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# Calmodulin inhibits inositol 1,4,5-trisphosphate-induced calcium release through the purified and reconstituted inositol 1,4,5-trisphosphate receptor type 1

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**Abstract Our previous studies have demonstrated that calmodulin binds to $IP_3R$ type 1 ($IP_3R1$) in a $Ca^{2+}$ dependent manner, which suggests that calmodulin regulates the $IP_3R1$ channel. In the present study, we investigated real-time kinetics of interactions between calmodulin and $IP_3R1$ as well as effects of calmodulin on $IP_3$-induced $Ca^{2+}$ release by purified and reconstituted $IP_3R1$. Kinetic analysis revealed that calmodulin binds to $IP_3R1$ in a $Ca^{2+}$ dependent manner and that both association and dissociation phase consist of two components with time constants of $k_a = 4.46 \times 10^2$ and $> 10^4$ $M^{-1}$ $s^{-1}$, $k_d = 1.44 \times 10^{-2}$ and $1.17 \times 10^{-1}$ $s^{-1}$. The apparent dissociation constant was calculated to be 27.3 μM. The $IP_3$-induced $Ca^{2+}$ release through the purified and reconstituted $IP_3R1$ was inhibited by $Ca^{2+}$/calmodulin, in a dose dependent manner. We interpret our findings to mean that calmodulin binds to $IP_3R1$ in a $Ca^{2+}$ dependent manner to exert inhibitory effect on $IP_3R$ channel activity. This event may be one of the mechanisms governing the negative feedback regulation of $IP_3$-induced $Ca^{2+}$ release by $Ca^{2+}$.**



*Key words:* Calmodulin; Inositol 1,4,5-trisphosphate; Inositol 1,4,5-trisphosphate receptor; Surface plasmon resonance

## 1. Introduction

Inositol 1,4,5-trisphosphate ($IP_3$) receptor ($IP_3R$), an $IP_3$-induced $Ca^{2+}$ releasing channel located on intracellular $Ca^{2+}$ stores, plays a crucial role in a variety of cell functions, including fertilization, cell proliferation, metabolism, secretion, contraction of smooth muscle and neural signals [1,2]. $IP_3$-induced $Ca^{2+}$ release (IICR) is regulated by cytosolic free $Ca^{2+}$, in a biphasic manner [3,4]. Open probability of $IP_3R$ induced by $IP_3$ showed a bell-shaped curve, depending on cytosolic $Ca^{2+}$ concentrations, determined using the cerebellar microsomal fraction incorporated into lipid bilayer. Increasing cytosolic $Ca^{2+}$ concentration up to 300 nM increased the open probability of $IP_3R$, whereas higher $Ca^{2+}$ concentration led to inhibition [3]. In addition, rapid increases in $Ca^{2+}$ concentration induced by flash photolysis of the caged $Ca^{2+}$ during IICR immediately altered the rate of IICR, in either a positive or negative manner. This positive or negative feedback regulation depended on increases in cytosolic $Ca^{2+}$. Although the bell-shaped $Ca^{2+}$ dependence of $IP_3$-induced $Ca^{2+}$ release is considered to be fundamental to generate a complex pattern of spatio-temporal $Ca^{2+}$ increase in cells, such as $Ca^{2+}$ wave and $Ca^{2+}$ oscillation, the mechanism of the bell-shaped dependence of IICR has remained unknown. The inhibitory effects of cytosolic $Ca^{2+}$, however, were absent, when $IP_3R$ was purified and reconstituted [5,6]. In addition, inhibitory effects of cytosolic $Ca^{2+}$ on IICR depend on the isoform of $IP_3R$, in which high concentrations of $Ca^{2+}$ inhibited $IP_3R$ type 1 ($IP_3R1$) and $IP_3R$ type 2 ($IP_3R2$), but not $IP_3R$ type 3 ($IP_3R3$) [7]. These observations suggest that such effects by $Ca^{2+}$ on IICR may mediate effect of some $Ca^{2+}$ sensing proteins.

Our previous studies demonstrated that $IP_3R1$ binds calmodulin in a $Ca^{2+}$ dependent manner suggesting that calmodulin regulates the function of $IP_3R1$ [8,9]. The calmodulin binding domain identified in $IP_3R1$ is conserved in $IP_3R$ type 2 ($IP_3R2$) but not in $IP_3R3$ [9]. To test the thesis that calmodulin may regulate $IP_3R1$ channel function, we investigated kinetics of interaction between $IP_3R1$ and calmodulin, using Biomolecular Interaction Analysis system, BIAcore 2000 TM (BIAcore AB). This system is a biosensor which makes use of the phenomenon of the surface plasmon resonance and enables us a real-time analysis of specific protein-protein interaction. Effects of calmodulin on the purified and reconstituted $IP_3R1$ channel activity were also investigated.

## 2. Materials and methods

### 2.1. Materials

The following reagents were purchased. $IP_3$ from Dojindo Laboratories (Kumamoto, Japan), Chelex-100 from Bio-Rad Laboratories (Hercules, CA, USA) and $^{45}Ca^{2+}$ from NEN (Boston, MA, USA). All the other reagents were of analytical grade or the highest grade available.

### 2.2. Purification of $IP_3R$ type 1 ($IP_3R1$) and calmodulin

$IP_3R1$ was purified type specifically from mouse cerebellar microsomal fractions, using an immunoaffinity column conjugated with a polyclonal antibody against $IP_3R1$ as described [10]. Calmodulin was

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*Abbreviations:* $IP_3$, D-*myo*-inositol 1,4,5-trisphosphate; $IP_3R$, $IP_3$ receptor; $IP_3R1$, $IP_3R$ type 1; $IP_3R2$, $IP_3R$ type 2; $IP_3R3$, $IP_3R$ type 3; HEPES, *N*-(2-hydroxyethyl) piperizine-*N*′-2-ethanesulfonic acid; EGTA, ethylene glycol-bis(β-amino-ethyl ether) *N*,*N*,*N*′,*N*′-tetra acetic acid; IICR, $IP_3$-induced $Ca^{2+}$ release; NHS, *N*-hydroxysuccinimide; EDC, *N*-ethyl-*N*′-(3-diethylaminopropyl)-carbodiimide

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purified from bovine brain by TCA precipitation and column chromatography as described previously [11].

*2.3. Immobilization of the purified $IP_3R1$ on a sensor chip*

The purified $IP_3R1$ was covalently immobilized on the surface of a sensor chip through its polysaccharide chain in order to maintain the same orientation against the sensor chip, according to the manufacturer's protocol (BIAcore AB) (Fig. 1). Briefly, the purified $IP_3R1$ was oxidized with 1 mM sodium metaperiodate in 100 mM sodium acetate buffer pH 5.5 for 20 min on ice to introduce aldehyde groups into the polysaccharide chain. The reaction mixture was passed over the desalting column, NAP5 (Amersham Pharmacia Biotech), to remove unreacted reagents. The NHS/EDC-activated carboxymethylated dextran sensor chip, CM5 (BIAcore AB), was reacted with 5 mM hydrazine hydroxide to introduce hydrazide on the sensor chip, and the residual unreacted esters were inactivated with 1 M ethanol amine hydrochloride. The activated sensor chip was then covalently conjugated with the oxidized $IP_3R1$, by aldehyde coupling. Following the conjugation, the resulting hydrazone bond was reduced with 100 mM of sodium cyanoborohydride in 10 mM sodium acetate buffer pH 4.5 to form a stable hydrazide bond. All solutions used in these procedures contained 0.5% Triton X-100.

*2.4. Kinetic analysis of interaction between $IP_3R1$ and calmodulin*

The interaction between $IP_3R1$ and calmodulin was investigated using BIAcore 2000 (BIAcore AB) in running buffer (100 mM KCl, 1 mM 2-mercaptoethanol and 20 mM HEPES/KOH 7.4) supplemented with 2 mM $CaCl_2$ or 2 mM EGTA at 25°C. For the association phase, various concentrations of calmodulin were injected over the surface of the $IP_3R1$ sensor chip with a flow rate of 30 μl/min for 60 s. Dissociation of the calmodulin from the immobilized $IP_3R1$ was then observed. The amount of binding protein is represented as an arbitrary unit (resonance unit, RU). There is a linear relationship between the mass of protein bound and the observed RU (1000 RU = 1 ng/mm$^2$).

*2.5. Reconstitution of purified $IP_3R1$ and $IP_3$-induced $^{45}Ca^{2+}$ influx measurements*

The purified $IP_3R1$ was reconstituted into liposomes as described [10]. $IP_3$-induced $^{45}Ca^{2+}$ influx into the purified and reconstituted $IP_3R$/liposome was measured as described [10] in the presence of 200 μM free $Ca^{2+}$ and 10 μM $^{45}Ca^{2+}$ together with various concentrations of calmodulin. The $IP_3R1$/liposome was incubated with 1 μM of $IP_3$ for 1 min at room temperature in order to induce $Ca^{2+}$ influx into the liposome. After removal of extravesicular $Ca^{2+}$ and $^{45}Ca^{2+}$ by Chelex-100, $^{45}Ca^{2+}$ trapped inside the liposome was measured using a liquid scintillation counter.

## 3. Results and discussion

$IP_3$-induced $Ca^{2+}$ release (IICR) is regulated by cytosolic free $Ca^{2+}$ in a biphasic manner [3,4], a process considered to be a fundamental mechanism for generation of a complex spatio-temporal $Ca^{2+}$ increase in cells, such as $Ca^{2+}$ wave and $Ca^{2+}$ oscillation. The inhibitory effects of cytosolic $Ca^{2+}$ were absent or faint when $IP_3R1$ was purified and reconstituted [5,6], which suggests the possibility that such effects may mediate some $Ca^{2+}$ sensing proteins. Recently, it was reported that the bell-shaped dependence of $Ca^{2+}$ of IICR depended on the isoform of $IP_3R$, in which inhibitory effects of high concentration of $Ca^{2+}$ were observed in both $IP_3R1$ and $IP_3R2$ but not in $IP_3R3$ [7]. Calmodulin is a $Ca^{2+}$ binding/sensing protein which exerts cellular function in response to $Ca^{2+}$ increase by binding to target proteins. Earlier studies demonstrated that $IP_3R1$ binds calmodulin in a $Ca^{2+}$ dependent manner [8,9], which suggests that calmodulin regulates the function of $IP_3R1$. To examine the possibility that calmodulin regulates $IP_3R1$ channel function, we investigated kinetics of the interaction between $IP_3R1$ and calmodulin and effects of calmodulin on the purified $IP_3R1$ channel activity.

To study kinetics of the interaction, we used Biomolecular Interaction Analysis system, BIAcore 2000 TM (BIAcore AB), which allows one to analyze a real-time kinetics of specific protein-protein interactions. We analyzed kinetics of the interaction between $IP_3R1$ and calmodulin by injecting various concentrations of calmodulin in the presence or absence of $Ca^{2+}$ on the $IP_3R1$ immobilized sensor chip.

Fig. 1 shows the scheme of preparation of the orientation specific immobilization of $IP_3R1$ on sensor chip. As the $IP_3R1$ has *N*-glycosylation sites within the luminal portion (residues 2475 and 2503 of mouse $IP_3R1$) [12], immobilization of $IP_3R1$ through its sugar chain could maintain $IP_3R1$ in the same orientation against the sensor chip. The $IP_3R1$ sensor chip prepared using this procedure was stable for repetitive use and responses were reproducible.

To examine effects of the concentration of $IP_3R1$ immobilized on binding responses, we prepared three flow cells with different amounts of $IP_3R1$. Flow cells (Fc) 1, 2, and 3 were conjugated with different amounts of $IP_3R1$ by changing reaction time during aldehyde coupling, and Fc4 served as a blank (without $IP_3R1$). For the association phase, calmodulin was injected into the Fc1–Fc4 for 60 s with a flow rate of 30 μl/min. The dissociation of calmodulin from the immobilized $IP_3R1$ was then observed. Fig. 2A shows typical sensorgrams of the interaction between the immobilized $IP_3R1$ and 10 μM

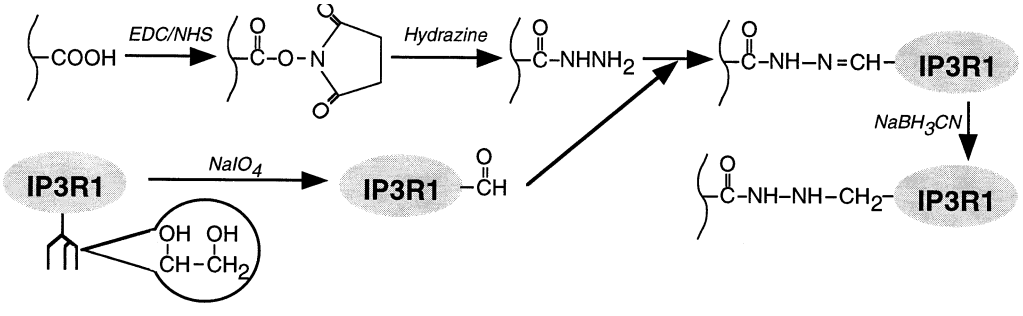


Fig. 1. Schematic representation of the orientation specific immobilization of $IP_3R1$. Schematic representation of the reaction for the immobilization of $IP_3R1$ through the polysaccharide chain. Carboxymethylated dextran sensor chip, CM5 (BIAcore AB) was activated by a series of the reactions with EDC/NHS and hydrazine to introduce hydrazide on the sensor chip. The purified $IP_3R1$ was oxidized with metaperiodate to introduce aldehyde groups to the polysaccharide chain located on the luminal portion of $IP_3R1$. $IP_3R1$ was then immobilized on the sensor chip by aldehyde coupling, which followed reduction of hydrazone bond by cyanoborohydride. This procedure allows one to immobilize $IP_3R1$ in the same orientation.

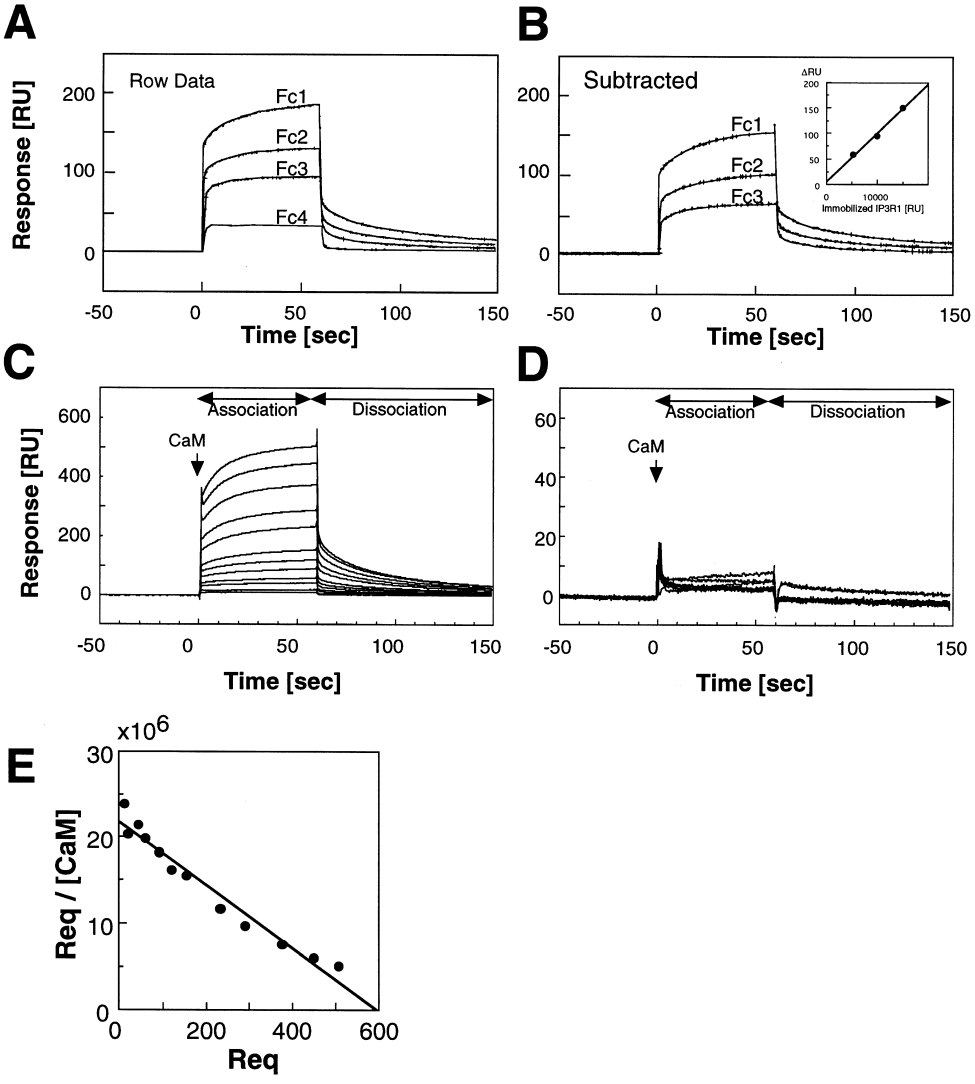


Fig. 2. Sensorgram for calmodulin binding to immobilized $IP_3R1$. A: Typical sensorgram for calmodulin binding. Calmodulin (10 μM) was injected over three flow cells (Fc1, 2 and 3) immobilized with different concentrations of $IP_3R1$ and one flow cell (Fc4) without $IP_3R1$ as blank in the presence of 2 mM $Ca^{2+}$. B: Net sensorgrams were obtained by subtracting the blank (Fc4) from each profile (Fc1, 2 and 3). Inset: The ΔRU values of the end of injections were plotted against the concentration of the immobilized $IP_3R1$ to examine the crowded effect of immobilized $IP_3R1$ on the sensor chip. C: Net sensorgram for different concentrations of calmodulin (from bottom to top; 0.5, 1.0, 2.0, 3.0, 5.9, 7.5, 10, 20, 30, 50, 75, 100 μM of calmodulin injected) in the presence of $Ca^{2+}$. D: Net sensorgram for different concentrations of calmodulin (from bottom to top; 0.5, 1.0, 2.0, 3.0, 5.9, 7.5, 10, 20, 30, 50, 75, 100 μM of calmodulin injected) in the absence of $Ca^{2+}$. E; Schatchard plot. Equilibrium responses (Req) of each concentration of calmodulin were obtained by curve fitting of the association phase. Apparent dissociation constant of 27.3 μM for calmodulin $IP_3R1$ was calculated from the slope of Scatchard plot.

of calmodulin in the presence of $Ca^{2+}$. Net sensorgrams were obtained by subtracting the blank (Fc4) as non-specific binding from each profile (Fc1, 2 and 3) (Fig. 2B). Specific calmodulin binding to the $IP_3R1$ was observed and the response in resonance unit (RU/arbitrary unit) depended on the amount of $IP_3R1$ immobilized. The ΔRU values (net response) of the end of injections were plotted against the concentration of the immobilized $IP_3R1$ (Fig. 2B, inset), in which ΔRU values are proportional to the amounts of $IP_3R1$ immobilized, confirming there is no crowded effect of immobilized $IP_3R1$ on the interaction. We then used Fc2 for further kinetic analysis.

Various concentrations of calmodulin (from 0.5 to 100 μM) were injected over the $IP_3R1$ immobilized flow cell in the presence or absence of $Ca^{2+}$ (Fig. 2C and D). In the presence of $Ca^{2+}$, calmodulin binds to $IP_3R1$, in a dose dependent manner (Fig. 2C), whereas, in the absence of $Ca^{2+}$, there was no specific binding of calmodulin to $IP_3R1$ (Fig. 2D). These results indicate that calmodulin binds to $IP_3R1$, in a $Ca^{2+}$ dependent manner, thus confirming our previous observation. On the contrary to our observation, $Ca^{2+}$ independent calmodulin binding to $IP_3R1$ and $Ca^{2+}$ independent inhibition of $IP_3$ by calmodulin were reported [13,14]. Patel et al. reported that calmodulin binds to partially purified cerebellar $IP_3R$, in both $Ca^{2+}$ dependent and independent manner [13]. They measured $IP_3R$/calmodulin binding kinetics by using a scintillation proximity assay system under the condition of a low ionic strength (20 mM PIPES pH 7.2, 1 mM EGTA, 5 mM $K_2HPO_4$, 0.1% Surfact-Amps X-100, 1% BSA and 0–

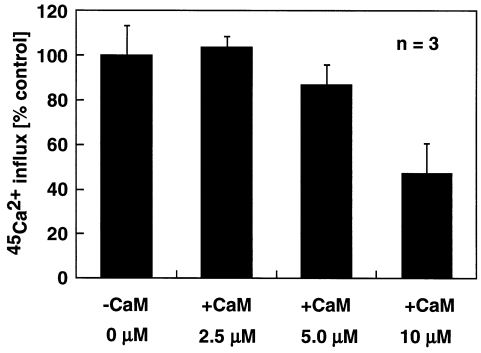


Fig. 3. Calmodulin inhibits $IP_3$-induced $Ca^{2+}$ release, in a dose dependent manner. Effects of $Ca^{2+}$/calmodulin on $IP_3$-induced $Ca^{2+}$ release were investigated using purified and reconstituted $IP_3R1$. $IP_3$-induced $Ca^{2+}$ influx into the liposome was measured in the presence of various concentrations of calmodulin. The $IP_3R1$ reconstituted liposome was incubated with 1 μM of $IP_3$ for 1 min to induce $Ca^{2+}$ influx into liposome. $^{45}Ca^{2+}$ trapped in the liposome was measured after removal of extravesicular $Ca^{2+}$ by Chelex-100.

1 mM $CaCl_2$). Simpa et al. reported that calmodulin inhibited $IP_3$ binding to a recombinant ligand binding domain of $IP_3R1$ (N-terminal 581 amino acids) in the absence of $Ca^{2+}$ [14]. They suggested that the N-terminal $IP_3$ binding region of $IP_3R1$ might contain a $Ca^{2+}$ independent calmodulin binding site. However, this effect was completely abolished by changing pH from 7.0 to 7.8. The reason for the discrepancy on the $Ca^{2+}$ dependency of calmodulin binding to the $IP_3R1$ may be due to a different buffer composition or system used.

Kinetic analysis of the binding curves revealed that both the association and dissociation phases consist of two components with fast and slow time constants. From slopes of dRU/d$t$ versus RU plot of each profile, apparent time constant for slow association phase, $k_{a(slow)}$, was calculated to be $4.46\times10^2$ $M^{-1}$ $s^{-1}$. Time constant for fast phase, $k_{a(fast)}$, was not determined, because it is too rapid to measure ($>10^4$ $M^{-1}$ $s^{-1}$). In case of the dissociation phase, the sensorgram of each dissociation phase is well fitted by a biexponential curve. Time constants were calculated to be $k_{d(slow)} = 1.44\times10^{-2}$ and $k_{d(fast)} = 1.17\times10^{-1}$ $s^{-1}$ as mean of values at different concentrations of calmodulin. As the precise time constant for fast association phase was not determined, we calculated the apparent dissociation constant for $Ca^{2+}$/calmodulin-$IP_3R1$ to be 27.3 μM, based on equilibrium responses (Fig. 2E). This value is lower than the previously determined value of 700 nM for the calmodulin binding peptide of $IP_3R1$ and calmodulin. The reason for this difference may be due to different materials used, i.e. purified and immobilized $IP_3R1$ was used in this study. The low affinity binding of calmodulin to $IP_3R1$ but with fast association/dissociation kinetics enables one to speculate that feedback regulation of $IP_3R1$ by $Ca^{2+}$ could be mediated by $Ca^{2+}$/calmodulin.

To investigate effects of $Ca^{2+}$/calmodulin on $IP_3R1$ channel activity, $IP_3$-induced $^{45}Ca^{2+}$ influx was measured in the presence of various concentrations of calmodulin (Fig. 3). In the absence of calmodulin, the $IP_3$-induced $^{45}Ca^{2+}$ influx by the purified and reconstituted $IP_3R1$ was observed at high concentration of free $Ca^{2+}$ (210 μM). In the presence of calmodulin, however, this $IP_3$-induced $^{45}Ca^{2+}$ influx was apparently inhibited in a calmodulin dose dependent manner, thereby strongly indicating that one of the pathways of $Ca^{2+}$ feedback inhibitory regulation of $IP_3R1$ could be mediated by $Ca^{2+}$ calmodulin. Miyakawa et al. reported that both $IP_3R1$ and $IP_3R2$ mediated $Ca^{2+}$ release exhibited a clear biphasic $Ca^{2+}$ dependence, whereas $IP_3R3$ showed a flatter $Ca^{2+}$ dependence [7]. If inhibition of IICR by $Ca^{2+}$/calmodulin is a mechanism of negative feedback regulation, this isoform specific inhibitory effect of high $Ca^{2+}$ concentration on IICR might be explained by $Ca^{2+}$/calmodulin binding property to $IP_3R$. Our previous studies demonstrated that the calmodulin binding domain identified in $IP_3R1$ was conserved in $IP_3R$ type 2 ($IP_3R2$) but not in $IP_3R3$ and that $Ca^{2+}$/calmodulin binds to both $IP_3R1$ and $IP_3R2$ but not to $IP_3R3$ [9,15]. Therefore, we propose that inhibitory effects of high $Ca^{2+}$ concentrations on IICR are mediated by $Ca^{2+}$/calmodulin in an isoform of $IP_3R$ specific manner.

Recently, Missiaen et al. reported that the bell-shaped $Ca^{2+}$ dependence of the IICR is modulated by $Ca^{2+}$/calmodulin [16]. They demonstrated that IICR in permeabilized A7r5 cells is inhibited by calmodulin at cytosolic free $Ca^{2+}$ concentrations of 0.3 μM or higher. At a cytosolic free $Ca^{2+}$ concentration of below 0.3 μM, this inhibitory effect was not observed, indicating that inhibitory effects mediated by calmodulin on IICR are $Ca^{2+}$ dependent. In addition, we observed that the inhibitory effect of high concentration of $Ca^{2+}$ on the open probability of cerebellar $IP_3R1$ is abolished by addition of calmodulin antagonist, W-7, indicating that inhibitory phase could be mediated by $Ca^{2+}$/calmodulin [17]. Our study using purified and reconstituted $IP_3R1$ strongly supports these observations of inhibitory effect of $Ca^{2+}$/calmodulin on IICR, and provides new evidence that $Ca^{2+}$/calmodulin directly regulates the $IP_3R1$ channel activity with rapid binding kinetics. Therefore, inhibitory effects of $Ca^{2+}$/calmodulin could be one of the fundamental mechanisms of immediate negative feedback regulation of IICR by $Ca^{2+}$ which could generate a complex spatio-temporal pattern of $Ca^{2+}$ increase such as $Ca^{2+}$ wave and $Ca^{2+}$ oscillation.

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## References

[1] Berridge, M.J. (1993) Nature 361, 315–325.
[2] Mikoshiba, K. (1997) Curr. Opin. Neurobiol. 7, 339–345.
[3] Bezprozvanny, I., Watras, J. and Ehrlich, B.E. (1991) Nature 351, 751–754.
[4] Iino, M. and Endo, M. (1992) Nature 360, 76–78.
[5] Hirota, J., Michikawa, T., Miyawaki, A., Furuichi, T., Okura, I. and Mikoshiba, K. (1995) J. Biol. Chem. 270, 19046–19051.
[6] Thrower, E.C., Lea, E.J. and Dawson, A.P. (1998) Biochem. J. 330, 559–564.
[7] Miyakawa, T., Maeda, A., Yamazawa, T., Hirose, K., Kurosaki, T. and Iino, M. (1999) EMBO J. 18, 1303–1308.
[8] Maeda, N., Kawasaki, T., Nakade, S., Yokota, N., Taguchi, T., Kasai, M. and Mikoshiba, K. (1991) J. Biol. Chem. 266, 1109–1116.
[9] Yamada, M., Miyawaki, A., Saito, K., Nakajima, T., Yamamoto-Hino, M., Ryo, Y., Furuichi, T. and Mikoshiba, K. (1995) Biochem. J. 308, 83–88.
[10] Nakade, S., Rhee, S.K., Hamanaka, H. and Mikoshiba, K. (1994) J. Biol. Chem. 269, 6735–6742.
[11] Yazawa, M., Sakuma, M. and Yagi, K. (1980) J. Biochem. (Tokyo) 87, 1313–1320.

[12] Michikawa, T., Hamanaka, H., Otsu, H., Yamamoto, A., Miyawaki, A., Furuichi, T., Tashiro, Y. and Mikoshiba, K. (1994) J. Biol. Chem. 269, 9184–9189.
[13] Patel, S., Morris, S.A., Adkins, C.E., O'Beirne, G. and Taylor, C.W. (1997) Proc. Natl. Acad. Sci. USA 94, 11627–11632.
[14] Sipma, H., De Smedt, P., Sienaert, I., Vanligen, S., Missiaen, L., Parys, J.B. and De Smedt, H. (1999) J. Biol. Chem. 274, 12157–12162.
[15] Cardy, T.J. and Taylor, C.W. (1998) Biochem. J. 334, 447–455.
[16] Missiaen, L., Parys, J.B., Weidema, A.F., Sipma, H., Vanlingen, S., De Smedt, P., Callewaert, G. and De Smedt, H. (1999) J. Biol. Chem. 274, 13748–13751.
[17] Michikawa, T., Hirota, J., Kawano, S., Hiraoka, M., Yamada, M., Furuichi, T. and Mikoshiba, K. (1999) Neuron, in press.