

# Primary structure and functional expression of a high voltage activated calcium channel from rabbit lung

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The complete amino acid sequence of the receptor for organic calcium channel blockers (CaCB) from rabbit lung has been deduced by cloning and sequence analysis of the cDNA. Synthetic RNA derived from this cDNA induces the formation of a functional CaCB-sensitive high voltage activated calcium channel in *Xenopus* oocytes.

Ca<sup>2+</sup> channel; Smooth muscle; Primary structure; Expression

## 1. INTRODUCTION

Voltage activated calcium channels are membrane spanning proteins that allow the controlled entry of Ca<sup>2+</sup> ions into the cytoplasm of cells. This class of channels comprises a group of very similar yet distinct proteins or protein complexes that differ in kinetics, voltage dependence and their relative sensitivity to organic calcium channel blockers [1,2]. The principal subunit of a high voltage activated (L-type) calcium channel is the CaCB-receptor or  $\alpha_1$  subunit [3–11]. So far, only the rabbit skeletal [12,13] and cardiac muscle CaCB-receptors [14] have been cloned. Pharmacologically, the primary target for CaCBs is smooth muscle [15]. Assuming that airway smooth muscle cells are abundant in lung we have now isolated complementary DNAs for the CaCB-receptor from rabbit lung on the basis of sequence homology with its skeletal muscle counterpart.

## 2. MATERIALS AND METHODS

### 2.1. Generation and screening of rabbit lung cDNA libraries

A randomly primed cDNA library was constructed in pUC 9 using poly(A)+RNA prepared from adult rabbit lung. Screening with a rabbit skeletal muscle CaCB-receptor cDNA probe, that is *EcoRI* (nt 1007)-*SacI*(nt 5315) fragment from pCCH102 [12] gave 8 positive clones including pCaKL5 (nt 3957 to 5248) and pCaKL6 (nt 2452 to 4193 (Fig. 1)). The *PstI* (nt 4899)-*PstI*(nt 5030) fragment from clone pCaKL5 was used for screening of an oligo(dT)library and six positive clones including pCaKL34 (4792 to 6830 and a stretch of 10 dA residues), pCaK135 (4830 to 6826) and pCaK125 (nt 4847 to 6829) were obtained. Elongation of a synthetic primer complementary to nt 2756 to 2773 and screening of the resulting clones with the *PvuII* (nt 2506)-

*AflIII*(nt 2674) fragment yielded 4 positive clones including pCaKL14 (nt 2769 to nt-544). Sequencing of the cDNA was performed on both strands. In addition pCaKL35 and pCaKL25 were sequenced. One nucleotide difference occurred among the individual clones: at nt 5422, G (pCaKL34 and pCaKL35) or T (pCaK125). The difference results in a substitution of Gly to Cys. Cloning procedures, if not stated otherwise, were carried out as described [16].

### 2.2. Construction of recombinant plasmid for cDNA expression

The recombinant plasmid pSCaL carrying the entire protein-coding sequence of the rabbit lung CaCB-receptor cDNA was constructed as follows. The 2.1 kb *HindIII*-*BglIII* fragment from pCaKL34, the 0.7 kb *BglII*-*SacII* fragment from pCaKL5, the 1.4 kb *SacII*-*AflIII* fragment from pCaK16 and the 3.0 kb *AflIII*-*XmaI* fragment from pCaKL14 were subcloned in the 2.9 kb *HindIII*-*XmaI* fragment of pBluescript KS to yield pBCaL. The 0.3 kb *BglII*-*HindIII* fragment (carrying a poly(dA) poly(dT) tract from pSPCA1 [14] was blunted by Klenow fragment, cleaved by *BamHI* and ligated with the 6.9 kb *BamHI*-*HpaI* fragment from pBCaL and the *BamHI*-cleaved pSP72 (Promega) to yield pSCaL.

### 2.3. Expression of calcium channels in *Xenopus* oocytes

The mRNA specific for the lung CaCB-receptor was synthesized *in vitro* using Asp718-cleaved pSCaL as template. *Xenopus laevis* oocytes were injected with the CaCB-receptor specific mRNA (0.4  $\mu\text{g}/\mu\text{l}$ ); the average volume injected was 50 nl. The injected oocytes were incubated as described [17] and the follicular cell layer was removed from oocytes before electrophysiological measurements. Whole cell currents were recorded at room temperature in (mM) 40 Ba<sup>2+</sup>, 50 Na<sup>+</sup>, 2 K<sup>+</sup>, 5 HEPES (pH 7.4 with methanesulfonic acid). Current records were sampled at 0.5 ms intervals after low-pass filtering at 500 Hz.

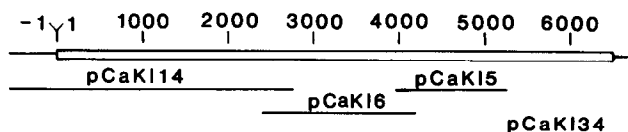


Fig. 1. Cloning strategy for the CaCB-receptor. The protein-coding region is indicated by a closed box. The extent of cDNA inserts of the individual clones used for sequence analysis are shown by thick lines.

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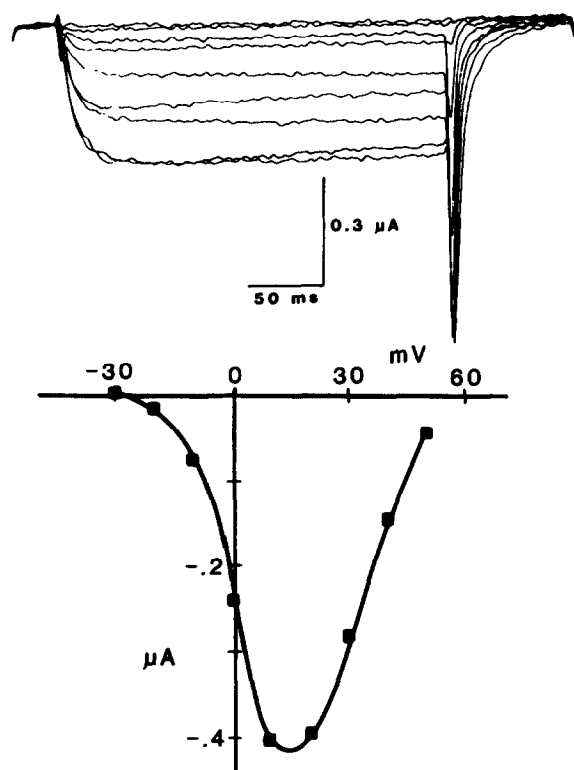


Fig. 4. (A) Current responses ( $I_{Ba}$ ) in *Xenopus* oocytes injected with mRNA specific for the lung CaCB-receptor. The depolarizations ranged from  $-30$  to  $50$  mV in  $10$  mV steps from a holding potential of  $-80$  mV. (B) Current-voltage relation corresponding to the data shown in A.

kinase in vivo. However, the biochemical modulation of both channels appears to be different. cAMP-dependent phosphorylation increases the cardiac calcium current [19] whereas it has little or no effect on the smooth muscle current [20]. Therefore stimulation of the calcium current might be not due to phosphorylation of the CaCB-receptor itself. Further work involving the stable expression of the CaCB-receptor in cells exhibiting appropriate signal transduction pathways will be required to test this hypothesis.

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