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Involvement of the brain type of ryanodine receptor in T-cell proliferation

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Abstract Cloning and sequence analysis of cDNA showed that the brain type of ryanodine receptor (RYR) is expressed in human Jurkat T-lymphocyte cells. Fura-2 measurements revealed that the RYR in T-cells functions as a ryanodine-sensitive, caffeine-insensitive Ca^{2+} release channel. Furthermore, ryanodine stimulated proliferation and altered the growth pattern of cultured human T-cells when added together with FK506.

Key words: Ryanodine receptor; cDNA cloning; Calcium release; Cell proliferation; FK506; Jurkat T-lymphocyte

1. Introduction

Ca^{2+} release from endoplasmic reticulum stores is critical in the regulation of a diverse range of cellular functions [1]. The ryanodine receptor (RYR) is abundantly present in skeletal muscle and is one of the major Ca^{2+} release channels [2,3]. Cloning and sequence analysis of complementary DNA has revealed the primary structure of the RYR from skeletal muscle [4,5] as well as from heart [6,7]. Recently we have cloned and sequenced cDNA of the third type of RYR from rabbit brain [8]. The skeletal muscle RYR is suggested to be directly coupled with the voltage-gated calcium channel, whereas the cardiac RYR is activated by Ca^{2+} itself, causing Ca^{2+}-induced Ca^{2+} release (CICR) from intracellular stores [2,9]. The third type of RYR is expressed also in smooth muscle tissues [8], but its functional properties have not been characterized.

Several lines of evidence have suggested that CICR occurs not only in excitable cells but also in inexcitable cells [10,11]. However, little is known about the molecular mechanism of CICR in inexcitable cells. Also it has been recently reported that the RYR in skeletal muscle is tightly associated with FK506-binding protein (FKBP), the cytosolic receptor for the immunosuppressive agent FK506 [12], and that calcium release of the RYR is modulated by FK506 [13]. We report here that the brain type of RYR is functionally expressed in human Jurkat T-lymphocyte cell line. We also studied effects of ryanodine and FK506 on cell growth.

2. Materials and methods

2.1. Reverse PCR analysis and cloning of partial cDNAs from T-cells

cDNA of Jurkat lymphocytes was synthesized as previously [8]. Its PCR analysis was carried out through 40 cycles at 95°C for 1 min, 55°C for 2 min and 72°C for 3 min. The upstream primer, 5′-dT-TCATCCTGCTGTTTTATAAGGT-3′, corresponding to nucleotide residues 13,724–13,746, and the downstream primer, 5′-dCA-GATGAAGCATTTGGTCTCCAT-3′, is complementary to 14,860–14,882 of the skeletal muscle RYR cDNA [4]. These sequences are common to the rabbit cardiac and brain RYRs [6–8] as well as the human skeletal muscle RYR [5]. RYR cDNAs used as control were pNRR42 [8] for the rabbit brain RYR, pHRR12 [6] for the rabbit cardiac RYR and pSRR3 for the rabbit skeletal muscle RYR; pSRR3 is a pBluescript SK(−) recombinant carrying nucleotide residues 13,481–15,230 of the rabbit skeletal muscle RYR cDNA [4]. PCR products were resolved on a 1.2% agarose gel and were subjected to Southern blot analysis, which was by the procedure of Southern [14] except that the filters were washed at 50°C with 0.1 × SSC containing 0.1% SDS. cDNA probes used were as follows; the 2.0-kb *Eco*RI(vector)/*Eco*RI(vector) fragment derived from pBRR110 [8], the 4.7-kb *Eco*RI(vector)/*Eco*RI(vector) fragment from pHRR12; the 1.7-kb *Eco*RI(vector)/*Eco*RI(vector) fragment from pSRR3.

cDNA cloning was carried out as previously described [8]. A cDNA library derived from poly(A)$^+$ RNA of Jurkat T-cells were screened using the reverse PCR product as a probe. Sequencing was carried out on both strands as previously described except that and A.L.F. DNA Sequencer II (Pharmacia LKB) was used in some cases. The amino acid sequence was deduced by using the open reading frame corresponding to the amino acid sequence of the rabbit brain RYR [8].

2.2. Ca^{2+} release from intracellular calcium stores

The Ca^{2+} release experiments were performed as described previously [15,16]. 5×10^5 of Jurkat cells loaded with Fura-2-AM in a cuvette were tested for response to caffeine and ryanodine. The fluorescence signal was monitored with the excitation wavelength alternating at 340 and 380 nm, using an intracellular ion analyzer (CAF-110, JASCO). The fluorescence baseline was not stable enough to allow reliable measurements in Jurkat cells preincubated with FK506.

2.3. Effects of ryanodine and FK506 on cell growth

Jurkat T-cells were seeded at 5×10^5/well in 200 μl of RPMI 1640 medium/10% fetal bovine serum in flat-bottomed 96-well plates. FK506 was dissolved in methanol at a concentration of 10 mM. Methanol at low concentrations did not affect the measurements. Ryanodine, FK506 and [^{3}H]thymidine were added at the initiation of the cultures. Assays were performed in duplicate and repeated at least four times. Cells were photomicrographed after 36 h. Uptake of [^{3}H]thymidine was measured after between 36 and 48 h of culture.

3. Results

Southern blot analysis of reverse PCR products from Jurkat T-cells, amplified using common primers for the rabbit skeletal muscle, cardiac and brain RYRs, revealed that an RNA species hybridizable with the brain RYR cDNA is expressed in human T-lymphocytes (Fig. 1). Northern blot analysis of mRNA also showed a very small amount of hybridizable RNA species of about 16 kb, a size similar to those observed for the RYRs [4–8]

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Abbreviations: RYR, ryanodine receptor; CICR, calcium-induced calcium release; FKBP, FK506-binding protein; $[Ca^{2+}]_i$, intracellular calcium concentration.

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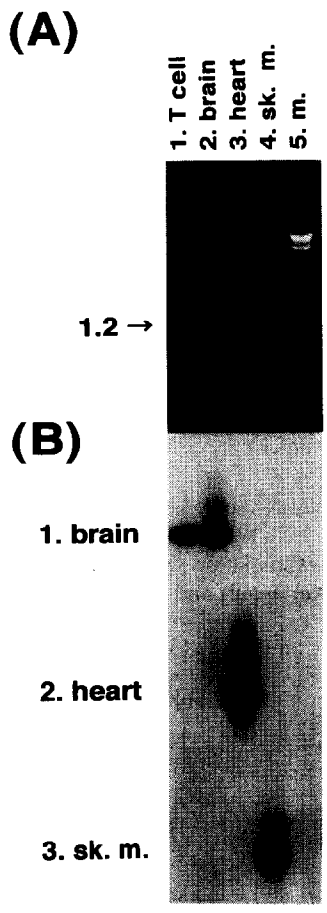

Fig. 1. mRNA of the brain-type RYR is expressed in human Jurkat T-cells. Ethidium stained agarose gel (A) and Southern blot analysis (B) of reverse PCR products of Jurkat T-cell RNA (lane 1) and PCR products of the rabbit brain (lane 2), cardiac (lane 3) and skeletal muscle (sk. m.) (lane 4) RYR cDNAs. cDNA probes used in (B) were specific for the brain (B-1), cardiac (B-2) and skeletal muscle (B-3) RYRs. The size markers (m.) (lane 5) were the *Hin*dIII cleavage products of phage λ DNA (sizes in kilobases). Autoradiography was performed at −70°C for 12 h with an intensifying screen.

(data not shown). To identify the hybridizable RNA species present in human T-cells, we screened a cDNA library derived from poly(A)$^+$ RNA of Jurkat T-cells using the reverse PCR product as a probe. Several of the cDNAs cloned contained an open reading frame of 928 amino acid residues, which corresponds to the sequence of the C-terminal one fifth of the RYRs (Fig. 2). Comparison of the deduced amino acid sequence with those of the rabbit brain, cardiac and skeletal muscle RYRs reveals 95%, 66% and 61% amino acid identities, respectively. The amino acid sequence from T-cells is almost identical to that of the rabbit brain RYR in the region containing the four putative transmembrane segments (M1–M4) [4,6,8], whereas the two sequences are relatively divergent in the putative modulator binding region immediately preceding the M1 segment [4,17]. This minor diversity probably results from the species difference, since the 3′ non-coding nucleotide sequences are also homologous to each other (data not shown). From the similarity, we conclude that a human counterpart of the rabbit brain RYR is expressed in Jurkat T-cells.

To confirm functional expression of the RYR in T-cells, first we measured changes in $[Ca^{2+}]_i$ in response to application of ryanodine (Fig. 3). Application of ryanodine (200 μM) caused a slow increase in $[Ca^{2+}]_i$ of long duration. Interestingly, caffeine (10 mM) failed to evoke a similar response, while both ryanodine and caffeine always resulted in a clear rise in $[Ca^{2+}]_i$ in control experiments where we used CHO cells transfected with the cardiac RYR cDNA [15] (data not shown). Secondly, we studied effects of ryanodine on growth of Jurkat T-cells. When ryanodine was added into culture medium, cell growth was only minimally stimulated as judged by cell count and [^{3}H]thymidine uptake, although cells showed a tendency to form large clusters (Fig. 4). However, when an immunosuppressive agent FK506 was added in culture medium at a low concentration (1 μM) where FK506 itself does not affect cell growth significantly, ryanodine exerted a biphasic effect on cell growth as its concentration was increased. When the concentration of ryanodine was low (100 μM), [^{3}H]thymidine uptake was markedly increased and extensive cluster formation was observed. Cluster formation appeared correlated well with

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Human  EFQKAMEGQKQYTQSEIDFLLSCAEADENDMFNYVDFVDRFHEPAKDIGFNVAVLLTNLSEHMPNDSRLK   70
Rabbit ..............................I.................................... 4014

Human  CLLDPAESVLNYFGPYLGRIEIMGGAKKIERVYFEISESSRTQWEKPQVKESKRQFIFDVVNEGGEQEKM  140
Rabbit .............E..................................................... 4084

Human  GLFVNFCEDTIFEMQLASQISESDSADRPEEEEEDEDSSYVLEIAGEEEEDGSLEPASAFAMACASVKRN  210
Rabbit E.............................G..E.......N......K.F.S.........L... 4154

Human  VTDFLKRATLKNLRKQYRNVKKMTAKELVKVLFSFFWMLFVGLFQLLFTILGGIFQILWSIVFGGGLVEG  280
Rabbit I.NL.RK.....................F..............F...V..................... 4224
Mink                                                ..........N.......... 22

Human  AKNIRVTKILGDMPDPTQFGIHDDTMEAERAEVMEPGITTELVHFIKGEKGDTDIMSDLFGLHPKKEGSL  350
Rabbit .....................A.........A.A.........V...R...EL.............GV 4274
Mink   .....................ALD......T...VPP..I..V......A........F......G. 92

Human  KHGPEVGLGDLSEIIGKDEPPTLEGTVQKKRKAQAAEMKAANEAEGKVESEKADMEDGEKEDKDKEEEQA  420
Rabbit .....................S..R.........T..EH.............L.........A....R. 4364
Mink   .....G.................S..R.............H.......P..T.L.......TA..... 162

Human  EYLWTEVTKKKKRRCGQKVEKPEAFTANFFKGLEIYQTKLLHYLARNFYNLRFLALFVAFAINFILLFYK  490
Rabbit ....A.........R.........M..................................................  4434
Mink   .A..AD........R.........M.....------------------------------------------  203
                                                    └──── M1 ────┘

Human  VTEEPLEEETEDVANLWNSFNDEEEEEAMVFFVLQESTGYMAPTLRALAIIHTIISLVCVVGYYCLKVPL  560
Rabbit .....................L.....................VV.....................  4504
Mink   .....V..............T.....................A.........V.....................  273
                                                  └──────── M2 ────────┘

Human  VVFKREKEIARKLEFDGLYITEQPSEDDIKGQWDRLVINTPSFPNNYWDKFVKRKVINKYGDLYGAERIA  630
Rabbit .....................................................H.....................  4574
Mink   ..........................................................................V 343

Human  ELLGLDKNALDFSPVEETKAEAASLVSWLSSIDMKYHIWKLGVVFTDNSFLYLAWYTTMSVLGHYNNFFF  700
Rabbit .....................T.....................................................  4644
Mink   ................AS..........................................................  413

Human  AAHLLDIAMGFKTLRTILSSVTHNGKQLVLTVGLLAVVVYLYTVVAFNFFRKFYNKSEDDDEPDMKCDDM  770
Rabbit .....................................................................  4714
Mink   .....................................................................  483
                                                  └──────── M3 ────────┘

Human  MTCYLFHMYVGVRAGGGIGDEIEDPAGDPYEMYRIVFDITFFFFVIVILLAIIQGLIIDAFGELRDQQEQ  840
Rabbit .....................................................................  4784
Mink   .....................................................................  553
                                                              └──── M4 ────┘

Human  VREDMETKCFICGIGNDYFDTTPHGFETHTLQEHNLANYLFFLMYLINKDETEHTGQESYVWKMYQERCW  910
Rabbit .....................................................................  4854
Mink   .....................................................................  623

Human  DFFPAGDCFRKQYEDQLG                                                    928
Rabbit .................                                                    4872
Mink   .................                                                    641
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Fig. 2. Partial amino acid sequence (in one-letter code) of human Jurkat T-cell RYR (top), deduced from the cDNA sequence, and its alignment with those of the rabbit brain RYR [8] (middle) and the mink lung epithelial cell RYR [10] (bottom). Numbers of the amino acid residues at the right-hand end of the individual lines are given. Dots represent residues identical to those of the T-cell RYR. Gaps (−) have been inserted to achieve maximum homology and a continuous stretch of gaps was counted as one substitution regardless of its length. The putative transmembrane segments M1–M4 are indicated; the termini of each segment are tentatively assigned by comparison with the rabbit RYRs [4,6,8].

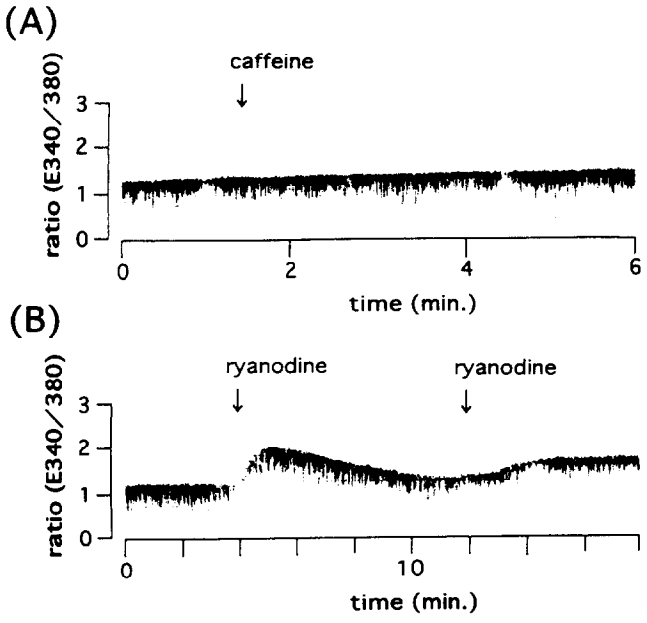

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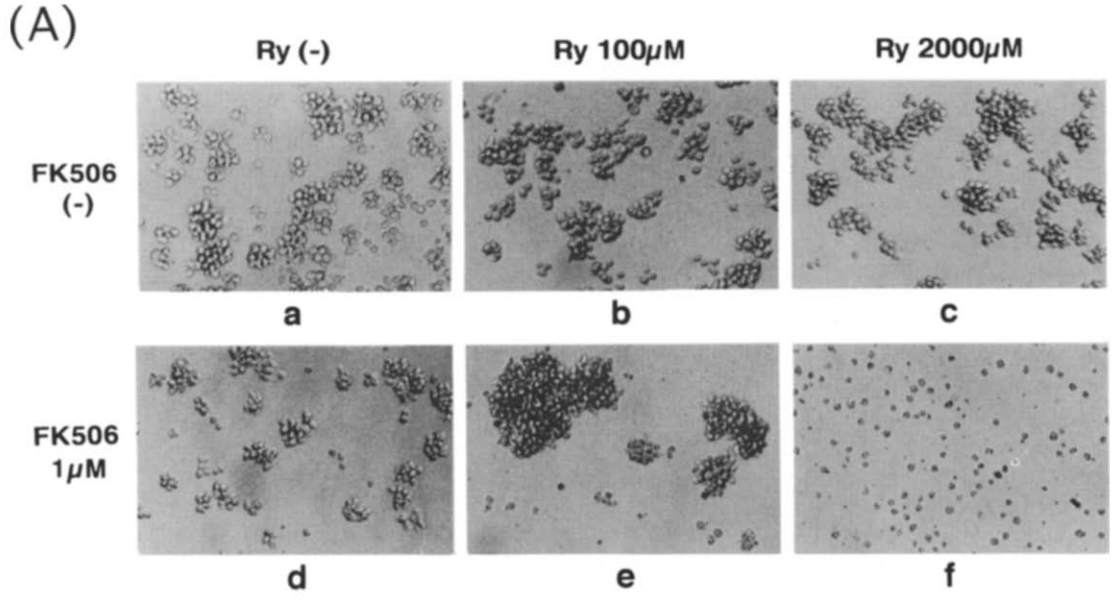
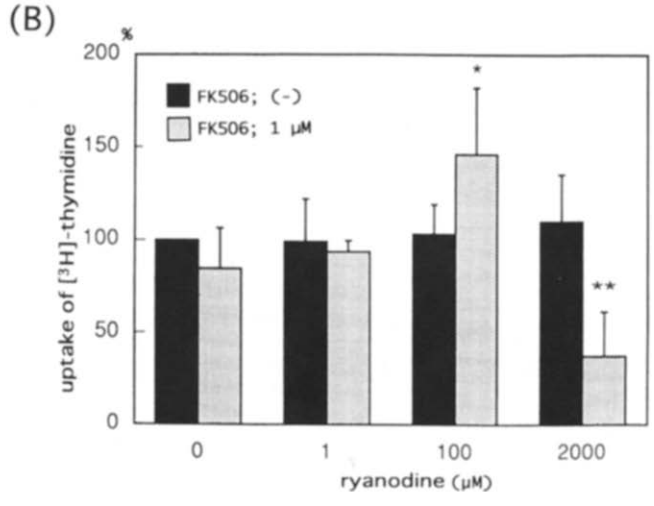
Fig. 3. Response of intracellular $[Ca^{2+}]_i$ in Jurkat T-cells to application of caffeine (A) and ryanodine (B). Whereas caffeine caused no effect, ryanodine evoked an increase in $[Ca^{2+}]_i$ of long duration. Subsequent application of ryanodine caused an increase smaller in size, presumably because of depletion of Ca^{2+} in intracellular stores. Addition of caffeine and ryanodine is indicated by arrows. The trace shown is raw data and not corrected for baseline shift. The peak change in fluorescence ratio caused by the first application of ryanodine roughly corresponds to 150 nM in $[Ca^{2+}]_i$.

[^{3}H]thymidine uptake. Cell growth was almost completely suppressed at a higher concentration of ryanodine (2,000 μM). When the concentration of FK506 was higher (10 μM), cell growth was suppressed regardless the concentration of ryanodine (data not shown).

4. Discussion

Our present results show that the brain type of RYR is expressed in Jurkat T-cells and functions as a ryanodine-sensi-





tive, caffeine-insensitive Ca^{2+} release channel. The RYR has been identified in skeletal [2], cardiac [9], smooth muscles [18] and neurons [19,20]. Although a direct interaction between the voltage-gated calcium channel and the RYR has been suggested in skeletal muscle, it is generally accepted that the RYR contributes to CICR from intracellular stores in these excitable cells [2,9]. However, CICR is not specific to excitable cells but has been observed or suggested in inexcitable cells, including lung epithelial cells [10] and T-cells [11]. Failure to recognize importance of the RYR in inexcitable cells may be partly accounted for by the fact that the brain type of RYR is insensitive to caffeine [10], which has been considered as a defining agent of the RYR [2]. In inexcitable cells, the RYR is presumably activated by Ca^{2+} transients and contributes to maintaining an elevated $[Ca^{2+}]_i$. Recently several lines of evidence have shown that cyclic ADP-ribose is a second messenger to release Ca^{2+} from intracellular calcium stores [21–23] and that CD38, a surface antigen of human lymphocytes, has a cyclic ADP-ribose forming activity [24,25]. It is an intriguing possibility that CD38 and cyclic ADP-ribose contribute to a Ca^{2+} signalling pathway unique to lymphocytes.

Increase in $[Ca^{2+}]_i$ is necessary for lymphocyte proliferation [26,27]. Ca^{2+} activates the Ca^{2+}/calmodulin-dependent protein phosphatase, calcineurin, which in turn affects transcription of the interleukin-2 gene [28]. FK506, a potent immunosuppressive agent highly effective in preventing rejection in human organ transplantations [29], binds to a family of FKBP, and the resulting complexes inhibit calcineurin, thereby blocking T-cell activation [28,30]. Recently it has been reported that the skeletal muscle RYR is tightly associated with FKBP [12] and that calcium release channel activity is modulated by FK506, which reduces the caffeine threshold required for CICR [13]. Together with these findings, our present results suggest a possibility that interaction between FK506 and the RYR takes place also in T-cells, since either of FK506 (1 μM) and ryanodine alone did not exert significant effects on cell growth. We assume that FK506 renders the T-cell RYR more sensitive to ryanodine. In the presence of FK506 at a low concentration, application of a small amount of ryanodine would cause a sustained $[Ca^{2+}]_i$ elevation, leading to accelerated cell growth. With higher doses of ryanodine, however, cell growth would be rather suppressed probably because of cell death caused by Ca^{2+} toxicity. Alternatively, FK506-FKBP complexes may inhibit calcineurin, thereby keeping the RYR in a more phosphorylated and activated state, as is observed in the cardiac RYR [31]. In conclusion, our observation suggests that the RYR plays a critical role in the propagation of the Ca^{2+} signalling in T-cell proliferation and that it is involved in the pharmacological action of FK506.

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References

[1] Berridge, M.J. (1993) Nature 361, 315–325.
[2] Endo, M. (1977) Physiol. Rev. 57, 71–108.
[3] McPherson, P.S. and Campbell, K.P. (1993) J. Biol. Chem. 268, 13765–13768.
[4] Takeshima, H., Nishimura, S., Matsumoto, T., Ishida, H., Kangawa, K., Minamino, N., Matsuo, H., Ueda, M., Hanaoka, M., Hirose, T. and Numa, S. (1989) Nature 339, 439–445.
[5] Zorzato, F., Fujii, J., Otsu, K., Phillips, M., Green, N.M., Lai, F.A., Meissner, G. and MacLennan, D.H. (1990) J. Biol. Chem. 265, 2244–2256.
[6] Nakai, J., Imagawa, T., Hakamata, Y., Shigekawa, M., Takeshima, H. and Numa, S. (1990) FEBS Lett. 271, 169–177.
[7] Otsu, K., Willard, H.F., Khanna, V.K., Zorzato, F., Green, N.M. and MacLennan, D.H. (1990) J. Biol. Chem. 265, 13472–13483.
[8] Hakamata, Y., Nakai, J., Takeshima, H. and Imoto, K. (1992) FEBS Lett. 312, 229–235.
[9] Inui, M., Saito, A. and Fleischer, S. (1987) J. Biol. Chem. 262, 15637–15642.
[10] Giannini, G., Clementi, E., Ceci, R., Marziali, G. and Sorrentino, V. (1992) Science 257, 91–94.
[11] Guse, A.H., Roth, E. and Emmrich, F. (1993) Biochem. J. 291, 447–451.
[12] Jayaraman, T., Brillantes, A.M., Timerman, A.P., Fleischer, S., Erdjument-Bromage, H., Tempst, P. and Marks, A.R. (1992) J. Biol. Chem. 267, 9474–9477.
[13] Timerman, A.P., Ogunbumni, E., Freund, E., Wiederrecht, G., Marks, A.R. and Fleischer, S. (1993) J. Biol. Chem. 268, 22992–22999.
[14] Southern, E. (1979) Methods Enzym. 68, 152–176.
[15] Imagawa, T., Nakai, J., Takeshima, H., Nakasaki, Y. and Shigekawa, M. (1992) J. Biochem. (Tokyo) 112, 508–513.
[16] Penner, R., Neher, E., Takeshima, H., Nishimura, S. and Numa, S. (1989) FEBS Lett. 259, 217–221.
[17] Chen, S.R.W., Zhang, L. and MacLennan, D.H. (1992) J. Biol. Chem. 267, 23318–23326.
[18] van Breemen, C. and Saida, K. (1989) Annu. Rev. Physiol. 51, 315–329.
[19] Lipscombe, D., Madison, D.V., Poenie, M., Reuter, H., Tsien, R.W. and Tsien, R.Y. (1988) Neuron 1, 355–365.
[20] Thayer, S.A., Perney, T.M. and Miller, R.J. (1988) J. Neurosci. 8, 4089–4097.
[21] Galione, A., Lee, H.C. and Busa, W.B. (1991) Science 253, 1143–1146.
[22] Lee, H.C. (1993) J. Biol. Chem. 268, 293–299.
[23] Takasawa, S., Nata, K., Yonekura, H. and Okamoto, H. (1993) Science 259, 370–373.
[24] States, D.J., Walseth, T.F. and Lee, H.C. (1992) Trends Biochem. Sci. 17, 495.

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Fig. 4. Effect of ryanodine and FK506 on proliferation of Jurkat T-cells. (A) In the absence of FK506, cell growth was not affected by ryanodine (Ry) at a low (100 μM) (b) or at a high concentration (2,000 μM) (c), as compared with control (a). In the presence of FK506 (1 μM), which alone did not affect cell growth (d), remarkable cell proliferation and large cluster formation were observed at a low concentration of ryanodine (100 μM) (e), whereas cells were completely dispersed at a higher concentration of ryanodine (2,000 μM) (f). Magnification, ×1400. Assays were performed in duplicate and repeated at least five times, all giving similar results. (B) In the absence of FK506, uptake of [^{3}H]thymidine was not affected by ryanodine at a low (100 μM) or at a high concentration (2,000 μM), as compared with controls. In the presence of FK506 (1 μM), uptake of [^{3}H]thymidine was remarkably increased at a low concentration of ryanodine (100 μM), but suppressed at a higher concentration of ryanodine (2,000 μM). FK506 (1 μM) alone did not affect [^{3}H]thymidine uptake. In the presence or absence of FK506, uptake of [^{3}H]thymidine was not significantly affected by ryanodine at a lower concentration (1 μM). $^*P < 0.05$ and $^{**}P < 0.01$ compared to controls by Student's t-test. Each vertical line represents the mean ± S.E.M.

[25] Howard, M., Grimaldi, J.C., Bazan, J.F., Lund, F.E., Santos-Argumedo, L., Parkhouse, R.M.E., Walseth, T.F. and Lee, H.C. (1993) Science 262, 1056–1059.
[26] Gardner, P. (1990) Annu. Rev. Immunol. 8, 231–252.
[27] Weiss, A., Imboden, J., Hardy, K., Manger, B., Terhorst, C. and Stobo, J. (1986) Annu. Rev. Immunol. 4, 593–619.
[28] Sigal, N.H. and Dumont, F.J. (1992) Annu. Rev. Immunol. 10, 519–560.
[29] Starzl, T.E., Todo, S., Fung, J., Demetris, A.J., Venkataramman, R. and Jain, A. (1989) Lancet ii, 1000–1005.
[30] Schreiber, S.L. (1991) Science 251, 283–287.
[31] Witcher, D.R., Kovacs, R.J., Schulman, H., Cefali, D.C. and Jones, L.R. (1991) J. Biol. Chem. 266, 11144–11152.