Supplemental Data

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Functional Expression of IP$_3$R2 – The cDNA of both rat and mouse IP$_3$R2 is unstable when propagated in *E. coli*. Typically, colonies were very small and they were not improved by systematic changes of the plasmid (pBlueScript from Stratagene, pEHTR1a, pcDNA3 or pcDNA3.2/DEST from Invitrogen, or pEYFP-C2 from Clontech), temperature (22-37°C), the antibiotics used for selection (kanamycin or ampicillin), growth medium (Luria-Bertani medium, Terrific Broth or SOC medium from Invitrogen) or the strain of *E. coli* (XL1B, XL10, DH5α, JM109, or JM110). About 90 % of selected colonies failed to grow when cultured in LB medium, and those that grew did so only after a lag of more than 36 h. Rarely, we succeeded in obtaining useable yields of plasmid, but even 200 mL cultures provided only 200-300 µg of plasmid DNA. In rare cases, colonies grew normally, but sequencing of the plasmid then invariably revealed large scale deletions or insertions of unrelated sequences within the ORF of IP$_3$R2 or single base deletions that introduced premature termination codons. These aberrant IP$_3$R2 sequences were apparently better tolerated by *E. coli* because the colonies grew normally after transformation. We identified two such base pair deletions in the plasmid for rat IP$_3$R2 (positions 6312 and 6505) and then corrected the mutations by site-directed mutagenesis. After transformation of *E. coli* with the corrected full-length cDNA for rat IP$_3$R2, the resulting colonies were small and unstable. We encountered none of these problems when propagating plasmids for full-length IP$_3$R1 or IP$_3$R3, or smaller fragments of IP$_3$R2 (e.g., bases 2291-8352 encoding residues 682-2701). We had similar problems with the cDNA for mouse IP$_3$R2 (GenBank accession number AB182990) (14). The problems, however, appeared less severe with the mouse IP$_3$R2, and we were able to propagate the plasmid and achieve expression of IP$_3$R2 in both DT40 and Sf9 cells without introducing mutations.

These observations suggest that *E. coli* select against full-length IP$_3$R2 cDNA. We suspect that a cryptic bacterial promoter within the 5' region of the full-length IP$_3$R2 ORF may allow expression of a protein that is toxic to *E. coli*. Similar problems were experienced with the cDNA for full-length cystic fibrosis transmembrane conductance regulator (48). Our problems highlight the necessity for complete sequencing of plasmids encoding IP$_3$R.

SUPPLEMENTAL DISCUSSION

How Many Gαs are There in an AC-IP$_3$R Junction? – In HEK-PR1αs⁻ cells, we assume that 34 % of AC-IP$_3$R junctions entirely lack Gαs (see text). Assuming the distribution of αs among these junctions is random, then from the Poisson distribution:

$$P_0 = e^{-z}$$

where,

P_0, probability of 0 Gαs/junction (= 0.34 in HEK-PR1αs⁻ cells)
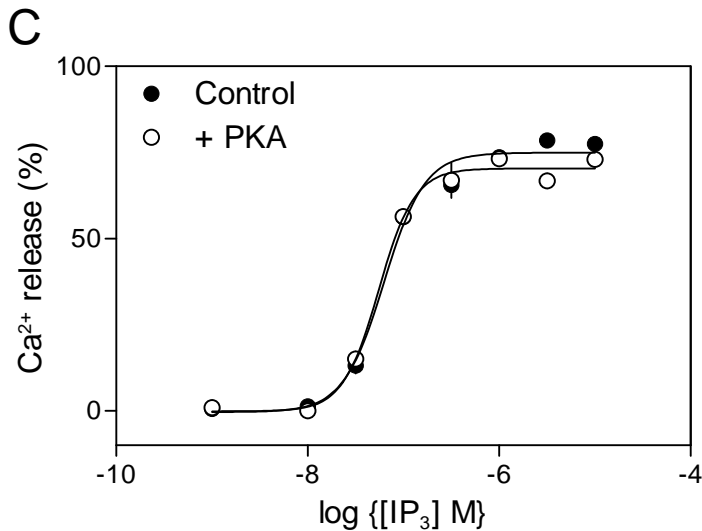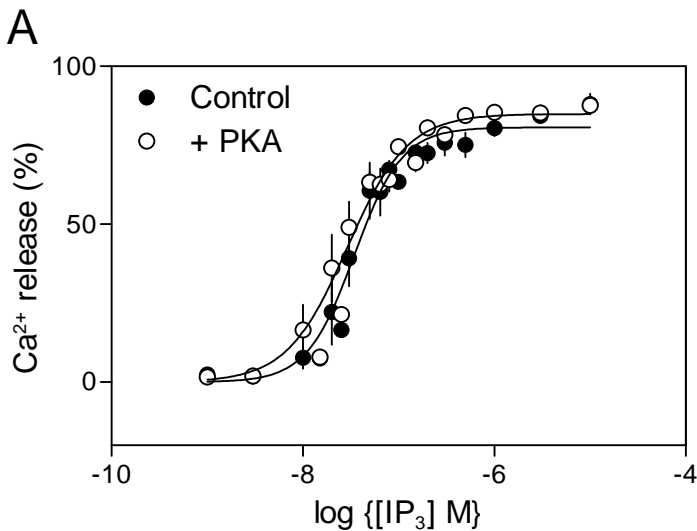
z, mean number of Gαs/junction.

The average number of Gαs/junction is thus 1.1 in HEK-PR1αs⁻ cells. HEK-PR1αs⁻ cells express only ≤ 5 % of the Gαs expressed in HEK-PR1 cells (7), from which we infer that there are an average of 22 Gαs/junction in these cells.

SUPPLEMENTAL REFERENCE

48. Gregory, R. J., Cheng, S. H., Rich, D. P., Marshall, J., Paul, S., Hehir, K., Ostedgaard, L., Klinger, K. W., Welsh, M. J., and Smith, A. E. (1990) *Nature* **347**, 382-386

SUPPLEMENTAL TABLE S1
Primers used

Primer	Sequence (5'-3')
1	GATCCCCGTTCAGAGTGGACTACATCTTCAAGAGAGATGTAGTCCACTCTGAACTTTTTGGAAA
2	AGCTTTTCCAAAAAGTTCAGAGTGGACTACATCTCTCTTGAAGATGTAGTCCACTCTGAACCGGG
3	GTAACGAATTCGCCACCATGTCTGACAAAATGTCTAGT
4	CCGGTACCGAATTCTTAGGCTGGCTGCTGT
5	GCGTCGACGCCACCATGTCTGACAAAATGT
6	TATCGCTCGAGTAACTGCACCTGTTTAAAGGCCTG
7	GGCCTCGAGTCAGTGCGGTGGCAT
8	AGGAATTCGCCACCATGAATGAAATGTCCAGC
9	CCGGTACCGAATTCAGCGGCTCATGCAGTT

A

C

B

	IP$_3$R1		IP$_3$R2	
	Control	+PKA	Control	+PKA
EC$_{50}$ (nM)	35	26	37	35
	50	40	65	59
	22	15	33	36
	36 ± 8	27 ± 7	45 ± 10	43 ± 8
EC$_{50}$, Control relative to PKA (%)	135 ± 6*		103 ± 6	
Ca^{2+} release (%)	80	84	70	67
	85	84	76	73
	84	83	72	74
	83 ± 2	84 ± 1	73 ± 2	71 ± 2
Ca^{2+} release, Control relative to PKA (%)	99 ± 2		102 ± 2	

SUPPLEMENTAL FIGURE S1. **Effects of PKA on IP$_3$R subtypes expressed in DT40 cells.** *A*, Concentration-dependent effects of IP$_3$ on Ca^{2+} release from DT40-IP$_3$R1 cells alone and after pre-incubation with the catalytic subunit of PKA (200 units/mL, 10 min). The results with IP$_3$R2 are shown in figure 6*A*. *B*, Paired comparisons of the effects of PKA on IP$_3$-evoked Ca^{2+} release from 3 independent experiments and the overall summary. *denotes values significantly different from control (P < 0.05). Analysis of paired EC$_{50}$ values from individual experiments reveals a modest (~30 %) sensitization of IP$_3$-evoked Ca^{2+} release by PKA from DT40-IP$_3$R1 cells, but no effect in DT40-IP$_3$R2 cells. Results, where shown with error bars, are means ± SEM, n = 3. *C*, Intact DT40-IP$_3$R2 cells were treated with H89 (10 µM, 60 min) to inhibit endogenous PKA and perhaps thereby allow any endogenous phosphorylation of IP$_3$R to be reversed. Cells were then permeabilized and their responses to IP$_3$ with or without the catalytic subunit of PKA (200 units/mL, 10 min) were assessed. Results (means ± SEM, n ≥ 3) show the concentration-dependent release of intracellular Ca^{2+} stores by IP$_3$.