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Endogenous expression of the β1A sodium channel subunit in HEK-293 cells

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Abstract The expression of the sole α-subunit of muscle or brain sodium channels in frog oocytes mediates currents with a bimodal inactivation with an abnormal slow mode that is strongly depressed only by co-expression of the β1-subunit. In contrast, in the expression of the α-subunit in the human embryonic kidney cell line, HEK-293, the slow mode is almost absent, suggesting an endogenous expression of the β1-subunit. We have tested this hypothesis by reverse transcriptase-polymerase chain reaction. We found an abundant expression of mRNA encoding the β1A splicing of the putative regulatory sodium channel subunit but no mRNA encoding the β1-subunit in HEK cells. This finding is consistent with the idea that the endogenous β1A-subunit is sufficient for suppressing the slowly inactivating mode of sodium currents by co-assembly with α-subunits, and calls attention to the reliability of effects attributed in HEK cells to α–β1 co-expression.

Key words: Sodium channel; β1-Subunit; Alternative splicing; Heterologous expression; HEK-293

1. Introduction

The sodium channel is a heteromultimeric membrane protein responsible for the upstroke of the action potential in most excitable cells. Characterisation of the electrophysiological properties of the pore-forming α-subunit has been done in various heterologous expression systems. While the expression in cell systems like the human embryonic kidney cell line, HEK-293, have shown a kinetic behaviour similar to that of the native preparations [1,2], injection of the α-subunit cRNA in *Xenopus* oocytes shows an abnormally large component of a slowly inactivating mode [3–7]. This is consistent with the hypothesis that the channels are statistically distributed between two modes of gating, one with faster inactivation and a slower mode with an inactivation time constant more than 10-fold larger [3–5,8]. This slowly inactivating mode is substantially reduced by co-injection of cRNA encoding the β1-subunit [3,8–12], yielding sodium currents similar to those recorded in transfected cells.

The fairly normal expression of the sodium channel in transfected cells has been hypothesised to be due to an endogenous expression of the sodium channel β1-subunit [13], either constitutional or induced by the heterologous expression of the α-subunit. No direct demonstration of the presence of such an ancillary subunit in the HEK cells has been reported. When searching for the presence of the mRNA encoding the β1-subunit in the HEK cells using reverse transcriptase-polymerase chain reaction (RT-PCR), we were first surprised to find a clearly negative result, although the same technique reveals β1-mRNA in control neuroblastoma cells. Here we show that HEK cells do instead express an alternative splice variant of the β1-subunit, the β1A, which has recently been reported in adult adrenal gland and heart and in embryonic brain cells [14]. Evidently, β1A can co-assemble equally well with α-subunit [14] and modulate thereby the sodium channel inactivation, reducing the slow mode significantly.

2. Materials and methods

2.1. Cell cultures

HEK-293 was grown under standard conditions. To induce the expression of the sodium channel α-subunit, the cells were transfected by electroporation using the plasmid pcDNA3 (Invitrogen) containing the cDNA encoding the rat skeletal muscle sodium channel [7]. Transient expression was detected electrophysiologically 48–72 h after transfection. Monoclonal cell lines were obtained by selection for resistance to the antibiotic geneticin (G-418; Sigma). Twenty hours after transfection, the cells were incubated in medium supplemented with geneticin (800 μg/ml). After 8–10 days, cell colonies were isolated, grown separately and tested electrophysiologically. Three high expression clones, named HEK-μ1-0, HEK-μ1-9 and HEK-μ1-29, were selected. During later stages, cells were maintained in the absence of geneticin without significant loss of expression levels.

2.2. RT-PCR

Total RNA was extracted from parental and transfected cells using the RNeasy kit (Qiagen). The cDNA for each cell line was obtained by retrotranscription of 1 μg of total RNA using an Advantage RT-PCR kit (Clontech). To verify if either β1 or β1A were expressed in HEK cells, we amplified a region of cDNA that is presumably common to β1 and its slice variant β1A, assuming a homology with the gene splicing described on rat [14]. We used the forward primer β1/β1A-3 (5′-GGGAGGCTGCTGGCCTTAGTGGTC-3′) and the reverse primer β1/β1A-5 (5′-GTCCACTACCTCAATGTGGATCTTC-3′). For the amplification of the sole β1-subunit, we used the forward primer β1/β1A-3 and the reverse primer β1-5 (5′-CTATTCGGC-CACCTGGACGCCCGT-3′), which should anneal with the human β1-cDNA but not with the β1A. A specific β1A primer could not be used because the nucleotide sequence for the human isoform β1A is not yet known. To confirm the presence of the transfected α-subunit in HEK-μ1 cells, we amplified the corresponding cDNA using the forward primer μ1α-3 (5′-GGCTATCTCTCCCTCCTTCAGGTGG-3′) and the reverse primer μ1α-5 (5′-CCGAGCCAAGCGGATCA-CACGGAAC-3′). To control the presence of an endogenous sodium channel α-subunit in HEK cells, parental and transfected cell cDNA was checked with the forward primer h-α-3 (5′-GGATATCTGTCTC-TACTTCAAGTAG-3′) and the reverse primer h-α-5 (5′-CTTGG-CAAGACGGATCACTCGGAAC-3′), which should anneal to the cDNA of type 2 and type 8 sodium channels (SCNA2A and SCNA8A). As a control for the primers human h-α, β1 and β1/β1A, we amplified the corresponding cDNA fragments from the total RNA purified from a human neuroblastoma cell line, SH-SY5Y, using the same protocols. Primers for the housekeeping enzyme

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G3PDH, provided by the RT-PCR kit, were used as a control in all experiments. The PCR of the retrotranscription product was carried out using Taq polymerase (Promega), with the following cycling parameters: 45 s at 94°C, 45 s at 60°C, 120 s at 72°C for 28 cycles and then 10 min at 72°C. PCR products were analysed by electrophoresis on a 2% agarose gel.

2.3. Sodium channel functional expression

Xenopus laevis oocytes were injected with cRNA encoding the rat skeletal muscle α-subunit alone or co-injected with β1-cRNA as described [10,15]. Sodium currents were measured from membrane macro-patches in the cell-attached configuration [15,16]. The sodium channel expression in transfected cells was measured using the patch-clamp technique in the whole-cell configuration [16].

3. Results

As already described, the expression of the α-subunit cRNA expressed in oocytes (Fig. 1A) mediates the sodium current response to step depolarisations that are characterised by a rapid rise followed by a biphasic inactivation [3–7]. The falling phase of the current can be well fitted with a double-exponential function, $I(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2)$, where the two time constants, $\tau_1 < \tau_2$, differ by about one order of magnitude [9–11]. To quantify the propensity of the channels to gate in either mode, we define the parameter $P_{slow} = a_2/(a_1+a_2)$ as a measure of the probability of finding a channel in the slow mode during the test stimulus. For the expression of the sole α-subunit, $P_{slow} = 10.4 \pm 2.6\%$ (mean ± S.D.; $n = 15$). The co-expression of the β1-subunit reduces P_{slow} by a factor of about three, to $3.5 \pm 0.5\%$ ($n = 21$) (Fig. 1B), but does not alter the kinetics and voltage dependence of the two modal components [10,11]. This suggests that the main effect of the α–β1 interaction in oocytes is a reduction of the propensity of the channels for the slow mode. In contrast, the current expressed by the same α-subunit sodium channel in transiently (Fig. 1D) or permanent transfected HEK cells (Fig. 1E) showed a less prominent or even absent slow mode. The mean estimate of P_{slow} in permanently transfected HEK cells was of $1.08 \pm 0.99\%$ ($n = 9$), even closer than in the case of α–β1 co-expression to the vanishingly small value observed in neuronal cell lines (Fig. 1F).

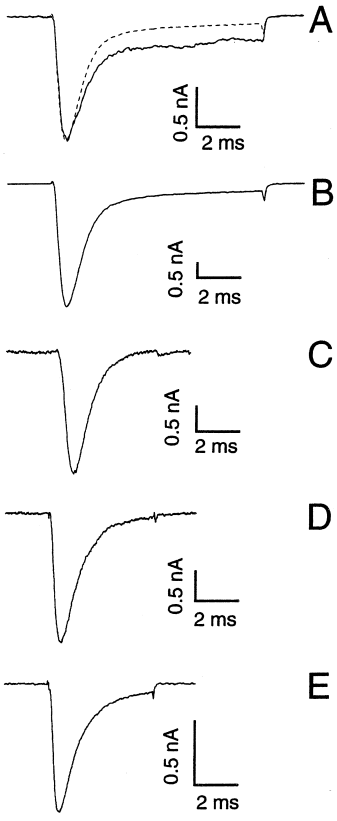

Fig. 1. Comparison of the heterologous expression of sodium current in frog oocytes and in mammalian cells. Injection of the sole α-subunit cRNA in oocytes yields sodium currents (A) comprising a slowly inactivating component that is strongly depressed by the co-expression of the β1-subunit (B and dotted line in A, scaled to the same peak value). In contrast, transient (C) or permanent (D) transfection of HEK-293 cells with sodium channel α-subunit alone leads to the expression of a functional phenotype with a vanishingly same slow inactivation component, similar to that observed on SH-SY5Y human neuroblastoma cells (E).

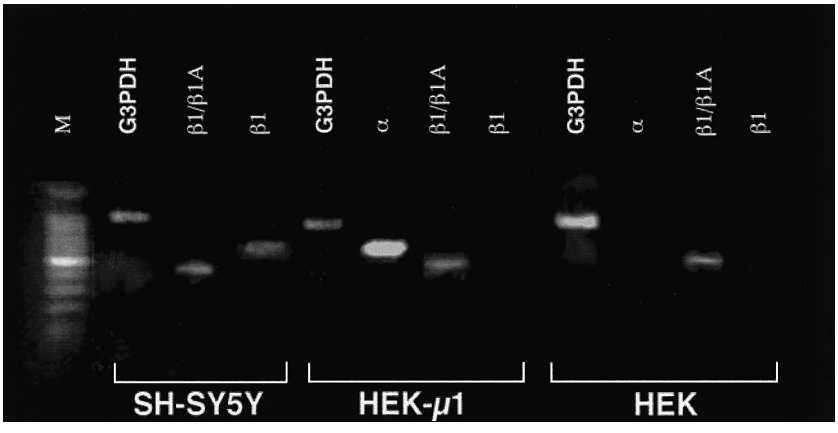

Fig. 2. Electrophoresis of RT-PCR products obtained from human neuroblastoma (SH-SY5Y), from HEK cells transfected with rat skeletal muscle sodium channel α-subunit (HEK-μ1), and from parental HEK cells (HEK). M: DNA markers; G3PDH: amplification of the cDNA encoding the enzyme glycerol-3-phosphate dehydrogenase; α: amplification of a cDNA segment of the sodium channel α-subunit with the primers μ1α-3 and μ1α-5; β1/β1: amplification of the cDNA segment encoding the first 400 bp, shared by the 5′-end of the β1-subunit and the 5′-end of the putative splice variant β1A, using the primers β1/β1A-3 and β1/β1A-5; β1: amplification of the 600 bp segment of the human β1-subunit cDNA, using the primers β1/β1A-3 and β1-5.

Similar results were obtained from HEK cells microinjected with cRNA encoding the α-subunit [17].

To explain the absence of the slow mode in the heterologous cell expression of the sole α-subunit, it has been suggested that the β1-subunit is expressed endogenously by the HEK cells [13]. In order to control this hypothesis, we attempted to identify the β1-subunit cDNA in the total RNA extracted from either parental HEK cells or transfected HEK-μ1 cells. Fig. 2 shows that in both cases, the β1-subunit is virtually absent, whereas the same method of analysis reveals, as expected, a strong expression of the rat α-subunit in transfected cells. On the other hand, the PCR amplification of the cDNA sequence expected to be shared by β1 and β1A yielded that β1A splice variant is constitutively present in HEK cells, and expresses independently of the α-subunit presence. Differently, and as a test of validity of our testing method, we obtained in human neuroblastoma cells the signals from the PCR amplification using either β1 primers, the β1/β1A primers and the α primers, indicating neuronal cells express as expected [14] least the β1-subunit together with the α-subunit (Fig. 2). The human α-subunit cDNA was not detected from HEK cells.

4. Discussion

On searching an endogenous expression of auxiliary β1-subunit of the human sodium channel in epithelial cells like HEK-293, we found the presence of the putative splice variant β1, analogous to β1A-subunit recently characterised and sequenced in rat [14]. This alternative splicing of the ancillary sodium channel subunit has been described to have similar functional properties than the β1-subunit, in particular considering that the putative functional region, i.e. the N-terminal region of the polypeptide [18], is well conserved among the two described splicings in rat [14], and thus most likely also in human. The presence of the sole β1A variant in an epithelial cell line is consistent with the embryonic localisation of β1A in rats, but the functional meaning of the abundant β1A expression in a non-excitable cell line remains obscure. Our present finding supports the hypothesis that the absence of the slow inactivation mode of sodium currents in α-transfected HEK cells is due to the co-assembly of the α-subunit with the endogenous β1A-subunit, and that the absence of any type of β1-subunit allows the sodium channels formed by the sole α-subunit in frog oocytes with a relatively high probability of conformation in which they inactivate more slowly than normal.

While giving experimental support to the widely accepted explanation of the different properties of sodium channels expressing heterologously in frog oocytes or in mammalian cell lines, our results provide a 'caveat' for studies of α–β1 interactions in HEK cells [19], because it is clear that the effect of an exogenous introduction of β1 in this preparation can only be attributed to an over-expression, that is difficult to quantify.

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