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Dynamic Control of Inositol 1,4,5-Trisphosphate-Induced Ca^{2+} Release: A Theoretical Explanation for the Quantal Release of Ca^{2+}

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SUMMARY

A theoretical model has been elaborated in order to describe the kinetics of Ca^{2+} release induced by inositol 1,4,5-trisphosphate (IP_3). The model is based on the existence of a key molecule that controls the interconversion of open and closed forms of the Ca^{2+} channel. The model can quantitatively explain the previously obtained experimental observations that showed that a continuous IP_3 stimulus leads to a biphasic Ca^{2+} release and that successive IP_3 additions provoke repetitive bursts of Ca^{2+} release. Other published interpretations of these observations are discussed.

IP_3 propagates a specific hormone stimulus by mobilizing Ca^{2+} essentially from intracellular stores (see Ref. 1 for review). The membrane of these IP_3-sensitive vesicles contains Ca^{2+} channels composed of four identical monomers that specifically bind IP_3 (2). The IP_3-induced opening of the channel allows the passive transport of Ca^{2+} across the membrane of the vesicle. An ATP-driven Ca^{2+} pump ensures the refilling of the store. In the absence of ATP, the rate of Ca^{2+} efflux depends at least on the IP_3 concentration and on the gradient of free Ca^{2+} concentration across the membrane. Although a monoexponential decay curve of intravesicular Ca^{2+} is observed in the presence of the Ca^{2+} ionophore ionomycin, IP_3-induced Ca^{2+} release exhibits biphasic kinetics (3, 4); a short phase of fast Ca^{2+} release is followed by a prolonged phase of slow efflux, which is mainly accounted for by an IP_3-independent leak across the membrane. Moreover, the amount of Ca^{2+} released during the first phase, which thus represents the apparent size of the IP_3-sensitive Ca^{2+} store, depends on IP_3 concentration (3, 4). The term "quantal Ca^{2+} release" has been proposed (3) to describe the experimental fact that a submaximal IP_3 concentration mobilizes only a fraction of the stored Ca^{2+}. According to these observations, another study (5) showed that, when the ATP-driven Ca^{2+} pump is active, successive additions of IP_3 provoke short pulses of Ca^{2+} release, leading to the concept of "increment detection."

Biphasic IP_3-induced Ca^{2+} release observed in the absence of ATP may be explained by a transient activation of the Ca^{2+} channel, which could be due to a time-dependent decrease of IP_3 concentration or to desensitization of the IP_3 receptor. However, experimental observations do not support these interpretations (3–5). Two other explanations, based on the elaboration of equilibrium models, have been proposed and suggest either the existence of a heterogeneous population of Ca^{2+} stores with different sensitivities to IP_3 (3, 4) or the regulation of Ca^{2+} release by intravesicular Ca^{2+} (6). Our study shows that the latter explanation cannot account for the successive bursts of Ca^{2+} release induced by repetitive additions of IP_3 and that the model supporting the former explanation is unlikely because of its high degree of complexity.

The aim of this paper is to propose a model, devoid of unnecessary complexity, that can simulate the kinetic experimental observations on the IP_3-induced quantal Ca^{2+} release. This model involves a fast catalytic interconversion of open and closed forms of the Ca^{2+} channel. The net interconversion rate is controlled by a molecule that, as a sensor of the channel activity, should be able to remember that the Ca^{2+} efflux has been recently stimulated. For this purpose, it is assumed that the activation of this molecule resulting from the opening of Ca^{2+} channels is a slow catalytic process. Thus, the key feature of this model is the dynamic control of the number of open Ca^{2+} channels by cross-coupled catalytic processes. It is demonstrated by numerical simulations that the model is quantitatively compatible with the published evoked data.

Rationale

The model is based on the postulate that the transient IP_3-induced Ca^{2+} efflux is due to a conformational change of the IP_3 receptor-Ca^{2+}

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ABBREVIATIONS: IP_3, D-*myo*-inositol 1,4,5-trisphosphate; ISP_3, DL-inositol 1,4,5-trisphosphorothioate; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; GDPβS, guanosine-5′-*O*-(2-thio)diphosphate.

channel molecule. This molecule may thus exist in two interconvertible forms, termed A and B. The IP_3-bound forms are denoted A_I and B_I, respectively. The A_I form represents the open Ca^{2+} channel, whereas the B_I form is inactive despite the presence of IP_3. The biphasic kinetics of IP_3-induced Ca^{2+} release could result from a fast activation of the channel due the binding of IP_3 to the A form, followed by a slow conversion of the active A_I form to the inactive B_I form, leading to channel desensitization. This conversion should be reversible, in order to allow the resensitization of the system. However, the interconversion reaction must favor the accumulation of the B_I form at the expense of the A_I form, because the experimental observations have shown that the IP_3-dependent Ca^{2+} efflux virtually vanishes in the second phase of the Ca^{2+} release (3, 4). Therefore, the conversion B_I to A_I must be much slower than the conversion of A_I to B_I and, thus, active channel molecules cannot be rapidly mobilized because the resensitization process is slower than the desensitization process. Thus, as suggested by others (3–5), the classic concept of desensitization does not apply to the IP_3 receptor-Ca^{2+} channel system, because it cannot explain the experimentally observed succession of Ca^{2+} bursts induced by repetitive submaximal additions of IP_3 (5). As a consequence, one has to conclude that the interconversion rates are fast, despite the fact that the accumulation of the B_I form is slow. In order to fulfill these requirements, dynamic control of the interconversion rates is introduced in the model. This control is provided by a system that slowly increases its activity as long as the channel is in the active form A_I and that regulates the balance of the channel interconversion rates in order to favor the accumulation of the closed channel form.

The simplest description of such a system is given in Fig. 1. The model assumes the existence of a "memory" molecule M, which is slowly converted by the open channel A_I to an active form, M*. This form, M*, is capable of stimulating the rate, v_b, of B_I formation. Thus, this memory molecule is operative when it remembers that the channel has been recently activated. More explicitly, the Ca^{2+} flux across the vesicle membrane increases due to the fast accumulation of the A_I channel form induced by IP_3. At the same time, the active A_I form catalytically converts M to M*. M* slowly accumulates and accelerates the conversion of A_I to B_I, leading to the decrease of Ca^{2+} efflux. In response to the addition of a submaximal IP_3 concentration, the system reaches a steady state characterized by a low A_I concentration and, thus, by a low M* concentration. A further addition of IP_3 displaces the binding equilibrium in favor of B_I. Because the B_I concentration increases, the rate (v_a) of A_I formation may overwhelm the rate (v_b) of B_I formation and, thus, the excess B_I molecules are converted to the active A_I form, allowing an increase of Ca^{2+} efflux. More active M* molecules are then slowly produced, leading to a further delayed inactivation of the Ca^{2+} channel.

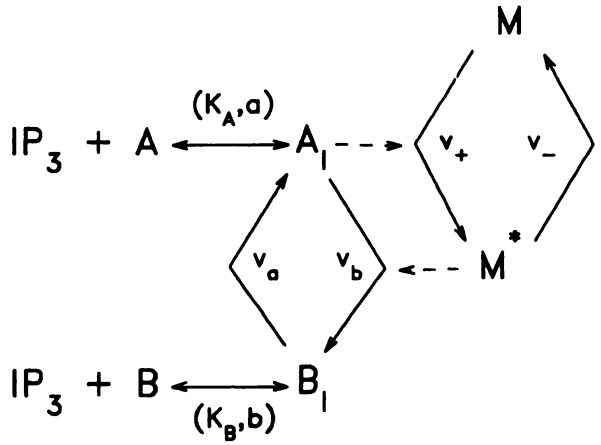

Fig. 1. Description of the dynamic control of IP_3-induced Ca^{2+} release by the memory molecule M. The binding of IP_3 to both forms A and B of the receptor obeys a Hill model characterized by a dissociation constant (K_A and K_B) and a Hill coefficient (a and b). The conversion of the active channel A_I to the closed form B_I is accelerated by the active form M* of the memory molecule. This molecule is activated by the active A_I form of the channel.

This description of the behavior of the proposed system requires a theoretical demonstration showing that the experimental kinetic observations can be explained by the model. For that purpose, numerical simulations must be performed on the basis of the mathematical equations associated with the reaction scheme.

It is assumed that the binding of IP_3 to both forms of the receptor is always at equilibrium and is described by the Hill equation:

$$A_I = \frac{A_{tot}}{r_a} \quad (1)$$

$$B_I = \frac{B_{tot}}{r_b} \quad (2)$$

with

$$r_a = 1 + (K_A/[IP_3])^a \quad (3)$$

$$r_b = 1 + (K_B/[IP_3])^b \quad (4)$$

A_{tot} is the total concentration of the channel in the A form; K_A and a are the equilibrium dissociation constant and the Hill coefficient of IP_3 binding to A, respectively. Equivalent notation is used for IP_3 binding to the B form (eqs. 2 and 4). The total concentration of the IP_3-sensitive channel is given by

$$Ch_{tot} = A_{tot} + B_{tot} \quad (5)$$

Because the interconversion rates v_a and v_b are fast, it is assumed that the steady state is instantaneously attained, i.e., $v_a = v_b$ at any time. For the sake of simplicity, it is assumed that the formation of A_I is a first-order process

$$v_a = k_a B_I \quad (6)$$

The rate of B_I formation, which is controlled by M*, obeys michaelian kinetics, characterized by the turnover number k_t and the Michaelis constant K_M:

$$v_b = \frac{k_t M^* A_I}{K_M + A_I} \quad (7)$$

On the basis of these equations, the concentration of A_I at any time can be computed by

$$A_I(t) = \frac{1}{2r_a}\left[-s + (s^2 + 4r_a K_M Ch_{tot})^{1/2}\right] \quad (8)$$

with

$$s = r_a K_M - Ch_{tot} + \frac{r_b k_t M^*(t)}{k_a} \quad (9)$$

The variation of M* concentration with respect to time is given by

$$\frac{dM^*}{dt} = v_+ - v_- \quad (10)$$

where v_+ and v_- are the rates of formation of active and inactive forms of the memory molecule, respectively. It is assumed that the inactivation of the molecule is a first-order process and that the concentration of the active form is always much lower than the total concentration of the memory molecule. Therefore,

$$v_- = k_- M^* \quad (11)$$

$$v_+ = k_+ A_I \quad (12)$$

Considering an initial concentration of M*, the numerical integration of eq. 10 gives $M^*(t)$ and thus $A_I(t)$, by using eqs. 8 and 9.

The kinetics of variation of the intravesicular Ca^{2+} concentration $Ca_{in}(t)$ are described by the differential equation that involves the

ATP-driven Ca^{2+} pump, the IP_3-stimulated Ca^{2+} efflux, and the Ca^{2+} leak. Assuming that the pump is a michaelian process characterized by the maximal velocity V_p and by the Michaelis constant K_p, one obtains

$$\frac{dCa_{in}}{dt} = \frac{V_p Ca_{ex}}{K_p + Ca_{ex}} - (k_c A_I(t) + k_l)Ca_{in} \qquad (13)$$

where k_c and k_l are the second-order constant of the IP_3-sensitive Ca^{2+} efflux and the first-order constant of the leak, respectively. Assuming that the total concentration of Ca^{2+} is constant and equal to Ca_{tot}, the cytosolic concentration Ca_{ex} can be replaced by $Ca_{tot} - Ca_{in}$. The numerical integration of eq. 13 gives $Ca_{in}(t)$ and thus $Ca_{ex}(t)$.

All numerical integrations are based on the fourth-order Runge-Kutta method.

Results and Discussion

The demonstration that the kinetic concepts involved in the proposed model may explain IP_3-induced quantal Ca^{2+} release requires that the experimentally observed data can be generated by the model. For that purpose, several kinetic experiments were considered, in particular, those of Taylor and Potter (4) describing the efflux of Ca^{2+} from permeabilized hepatocytes. These authors presented several decay curves for intravesicular Ca^{2+} content obtained with different concentrations of ISP_3, in the absence of ATP in order to avoid the refilling of the Ca^{2+} store (Fig. 2 of Ref. 4). Fig. 2 shows the results of the simulation. As expected, the model is able to generate biphasic kinetics of Ca^{2+} release. The amount of Ca^{2+} released during the first phase, i.e., the apparent size of the Ca^{2+} pool, is dependent on the concentration of ISP_3. Indeed, a higher ISP_3 concentration leads to a higher initial A_I concentration and, thus, to a higher initial Ca^{2+} efflux. It was also shown in the same experimental study (Fig. 4 of Ref. 4) that a further addition of ISP_3 in the second phase of the Ca^{2+} release previously induced by a submaximal ISP_3 concentration provokes a new fast Ca^{2+} efflux. This observation suggested that the quantal release is not due to IP_3 receptor desensitization. As expected, simulation showed that the model can quantitatively explain these data (Fig. 2). It has to be noted that the slope of the decay curve in the semilogarithmic representation does not significantly depend on the Ca^{2+} content of the store. Indeed, the addition of 10 μM ISP_3 at zero time or after 3.83 min leads to the same initial slope of the curve, although the Ca^{2+} contents at the beginning of the two stimulations are different because of the leak. Again, this theoretical result is compatible with the experimental observation (Figs. 2 and 4 of Ref. 4).

Biphasic IP_3-induced Ca^{2+} release was also observed in permeabilized RBL cells at 37° (5). As shown in Fig. 3, the model can simulate the reported experimental data obtained in the absence of Ca^{2+} reuptake by the store (Fig. 3 of Ref. 5). Using the same parameter values, the model can simulate the response of the IP_3-sensitive Ca^{2+} store to sequential additions of IP_3 when the ATP-driven pump is active. The theoretical curves shown in Fig. 4 correspond to different sequences of IP_3 additions and present successive bursts of Ca^{2+} release reminiscent of those observed in the experimental study (Fig. 5 of Ref. 5).

The interpretation of the evoked observations has led to other possible explanations. It was suggested (3) that the quantal Ca^{2+} release could be due to the existence of a heterogeneous population of IP_3-sensitive stores exhibiting different sensitivities to IP_3. Thus, at a given submaximal IP_3 concentration, the more sensitive stores may release their Ca^{2+} content, whereas the less sensitive stores cannot respond to the IP_3 stimulus. Because the IP_3-induced Ca^{2+} efflux observed in the second phase is not different from the basal Ca^{2+} efflux, it must be concluded that the stores respond in a strict all-or-nothing manner. This kind of behavior requires a highly sophisticated regulatory mechanism. Even a high degree of cooperativity associated with the binding of IP_3 would not be sufficient to meet this requirement. Moreover, the distribution of the sensitivity to IP_3 and of the Ca^{2+} content of the different stores should be under fine-tuned control of an undefined cellular mechanism, in order to account for the observed continuous

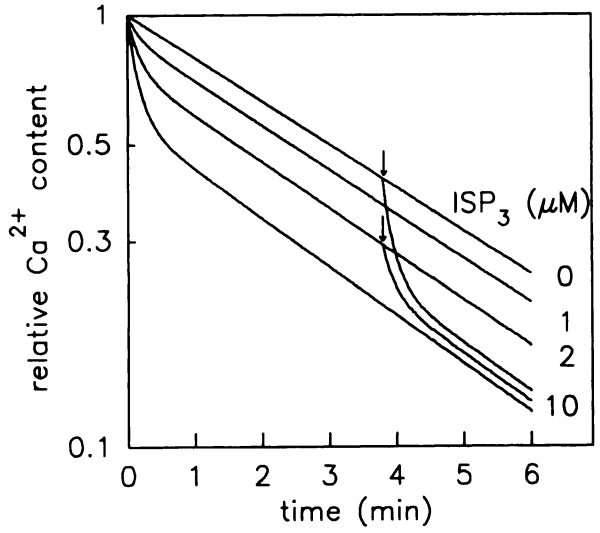

Fig. 2. Numerical simulation of Ca^{2+} efflux stimulated by ISP_3. ISP_3 (different concentrations from 0 to 10 μM, as indicated) is added at zero time. After 3.83 min (*arrows*), a further ISP_3 addition brings the final concentration to 10 μM. The parameters were chosen in order to generate decay curves compatible with experimental data obtained by Taylor and Potter (Figs. 2 and 4 of Ref. 4): $K_A = K_B = 2$ μM; $a = b = 2$; $k_l/k_e = 10{,}000$; $K_M/Ch_{tot} = 10^{-7}$; $k_+ = 0.0005$ min^{-1}; $k_- = 0.05$ min^{-1}; $k_cCh_{tot} = 3$ min^{-1}; $k_1 = 0.23$ min^{-1}; $V_p = 0$ (no Ca^{2+} reuptake).

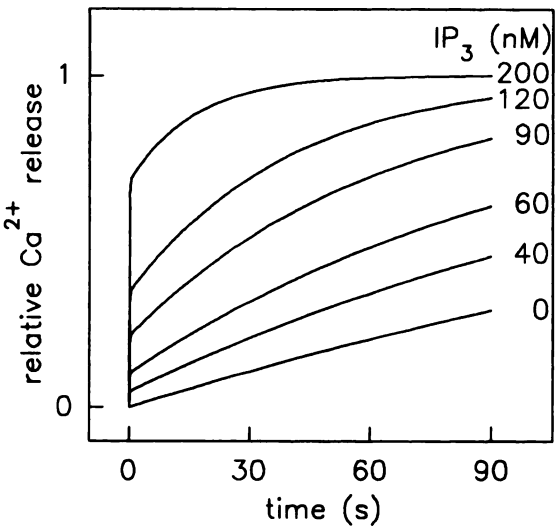

Fig. 3. Numerical simulation of Ca^{2+} efflux stimulated by IP_3. IP_3 (different concentrations from 0 to 200 nM, as indicated) is added at zero time. The parameters were chosen in order to generate Ca^{2+} release curves compatible with experimental data obtained by Meyer and Stryer (Fig. 3 of Ref. 5): $K_A = K_B = 1$ μM; $a = b = 2$; $k_l/k_e = 20{,}000$; $K_M/Ch_{tot} = 10^{-7}$; $k_+ = 0.0005$ sec^{-1}; $k_- = 0.05$ sec^{-1}; $k_cCh_{tot} = 300$ sec^{-1}; $k_1 = 0.0038$ sec^{-1}; $V_p = 0$ (no Ca^{2+} reuptake).

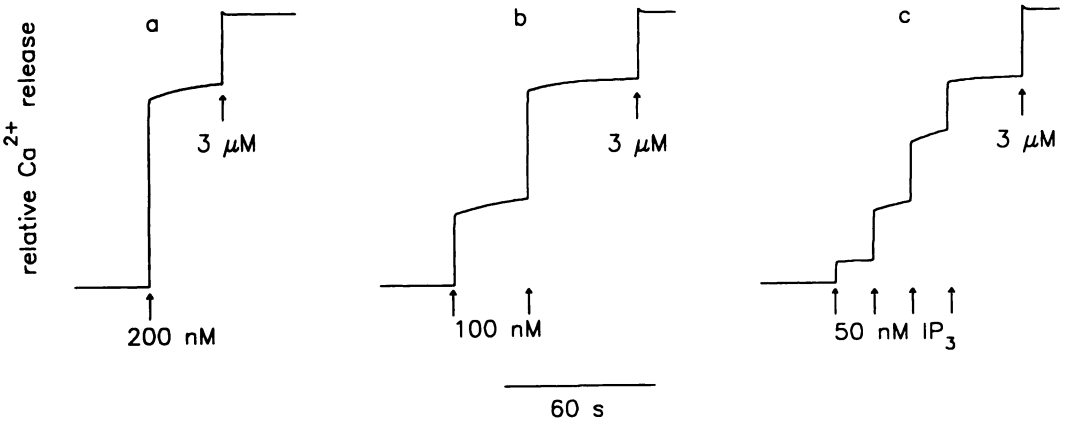

Fig. 4. Numerical simulation of Ca^{2+} efflux stimulated by sequential additions of IP_3. Different concentrations of IP_3 are added at different times (*arrows*). The Ca^{2+} store is virtually emptied with 3 μM IP_3. a, Single addition of 200 nM IP_3. b, Additions of 2 × 100 nM. c, Additions of 4 × 50 nM. The parameters were chosen in order to generate Ca^{2+} release curves compatible with experimental data obtained by Meyer and Stryer (Fig. 5 of Ref. 5): $K_A = K_B = 1$ μM; $a = b = 2$; $k_i/k_a = 20{,}000$; $K_M/Ch_{tot} = 10^{-7}$; $k_+ = 0.0005$ sec^{-1}; $k_- = 0.05$ sec^{-1}; $k_c Ch_{tot} = 300$ sec^{-1}; $k_1 = 0.0038$ sec^{-1}; V_p/Ca_{tot} 0.02 sec^{-1}; $K_p/Ca_{tot} = 0.3$.

relationship between rate of Ca^{2+} release and IP_3 concentration (5). The high degree of complexity implicitly contained in such a system renders this explanation rather unlikely.

Another proposed hypothetical model (6) assumed that the biphasic kinetics of Ca^{2+} release would be the consequence of control of the efflux by the intravesicular Ca^{2+} concentration. During the IP_3-induced Ca^{2+} release, the affinity of the IP_3 binding site would become lower because the intravesicular Ca^{2+} concentration decreases. In fact, numerical simulations have shown that this model can account for the biphasic kinetics (7). However, it also predicts that, for a given IP_3 concentration, the initial slope of the decay curve in the semilogarithmic representation should depend on the Ca^{2+} content of the store. This prediction is in contraction to the experimental observation (4) mimicked in Fig. 2 and discussed above.

The model presented in our study was initially proposed to account quantitatively for the experimental data obtained by Taylor and Potter (4) and by Meyer and Stryer (5). Because the previously reported interpretations seem to be unlikely or inadequate, this model would be a first acceptable theoretical explanation of the concepts of "quantal Ca^{2+} release" (3) and "increment detection" (5). The main feature of the model is the existence of dynamic control of the channel activity by a memory molecule. It was assumed that this molecule is activated by the open channel A_I and catalyzes the conversion of A_I to the closed form B_I. However, another possible model could involve a catalyst that converts B_I to A_I and that would be inactivated by A_I. Similar results could be obtained with both models. In both cases, the quantal Ca^{2+} release requires that the activity of the Ca^{2+} channel is controlled by catalytic processes. Interestingly, biphasic kinetics of IP_3-induced Ca^{2+} release in RBL cells can be observed at 37° but not at 11° (5). On the other hand, the IP_3-sensitive Ca^{2+} channel can be phosphorylated at different sites by specific protein kinases (8), suggesting a possible control of the channel activity by covalent modifications of the IP_3 receptor. However, it must be emphasized that the biphasic kinetics can be observed in the absence of ATP (4, 5). In this context, one can also mention the fact that GTP, but neither GTPγS nor GDPβS, abolishes the apparent desensitization of IP_3-induced Ca^{2+} release in pancreatic acinar cells (9).

The model attributes to the open form of the channel (A_I) a crucial role in the regulation of the activity of the memory molecule M. Because A_I induces the accumulation of M*, the activation of this molecule can be correlated with the Ca^{2+} flux and, thus, with the local cytosolic Ca^{2+} concentration in the vicinity of the channel. Therefore, an alternative description of the system could be that the rate of M activation is controlled by the local Ca^{2+} concentration. Such regulation is supported by experimental evidence (10) showing that Ca^{2+} may inhibit IP_3-induced Ca^{2+} release in AR42J cells and that this inhibition is more pronounced when the cells are exposed to a high Ca^{2+} concentration before stimulation by IP_3. It appears that some component of the system is able to remember that Ca^{2+} was recently mobilized.

In the proposed model, the memorization process consists of the catalytic activation of the memory molecule M responsible for the dynamic regulation of the accumulation of the closed form B_I. All the simulation results assumed that the IP_3 binding affinity is the same for both channel forms, i.e., $K_A = K_B$. However, experimental observations suggested that, in rat hepatocytes, the IP_3 binding component associated with the phase of fast Ca^{2+} efflux has a lower apparent affinity for IP_3 than the one associated with slow efflux (11) and that the IP_3 stimulation of Ca^{2+} release induces a reversible transformation of the receptor from a low affinity state, related to Ca^{2+} release, to a desensitized high affinity state (12). The concept of IP_3 receptor interconversion is confirmed by the Ca^{2+}-induced increase of IP_3 binding observed in liver membrane preparations (12). However, cerebellar membranes studied under the same experimental conditions showed a Ca^{2+}-induced decrease of IP_3 binding (12), suggesting that the change of binding affinity is perhaps not crucial for regulating Ca^{2+} efflux.

It has been proposed (5, 8, 12) that the IP_3 receptor interconversion and the biphasic IP_3-induced Ca^{2+} release could explain the sustained Ca^{2+} oscillations observed in many cell types (1). The concept of quantal Ca^{2+} release involves, in fact, a negative feedback loop that causes a transient mobilization of Ca^{2+} from IP_3-sensitive stores. However, theoretical simulation can show that the existence of a negative feedback loop is not sufficient to account for Ca^{2+} oscillations. An intracellular Ca^{2+} oscillator must also contain a positive feedback loop, such as, for instance,

the activation of Ca^{2+} release by intracellular Ca^{2+}. The concept of Ca^{2+}-induced Ca^{2+} release has been documented, especially in the case of IP_3-insensitive Ca^{2+} stores that contain ryanodine receptors (1). More recent observations showed that cytosolic Ca^{2+} may act as a coagonist of IP_3-induced Ca^{2+} release (13). The existence of such a positive feedback of Ca^{2+} on its own release has been shown to allow cytosolic Ca^{2+} oscillations (14).

In conclusion, this theoretical work proposes a plausible explanation for the quantal Ca^{2+} release from IP_3-sensitive Ca^{2+} stores and for other related kinetic properties. It predicts the existence of a memory molecule that controls the rate of channel interconversion and that is catalytically transformed when the channel is open. The complexity of the model due to the existence of cross-coupled catalytic interactions between this molecule and the receptor is reminiscent of β-adrenergic receptor desensitization induced by the phosphorylation of the receptor by β-adrenergic receptor kinase or by cyclic AMP-dependent protein kinase (15).

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