Ca²⁺ and calmodulin differentially modulate *myo*-inositol 1,4,5-trisphosphate (IP₃)-binding to the recombinant ligand-binding domains of the various IP₃ receptor isoforms

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We have expressed the N-terminal 581 amino acids of type 1 *myo*-inositol 1,4,5-trisphosphate receptor (IP₃R1), IP₃R2 and IP₃R3 as recombinant proteins [ligand-binding site 1 (lbs-1), lbs-2, lbs-3] in the soluble fraction of *Escherichia coli*. These recombinant proteins contain the complete IP₃-binding domain and bound IP₃ and adenophostin A with high affinity. Ca²⁺ and calmodulin were previously found to maximally inhibit IP₃ binding to lbs-1 by 42 ± 6 and 43 ± 6 % respectively, and with an IC₅₀ of approx. 200 nM and 3 μM respectively [Sipma, De Smet, Sienaert, Vanlingen, Missiaen, Parys and De Smedt (1999) J. Biol. Chem. 274, 12157–12562]. We now report that Ca²⁺ inhibited IP₃ binding to lbs-3 with an IC₅₀ of approx. 700 nM (37 ± 4 % inhibition at 5 μM Ca²⁺), while IP₃ binding to lbs-2 was

not affected by increasing [Ca²⁺] from 100 nM to 25 μM. Calmodulin (10 μM) inhibited IP₃ binding to lbs-3 by 37 ± 4 %, while IP₃ binding to lbs-2 was inhibited by only 11 ± 2 %. The inhibition of IP₃ binding to lbs-3 by calmodulin was dose-dependent (IC₅₀ ≈ 2 μM). We conclude that the IP₃-binding domains of the various IP₃R isoforms differ in binding characteristics for IP₃ and adenophostin A, and are differentially modulated by Ca²⁺ and calmodulin, suggesting that the various IP₃R isoforms can have different intracellular functions.

Key words: calcium, *myo*-inositol 1,4,5-trisphosphate receptor, IP₃ binding.

INTRODUCTION

The *myo*-inositol 1,4,5-trisphosphate receptor (IP₃R) is an intracellular Ca²⁺-release channel, of which three isoforms are identified [1]. The three isoforms are co-expressed in a cell- and tissue-type specific way, and each one may be predominant in a particular cell or tissue [2–5]. It is therefore conceivable that different isoforms exhibit different intracellular properties. However, until now limited progress was made concerning the characterization of the differences between the isoforms [6–9]. The main problem hereby is the difficulty to achieve stable, high levels of a particular IP₃R isoform above endogenous levels [9]. Moreover, the presence of multiple receptor subtypes in a cell as well as the formation of IP₃R heterotetramers and the existence of cell-type specific accessory proteins which allow for fine-tuning of the intracellular Ca²⁺-signals, makes it extremely difficult to resolve in cellular systems the effects of modulators on distinct receptor subtypes.

Two important modulators of the IP₃R that affect IP₃ binding are Ca²⁺ and calmodulin. Despite the fact that the regulation of the IP₃R by Ca²⁺ has been extensively studied (reviewed in [10]), the relation between Ca²⁺ and IP₃ binding is not yet clarified. Studies on full-length IP₃R1 and IP₃R3 expressed in insect cells led to partially conflicting results [11,12]. The inhibition of IP₃ binding to IP₃R1 may be mediated by an accessory Ca²⁺-binding protein like calmodin [13] or by a Ca²⁺-binding site on the receptor itself [14,15]. The Ca²⁺-dependent increase in IP₃ binding to IP₃R2 was also proposed to be mediated by an accessory protein [14].

Recently, calmodulin was reported to cause a Ca²⁺-independent inhibition of IP₃ binding to IP₃R1, but not to IP₃R3 [16,17]. This Ca²⁺-independent effect of calmodulin is probably not mediated by the earlier described Ca²⁺-dependent calmodulin-binding site [18]. There is evidence that a Ca²⁺-independent calmodulin-binding site would reside in the N-terminal portion of the IP₃R1 since calmodulin inhibited IP₃ binding to the recombinant first 581 amino acids of IP₃R1 [15]. The effect of calmodulin on IP₃ binding to IP₃R2 has not yet been investigated.

The binding of IP₃ to its receptor, which is a key step in the opening of the Ca²⁺-release channel, can be investigated *in vitro*. Since the N-terminal part of the IP₃R contains all the structural determinants responsible for the specific and selective binding of IP₃ [4,19,20], we have expressed the N-terminal 581 amino acids forming the IP₃-binding domain of the three IP₃R isoforms in the soluble fraction of *Escherichia coli*. This approach allowed us to directly compare the binding affinity of the three IP₃R isoforms under identical conditions and to demonstrate that IP₃ binding to these IP₃R isoforms was differentially modulated by Ca²⁺ and calmodulin.

MATERIALS AND METHODS

Construction of expression vectors encoding the IP₃-binding domain of IP₃R1, IP₃R2, and IP₃R3

A bacterial expression vector (pET21b/+; Novagen Inc., Madison, WI, U.S.A.) was used to express the N-terminal 581

Abbreviations used: IP₃, *myo*-inositol 1,4,5-trisphosphate; IP₃R, IP₃ receptor; lbs, ligand-binding site.

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amino acids of the mouse IP₃R1, rat IP₃R2 and rat IP₃R3. The construction of the vector containing the coding sequence of the IP₃R1 ligand-binding domain (pET-581/1) was described previously [15]. PCR was performed to produce DNA fragments encoding amino acids 1–581 of IP₃R2 and IP₃R3, using as templates CMV1-C102 containing a partial cDNA of rat IP₃R2 [21] and pCB6+IP₃R3 containing cDNA encoding the rat IP₃R3 [22] respectively. The IP₃R2-fragment was synthesized with forward primer 5'-GGGGCCCCATATGTCTGACAAAATG-TCCAGC-3' and reverse primer 5'-GGGGAATTCTTACAT-GACGCAGAAGTTCTTAGC-3'. The IP₃R3-fragment was synthesized with forward primer 5'-GGGGCCCCATATGAA-TGAAATGTCCAGCTT-3' and reverse primer 5'-GCCGAA-TTCTTACATCATCCCGAAGTCTTGGC-3'. Both forward primers contained an *Nde*I site including the start codon, while both reverse primers contained the codons for amino acids 575–581, a stop codon and an *Eco*RI site. The PCR-amplified fragments of IP₃R2 and IP₃R3 were ligated into the *Nde*I/*Eco*RI site of the bacterial expression vector pET21b/+, yielding pET-581/2 and pET-581/3 respectively. The sequences were confirmed by double-stranded sequencing using the Automated Laser Fluorescent[®] sequencing system (Amersham Pharmacia Biotech, Uppsala, Sweden).

Expression in *E. coli*, preparation of the soluble fraction and partial purification

The expression of the recombinant N-terminal 581 amino acids of the IP₃R1, IP₃R2 and IP₃R3 (lbs-1, lbs-2 and lbs-3 respectively) was essentially as described [15,19]. Preparation of the soluble fraction of *E. coli* and partial purification of the recombinant proteins on a heparin-agarose column were as described previously [15].

IP₃ binding

Binding studies were performed essentially as described [23]. [³H]IP₃ binding was performed at 0 °C in 100 µl of a solution containing 50 mM Tris/HCl, 1 mM EDTA, 10 mM 2-mercaptoethanol. The pH of the various solutions at 0 °C and the [³H]IP₃ concentration are indicated in the legends to the figures. In the experiments with varying [Ca²⁺] or [calmodulin], EDTA was replaced by EGTA (1 mM). The free [Ca²⁺] was calculated using Maxchelator Software (Dr C. Patton, Stanford University, CA, U.S.A.). After 30 min of incubation, 10 µl of γ-globulin (20 mg/ml) and 110 µl of 20% (w/v) poly(ethylene glycol) in IP₃-binding solution were added and the samples were rapidly filtered through glass fibre filters. The amount of lbs-1, lbs-2 and lbs-3 used ranged between 2.5 and 8 µg. Non-specific binding was determined in the presence of 12.5 µM unlabelled IP₃. Scatchard analyses were evaluated using the Kell Radlig program (version 6.0.2, Biosoft, Cambridge, U.K.). Statistical analysis was performed using the paired Student's *t* test. Values were considered significantly different when *P* < 0.05.

Microsomal preparations, antibodies and Western blotting

The rabbit cerebellar, rat aortic smooth muscle cells (A7r5) and Chinese hamster ovary cells (CHO-K1) microsomal preparations [23] and the rat basophilic leukaemia cells (RBL-2H3) F3-fraction [24] were prepared as described previously. The polyclonal antibody Rbt03 against the C-terminus of IP₃R1 and the mouse monoclonal antibody against an N-terminal epitope of human IP₃R3 (Transduction Laboratories, Lexington, KY, U.S.A.) were characterized previously [3,23,24]. A new antibody was raised against the N-terminal part of IP₃R2. A synthetic peptide

consisting of amino acids 330–344 (KTVRDGELPTSKKKH) of the rat IP₃R2 [21] was coupled to keyhole-limpet haemocyanin through an additional C-terminal cysteine and using *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester as a cross-linking reagent. Two rabbits (Rbt226 and Rbt227) were repeatedly immunized with the coupled peptide and both developed an antibody specifically recognizing IP₃R2. The various microsomal preparations and partially purified fractions of *E. coli* were analysed on Laemmli-type gels and either stained with Sypro[®] Orange (Bio-Rad, Hercules, CA, U.S.A.) or transferred to Immobilon-P (Millipore Corporation, Bedford, MA, U.S.A.). Immunodetection of the proteins on the transfers was exactly as described previously [24,25].

Materials

[³H]IP₃, the anti-mouse and anti-rabbit alkaline phosphatase-coupled secondary antibodies and the Vistra enhanced chemi-fluorescence substrate were from Amersham Pharmacia-Biotech (Sweden). Adenophostin A was isolated as described previously [26].

RESULTS AND DISCUSSION

Expression of the IP₃-binding domain of IP₃R1, IP₃R2 and IP₃R3

We constructed bacterial expression vectors encoding the N-terminal 581 amino acids of mouse IP₃R1 (pET-581/1), rat IP₃R2 (pET-581/2) and rat IP₃R3 (pET-581/3). The resulting recombinant proteins expressed in *E. coli* and partially purified on a heparin-agarose column, migrated with an apparent molecular mass of 66 kDa (lbs-1, [15]), 63 kDa (lbs-2) and 64 kDa (lbs-3), as determined by Sypro Orange[®] staining after SDS/PAGE (results not shown). The identity of the recombinant proteins was determined using domain- and isoform-specific antibodies. Lbs-1 was previously characterized [15]. For the identification of lbs-2 a new antibody was developed that has its epitope in the N-terminal domain of IP₃R2. This antibody, named Rbt226, recognized IP₃R2 in hamster CHO-K1 and in rat RBL-2H3 cells, and did not cross-react with IP₃R1 or IP₃R3 (Figure 1A). It recognized lbs-2 but neither lbs-1 nor lbs-3 (Figure 1B). The anti-IP₃R3 antibody is a monoclonal antibody, recognizing specifically IP₃R3 in cell types from various species [3,24,27]. Since its epitope is located in the N-terminal part of the receptor, it was used for identification of lbs-3 (Figure 1C). A weak signal was, however, also observed in mock-transfected cells as well as in cells expressing lbs-1 or lbs-2. Some degradation was observed for lbs-2 (Figure 1B). However, it can be expected, from comparison with what is reported for IP₃R1 [19,20], that degraded proteins will probably not bind IP₃. This is consistent with our finding that binding data to the recombinant IP₃-binding domains could best be fitted using a one-site Scatchard analysis.

Ligand-binding properties of lbs-1, lbs-2 and lbs-3

The recombinant lbs-1, lbs-2 and lbs-3 specifically bound [³H]IP₃ at pH 7.4 with high affinity (Figures 2A–2C). No specific binding of IP₃ could be detected in the partially purified fractions of *E. coli* transfected with the control expression vector pET21b/+. The Scatchard transformation of each displacement curve is shown in an insert. The *K*_d and *B*_{max} values of the recombinant proteins for IP₃ were determined (Table 1). This is the first report in which the affinities of the ligand-binding domains of the three IP₃R isoforms are measured under identical conditions. The observed *K*_d and IC₅₀ values fall in a quite narrow range and are

Figure 1 Expression of the recombinant IP_3 -binding domain of $\text{IP}_3\text{R1}$, $\text{IP}_3\text{R2}$, and $\text{IP}_3\text{R3}$ in *E. coli*

(A) Microsomal proteins (200 μg) of CHO-K1 cells (lane 1), RBL-2H3 cells (lane 2), A7r5 cells (lane 3) and of rabbit cerebellum (lane 4) were separated by SDS/PAGE [3–12% (w/v) gel] and transferred to Immobilon-P. The blot was probed with the Rbt226 antibody against $\text{IP}_3\text{R2}$ (1:500). The position of $\text{IP}_3\text{R2}$ is indicated by the arrow. (B and C) Partially purified soluble fractions of *E. coli* (2 μg) transfected with pET21b/+ (lane 1), pET-581/1 (lane 2), pET-581/2 (lane 3) or with pET-581/3 (lane 4) were separated by SDS/PAGE (3–12% gel) and transferred to Immobilon-P. The blot in (B) was probed with Rbt226 (1:2500). The position of lbs-2 (arrow) is indicated. The blot in (C) was probed with the monoclonal antibody against part of the IP_3 -binding domain of $\text{IP}_3\text{R3}$ (1:800). The position of lbs-3 (arrow) is indicated.

in good agreement with previous reports [4,21,23,28,29]. Adenophostin A is a more potent agonist than IP_3 in stimulating the IP_3R [26,30]. We examined the binding properties of adenophostin A to lbs-1, lbs-2 and lbs-3. The displacement of [^3H] IP_3 binding to lbs-1, lbs-2 and lbs-3 by adenophostin A occurred with an IC_{50} of 41 ± 4 nM, 19 ± 3 nM, and 39 ± 7 nM (mean $\pm$ S.E.M.) respectively (Figure 3). When comparing these values with the corresponding IC_{50} values for the displacement of [^3H] IP_3 by cold IP_3 for each recombinant protein (Table 1) it is clear that adenophostin A bound to the recombinant proteins 2–3 times more efficiently than IP_3 itself. This is comparable with results obtained with intact mouse cerebellar $\text{IP}_3\text{R1}$ [30] and with hepatic membranes which predominantly express $\text{IP}_3\text{R2}$ [31]. Furthermore, the IC_{50} values had the same ranking order as those obtained for IP_3 . Unlike IP_3 ([30] and this study), adenophostin A interacted with the ligand-binding domains with positive co-operativity. Moreover, the level of co-operativity was different between lbs-1, lbs-2 and lbs-3 with the highest degree of co-operativity for the former and the lowest for the latter, demonstrating that the interaction was isoform-specific (Figure 2).

In this study, lbs-2 has a significantly higher affinity than lbs-3 for IP_3 (K_d , Table 1) and than both lbs-1 and lbs-3 for adenophostin A (IC_{50} , Figure 3). No significant differences exist between lbs-1 and lbs-3. The high affinity of lbs-2 and the lower affinity of lbs-3 concur with most other binding studies [4,11,21,23,28,29] as well as with the IP_3 sensitivity observed by analysing IP_3 -induced Ca^{2+} release in genetically modified B-cells, each expressing a single IP_3R isoform [9]. Differences between the studies, however, exist with respect to the affinity of the $\text{IP}_3\text{R1}$ that has already been described as of very high [29], intermediate [4,9,21] or of quite low [23] affinity, when compared with the other IP_3R isoforms. Reasons for this variance may include the exact conformation of the ligand-binding domain (fusion proteins versus monomeric versus tetrameric structures), the possible presence of regulatory proteins associated with immunoprecipitated IP_3R isoforms, and the different sensitivity

Figure 2 Binding of IP_3 to (A) lbs-1, (B) lbs-2 and (C) lbs-3

IP_3 binding to 6 μg of lbs-1, lbs-2 and lbs-3 was measured in the presence of 3.5–5.5 nM [^3H] IP_3 at pH 7.4 and increasing concentrations of unlabelled IP_3 . Total incubation volumes were 85 μl (A and B) or 100 μl (C). Results from a typical experiment (representative of five to seven independent experiments) are shown and each data point is the mean $\pm$ S.D. of three to six values. Corresponding Scatchard analyses for each displacement curve are shown in the inserts.

of the various isoforms to parameters such as pH and redox status.

Taken together, the very good correspondence with the properties of the intact receptors indicates that lbs-1, lbs-2 and lbs-3 were in the right conformation to act as bona fide IP_3 -binding pockets.

Table 1 IP₃-binding characteristics of lbs-1, lbs-2 and lbs-3 at pH 7.4

Binding parameters were determined from Scatchard analyses as described in Figure 2. Results are the means $\pm$ S.E.M. for (*n*) independent experiments, each performed in triplicate. From the IC₅₀ and *K_d* values mentioned in the Table, only the *K_d* values for lbs-2 and lbs-3 are statistically different (*P* < 0.05, unpaired Student's *t* test).

	IC ₅₀ (nM)	<i>K_d</i> (nM)	<i>B_{max}</i> (pmol/mg)
Lbs-1	98 $\pm$ 16 (6)	58 $\pm$ 4 (6)	214 $\pm$ 32 (6)
Lbs-2	71 $\pm$ 10 (5)	39 $\pm$ 7 (5)	128 $\pm$ 42 (5)
Lbs-3	101 $\pm$ 13 (7)	74 $\pm$ 12 (3)	79 $\pm$ 18 (3)

Figure 3 Binding of adenophostin A to lbs-1, lbs-2 and lbs-3

IP₃ binding to lbs-1 (●; 4 μ g), lbs-2 (■; 3 μ g) and lbs-3 (▲; 6 μ g) was measured in the presence of 3 nM [³H]IP₃ at pH 7.4 and increasing concentrations of adenophostin A. Results from a typical experiment (representative of three experiments) are shown and each data point is the mean $\pm$ S.D. of three to six values. The IC₅₀ values determined in three independent experiments were 41 $\pm$ 4 nM, 19 $\pm$ 3 nM and 39 $\pm$ 7 nM for lbs-1, lbs-2 and lbs-3 respectively. The value found for lbs-2 is statistically different from those found for lbs-1 or lbs-3 (*P* < 0.05, unpaired Student's *t* test). The Hill coefficients were determined by fitting the curves with the Hill equation using Origin 6.0 and were 7.4 $\pm$ 0.6, 4.7 $\pm$ 2.0 and 2.0 $\pm$ 0.5 (mean $\pm$ S.E.M.) for lbs-1, lbs-2 and lbs-3 respectively.

Effect of Ca²⁺ on IP₃ binding to lbs-2 and lbs-3

Ca²⁺ reduced the affinity of lbs-1 for IP₃ [15], similarly to its effect on the cerebellar or overexpressed full-length IP₃R1 [11,14,32]. We now investigated the effect of Ca²⁺ on [³H]IP₃ binding to lbs-2 and lbs-3. In the physiological range between 0.1 and 25 μ M and at pH 7.0, Ca²⁺ modulated IP₃ binding to lbs-2 and lbs-3 in a strikingly different way (Figure 4). While [³H]IP₃ binding to lbs-2 was not significantly affected by Ca²⁺, [³H]IP₃ binding to lbs-3 was markedly decreased, reaching a maximal inhibition of 37 $\pm$ 4 % at 5 μ M Ca²⁺. This inhibition is quantitatively comparable to what was previously observed for lbs-1 [15], but occurred with a lower Ca²⁺ sensitivity (an IC₅₀ of 700 nM compared with 200 nM). From a kinetic model, based on IP₃ binding data and on electrophysiological analysis, it was suggested that the Ca²⁺-dependent inhibition of IP₃ binding to IP₃R1 is part of the mechanism which leads to the well-known inhibition of Ca²⁺ release through the IP₃-sensitive channel at high cytosolic Ca²⁺ concentrations [33]. Moreover, the Ca²⁺-

Figure 4 The effect of Ca²⁺ on IP₃ binding to lbs-2 and lbs-3

The concentration of [³H]IP₃ was 5 nM and the pH was 7.0. Ca²⁺ was added to lbs-2 (■; 3 μ g) or lbs-3 (●; 6 μ g) 5 min before adding the binding buffer. [³H]IP₃ binding was expressed as the percentage of [³H]IP₃ binding measured in the presence of 100 nM Ca²⁺. Each data point is the mean $\pm$ S.E.M. for at least three experiments, each performed in quadruplicate. *Different from [³H]IP₃ binding in the presence of 100 nM Ca²⁺ (*P* < 0.05 as determined with the paired Student's *t* test).

dependent inhibition of both IP₃ binding and IP₃-induced Ca²⁺ release displays the same selectivity for various divalent cations, suggesting that a common Ca²⁺-binding site is involved in both functions [34].

Our results suggest that a similar inhibition occurs for IP₃R3, but not for IP₃R2. The interaction of Ca²⁺ with IP₃ binding to IP₃R3 was previously studied in insect Sf9 cells overexpressing this isoform [11,12] and led to conflicting results. In both studies, low Ca²⁺ concentrations led to an increase in IP₃ binding, which was explained either by an increase in affinity [11] or in *B_{max}* [12]. High Ca²⁺ concentrations, however, led to a decrease in the IP₃-binding affinity and therefore to an inhibition of the IP₃-binding activity [12]. Our results suggest that this inhibition is due to the interaction of Ca²⁺ with a binding site localized in the IP₃-binding domain itself. Lbs-3 contains a region with very high homology to the Ca²⁺-binding site 3b that was detected for IP₃R1 [35,36]. The inhibition of IP₃ binding to IP₃R3 could therefore contribute to the descending phase of the bell-shaped Ca²⁺ dependence of IP₃-induced Ca²⁺ release in cell types expressing predominantly IP₃R3 [8,37].

The relation between Ca²⁺ and IP₃ binding to IP₃R2 was previously studied in hepatocytes [38,39] and in RBL-2H3 cells [40,41], two cell types expressing predominantly this isoform. Since these cell types also express significant amounts of IP₃R1 and/or IP₃R3, a contribution of those isoforms or of heterotetramers can not be excluded. The available results indicate for IP₃R2 a significant increase in IP₃ binding by Ca²⁺, which is due to the conversion of the receptor to a high-affinity inactive state [38,39]. Extraction of peripheral membrane proteins abolished the effect of Ca²⁺ on liver membranes, suggesting that Ca²⁺ regulates liver IP₃R2 by interacting with membrane-associated proteins [14,42]. This is consistent with our observation that Ca²⁺ had no direct effect on IP₃ binding to lbs-2.

Figure 5 The effect of calmodulin on IP₃ binding to (A) lbs-2 and (B) lbs-3

The concentration of [³H]IP₃ was 5 nM, the pH was 7.0 and the medium contained 1 mM EGTA and no added Ca^{2+} . Calmodulin was added to the lbs-2 (3 μg) or lbs-3 (6 μg) 5 min before adding the binding buffer. [³H]IP₃ binding was expressed as the percentage of [³H]IP₃ binding measured in the absence of calmodulin. The means ± S.E.M. are shown for at least four experiments, each performed in quadruplicate. *Different from [³H]IP₃ binding in the absence of calmodulin ($P < 0.05$ as determined with the paired Student's *t* test).

Effect of calmodulin on IP₃ binding to lbs-2 and lbs-3

Apart from the Ca^{2+} -dependent binding of calmodulin to the regulatory domain of IP₃R1 and IP₃R2 [18], recent evidence demonstrated that calmodulin caused a Ca^{2+} -independent inhibition of IP₃ binding to the purified cerebellar IP₃R1 [16] and to microsomes of Sf9 insect cells overexpressing IP₃R1 [17]. Calmodulin inhibits IP₃ binding to lbs-1, both in the presence and absence of Ca^{2+} [15], suggesting that a Ca^{2+} -independent interaction site for calmodulin is present within the N-terminal ligand-binding domain of IP₃R1. To allow comparison with the other IP₃R isoforms and to better understand the role of calmodulin on the IP₃R, we examined the effect of calmodulin on lbs-2 and lbs-3 in the absence of Ca^{2+} (Figure 5). Interestingly, calmodulin differently modified IP₃ binding to lbs-2 and lbs-3. Calmodulin (10 μM) decreased IP₃ binding to lbs-3 by $37 \pm 4\%$, whereas IP₃ binding to lbs-2 was only decreased by $11 \pm 2\%$. The value for lbs-3 was identical to the maximal inhibition reported for the type 1 isoform [15–17]. The effect of calmodulin on IP₃ binding to lbs-3 was concentration-dependent with a half-maximal effect at about 2 μM. Our finding for lbs-3 contrasts with the previously published lack of effect of calmodulin on the full-length IP₃R3 overexpressed in Sf9 insect cells [17]. It may

indicate that, in analogy to the type 1 IP₃R, a calmodulin-binding site is present in the N-terminal part of the type 3 IP₃R, but that in the full-length receptor the tertiary or quaternary structure of the receptor prevents access of calmodulin. Alternatively, it may represent differences in the type of expression system used. In conclusion, our results suggest that a Ca^{2+} -independent interaction site for calmodulin is located in the N-terminal ligand-binding domain of the various IP₃R isoforms. It may depend on the full-length conformation whether this site is functionally relevant or not.

CONCLUSIONS

We have expressed in *E. coli* the N-terminal 581 amino acids of IP₃R1, IP₃R2 and IP₃R3, containing the complete ligand-binding domain. The recombinant proteins all had a high affinity for IP₃ and adenophostin A. IP₃ binding to the various isoforms was differentially modulated by Ca^{2+} and calmodulin. The ligand-binding domain of IP₃R2, in contrast to that of the other two isoforms, was not affected by Ca^{2+} and only minimally by calmodulin. These differences in IP₃-binding properties between the various IP₃R isoforms are indicative for an isoform-specific cellular function of these channel proteins.

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