytes favors the hypothesis that $InsP_3$ would be the primary coordinating messenger [80]. This model, schematized in Fig. 1, ascribes an essential role to the gradient of hormonal sensitivity which determines both the direction of wave propagation and the propagation velocity. In fact, this model proposes that such intercellular Ca^{2+} waves are only apparent in the sense that no Ca^{2+} does really flow from one cell to the other to generate the waves. The sensitivity gradient is responsible for the intrinsic different levels of $InsP_3$, which in turn induce different latencies of response to the hormonal stimulation. Although these differences are reduced by the passage of small amounts of $InsP_3$ through gap junctions, each hepatocyte of the multiplet displays repetitive Ca^{2+} spikes with a slight phase-shift with respect to its neighboring cell; such sequential spiking gives the appearance of a phenomenon of wave propagation, which is known as 'phase wave'. This model allowed us to perform theoretical predictions as to the effect of applying very low doses of stimuli or of inhibiting the gap junctions before the application of the hormone, which were confirmed experimentally [80].

This model for the propagation of intercellular calcium signals provides evidence for an unusual aspect of gap junctions. Indeed, usually, the gap junctions are thought to create a syncytium since molecules – less than 1.5 nm diameter or 1200 Da – are able to pass through the junction. In excitable cells (essentially neurons and smooth and myocardial muscle fibers), a major function of gap junctions is the rapid relay of currents generated at the plasma membrane by the passage of K^+, Na^+ or Cl^- ions between cells. This electrical coupling is involved in the synchronization of calcium signals in certain types of cell [81,82]. In unexcitable cells, the principal

role of the gap junctions is the exchange of signals and metabolites as ions (facilitating the electrical coupling of cells), second messengers (facilitating synchronization of cell function), metabolites and substrates (which may be involved in intercellular 'metabolic collaboration'). In the liver, one of the essential functions of gap junctions is the creation of a cytosolic pseudo-syncytium in each hepatocyte plate. This optimizes the response of the parenchyma to toxic or metabolic loads. It would therefore be logical to assume that, despite the heterogeneous microenvironment along the porto-centrilobular axis, any stimulation of hepatocytes (particularly by hormones) is likely to be 'homogenized' in the cytosolic syncytium of the hepatic plate. However, our results [66,72,78], suggest that the gap junctions of hepatocytes do not homogenize throughout the plate the signals generated in each cell in response to an agonist that mobilizes Ca^{2+}. Instead, they appear to coordinate the individual cellular responses. The gap junctions therefore 'respect' the individuality of the cell (differences in hormone sensitivity for example) even though the stimulus (e.g., hormonal) is applied to the entire hepatocyte plate. This is an essential condition for the existence of an apparent intercellular calcium wave in a system in which all the cells are uniformly stimulated by the agonist [78,80].

Such an organization should be of major functional advantage. In certain tissues, such as the liver, intercellular gradients may themselves support one or several functions. The capacity of the various cells to respond to a stimulus (globally applied to all the cells) in a particular order, despite the existence of junctional coupling, may allow the regulation of the direction of the hormonal response, from cell to cell. These functional characteristics may be of importance, not only in the liver, but also in other epithelial tissues, for the fine regulation of intercellular communication.

4. Calcium signaling at the subcellular level: Ca^{2+} blips and puffs

At the single cell level, Ca^{2+} accumulation during the rising phase of a Ca^{2+} peak is due to the Ca^{2+} flux produced by the simultaneous opening of several tens of $InsP_3$-sensitive channels. In order to synchronize their period of activity, these channels have to communicate with one another. Since the activation of an $InsP_3$-bound channel requires the binding of Ca^{2+} to the activatory sites facing the cytosolic medium, an open channel is capable of signaling its active state through the resulting local elevation of Ca^{2+} in the vicinity of a neighboring channel. The efficiency of this Ca^{2+}-mediated communication highly depends on the amount of calcium ions flowing through the open channel and on the rate at which these ions propagate in the cytosol. On the basis of electrophysiological studies [47] and of the measure of the Ca^{2+} concentrations in the ER [1], a physiological value of about 0.1 pA was estimated for the Ca^{2+} current of a single $InsP_3$-sensitive channel [83]. It is interesting to note that during the mean open time of the channel (about 3 ms), such a current generates a cytosolic Ca^{2+} mobilization of about 1000 ions. Amongst this thousand of ions, only a few of them (between 10 and 40, depending on the buffer capacity due to both endogenous buffers and Ca^{2+}-sensitive fluorescent dye) can freely diffuse and bind to a neighboring channel. As discussed in this section, such a low amount of free Ca^{2+}, hypothetically able to synchronize channels, can have, at most, a local effect.

The study of elementary Ca^{2+} signals induced by low $InsP_3$ concentrations and leading to subcellular Ca^{2+} rises has shed some light upon the spatial range over which interchannel communication may occur. Essentially two cell types, namely the *Xenopus* oocyte [84] and the HeLa cell [85] have been used for measuring these elementary $InsP_3$-induced Ca^{2+} signals. However, the general description of these events may likely apply to other electrically non-excitable cell types. Elementary Ca^{2+} signals have been first thought to outline two stereotypic classes of events: the blips, which would be true elementary events resulting from transient activation of a single channel, and the puffs exhibiting amplitudes about 5-fold higher that would be generated by concerted opening of a few clustered channels. As demonstrated by confocal microfluorimetry, these clustered channels formed a Ca^{2+} releasing site contained in a volume smaller than 1 μm^3, which was the limit of detection of this technique [86]. However, it was subsequently observed that a single Ca^{2+} releasing site was able to produce puff events exhibiting a whole spectrum of

Fig. 9. Stochastic simulation of the activity of a single $InsP_3$-sensitive Ca^{2+} channel. The model and the parameter values used to generate this pseudo-electrophysiological trace are the same as those used in Fig. 5 of [83]. The trace shows repetitive opening of the channel, which is supposed to be placed in a cytoplasmic-like environment, leading to an activity burst lasting about 100 ms and surrounded by silent periods.

amplitudes [85,87]. For both blips and puffs, Ca^{2+} rose in a few tens of milliseconds [85,86].

In order to explain the characteristics of these elementary Ca^{2+} signals, numerical simulations of a minimal model of the $InsP_3$-sensitive Ca^{2+} channels were performed, taking Ca^{2+} diffusion in a buffered cytosolic medium into account. Because the simulation had to consider a few number of channels (i.e., one channel for blips and about five channels for puffs), a stochastic approach was used, which allowed to follow the successive random transitions between the different channel states characterized by the numbers of $InsP_3$ and Ca^{2+} molecules bound to their respective binding sites (see inset of Fig. 4 and [83] for more details). Since the probability of transition was a function of the Ca^{2+} concentration in the vicinity of the channel, it was necessary to combine the simulation of the channel and the computation of the spatial distribution of Ca^{2+} concentration which depended on the Ca^{2+} flux through the open channel and on the Ca^{2+} diffusion in the cytosol.

This theoretical study predicted that an isolated $InsP_3$-bound channel would exhibit a prolonged period of activity resulting from the positive feedback exerted by the elevated Ca^{2+} concentration at the mouth of the channel (Fig. 9). Thus, the blip event, which lasts much longer than the mean open time of a single channel, can be explained by the Ca^{2+}-induced repetitive opening of the channel, a phenomenon that can be qualified of 'bursting'. The termination of such a bursting period is essentially controlled by the Ca^{2+}-sensitive inhibitory sites of the channel. Due to the combined effects of Ca^{2+} diffusion and buffering, the amplitude of a blip resulting from the repetitive opening of a single channel reaches a mean value of about 40 nM Ca^{2+} in a volume of 1 fl around the channel. In agreement with experimental observations, this Ca^{2+} increment is rather low as compared to a cytosolic basal concentration ranging from 40–100 nM. It was important to verify in which condition an open channel might induce the activation of other neighboring channels leading to puff production.

As observed in *Xenopus* oocytes [87] and in HeLa cells [8], the distribution of Ca^{2+} amplitudes of puffs presented a remarkable characteristic: at an $InsP_3$ concentration able to produce Ca^{2+} puffs involving the concerted activation of about five channels, the occurrence of blips was very rare, as if, once a channel opened, it readily recruited several other channels in the cluster. Such an efficient channel synchronization requires that, during the burst of activity of the leader channel (i.e., the first one to open), the Ca^{2+} signal is able in most cases to propagate up to a neighboring $InsP_3$-bound channel of the same cluster and activate it. This quantitative observation is fundamentally important: it allows to delineate the constraints that a channel cluster must meet in order for the probability that the first opened channel leads to the opening of the other $InsP_3$-bound channels of the cluster to be close to 1. A first constraint concerns the distance between two neighboring channels. As shown by simulation [88], the concerted synchronization of two channels in a cluster may occur with a sufficiently high probability only when the channels are in close contact, i.e., when the centers of the two channels are separated by a distance of 12 nm, which corresponds to the channel diameter. This theoretical prediction is explained by the low range of Ca^{2+}-mediated interchannel communication, as evoked above. This spatial constraint on the interchannel distance has in turn an interesting consequence concerning the prediction of the number of channels contained in a cluster. Remember first that in the experimental conditions producing Ca^{2+} puffs, whose amplitude is about 170 nM Ca^{2+} in a volume of 1 fl around the releasing site, the $InsP_3$ concentration is such that only about five channels of a cluster are $InsP_3$-bound. Also, we have just seen that it is necessary that at least one $InsP_3$-bound channel is adjacent to the leader active channel in order to allow synchronization. Since the five $InsP_3$-bound channels are randomly distributed in the cluster, the total number of channels in the cluster must be restricted

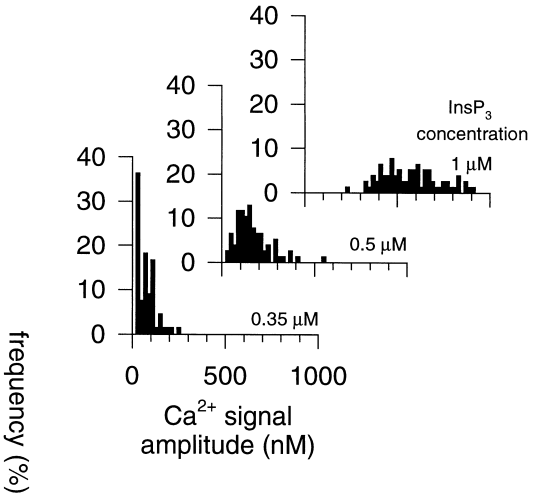

Fig. 10. Effect of $InsP_3$ concentration on the distribution of Ca^{2+} signal amplitudes arising from the activity of a 25 closely packed channel cluster. The model and the parameter values used in this stochastic simulation are the same as those used in Fig. 4 of [88]. At low $InsP_3$ concentration (0.35 μM), the Ca^{2+} signal amplitudes are low and blip events (characterized by an amplitude lower than 40 nM, i.e. generated by the opening of only one channel in the cluster) frequently occur. At higher $InsP_3$ concentrations, blip events are scarce even when only five channels on average participate in the Ca^{2+} signal (0.5 μM $InsP_3$), or virtually absent (1 μM $InsP_3$).

to some upper limiting value; if not, $InsP_3$-bound channels could be separated from each other by non-excitable $InsP_3$-free channels which would hinder the $InsP_3$-bound channels from effective communication through Ca^{2+}. In fact, simulations based on such a line of argument predict that a cluster likely consists of 20–30 channels [88]. Interestingly, this prediction is supported by the fact that the theoretical intercluster distance of 1.4 μm (calculated on the basis of the known density and distribution of $InsP_3$-receptors in the cytoplasm of *Xenopus* oocyte) is compatible with experimentally measured distances of about 2 μm [89]. It has to be noted that, based on the same hypotheses, the same simple calculation predicts, in the hepatocyte, an intercluster distance of 1 μm.

In an attempt to verify the theoretical predictions concerning the spatial organization of a Ca^{2+} releasing site, stochastic simulations were performed and demonstrated that a typical cluster made of 25 closely packed channels exhibited an efficient interchannel communication and thus, an adequate synchronization of the channels participating to a puff event [88], as it was experimentally observed. The distributions of Ca^{2+} signal amplitudes at different $InsP_3$ concentrations were also in agreement with experimental observations (Fig. 10). Clusters consisting of a higher number of channels or of more distant channels cannot explain the concerted activation of the channels.

The physiological consequence of the existence of such channel clusters is that a Ca^{2+} releasing site operates under the control of a local Ca^{2+} concentration present in a cytosolic domain surrounding the site. This domain has a linear dimension of about 60 nm, corresponding to the size of the cluster, and is essentially supplied by Ca^{2+} ions mobilized by the site. Thus, as far as blips and puffs of moderate amplitudes are concerned, the Ca^{2+} releasing site is an autonomous functional entity which works independently of the other sites present in the cell, essentially because the distance between two adjacent clusters (about 1 or 2 μm) is about one order of magnitude higher than the Ca^{2+}-mediated communication range. Such an autonomous functional entity consisting of the cytosolic space surrounding about 25 closely packed Ca^{2+} channels could be viewed as a rational basis for the operational concept of intermediate domain which was previously introduced in an empirical manner to account for the so-called 'incremental detection' phenomenon (see Section 2.3 above). From the large distance between adjacent channel clusters, one can reasonably anticipate that, whereas the interchannel communication within a cluster is controlled by the local Ca^{2+} concentration, the cluster synchronization necessary to initiate a Ca^{2+} wave develops because a number of active sites independently and randomly contribute to the global elevation of cytosolic Ca^{2+}. This description is in fact in complete agreement with experimental observations [9,85] showing that the initiation of a Ca^{2+} wave is preceded by elementary events which occur more and more frequently as global cytosolic Ca^{2+} increases.

In conclusion, elementary events like blips and puffs, as well as oscillations and waves are governed by the positive feedback exerted by Ca^{2+} ions on the activity of the $InsP_3$-sensitive Ca^{2+} channels. At the subcellular level, the activity of a Ca^{2+} releasing site results from the concerted opening of the $InsP_3$-bound channels, due to the local increase of Ca^{2+}

concentration in a cytosolic domain around the site. On the contrary, at the cellular level, oscillations and waves are apparently under the control of a global cytosolic Ca^{2+} signal produced by the non-concerted activation of a number of Ca^{2+} releasing sites. It can be tentatively proposed that the silent period between two Ca^{2+} peaks, i.e., the oscillation period, could be explained by the time required by the cell to produce enough puff events leading to the global Ca^{2+}-induced burning of all excitable sites. However, this proposal has not yet been theoretically substantiated by quantitative simulations when oscillation periods of the order of the minute are to be explained.

5. Conclusions

Hepatocytes appear as a prototypic cell type to study the spatio-temporal organization of intracellular Ca^{2+} as well as its possible physiological implications. This organization appears from the subcellular to the intercellular level and ranges from the micron (for the Ca^{2+} increase around a single channel or a small group of Ca^{2+} channels) to about 1 mm (for the intercellular wave through the liver lobule). Studies on single cells as well as on small group of connected hepatocytes (multiplets) have allowed a rather clear understanding of such apparently complex phenomena, thanks to the synergy provided by an experimental and a theoretical approach. As to the organization of intracellular Ca^{2+} dynamics, the mechanisms responsible for Ca^{2+} blips, puffs, oscillations and waves mainly rely on fine regulations at the level of the Ca^{2+}-releasing entities (the $InsP_3Rs$) and at the level of Ca^{2+} diffusion; concerning the intercellular organization, the existence of oriented Ca^{2+} waves seems to rely on both a gradient of hormonal sensitivity and the passage of small amounts of $InsP_3$ through gap junctions.

As to the physiological implications of such repetitive Ca^{2+} increases, Ca^{2+} oscillations could increase both the robustness and the efficiency of the signal induced by hormonal stimulation. Such effects even appear in a coordinated and oriented manner at the level of the liver lobule, thanks to the propagation of intercellular Ca^{2+} waves. Such waves could also allow the orientation of other vital functions of the liver such as biliary secretion or canalicular contraction.

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