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Short communication

Heterologous expression of the human potassium channel Kv2.1 in clonal mammalian cells by direct cytoplasmic microinjection of cRNA

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Summary. The cloned human delayed rectifying K^+ channel Kv2.1 (*drk1*) was expressed in clonal mouse fibroblasts (L-cells) and rat basophilic leukemia cells (RBL-1) by direct cytoplasmic microinjection of complementary RNA (cRNA). Within six hours, cells microinjected with Kv2.1 cRNA expressed a large sustained outward current as determined from whole-cell patch-clamp recordings. Nearly 100% of cells injected with cRNA expressed outward current. Current density was 30–70 pA/pF when measured at a potential of +50 mV. Steady-state activation and inactivation parameters for Kv2.1 were similar when expressed in either L-cells or RBL-1 cells. These results are the first to demonstrate that functional ion channel proteins can be expressed in mammalian clonal cell lines by direct cytoplasmic microinjection of cRNA.

Introduction

Heterologous expression of ion channels by direct injection of mRNA into *Xenopus* oocytes was first reported by Sumikawa et al. (1981). Since this time, *Xenopus* oocytes have become the standard expression system for the study of cloned ion channel function. The popularity of the *Xenopus* oocyte as an expression host arises from several factors. First, the oocyte is large (approximately 1 mm in diameter) thus facilitating microinjection and intracellular recording. Secondly, the oocyte exhibits a robust translational capacity. Finally, the oocyte expresses relatively few endogenous voltage-gated ion channels. In contrast to the multitude of studies utilizing *Xenopus* oocytes to study ion channels expressed from microinjected cRNA, there are no reports of analogous experiments using mammalian cells. In this study, we show that the cloned human delayed rectifying K^+ channel Kv2.1 is expressed in two mammalian cell lines following direct cytoplasmic microinjection of cRNA. The potential advantages of using mammalian cells, as opposed to *Xenopus* oocytes, to study cloned ion channel function are discussed.

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Materials and Methods

Cell culture. L-cells (mouse fibroblast cell line) and RBL-1 cells (rat basophilic leukemia cell line) were purchased from the American Type Culture Collection (Rockville, MD, USA). RBL-1 cells were cultured in Minimal Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin and 1% glutamine. L-cells were maintained in an identical medium supplemented with non-essential amino acids.

RNA injection. The human Kv2.1 cDNA (GenBank L02840) was isolated from a human brain (cortex) cDNA library prepared in λZAP by probing with rat Kv2.1 DNA and using standard recombinant DNA procedures. Between the human and rat sequences there are only two amino acid differences from the N-terminus through S6. The *in vitro* transcription of cRNA was performed as described (Joho et al. 1990). Clonal cells were injected with cRNA using an Eppendorf (Madison, WI, USA) 5242 pressure (N_2) microinjector and 5171 micromanipulator system. Usually, cRNA was diluted from a stock solution of 200 ng/μl to a final concentration of 20 ng/μl with a solution containing 0.1% fluorescein dextran (10,000 MW, Molecular Probes, Eugene, OR, USA) in 100 mM KCl. The resulting solution was loaded into a hematocrit tube (sealed at one end by fire-polishing) and centrifuged at 16,000 x g for 10 minutes to remove particulates. About 1 μl of the supernatant was then removed and loaded into an Eppendorf Femtotip pre-pulled glass capillary (diameter of tip opening 0.5±0.2 μm) for injection. Cells were visualized on a Nikon Diaphot inverted microscope at 200-400x with phase contrast optics and a video system. In general, satisfactory injections were obtained with a pressure of 80-100 hPa and a duration of 0.3 s. Injections could be confirmed by observing the cells for fluorescence (Nikon B2A filter block). In some experiments, cRNA was diluted with 0.1% fluorescein dextran in water with equivalent results.

Electrophysiology. For the electrophysiological recordings, cells were bathed in an external solution containing (in mM): NaCl, 140; KCl, 5.4; HEPES, 10; $CaCl_2$, 2.0; $MgCl_2$, 0.8; glucose 15. The pH of the external solution was adjusted to 7.4 with NaOH and had an osmolality of 295 mosm kg^{-1}. K^+ currents were recorded with either an Axopatch 200 or an Axopatch 1D (Axon Instruments, Foster City, CA, USA) patch-clamp amplifier in the whole-cell configuration. Patch electrodes were pulled from Corning type 7052 glass and filled with an internal solution containing (in mM): KCl, 125; HEPES, 10; EGTA 11; $CaCl_2$, 1.0; $MgCl_2$, 2.0; MgATP, 4.0; Na_2GTP, 0.1. The pH of the internal solution was adjusted to 7.2 with KOH and had an osmolality of 270 mosm kg^{-1}. Electrode resistance was generally 2–4 MΩ when filled with internal solution. Previously injected cells were positively identified with epifluorescent

illumination. Generation of pulse protocols, acquisition and analysis of data, and production of figures was carried out with a Macintosh II computer using custom and commercial software.

Results

Cytoplasmic injections were carried out by "aiming" the pipette tip at the periphery of the cell—successful injection was indicated by a rippling "fluid wave" traversing the cell and a slight increase in cell size (≈10%) as observed on a video monitor. Microinjection of the cytoplasm was confirmed by examining the cells for fluorescence. Injected cells could be positively identified for at least three days post-injection although the fluorescence became progressively dimmer with time. Neither the injection procedure, fluorescein dextran, nor the cRNA appeared to interfere with vital cell processes as injected cells were still capable of division (i.e., the number of fluorescent L-cells approximately doubled, as determined for direct counting, after 24 hrs in culture; data not shown).

The result of injecting Kv2.1 cRNA into L-cells is shown in Fig. 1A. L-cells which were uninjected, injected with water, or injected with 0.1% fluorescein dextran displayed virtually no voltage-activated currents (Hosoi & Slayman 1991) in response to 70 ms command steps from a holding potential of -50 mV to potentials between -80 and +80 mV (Fig. 1A&C, control & ○). In contrast, L-cells injected 24 hrs previously with human Kv2.1 cRNA expressed large time- and voltage-dependent outward currents in response to depolarizing command pulses (Fig. 1A &C, injected & ●). The outward current activated at step potentials positive to -30 mV, exhibited the characteristic "delay" in activation which is the hallmark of "delayed-rectifier" type of K^+ currents, and produced outward tail currents when the potential was returned to -50 mV. Tail currents reversed polarity around -80 mV consistent with a current carried primarily by K^+ (data not shown). Characteristics of the outward current in injected L-cells were similar to those obtained in *Xenopus* oocytes injected with rat Kv2.1 cRNA (Frech et al. 1989). To assess the generality of the expression in clonal lines, human Kv2.1 cRNA was also injected into RBL-1 cells. Under normal conditions, RBL-1 cells express only an inwardly rectifying K^+ channel (Fig. 1B&C, control & □; Lindau & Fernandez, 1986). Thus, at potentials positive to about -40 mV there is a nearly complete absence of current. Twenty-four hrs following cytoplasmic injection of human Kv2.1 cRNA, RBL-1 cells exhibited a large time-dependent outward current at potentials positive to -30 mV in addition to the endogenous inwardly rectifying current evident at more hyperpolarized potentials (Fig. 1B&C, injected & ■).

Once conditions were optimized, expression was present in nearly 100% of the injected cells tested. For example, in one experiment, seven L-cells were recorded 24 hrs after cRNA injection. Outward current amplitudes, measured at +50 mV, ranged from 0.5–1.6 nA with a mean and S.E.M. of 1.02±0.17 nA. Thus even the smallest current was more

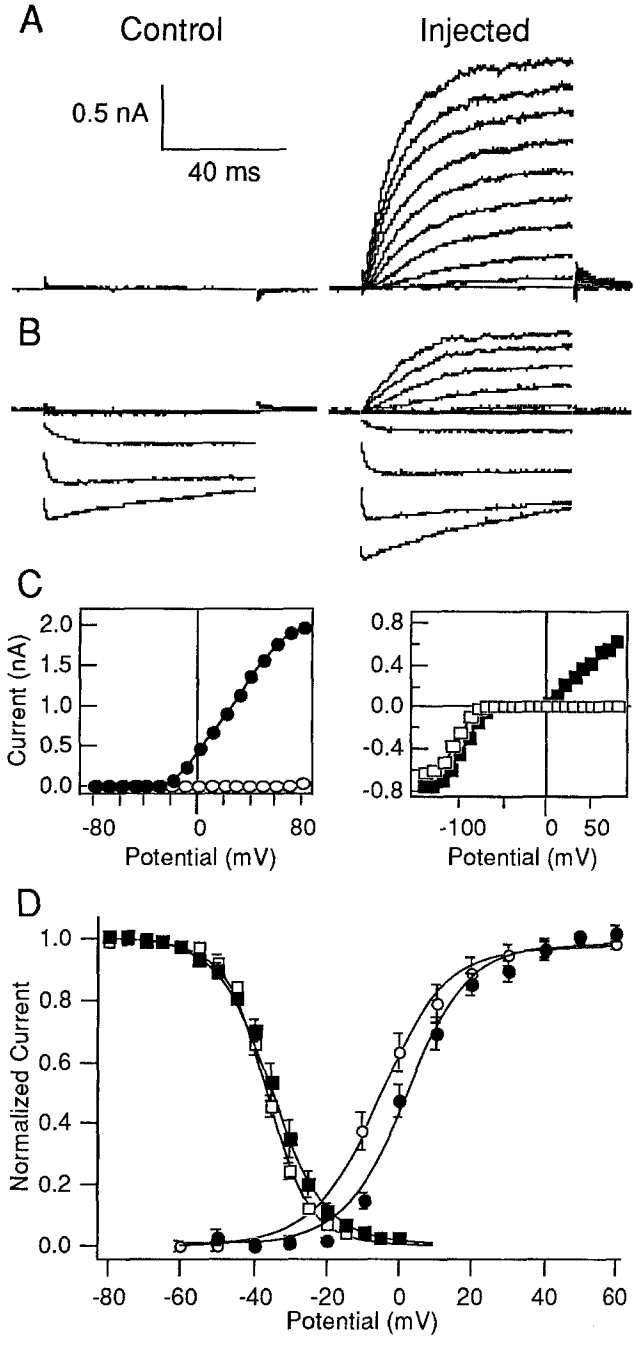

Fig 1. *A and B.* Superimposed current traces from an L-cell (*A*) or RBL-1 cell (*B*) which was either uninjected (control) or injected with human Kv2.1 cRNA (injected). The current traces illustrated were evoked by 70 ms depolarizing pulses to potentials between -30 and +60 mV (10 mV increments) from a holding potential of -50 mV in *A* and between -140 and +80 mV (20 mV increments) in *B*. *C*. Current-voltage relationships for L-cells (left; ○ control, ● injected) and RBL-1 cells (right; □ control, ■ injected) were determined from current amplitudes measured isochronally at the termination of the step. *D*. Normalized activation and inactivation curves in L-Cells (open, n=4–7) and RBL-1 cells (filled, n=5) twenty-four hrs after injection with human Kv2.1 cRNA. The solid lines represent the best fit to a modified Boltzmann equation as determined by least-squares nonlinear regression.

than an order of magnitude larger than background currents. Mean membrane capacitance, as determined from integration of the uncompensated capacitance transient, and current density were 23.7±3.0 pF and 43.1±5.8 pA/pF, respectively. Data pooled from three separate experiments showed that 32/32 cells expressed unambiguous time-dependent outward currents (> 200 pA). Although not studied in detail, a few observations concerning the time course of expression were made. In general, currents tended to be smaller 48 hrs after cRNA injection when compared to currents measured after 24 hrs. Somewhat surprisingly, very large (about 2 nA) outward currents were recorded in injected RBL-1 cells only 4–6 hrs post-injection.

Biophysical and pharmacological properties of the expressed human Kv2.1 K^+ channel were similar regardless of the expression host. Figure 1D illustrates the steady-state activation (circles) and inactivation (squares) characteristics of the expressed K^+ channel in both L-cells (open symbols) and RBL-1 cells (filled symbols). Activation was determined from tail currents arising from channel deactivation upon repolarization to -50 mV from step potentials between -60 and +60 mV. Tail current amplitudes were measured isochronally 5 ms after the step pulse and normalized to the current obtained following a step to +60 mV. The resulting mean data were fit to a modified Boltzmann equation:

$$i(V) = I_{max}[1 + \exp((V_{0.5} - V)/k))]^{-1}$$

where i is the current amplitude at voltage V, $Imax$ is the current amplitude used for normalization, $V_{0.5}$ is the half activation/inactivation voltage, and k is a slope factor. $V_{0.5}$ and k for activation of the outward current expressed in L-cells (-4.6 and 8.6 mV, respectively) and RBL-1 cells (2.6 and 8.3 mV, respectively) were similar. Inactivation was determined from step currents evoked by a depolarizing pulse to +50 mV from various holding potentials between -80 and 0 mV. Holding potentials were maintained for 10–30 s with similar results. Step current amplitudes were normalized to the amplitude obtained from a holding potential of -80 mV. The resulting data were fit to a modified Boltzmann equation. $V_{0.5}$ and k for inactivation of the outward current expressed in L-cells (-36.0 and -5.7 mV, respectively) and RBL-1 cells (-34.4 and -7.2 mV, respectively) were also similar. Finally, the ability of tetraethylammonium to block the expressed K^+ current was also similar in both clonal cell types. For example, 3 mM TEA inhibited the human Kv2.1 K^+ current (measured at +50 mV) 53.8±1.8 (n=4) and 52.0±1.2% (n=3) in L-cells and RBL-1 cells, respectively. In comparison, the IC_{50} for TEA of the rat Kv2.1 K^+ channel expressed in *Xenopus* oocytes is 5.5 mM (Taglialatela et al. 1992).

Discussion

These results are the first to demonstrate that functional ion channel proteins can be expressed in mammalian clonal cell lines by direct cytoplasmic microinjection of cRNA. When compared to the *Xenopus* oocyte expression system, the present system offers several distinct advantages. First, the technique can presumably be applied to a wide variety of mammalian clonal cell lines and ion channels. Thus one can optimize the expression host in regard to physical properties (e.g., cell size and ease of obtaining GΩ seals), endogenous ion channels, and signal transduction pathways. Although the applicability of the described system to other cloned ion channels remains to be tested, we have been able to express another K^+ channel, human Kv1.5, with equal success in both cell lines used in this study (data not shown). Secondly, the use of the whole-cell variant of the patch-clamp technique allows for greater recording fidelity and control of the intracellular milieu than conventional two-electrode voltage-clamping in oocytes. Thirdly, post-translational events in clonal cells may be more appropriate for expressed mammalian proteins. Finally, clonal cells are not subject to the seasonal variation in translational efficiency and expression of endogenous ion channels observed in *Xenopus* oocytes. On the negative side, equipment for the convenient injection of cRNA into mammalian cells can be expensive and in general recordings are not as stable in mammalian cells when compared to the *Xenopus* oocyte.

In summary, we have demonstrated that functional ion channel proteins can be reliably expressed in mammalian clonal cell lines by direct cytoplasmic microinjection of cRNA. This technique may provide a useful adjunct to the *Xenopus* oocyte and other expression systems currently being used to investigate cloned ion channel function.

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