SUPPLEMENTARY ONLINE DATA

Activation of IP₃ receptors requires an endogenous 1-8-14 calmodulin-binding motif

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Figure S1 A conserved 1-8-14 motif in all IP₃Rs and RyRs

Alignments (with first and last residues numbered) of the N-terminal region of rat IP₃R1–IP₃R3 (SwissProt accession numbers NP_001007236, NP_112308 and NP_037270 respectively), chicken IP₃R1–IP₃R3 (SwissProt accession numbers XP_414438, XP_001235613 and XP_418035 respectively), Xenopus IP₃R1–IP₃R3 (SwissProt accession numbers NP_001084015, ABP88141 and ABP88140 respectively), Drosophila IP₃R (SwissProt accession number NP_734042), Caenorhabditis elegans IP₃R (SwissProt accession number NP_001023170) and rabbit RyR1–RyR3 (SwissProt accession numbers P11716, P30957 and Q9TS33 respectively) highlighting the residues proposed to form a 1-8-14 CaM-binding motif. The consensus sequence for a 1-8-14 motif is shown in the first row, with its three critical (1, 8 and 14 hydrophobic residues) and net charge of +6. A similar 1-8-14 motif is conserved in all IP₃R, which closely resembles a type A (1-5-8-14) motif, where position 5 is also a large hydrophobic residue. The motif within IP₃Rs differs from a classic 1-8-14 consensus sequence by having a tyrosine residue at position 14. All subtypes of RyR also have a similar 1-8-14 motif within a similar position in the three-dimensional structure, although the sequence lacks the usual net positive charge of a consensus 1-8-14 motif.

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Figure S2  Mutation of a non-critical residue (K52E) within the 1-8-14 motif has no effect on IP₃ binding or IP₃-evoked Ca²⁺ release

(A) Structure of the SD of IP₃R1 (PDB code 1XZZ) highlighting the 1-8-14 motif (red), the critical 1-8-14 hydrophobic residues (blue) and Lys52 (yellow). (B) Equilibrium competition binding of IP₃ (with 0.75 nM [³H]IP₃) to native NT and NT^K52E. (C) IP₃-evoked Ca²⁺ release from DT40-IP₃R1 and DT40-IP₃R1^K52E cells. Results are means ± S.E.M. (n⩾3).
An essential 1-8-14 calmodulin-binding motif in IP₃ receptors

Table S1  Peptides used in the present study

All peptides were synthesised by Sigma or New England Peptide. The isoelectric point (pI) is shown for each peptide calculated from http://www.innovagen.se/custom-peptide-synthesis/peptide-property-calculator/peptide-property-calculator.asp. Ac, acetyl.

| Peptide | Sequence | Source |
|---------|----------|--------|
| MLCK    | Ac-RRKWQKTHAVRAGRL-NH₂ | Ca²⁺–CaM-binding site of smooth muscle MLCK |
| 1-8-14  | Ac-KKFRDALFKLAPMRV-NH₂ | Fragment of IP₃R1 (residues 51–66) containing the 1-8-14 motif |
| 1-8-14C | Ac-KKEFODALFKLAPMRE-NH₂ | Inactive form of 1-8-14 peptide (mutations highlighted in bold and underlined) |
| 1-8-14S | Ac-AMRFLKYLPKRFDKNA-NH₂ | Scrambled form of 1-8-14 peptide |
| 1-8-14L | Ac-LNNPPKKFRDALFKLAPMRVYSAQKFWKA-NH₂ | Longer fragment of IP₃R1 (residues 46–75) containing the 1-8-14 motif |

Table S2  Primers used in the present study

Primers used for introducing mutations in the N-terminal fragment or full-length IP₃R1. The mutated bases are highlighted.

| Primer | Sequence (5′→3′) |
|--------|-----------------|
| F53E Forward | GGGGACCTTAACAATCCACCCAAGAGAGAGACTGCTCTT |
| F53E Reverse | AAGAGCGAGTCTCCTCTTCTGCTGATGTTAGGTCGCC |
| L60E Forward | GAAATTCAGAGACTGCCTTTAAGGAGTGTCCTATGAATCTCATGCA |
| L60E Reverse | TGCAGAATATCGATTCATAGGACACTCCTTTAAAGAGGCAGTCTCTGAATTTC |
| Y66E Forward | CTCTTTAAGCTATGTCTTCTATAAGAGAGACTGCTGACACAGAG |
| Y66E Reverse | CTGCTTCTGTGACACTGATTACATGGCATATGCTTTAAAGAG |
| K52E Forward | AAACATCACCCACCAAGAGAGAGACACTGCCTC |
| K52E Reverse | GAGGCGACTGCTGACACTGCTTGGTGATGTT |

Received 26 June 2012/13 September 2012; accepted 26 September 2012
Published as BJ Immediate Publication 26 September 2012, doi:10.1042/BJ20121034

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