

Supplementary Materials

Supplementary Table S1 Primers used for quantitative PCR analysis

Target	Accession number	Forward primer (5'-3')	Reverse primer (5'-3)
TRPC 1	AF061266	2086- CACACCTCCAAAGGCAAGGTC	2213- CGCATGGAGGTCAGGTATCG
TRPC 2	AF136401	368- CAGCCAGCGGTAGTGCGTC	517- GCAGGCCAGTGTGAGTGGAG
TRPC 3	NM_021771	549- CGTAGTAGGTGTGCTGGACCTGTG	653- GCCTTGTGCCCCTGTCTCT
TRPC 4	NM_053434	2004- CCCTGCCTACACCTTTCAATGTC	2077- GTGCGTCCATATCCAATTGACC
TRPC 5	NM_080898	1584- AAATAAAGGAAATGTGGGATGGTG	1733- TTCCCTTGGACGAGAACCATT
TRPC 6	NM_053559	1328- TAGCACATGCGGCCTCCTT	1405- GCTTGGTGCCTTCAAATCTGTC
TRPC 7	NM_012035.1	1057- GTTTCGTTGCTCACCTAACTGTC	1165- CCAAAGACAGCCAGGAATTTCA

Supplementary Table S2 Validation of calibration methods for analysis of TRPC expression levels

We used both β -actin and TBP for calibration in our initial analysis of cell lines 244 and 264. The results ($\Delta(C_T^{TBP} - C_T^{\beta\text{-actin}})$) show that the relative level of the RNA for each housekeeping protein is the same in both cell lines. In all subsequent work, only β -actin was used for calibration. Results are means \pm S.E.means from 12 independent PCR reactions.

Cell line	$C_T^{\beta\text{-actin}}$	C_T^{TBP}	$\Delta(C_T^{TBP} - C_T^{\beta\text{-actin}})$
244	19.23 \pm 0.59	28.04 \pm 0.58	8.81 \pm 0.89
264	19.94 \pm 0.59	28.06 \pm 0.47	8.12 \pm 0.82

Supplementary Table S3 Expression of RNAs for TRPCs in different A7r5 cell lines

The TRPC expression profile is shown for 6 cell lines in which the effects of AVP on CCE and NCCE were characterized immediately before the cells were frozen and the RNA then extracted from the frozen cells (see Experimental). Cells are categorized according to whether AVP evoked Sr^{2+} entry via the NCCE pathway under conditions where CCE was inhibited. In the cells lacking NCCE, AVP failed also to inhibit CCE. For each TRPC, $\Delta C_T (= C_T^{\text{TRPC}} - C_T^{\beta\text{-actin}})$ was calculated using β -actin expression for calibration. Results are means \pm S.E.mean of 3 independent PCR reactions. ^aThe primers for TRPC3 apparently interfered with amplification of the β -actin PCR product. In only these reactions, therefore, TRPC3 and β -actin were quantified in parallel assays of the same RNA extracts. In the cell lines (264 and 381) where TRPC3 was not detected after 43 cycles, C_T values for β -actin were 25 and 27, respectively. ^bIn all cell lines, TRPC7 product was not detected after 40 cycles, and in line 381 TRPC2 was undetected after 40 cycles. C_T for β -actin in these reactions was 23 ± 2 (for TRPC7) and 24.9 ± 0.3 (for TRPC2).

Expression of NCCE	Cell code	ΔC_T						
		TRPC1	TRPC2	^a TRPC3	TRPC4	TRPC5	TRPC6	^b TRPC7
+	94	4.35 \pm 0.53	5.60 \pm 0.05	6.74 \pm 0.83	7.18 \pm 0.16	10.36 \pm 0.64	3.97 \pm 0.61	>17
+	114	4.03 \pm 0.02	4.14 \pm 0.36	5.34 \pm 0.66	6.64 \pm 0.81	7.91 \pm 0.80	3.52 \pm 0.36	>17
+	244	2.05 \pm 0.21	9.30 \pm 0.17	7.20 \pm 0.32	7.42 \pm 0.47	15.44 \pm 0.88	3.89 \pm 0.65	>17
+	250	4.93 \pm 0.33	3.13 \pm 0.27	4.41 \pm 0.19	5.64 \pm 0.54	8.47 \pm 0.17	2.62 \pm 0.14	>17
-	264	2.70 \pm 1.19	6.59 \pm 0.68	>18	10.13 \pm 0.25	14.24 \pm 0.10	8.89 \pm 0.62	>17
-	381	5.77 \pm 0.56	> ^b 15	>16	8.60 \pm 0.53	11.05 \pm 0.54	8.74 \pm 0.60	>17

Supplementary Table S4 Expression of RNA for TRPCs in A7r5 cell lines with and without NCCE: Summary

Summary of data presented in Table S3.

	ΔC_T						
	TRPC1	TRPC2	TRPC3	TRPC4	TRPC5	TRPC6	^b TRPC7
with NCCE	3.84±0.63	5.54±1.35	5.92±0.64	6.72±0.40	10.55±1.71	3.50±0.31	>17
no NCCE	4.24±3.07	>11	>17	9.36±1.54	12.65±3.19	8.82±0.15	>17