

Modulation of the skeletal muscle sodium channel α -subunit by the β_1 -subunit

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Received 3 November 1993

Co-expression of cloned sodium channel β_1 -subunit with the rat skeletal muscle-subunit ($\alpha_{\mu 1}$) accelerated the macroscopic current decay, enhanced the current amplitude, shifted the steady state inactivation curve to more negative potentials and decreased the time required for complete recovery from inactivation. Sodium channels expressed from skeletal muscle mRNA showed a similar behaviour to that observed from $\alpha_{\mu 1}/\beta_1$, indicating that β_1 restores 'physiological' behaviour. Northern blot analysis revealed that the Na^+ channel β_1 -subunit is present in high abundance (about 0.1%) in rat heart, brain and skeletal muscle, and the hybridization with untranslated region of the 'brain' β_1 cDNA to skeletal muscle and heart mRNA indicated that the different Na^+ channel α -subunits in brain, skeletal muscle and heart may share a common β_1 -subunit.

Sodium channel; α -Subunit; β_1 -Subunit; *Xenopus* oocyte; Expression; Translational control

1. INTRODUCTION

Sodium channels responsible for the fast spread of excitation consists of a large ion-conducting α -subunit and up to two smaller, associated β -subunits (β_1 and β_2) [1,2]. The α -subunits cloned from brain and skeletal muscle form functional channels when expressed in *Xenopus* oocytes, although with much slower macroscopic inactivation compared with oocytes injected with its respective tissue mRNA. Fast (normal) macroscopic inactivation was restored by co-injection with low molecular weight mRNA from brain or skeletal muscle [3–5]. The plausible factor in low molecular weight mRNA was found to be the β_1 -subunit [6]. Co-expression of a human brain β_1 -subunit with rat skeletal muscle Na^+ channel α -subunit yielded similar observations and it was concluded that the functional domains of interaction between α - and β -subunits are highly conserved [7]. Bennett et al. [8] attributed the effect of β_1 -subunit to the modulation of gating modes of Na^+ channel α -subunits. Interestingly, the α -subunit of the heart differs as the macroscopic current decay is fast upon expression in *Xenopus* oocytes [9].

We have studied the effect of co-expression of the Na^+ channel β_1 -subunit cloned from rat brain on the kinetic properties of $\alpha_{\mu 1}$ Na^+ channels by measuring whole cell currents and compared with those obtained from oocytes injected with rat skeletal muscle mRNA. Expression of β_1 mRNA was evaluated by Northern blot analysis using probes from translated and untranslated regions of β_1 cDNA.

2. MATERIALS AND METHODS

2.1. PCR cloning of the β_1 -subunit

Rat brain poly(A)⁺ RNA was reverse transcribed and amplified with the polymerase chain reaction using oligonucleotides specific for the sodium channel β_1 -subunit. The primers were designed in a way that only two nucleotides of the original GC-rich 5'-UTR were present after ligation into a modified version of the pGEM-9zf(–) vector containing a polyadenylation sequence at the 3' end [10] and a 184 bp 5'-UTR (SacII–NcoI) sequence from the Shaker locus [11]. The sequence of the coding region was identical to the β_1 sequence reported by Isom et al. [6].

2.2. Northern blot

mRNA was isolated from adult Sprague–Dawley rat tissues (brain, heart, skeletal muscle and small intestine) and *Xenopus* oocytes as described [12] and poly(A)⁺ RNA was obtained by purification on an oligo(dT)–cellulose column. RNA was separated on a formaldehyde agarose gel and transferred to a nylon membrane. The concentration and integrity of the RNA samples were confirmed by staining the gel. Dilutions of β_1 cRNA were used as an internal standard to estimate the abundance. For hybridization the β_1 sodium channel cDNA NcoI(218)–HindIII(1,474) or the 3'-UTR BanII(891)–HindIII(1,474) cDNA fragment was isolated, labelled with digoxigenin dUTP and hybridization was performed for 16 h at 50°C in 50% formamide/0.82 M NaCl. The membrane was washed twice in 0.2 × SSC, 1% SDS at 60°C for 15 min. The chemiluminescent detection was done according to the manufacturer's instructions (Boehringer, Mannheim, Germany).

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Abbreviations: β_1 , β_1 -subunit of the sodium channel; $\alpha_{\mu 1}$, rat skeletal muscle sodium channel α -subunit isoforms; α_{IIa} , IIa rat brain sodium channel α -subunit; UTR, untranslated region.

2.3. Oocyte expression and electrophysiological measurement

Plasmids were linearized with *NotI* and cRNA was transcribed with T7 RNA polymerase in the presence of capping nucleotide. After removal of unincorporated nucleotides by LiCl precipitation the concentration of the cRNA was measured on a photometer and confirmed by electrophoresis. Stage II–V oocytes [13] were injected with 50 nl RNA solution ($\alpha_{\mu I}$, 5 ng; $\alpha_{\mu I}/\beta_1$, 5 ng/1.5 ng (1:1 molar ratio); skeletal muscle mRNA, 25 ng) and were incubated in ND96 (composition in mmol/l: NaCl 96, KCl 2, MgCl₂ 1, CaCl₂ 1, HEPES/Na⁺ 10, pH 7.5) supplemented with 100 U/ml penicillin, 100 μ g/ml streptomycin and 2.5 mM pyruvate, for 1–4 days at 21°C.

Two electrode voltage-clamp recordings were performed with a Dagan 8500 amplifier (Dagan, Minneapolis, USA) in ND96 as external bath solution at 22°C. Electrodes were filled with 3 M KCl and had a resistance of 0.3–1 M Ω . Currents were filtered at 3 kHz (Bessel type) and sampled at 15–30 kHz (TL-125 interface) using the PClamp 5.5 software (Axon Instruments, Burlingame, USA). For analysis oocytes with peak currents between 1 μ A and 3 μ A were taken.

3. RESULTS

Similar effects on the kinetic behaviour to those described by Isom et al. [6] were observed on co-injection of α_{IIa} with β_1 except that we used a lower concentration of β_1 in a 1:1 molar ratio of α_{IIa} to β_1 (not shown). Subsequently sodium channels were expressed following injection of cRNAs encoding the $\alpha_{\mu I}$ -subunit, a combination of $\alpha_{\mu I}$ and β_1 cRNAs ($\alpha_{\mu I}/\beta_1$) and rat skeletal muscle mRNA into *Xenopus laevis* oocytes. The electrophysiological properties are as given below.

3.1. Sodium current activation and current decay

The prominent effect of co-expression of β_1 was the acceleration of macroscopic current decay at all potentials. Sodium currents expressed from skeletal muscle mRNA were similar to those expressed from $\alpha_{\mu I}/\beta_1$ (Fig. 1A and B). The τ -values of macroscopic current decay plotted against the command potential are illustrated in Fig. 1B. Na⁺ currents expressed from skeletal

muscle mRNA are very close to the Na⁺ currents produced by $\alpha_{\mu I}/\beta_1$.

Peak currents elicited from $\alpha_{\mu I}/\beta_1$ were larger than those expressed from the same amount of $\alpha_{\mu I}$ cRNA alone (Fig. 1C), although there was a considerable variation among oocytes and also on the time of measurement following injection.

3.2. Steady-state inactivation

Currents from $\alpha_{\mu I}/\beta_1$ inactivated at more negative potentials than those expressed from $\alpha_{\mu I}$ alone (Fig. 2). Skeletal muscle mRNA induced currents with an inactivation behavior similar to the $\alpha_{\mu I}/\beta_1$. Currents expressed from $\alpha_{\mu I}$ alone showed two components with different inactivation behaviour. The fast decaying fraction inactivated at quite negative potentials, whereas only the slow decaying fraction showed the 'abnormal' insensitivity to depolarizing pulses in absence of the β_1 -subunit (Fig. 2). The co-expression of β_1 shifted the inactivation curve of $\alpha_{\mu I}$ to that derived after injection of skeletal muscle mRNA. Again a slight 'overshoot' is observed. The corresponding values are listed in Table I.

3.3. Recovery from inactivation

Sodium currents expressed from $\alpha_{\mu I}$ and to a lesser extent from skeletal muscle mRNA showed a biphasic time-course of recovery from inactivation ('fast' and 'slow' recovery from inactivation). The fraction of sodium channels exerting slow recovery (f_s) was 0.48 for sodium currents from $\alpha_{\mu I}$ alone. This fraction was hardly detectable in sodium currents expressed from $\alpha_{\mu I}/\beta_1$ (0.02) and was about 0.1 with mRNA (Fig. 3B). Time constants for fast recovery from inactivation were comparable in magnitude for $\alpha_{\mu I}$ (2.0 ± 0.4 ms), $\alpha_{\mu I}/\beta_1$ (2.3 ± 0.5 ms) and skeletal muscle mRNA (3.1 ± 0.7 ms) injected oocytes whereas the slow fraction in $\alpha_{\mu I}$ had

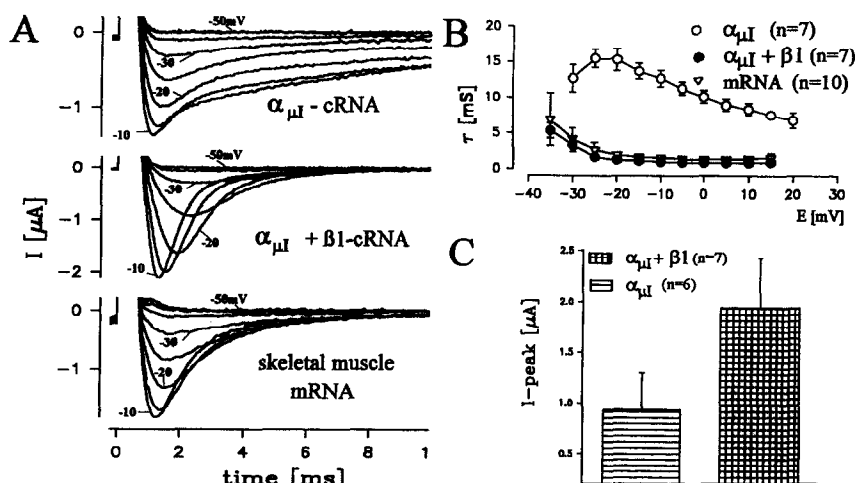


Fig. 1. (A) Representative current traces of skeletal muscle Na⁺ channels. Currents were evoked from a holding potential of -100 mV to the indicated potentials in increments of 5 mV. (B) Macroscopic current decay. Time constants of monoexponential current decay (τ) were determined by fitting a monoexponential function to the sodium current decay at different potentials. (C) Histogram showing the enhancement of the sodium peak current measured 4 days after injection of $\alpha_{\mu I}$ (5 ng) or $\alpha_{\mu I}/\beta_1$ (5 ng and 1.5 ng).

a time constant of 1.6 ± 0.5 s (see Fig. 3). The original traces illustrate that the fraction of $\alpha_{\mu I}$ sodium currents with slow recovery from inactivation is characterized by slow macroscopic current decay whereas the fast fraction showed fast recovery (Fig. 3A).

3.4. Northern blot

Signals were obtained with poly(A⁺) RNA of brain, heart and skeletal muscle. The hybridization with the 3' untranslated region of the brain β_1 cDNA showed signals of the same size and relative intensities for brain, heart and skeletal muscle mRNAs (Fig. 4). No specific signal was detectable from rat small intestine and from *Xenopus* oocyte total RNA. By comparison with the internal standard the signal obtained from 5 μ g of skeletal muscle, heart and brain poly(A⁺) RNA corresponded to 5 ng of cRNA. Assuming an average length of tissue mRNAs of 1,500 nucleotides the abundance is calculated as 0.1%.

4. DISCUSSION

The concentration of β_1 that induces functional effects with $\alpha_{\mu IIA}$ is much lower than reported by Isom et al. [6] and is most likely due to the removal of the GC-rich 5'-UTR, thereby improving the translational efficiency in the oocytes [6,7,15,16]. The 5'-UTR region from the Shaker potassium channel and an oligo(A)₄₀ tail present at the end of full-length β_1 transcripts might also contribute to this high expression level. Co-expression of $\alpha_{\mu I}/\beta_1$ produced an increased peak of the inward sodium current when compared to $\alpha_{\mu I}$ alone (Fig. 1C). A direct comparison with data obtained from single channel experiments [17] showed that the open probability at the peak current was similar in the presence and absence of β_1 , indicating that co-expression of β_1 led to more functional channels in the membrane.

Co-expression of β_1 shifted the steady-state inactivation curve to more negative potentials which is in accordance with earlier reports [6,7]. In addition we also observed a change in the slope of the inactivation curve. This discrepancy in the slope of the inactivation curve could be explained by differences in the resolution of the voltage clamp. The fast component which inactivates at

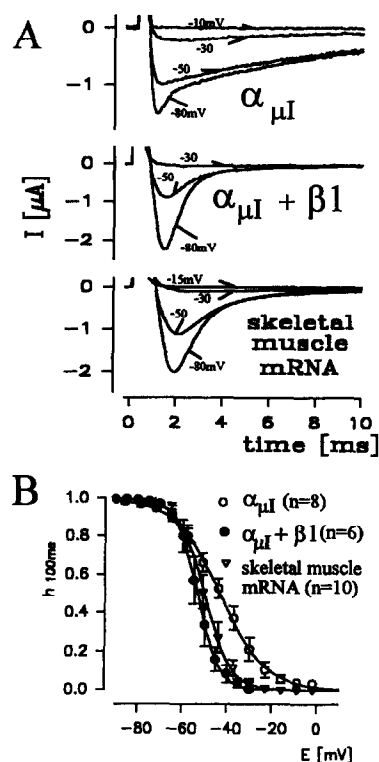


Fig. 2. Steady-state inactivation was measured by holding the oocyte's membrane for at least 5 s at -100 mV followed by an inactivating 100 ms prepulse to the indicated prepulse potentials and then evoking the Na⁺ current by jumping to -10 mV (mRNA, $\alpha_{\mu I}/\beta_1$) or 0 mV ($\alpha_{\mu I}$). (A) Representative current traces after holding at the indicated prepulse potentials. (B) Voltage dependence of steady-state inactivation. Standard deviations exceeding the symbol size are shown by vertical bars. The smooth curve is a fit to the means with a two-state Boltzmann distribution as described [14]; the corresponding values are listed in Table I.

negative potentials of the α current is lost if using current recordings with peak currents which are attained >2 ms after the voltage jump (see Fig. 2), and this results in no change in the slope of the inactivation curve. Changes in activation parameters are not shown due to the limitation of the resolution of the two electrode voltage clamp. For a reliable measurement of the activation parameters, macropatches or the cut open oocyte technique [18] has to be employed.

In contrast to channels expressed from $\alpha_{\mu I}$ alone that show bi-exponential recovery, the $\alpha_{\mu I}/\beta_1$ complex shows monoexponential recovery only. As the time constants for the fast recovery from inactivation do not differ significantly between $\alpha_{\mu I}$ and $\alpha_{\mu I}/\beta_1$ we conclude that in both cases sodium channels are in the same gating mode. Co-expression of β_1 practically abolishes the slow gating mode under our experimental conditions.

The kinetics of sodium channels induced by $\alpha_{\mu I}/\beta_1$ closely resembled the gating kinetics of sodium channels expressed from mRNA of the native tissue. The slight differences between the currents expressed from tissue mRNA and that expressed from $\alpha_{\mu I}/\beta_1$ may result from

Table I

Inactivation parameters derived from inactivation curve shown in Fig. 2

	$\alpha_{\mu I}$	$\alpha_{\mu I}/\beta_1$	poly(A) ⁺ RNA
$E_{0.5}$	-43.1 ± 2.7	$-53.3 \pm 2.7^*$	$-50.2 \pm 2.2^*$
k_i	10.2 ± 0.9	$4.8 \pm 0.4^*$	$6.0 \pm 0.9^*$
n	8	6	10

$E_{0.5}$ designates the membrane potential (mV) at half maximal steady state inactivation, k_i the slope at $E_{0.5}$ and n is the number of cells.

* $P < 0.05$ vs. $\alpha_{\mu I}$.

an inefficient translation of the tissue message of the native β_1 -subunit in the oocytes.

Northern blot analysis showed that mRNA encoding β_1 is present in high abundance in brain, skeletal muscle and heart. Further, it also reveals that the different sodium channel α -subunits may share a common β_1 -subunit. This is in accordance with Bennett et al. [8] who reported the cloning of a β_1 -subunit identical to the 'brain' β_1 -subunit from heart. These findings are in apparent contradiction to biochemical studies employing antibodies against the β_1 -subunit resulting in different sizes of β_1 -protein from different tissues after SDS-PAGE [2]. These differences in the migration behaviour of β_1 -subunits could be due to a different extent of glycosylation of the same protein in different tissues.

Co-expression of this β_1 with the rat heart sodium channel α -subunit [19] enhanced the peak current without significant alterations in the electrophysiological properties (L. Weigl, unpublished observations) indicating that the β_1 -subunit is also able to interact with the heart sodium channel α -subunit.

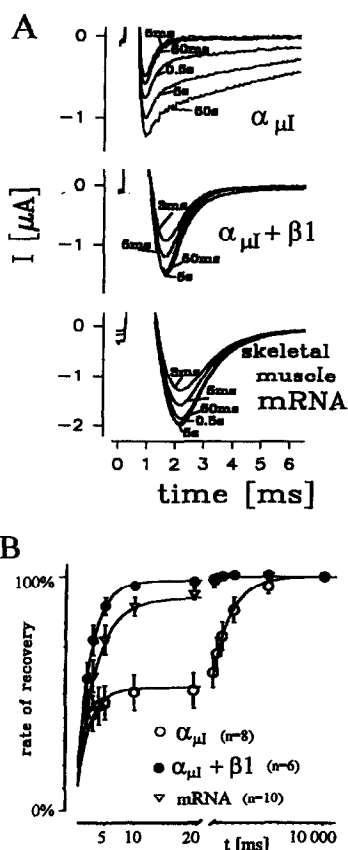


Fig. 3. Recovery from inactivation was measured by inactivating sodium channels for 100 ms with a supra-threshold pulse to -10 mV, then allowing recovery of sodium channels at -100 mV for a variable time interval (Δt), and then eliciting the peak sodium inward current by a pulse to -10 mV. (A) Representative current traces at indicated recovery time (Δt). (B) Rate of recovery vs. time. The vertical bars exceeding the symbol size are the standard deviation. The smooth curve is derived from a bi-exponential fit through the data points.

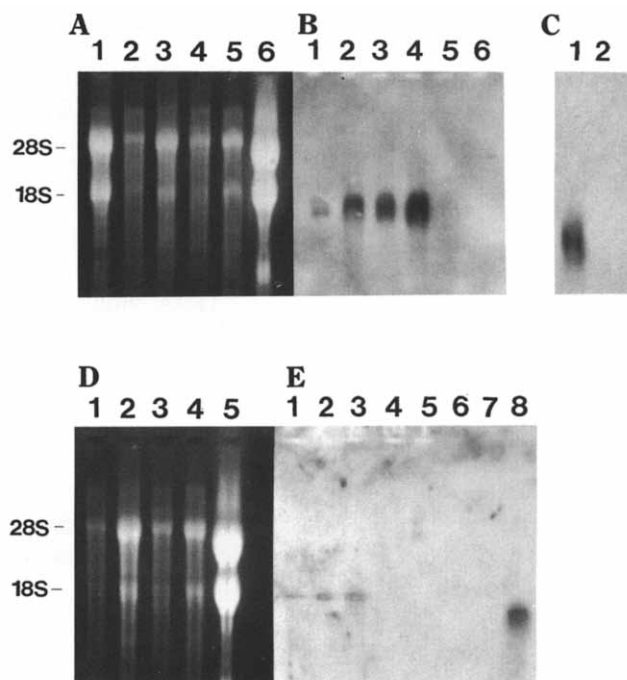


Fig. 4. Northern blot analysis. (A) Ethidium bromide-stained gel. (B) Corresponding chemiluminogram hybridized with the full-length β_1 cDNA probe exposed to film for 1 h. Lanes: 1, 15 μ g brain total RNA; 2–5, each 5 μ g rat poly(A)⁺ RNA; 2, brain; 3, heart; 4, skeletal muscle; 5, small intestine; 6, 25 μ g *Xenopus* oocyte total RNA. (C) Internal control exposed to film for 15 min; lane 1, 10 ng β_1 cRNA; lane 2, 1 ng β_1 cRNA. (D) Ethidium bromide-stained gel. (E) Corresponding chemiluminogram hybridized with a digoxigenin labelled fragment from the 3' untranslated region of the sodium channel (*Ban*II–*Hind*III fragment) (1 h exposure). Lanes: 1–4, each 5 μ g rat poly(A)⁺; 1, brain; 2, heart; 3, skeletal muscle; 4, intestine; 5, 25 μ g *Xenopus* oocyte total RNA; 6–8, β_1 cRNA (100 pg, 1 ng and 10 ng, respectively).

Although sodium channels induced by $\alpha_{\mu I}/\beta_1$ closely resemble sodium channels induced by skeletal muscle mRNA the amount of cRNA required to induce similar peak sodium currents is enormous when compared to the amount of sodium channel mRNA present in tissue. This could be a result of the artificial nature of the in vitro cRNA transcripts (inefficient capping, lack of a poly(A)⁺ tail) or additional factors present in tissue mRNA may be required for a high level expression of Na⁺ currents in *Xenopus* oocytes.

Surprising was the high abundance of the β_1 mRNA in brain, heart and skeletal muscle (about 0.1%) when compared to that of the sodium channel α -subunit mRNA (0.001–0.0001%; M. Wallner, unpublished observations). This high abundance of β_1 in skeletal, muscle and brain provokes the question of whether the translational regulation conferred by the GC-rich 5'-UTR region is also active in the native cells.

Acknowledgements: The authors are indebted to W. Agnew (New Haven) for providing a plasmid containing the $\alpha_{\mu I}$ construct, and to S. Goldstein (Yale) for the modified pGEM-9zf(–) vector. We thank W. Schreiber (Graz) for help and critical reading of the manuscript. Excellent technical assistance was provided by Mrs. B. Spreitzer.

(oocyte preparation and injection), Mr. A. Russ (electronics) and Mrs. E. Pichler (sequencing). This work was supported by the Austrian Research Foundation (P9249-MED), the US-Israel Binational Science Foundation, and a travelling fellowship to M.W. (Karl Franzens, University of Graz).

REFERENCES

- [1] Catterall, W.A. (1988) *Science* 242, 50–61.
- [2] McHugh Sutkowski, E.M. and Catterall, W.A. (1990) *J. Biol. Chem.* 265, 12393–12399.
- [3] Auld, V.J., Goldin, A.L., Krafte, D.S., Marshall, J., Dunn, J.M., Catterall, W.A., Lester, H.A., Davidson, N. and Dunn, R.J. (1988) *Neuron* 1, 449–461.
- [4] Trimmer, J.S., Coopermann, S.S., Tomiko, S.A., Zhou, J., Crean, S.M., Boyle, M.B., Kallen, R.G., Sheng, Z., Barchi, R.L., Sigworth, F.J., Goodman, R.H., Agnew, W.S. and Mandel, G., (1989) *Neuron* 3, 33–49.
- [5] Krafte, D.S., Goldin, A.L., Auld, V.J., Dunn, R.J., Davidson, N. and Lester, H. (1990) *J. Gen. Physiol.* 96, 689–706.
- [6] Isom, L.L., DeJongh, K.S., Patton, D.E., Reber, B.F.X., Offord, J., Charbonneau, H., Walsh, K., Goldin, A.L. and Catterall, W.A. (1992) *Science* 256, 839–842.
- [7] Cannon, S.C., McClatchey, A.I. and Gusella, J.F. (1993) *Pfluegers Arch* 423, 155–157.
- [8] Bennett, P. B., Makita, N. and George, A.L. (1993) *FEBS* 326, 21–24.
- [9] Satin, J., Kyle, J. W., Chen, M., Rogard, R.B. and Fozzard, H.A. (1992) *J. Membrane Biol.* 130, 11–22.
- [10] Swanson, R., Marshal, J., Smith, J.S., Williams, J.B., Boyle, M.B., Folander, K., and Kaczmarek, L.K. (1990) *Neuron* 4, 929–939.
- [11] Papazian, D., Schwarz, T.L., Tempel, B.L., Jan, Y.L. and Jan, L. Y (1987) *Science* 237, 749–753.
- [12] Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* 162, 156–159.
- [13] Krafte, D.S. and Lester, H.A. (1992) *Methods Enzymol.* 207, 339–345.
- [14] Schreibmayer, W. and Lindner, W. (1992) *J. Cardiovasc. Pharmacol.* 20, 324–331.
- [15] Fu, L., Ruiqiong, Y., Browder, L.W. and Johnston, R.N. (1991) *Science* 251, 807–810.
- [16] White, M.M., Chen, L., Kleinfeld, R., Kallen, R.G. and Barchi, R.L. (1991) *Mol. Pharmacol.* 39, 604–608.
- [17] Schreibmayer, W., Wallner, M. and Lotan, I. (1993) *Pfluegers Arch.* (submitted).
- [18] Taglialetela, M., Toro, L. and Stefani, E. (1992) *Biophys. J.* 61, 78–82.
- [19] White, M. M., Chen, L., Kleinfeld, R., Kallen, R.G. and Barchi, R.L. (1991) *Mol. Pharmacol.* 39, 604–608.