Normal targeting of a tagged Kv1.5 channel acutely transfected into fresh adult cardiac myocytes by a biolistic method

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We report the successful adaptation of biolistic transfection to target K+ channel activity into isolated adult rat ventricular myocytes. Expression is robust within 24 h of transfection, a time frame during which, in contrast to other methods, substantial dedifferentiation does not occur. An RNA encoding a tagged Kv1.5 channel was transfected into fresh rat ventricular myocytes by biolistic transfection. Ventricular myocytes transfected with a tagged Kv1.5 channel exhibited an increased sustained current component that is 40% sensitive to 100 μM 4-aminopyridine and which is absent in myocytes transfected with a fluorescent protein-encoding construct alone. Kv1.5 deletion mutations known to reduce the surface expression of the channel in heterologous cells similarly reduce the surface expression in transfected ventricular myocytes, although targeting to the intercalated disc per se is generally unaffected by both NH2- and COOH-terminal deletion mutants. Expressed current levels in wild-type Kv1.5, Kv1.5ΔSH3(1), Kv1.5ΔN209, and Kv1.5ΔN135 mutants were well correlated with apparent surface expression of the channel at the intercalated disc. These results conclusively demonstrate the function of channels present at the intercalated disc in native myocytes and identify determinants of trafficking and surface targeting in intact cells. Clearly, biolistic transfection of adult cardiac myocytes will be a valuable method to study the regulation of surface expression of channels in their native environment.

cardiomyocyte; transfection; potassium channel

A GREAT DEAL HAS BEEN LEARNED about the trafficking of cardiac ion channels in heterologous expression systems (reviewed in Ref. 25), and much has been learned about the roles of motifs within K+ channels that affect trafficking of the channels to the cell surface (12, 15, 31, 32). Several Rab GTPases (18, 23, 29) and dynamin (6, 20) have been implicated in voltage-gated potassium (Kv) channel trafficking, and the dynein motor has been proven important in the regulation of the functional expression levels of several Kv channels (6, 13). All of these findings, however, have relied on model expression systems, generally with only supportive and inferential evidence from cardiomyocytes themselves (6, 13, 18, 23, 29). This reliance on model systems has been a necessity because the transfection of adult cardiomyocytes has proved intractable, and, as for adult neurons, the introduction of cloned genes into adult cardiac myocytes has proven, to date, to be practical only with retroviruses.

Standard liposome-mediated protocols work for transfection of rat neonatal myocyte preparations, but the currents expressed in these cells are quite different from those of adult cardiomyocytes (22, 28). Viral transduction systems are effective in adult cells but require sophisticated containment facilities and either prolonged culture of the myocytes, during which time substantial dedifferentiation can occur (3, 30), or technically difficult in vivo transduction protocols followed by myocyte isolation (19, 26). On the other hand, so-called biolistic, or “gene gun,” methods have proved of limited use in transfecting surface myocardium in living heart with a nonviral vector (16) but have not been successfully adapted for transfection of isolated myocytes useful for electrophysiological or imaging studies. Balse et al. (4) have recently reported success with an adaptation of a lipofectamine-mediated transfection protocol (7, 27) for the transfection of adult atrial cardiomyocytes with standard mammalian expression vector constructs. However, while ionic currents conducted across the plasma membrane of these cells are broadly similar to those of acutely isolated myocytes, due to the necessity of a lengthier time in culture, these transfectants lack the normal rod-shaped myocyte morphology, so are therefore of limited use for studies of targeting of ion channels to plasmalemmal domains. As shown below, this limitation is true, also, of ventricular myocytes transfected by this method.

While transfection of adult ventricular myocytes is clearly effective by this method, the dedifferentiation that occurs concomitantly—including the loss of striation and intercalated disc structures—renders the method of limited use for the study of trafficking and localization of potassium channels in the myocytes. As our intent is to study this trafficking and localization, we tested whether another method, biolistic transfection, could be adapted for use in cardiomyocytes and, if so, whether myocytes transfected in this manner would retain the currents and structures of freshly isolated cells.

We report the successful adaptation of biolistic transfection methods for reliable, high-efficiency transfection of acutely isolated adult rat ventricular myocytes. Expression is robust within 24 h of transfection, a time frame during which, in control and enhanced green fluorescent protein (EGFP)-transfected myocytes, the currents remain essentially unchanged from the time of isolation. Transfection of these ventricular...
myocytes with Kv1.5 results in a selective increase in sustained current ($I_{\text{sus}}$), as expected in myocytes overexpressing Kv1.5. Confirming that the increase in $I_{\text{sus}}$ is indeed underlain by Kv1.5, the induced $I_{\text{sus}}$ is sensitive to 4-aminopyridine (4-AP). We further demonstrate the utility of this system in the study of the functional expression and localization of a number of Kv1.5 mutants in cardiomyocytes.

**MATERIALS AND METHODS**

*Plasmid constructs.* Human Kv1.5 was NH2-terminally tagged with EGFP or mCherry or double-tagged with a T7 tag at the NH2 terminus and a hemagglutinin (HA) tag within the S1–S2 linker of the channel. Plasmid constructs. Myocytes were transfected using Lipofectamine-mediated myocyte transfection. For transfection, freshly isolated rat ventricular myocytes in ~0.2 ml storage buffer [base Ca2+/free solution (see above) plus 20 mmol/l taurine, 1% BSA, 100 mmol/l CaCl2] were plated on laminin-coated coverslips at high density.

*Electrophysiology.* Whole cell voltage-clamp experiments were performed at room temperature using an Axopatch 200B amplifier and pClamp software (Axon Instruments, Foster City, CA). Patch pipettes had a resistance of 1 to 3 MΩ. Pipette solution (in mmol/l) 135 NaCl, 5 KCl, 13 MgCl2, 2.8 sodium acetate, 10 HEPES, 1 CaCl2, 0.2% Triton X-100 for 10 min followed by three 10-min washes with 100% ethanol that had been dried successively over two beds of silica gel. The beads were then suspended in 50 mmol/l spermidine and 1 to 2 μg of plasmid DNA per milligram of beads was added and precipitated onto the beads by dropwise addition of 1 mol/l CaCl2 to ~330 mmol/l. After 10 min at room temperature, the beads were washed four times with 100% ethanol that had been dried successively over two beds of silica gel. The beads were then suspended in 3 ml of 100% ethanol, sonicated in a Branson 1210 sonication bath, and loaded into plastic tubing using the Bio-Rad Tubing Prep Station and a Bio-Rad EconoPump peristaltic pump. For transfection, freshly isolated rat ventricular myocytes were incubated with appropriate primary and secondary antibodies. In other experiments, cells were incubated with 1 ml of lipofectamine (Invitrogen) were separately prepared in Opti-MEM I medium (500 μl final volume in each) and incubated 5 min at room temperature. The two solutions were mixed, incubated 20 min at room temperature, and then added to the culture dish. Transfection was allowed for 4 h in a 1% CO2 incubator. Cells were gently washed twice with M199 medium containing 5% FBS, 0.1× ITS and streptomycin-penicillin, and incubated 48 h in a 5% CO2 incubator before experiments.

*Biologic myocyte transfection.* Biologic gold beads coated with plasmid DNAs were prepared as directed in the Bio-Rad Helios Gene Gun System instruction manual with the exception that no polyvinylpyrrolidone was employed. Briefly, 25 mg of 0.6-μm gold beads were suspended in 50 mmol/l spermidine and 1 to 2 μg of plasmid DNA per milligram of beads was added and precipitated onto the beads by dropwise addition of 1 mol/l CaCl2 to ~330 mmol/l. After 10 min at room temperature, the beads were washed four times with 100% ethanol that had been dried successively over two beds of silica gel. The beads were then suspended in 3 ml of 100% ethanol, sonicated in a Branson 1210 sonication bath, and loaded into plastic tubing using the Bio-Rad Tubing Prep Station and a Bio-Rad EconoPump peristaltic pump. For transfection, freshly isolated rat ventricular myocytes were incubated with appropriate primary and secondary antibodies. In other experiments, cells were incubated with 1 ml of lipofectamine (Invitrogen) were separately prepared in Opti-MEM I medium (500 μl final volume in each) and incubated 5 min at room temperature. The two solutions were mixed, incubated 20 min at room temperature, and then added to the culture dish. Transfection was allowed for 4 h in a 1% CO2 incubator. Cells were gently washed twice with M199 medium containing 5% FBS, 0.1× ITS and streptomycin-penicillin, and incubated 48 h in a 5% CO2 incubator before experiments.

*Immunostaining and confocal imaging.* Myocytes were rinsed and fixed with 2% paraformaldehyde at room temperature. After 10 min, fixative was removed and the cells were washed with glycine buffer fixed with 2% paraformaldehyde at room temperature. After 10 min, fixative was removed and the cells were washed with glycine buffer.
512 resolution. The images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD).

Data statistics. Results are expressed as means ± SE. Statistical analyses were conducted using Student’s t-test (paired) or by one-way ANOVA, as appropriate.

RESULTS

Lipofectamine-mediated transfection of isolated adult rat ventricular myocytes. As an initial approach to adult rat cardiomyocyte transfection, we adapted the lipofectamine-mediated method employed for the transfection of adult atrial myocytes by Balse et al. (4). To test the effectiveness and effects of the procedure on ventricular myocytes, adult rat ventricular myocytes were isolated and cultured overnight under a low (1%) CO₂ atmosphere. The following morning, they were transfected by a lipofectamine procedure with either an NH₂-terminally T7-tagged, externally HA-tagged Kv1.5 construct in pcDNA3 (for imaging experiments) or, for electrophysiological studies, with a Kv1.5 construct plus an mCherry construct, both in pcDNA3. Similarly to atrial myocytes (4), ventricular myocytes transfected by this method express the introduced Kv1.5 at high levels (Fig. 1). However, also like atrial myocytes, substantial dedifferentiation is evident in these transfected myocytes. Even after only 2 days in culture, cells are rounded and lack striation and other myocyte-specific structures such as the intercalated disc (Fig. 1A). After longer culture times, the cells become flattened with extensive processes (Fig. 1B). Unlike fresh ventricular myocytes, where endogenous Kv1.5 localizes almost entirely to the intercalated disc (5, 8, 17) (Fig. 3A, right), Kv1.5-HA expression is apparent across the entire plasma membrane in these cells (Fig. 1, A and B).

Functional expression of the transfected channel was robust. At ±80 mV, sustained current densities were 9.5 ± 0.6 pA/pF in control, mCherry-transfected ventricular myocytes and 109.2 ± 22.8 pA/pF in those transfected with Kv1.5 plus mCherry (Fig. 1, C and D). Confirming that the increased sustained currents were due to Kv1.5 overexpression, these currents were reduced to 70.3 ± 14.5 pA/pF, a 40% reduction, by administration of 100 μM 4-AP (Fig. 1D; P < 0.01). The
sustained current in control myocytes was insensitive to 4-AP at this dose (data not shown). Although in some of the mCherry-transfected control cells currents typical of cardiomyocytes were present, in over 60% of these transfectants, as in Fig. 1C, little or no Na⁺ current and only small transient outward currents were seen upon depolarization from −80 mV. These changes occurred also in untransfected myocytes and are thus due to the prolonged culture times required to achieve transgene expression rather than to the lipofectamine-mediated transfection itself.

**Biollistic bombardment efficiently transfects freshly isolated adult rat ventricular myocytes.** To determine whether a biollistic protocol was capable of transfecting freshly isolated adult rat ventricular myocytes, we bombarded these cells with gold particles of sizes ranging from 0.6 to 1.6 μm coated with a pcDNA3 construct encoding mCherry fluorescent protein. Freshly isolated adult male rat ventricular myocytes were plated on laminin-coated coverglasses and incubated for 1 h at 37°C in an M199-based media (see MATERIAls AND METHODS). To minimize dedifferentiation in culture, no FBS was used. Whereas FBS was necessary for the survival of myocytes transfected by the lipofectamine-mediated method to the 24–48 h necessary to detect gene expression in that system, expression in bioollistically transfected myocytes is detectable in <24 h. Over this time frame, myocytes remain viable and, whether transfected or not, uniformly retain their morphological characteristics without the FBS (see below). After the 1-h incubation, the myocytes were bombarded with gold particles coated with pcDNA3/mCherry or pcDNA3/EGFP (1 μg DNA/mg gold beads) using a Bio-Rad Helios gene gun (Fig. 2A) and incubated overnight in the same media. The following morning they were assayed for transfection by fluorescence imaging and electrophysiological methods.

At pressures commonly used for other cell types, e.g., 400 psi, cardiomyocytes did not survive no matter which size gold particle was used (data not shown). Optimization of pressure, distance, and gold particle size, however, yielded mCherry expression in myocytes in practical numbers when 0.6-μm gold particles were used. No transformants were recovered in experiments employing 1.0-μm and 1.6-μm gold particles. Bombardment at 90–110 psi with 0.6-μm gold particles coated with 1 μg DNA/mg gold beads and a gene gun nozzle to myocyte distance of ~1 cm yielded the best results. Although roughly 90% of initially viable myocytes were killed in this pressure range, transfection efficiencies among surviving myocytes were quite high. An average of $28.2 \pm 5.7\%$ of surviving myocytes were transfected as assayed in several experiments by mCherry fluorescence. No transfecants were detectable when particles were fired at 60 psi. Pressures greater than ~130 psi killed essentially all of the bombarded myocytes.

Myocytes transfected under the optimized conditions retained typical myocyte morphology 24 h after bombardment. Transfected with expression vectors encoding EGFP (Fig. 2B, top) or encoding mCherry (Fig. 2B, middle), the transfected myocytes remained rod-shaped, striated and, as assayed by connexin 43 staining (Fig. 3A, left), with intercalated discs intact. Thus, this is the first successful transfection method for acutely isolated adult rat cardiomyocytes that yields transfecants with essentially normal myocyte morphology.

**Transfected Kv1.5 is targeted to the intercalated disc in biollistically transfected rat ventricular myocytes.** Having successfully adapted gene gun technology to the transfection of adult cardiomyocytes, we employed this technique in the study of Kv1.5 trafficking and localization in these cells. Kv1.5 has been reported to localize largely to the intercalated disc in rat and canine ventricular myocytes (2, 8, 17), although some argue that this apparent colocalization may be an artifact of nonspecific binding of antibody to this “sticky” region. To test whether or not the channel is truly targeted to that domain, we performed experiments in which we transfected freshly iso-
lated adult male rat ventricular myocytes with a Kv1.5 construct, in pcDNA3, NH2-terminally tagged with mCherry (Cherry-Kv1.5).

When examined under the confocal microscope, transfected cells were readily identified. Like wild-type Kv1.5 detected with anti-Kv1.5 (2, 8, 17), the Cherry-Kv1.5 fluorescence was concentrated at the intercalated disc regions of the cells and was quite faint elsewhere (Fig. 3A, middle). Little fluorescence was evident in the cell interior or at peripheral locations of the plasmalemma. This pattern was similar to that of endogenous Kv1.5. When freshly isolated ventricular myocytes were stained with an antibody directed to the COOH terminus of Kv1.5, the strongest staining for the endogenous channel was at the intercalated disc (Fig. 3A, right).

While these data clearly indicated that exogenous channels were targeted to the intercalated disc, it was not possible to discriminate between channel inserted in the plasma membrane or those located in a subsurface compartment. To test whether tagged Kv1.5 in transfected myocytes is expressed at the cell surface, experiments with a surface-tagged channel were conducted. Freshly isolated myocytes were transfected by the same method with a pcDNA3 construct in which Kv1.5 was tagged with an HA epitope in the extracellular S1–S2 linker and a T7-tag at the intracellular NH2 terminus (29). Staining of live cells for Kv1.5-HA (with mouse anti-HA, detected with Alexa Fluor 594-conjugated goat anti-mouse after fixation) clearly demonstrated cell surface expression of the tagged Kv1.5 in the transfected cells (Fig. 3B). While in a few cells, some channel was evident at peripheral locations, expression was highest at the intercalated disc in all cases. Figure 3B shows a cell in which peripheral localization of the channel was highest among all myocytes transfected with Kv1.5-HA in these initial experiments. Even in this case, the majority of Kv1.5 is localized to intercalated discs.

Transfection with Kv1.5 substantially alters myocyte current characteristics. To determine whether the transfected Kv1.5 was functional in the ventricular myocytes, freshly isolated myocytes were cotransfected, as described above, with a Kv1.5 construct in pcDNA3 plus another pcDNA3 construct encoding either EGFP or mCherry fluorescent protein (1 μg each per mg gold). The construct encoding the fluorescent protein (FP) allowed transfected myocytes to be readily identified for electrophysiology. As illustrated in Fig. 4, current profiles were altered as expected for myocytes overexpressing Kv1.5. Whereas peak transient outward current at +80 mV was unchanged by transfection with Kv1.5 (at 21.2 ± 2.4 pA/pF in
Kv1.5 + FP-transfected myocytes vs. 21.1 ± 3.5 pA/pF in myocytes transfected with FP alone), the sustained current was increased to 11.7 ± 1.0 pA/pF in the Kv1.5 + FP-transfected myocytes from 7.9 ± 0.7 pA/pF in myocytes transfected with FP alone (P < 0.05; Fig. 4, A and C). When the myocytes were subjected to a −40-mV prepulse for 75 ms to inactivate transient outward K⁺ current (Iₒ), the functional expression of Kv1.5 could be detected even at early depolarization times (Fig. 4B). Peak current at +80 mV in these prepulsed Kv1.5-transfected cells was 13.8 ± 1.8 pA/pF vs. 10.3 ± 0.9 pA/pF in control, FP-transfected myocytes (P < 0.05; Fig. 4, B and D). Sustained current densities in these prepulsed myocytes were 6.9 ± 0.7 pA/pF in the FP-transfected and 9.7 ± 1.3 pA/pF in the Kv1.5 + FP-transfected cells, respectively. Confirming that the increase in current density was due to expression of the transfected Kv1.5, the sustained currents in Kv1.5-transfected, but not in FP-transfected, myocytes were significantly reduced (P < 0.05) by 100 μM 4-AP (Fig. 4E); sustained currents in myocytes transfected with FP alone showed no such sensitivity to 4-AP.

Molecular determinants of Kv1.5 targeting to the intercalated disc of adult rat myocytes. Having established that tagged Kv1.5 is functionally expressed and trafficked to the intercalated disc in transfected rat ventricular myocytes, we next investigated the roles in channel trafficking played by several putative mechanisms already described using heterologous expression systems. A canonical PDZ-binding domain located at the COOH terminus of the channel has been proposed to be involved in the localization of Kv1.5 at the intercalated disc, where several anchoring proteins containing PDZ domains are located, including zona occludens-1 (ZO-1) (10), ZO-2 (24), ALP (21), and SAP97 (9). We studied the expression of an EGFP-tagged Kv1.5 in which the PDZ-binding domain was deleted (EGFP-Kv1.5ETDL). As illustrated in Fig. 5B, deletion of the PDZ-binding domain had no effect on the targeting of the transfected channel. Like full-length Kv1.5 (Fig. 5A), the channel localized almost entirely to the intercalated disc.

Deletion of the NH₂-terminal-most SH3-binding domain of the channel prevents internalization of the channel in human embryonic kidney (HEK)293 cells (6). We hypothesized that Kv1.5 localization at the intercalated disc may result from...
selective internalization from lateral membrane in the ventricular myocytes. When transfected with a EGFP-tagged SH3 deletion mutant, EGFP-Kv1.5/H9004 SH3(1), however, rat ventricular myocytes trafficked the mutant channel, like wild-type, to the intercalated disc (Fig. 5C).

Since there was no disruption of localization to the intercalated disc by the ETDL and SH3 mutants, we moved on to studies on the localization of tagged Kv1.5 channels with larger deletions in their NH2 termini, one known to have no effect on expression in heterologous cells, another known to not to traffic to the plasma membrane (D. Fedida laboratory, unpublished observation). As illustrated in Fig. 5D, when transfected into ventricular myocytes, this channel did indeed traffic normally to the cell surface, specifically to the intercalated disc. A larger deletion, Kv1.5ΔN209, results in substantially lower expression than the wild-type channel in heterologous expression systems. This pattern was recapitulated in the ventricular myocytes transfected with a GFP- and HA-doubly tagged Kv1.5ΔN209. Expression was detectable as GFP fluorescence at the intercalated disc, but only when brightness and contrast were considerably enhanced (Fig. 5E, GFP*). This was confirmed by staining for the external HA tag. Intercalated disc localization of the GFP-tag

An HA-tagged deletion of the NH2-terminal-most 119 amino acid residues, Kv1.5ΔN119-HA, was first tested. In heterologous cells, this mutant traffics normally to the plasma membrane (D. Fedida laboratory, unpublished observation). As illustrated in Fig. 5D, when transfected into ventricular myocytes, this channel did indeed traffic normally to the cell surface, specifically to the intercalated disc. A larger deletion, Kv1.5ΔN209, results in substantially lower expression than the wild-type channel in heterologous expression systems. This pattern was recapitulated in the ventricular myocytes transfected with a GFP- and HA-tagged Kv1.5ΔN209. Expression was detectable as GFP fluorescence at the intercalated disc, but only when brightness and contrast were considerably enhanced (Fig. 5E, GFP*). This was confirmed by staining for the external HA tag. Intercalated disc localization of the GFP-tag
and HA-tag overlapped precisely (compare Fig. 5E, top, panels 2 and 4).

We tested also a channel mutant, Kv1.5Δ135, which localizes entirely to the cell interior and fails to produce currents when transfected into HEK293 cells (D. Fedida laboratory, unpublished observation). This channel is deleted into the mid-T1 domain and likely fails to assemble properly in the HEK cells. When mCherry-tagged Kv1.5Δ135 was transfected into ventricular myocytes, it failed almost completely to traffic to the intercalated disc. In a few cases, slight punctate staining was evident at this structure (Fig. 5F), but in the great majority of transfectedants, no surface expression of the channel was evident. Instead, unlike all other mutants, punctate fluorescence was evident in the cell interior, visible especially when the image contrast was enhanced (Δ135-Cherry*). No such interior fluorescence was seen with other channel constructs; an enhanced view of Kv1.5-GFP from Fig. 5A is included in Fig. 5F to illustrate this point.

The functional expression of each of Kv1.5ΔSH3, Kv1.5ΔN209, and Kv1.5ΔN135 was tested electrophysiologically. The results of these analyses were consistent with the imaging data. Transfection with Kv1.5ΔSH3 increased the sustained current densities in rat ventricular myocytes to 10.8 ± 1.5 pA/pF at +80 mV (Fig. 6, A and B), a value not statistically different from the 11.8 ± 1.4 pA/pF seen in myocytes transfected with wild-type Kv1.5. Densities in both were significantly higher ($P < 0.05$) than the 7.7 ± 0.4 pA/pF of control, mCherry-alone-transfected myocytes at +80 mV (Fig. 6, B and G).

Transfection with Kv1.5ΔN209 (Fig. 6, C and D), on the other hand, had no detectable effect on the sustained currents. Current densities in myocytes transfected with Kv1.5ΔN209 averaged 8.8 ± 0.4 pA/pF at +80 mV, not significantly different than the 7.7 ± 0.4 pA/pF of control, mCherry-transfected myocytes and significantly lower ($P < 0.05$) than the 11.8 ± 1.4 pA/pF average density seen in wild-type Kv1.5-transfected myocytes. This failure to detect an increase in current density in myocytes transfected is broadly consistent with results from heterologous cells. While Kv1.5ΔN209 indeed produces detectable currents in transiently transfected HEK293 cells, these currents are small. Current densities in HEK293 cells transiently transfected with Kv1.5ΔN209 averaged 5.2 ± 1.4 pA/pF ($n = 9$) at +80 mV, roughly 10% of the $45.3 ± 8.5$ pA/pF ($n = 5$) produced by similar transfection with wild-type Kv1.5 ($P < 0.001$). Suggestively, the absolute

![Fig. 6. Effects of mutations in transfected Kv1.5 on sustained currents in rat ventricular myocytes. A: representative currents from rat ventricular cardiomyocytes cotransfected with Kv1.5ΔSH3 and mCherry. Traces shown are from −80 mV in 10-mV steps to +80 mV, from a holding potential of −80 mV. B: mean steady-state current density-voltage relationships from ventricular myocytes transfected with mCherry (control), Kv1.5 + mCherry, and Kv1.5ΔSH3 + mCherry stimulated as indicated in A. *$P < 0.05$, significant difference between control ss and both Kv1.5 ss and Kv1.5ΔSH3 ss (one-way ANOVA). C: representative currents from rat ventricular cardiomyocytes cotransfected with Kv1.5ΔN209 and mCherry stimulated as indicated in A. D: mean steady-state current density-voltage relationships from ventricular myocytes transfected with mCherry (control), Kv1.5 + mCherry, and Kv1.5ΔN209 + mCherry stimulated as indicated in A. *$P < 0.05$, significant difference between Kv1.5 ss and both Kv1.5ΔN209 ss and control ss (one-way ANOVA). E: representative currents from rat ventricular cardiomyocytes cotransfected with Kv1.5ΔN135 and mCherry stimulated as indicated in A. F: mean steady-state current density-voltage relationships from ventricular myocytes transfected with mCherry (control), Kv1.5 + mCherry, and Kv1.5ΔN135 + mCherry. *$P < 0.05$, significant difference between Kv1.5 ss and both Kv1.5ΔN135 ss and control ss (one-way ANOVA). G: representative currents from rat ventricular cardiomyocytes cotransfected with mCherry alone stimulated as indicated in A. H: 4-AP sensitivity of sustained currents in control, mCherry-transfected ventricular myocytes, and in ventricular myocytes transfected with the mutants described in A–F. The ratio of sustained current density at +80 mV after treatment with 4-AP to the sustained current density before 4-AP treatment is indicated. Values are means ± SE. *$P < 0.05$ (paired t-test).](http://ajpcell.physiology.org/)}
values detected in the transfected myocytes are consistent with these results from heterologous cells.

Like Kv1.5ΔN209, myocyte transfection with Kv1.5ΔN135 (Fig. 6, E and F) produced no significant effects on the sustained currents. Myocytes transfected with Kv1.5ΔN135 exhibited current densities of 8.6 ± 0.5 pA/pF, statistically equivalent to the 7.7 ± 0.4 pA/pF of control, mCherry-transfected myocytes and lower (P < 0.05) than the 11.8 ± 1.4 pA/pF seen in myocytes transfected with wild-type Kv1.5. When transiently transfected into HEK293 cells, Kv1.5ΔN135 produced no currents (data not shown).

Of the mutants tested electrophysiologically, only Kv1.5ΔSH3 conferred 4-AP sensitivity on the sustained currents in the myocytes. 4-AP (100 μM) significantly reduced current densities at +80 mV in Kv1.5ΔSH3-transfected myocytes (P < 0.05; Fig. 6H). Current densities for myocytes transfected with the partial T1-deletion mutants Kv1.5ΔN209 and Kv1.5ΔN135 were, like myocytes transfected with mCherry alone, not affected by 4-AP application. Thus, as in heterologous systems, an intact T1 domain appears to be necessary for normal trafficking of Kv1.5 in rat ventricular myocytes.

**DISCUSSION**

We have employed a new transfection protocol to introduce plasmid-encoded tagged Kv1.5 constructs into adult rat ventricular myocytes and have studied determinants of the surface localization of the Kv1.5 channel using this method. This biolistic transfection method has several major advantages. First, it shows a high efficiency: over 20% of surviving myocytes bombarded with the DNA-coated beads expressing tagged Kv1.5 as assayed by immunocytochemistry. Second, unlike the viral transduction methods generally used to express introduced proteins in myocytes, there is no need for complicated cloning strategies to incorporate the gene of interest into a large viral vector. Any gene in a mammalian expression vector can be introduced into the myocytes. Finally, one major advantage of this method is the rapid expression of the transgene—within 24 h—avoiding myocyte dedifferentiation in culture. Indeed, the expression of the transgene is rapid enough that the localization and effects of the introduced protein can be assayed in cells retaining the morphological and electrophysiological properties of freshly isolated myocytes. This method also eliminates the need for sophisticated containment facilities and prolonged culture of the myocytes or sophisticated surgical infection protocols. Taken together, this method represents a major breakthrough in the study of ion channel expression in their intact and native environment.

Indeed, using this method we provide for the first time unequivocal evidence that Kv1.5 localizes to the intercalated disc in adult cardiac myocytes. This was previously proposed using mainly immunohistochemical approaches for the study of endogenous channel expression in the myocardium (2, 8, 17), but it was still questioned due to the reportedly sticky nature of the intercalated disc. Using antibody to the channel, Abi-Char et al. (1) recently demonstrated apparent Kv1.5 partial localization at cell-cell contacts in cultured neonatal rat myocytes, but they were unable to detect such localization in neonatal myocytes transfected with a GFP-Kv1.5 fusion construct. This failure to detect GFP-Kv1.5 localization at the intercalated discs in these cells may be due to differences in channel expression patterns in neonatal myocytes from those of the adult or to the apparent dedifferentiation of the myocytes in culture. Dedifferentiation is much less problematic in myocytes transfected by our biolistic method. As evident in Figs. 2, 3, and 5, adult ventricular myocytes transfected by our method retain morphology, 24 h after transfection, very similar to that of freshly isolated cells. Currents recorded in fresh and GFP- or mCherry-transfected control cells are also very similar at both time points (Fig. 4A). Thus, results obtained with these cells are more representative of in vivo expression patterns than are neonatal myocytes cultured for several days. Our results also indicate that the NH2-terminal part of the Kv1.5 channel is a crucial determinant for its localization at the level of the intercalated disc. The PDZ-interacting domain located at the COOH terminus of the channel appears not to be necessary for this localization. However, it is possible that once addressed to the intercalated disc, anchoring proteins containing PDZ domains play an important role in the organization of Kv α-subunits in large protein complexes, including second messengers (7).

We did not find mistargeting to the lateral membrane by any of the channel mutants we tested. Of course, there may be specific motifs outside the regions of the channel that we have tested that are indeed required for correct surface localization. Further work is clearly required to answer this question.

The significance of the localization of Kv1.5 to the intercalated disc remains unknown. One possibility is that the intercalated disc functions somewhat analogously to the nervous system’s nodes of Ranvier, as suggested by Maier et al. (14). In this model, concentration of ion channels at the intercalated disc speeds conduction by allowing depolarization to jump in a saltatory fashion from intercalated disc to intercalated disc. Kucera et al. (11) have modeled the effects that the high density of Na,1.5 channels in the intercalated disc may have on conduction between cells. However, they found that under most plausible conditions, a high density of functional sodium channels at these sites impaired rather than enhanced conduction.

In summary, we have adapted a biolistic procedure to the transfection of acutely isolated rat cardiac myocytes. Transfection efficiencies among surviving myocytes are high and expression of the introduced genes is rapid and robust. Furthermore, the targeting of a tagged potassium channel, Kv1.5, introduced by this method recapitulates that of the endogenous channel. This method has substantial advantages over viral and lipofectamine-mediated methods and will facilitate the study of gene and protein expression as well as of protein trafficking in cardiac cell systems.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.
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