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K^+ channel expression in primary cell cultures mediated by vaccinia virus

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A recombinant vaccinia virus (VV) was used to express functional *Drosophila* Shaker H4 K^+ channels in primary cell cultures from rat heart (atrial and ventricular myocytes, fibroblasts), autonomic ganglia (SCG neurons) and CNS (hippocampal neurons, cerebral astroglia). In most cells the expressed currents possessed the typical characteristics of the native *Drosophila* muscle A currents, a few cells showed evidence of hetero-oligomers with new properties. The maximum current density corresponded to a channel density of 2 3/μm^2. Voltage recordings in heart cells showed altered action potential waveforms after successful infection. VV vectors thus are useful for studying altered excitability and cell-specific processing of ion channel proteins.

Heterologous expression, Shaker, Voltage-gated, Ion channel; Excitability

1. INTRODUCTION

The large DNA virus, vaccinia virus (VV), has been shown to be a useful vector for the expression of a voltage-gated *Drosophila* Shaker K^+ channel in various cell lines [1]. Several features, such as its large capacity for foreign cDNA inserts (>25 kb) [2], broad host range, high efficiency of infection, and cytoplasmic mRNA biogenesis render it an attractive system for heterologous expression of ion channels. We report here on the extension of this system to postmitotic mammalian cells in primary culture.

2. MATERIALS AND METHODS

Cardiac myocytes and fibroblasts were obtained from neonatal rats (postnatal days 2–3) after enzymatic dissociation of atrial or ventricular tissue [3] and used within the first 4 days. Glial cultures were prepared from the cerebral hemispheres, superior cervical ganglion (SCG) neuronal cultures were obtained from sympathetic ganglia [4], these were used during the first 2 weeks. Rats at embryonic days 18–19 were used to make hippocampal cultures [5].

Cultures were infected with the recombinant VV:H4 virus [1] at a multiplicity of infection (MOI) of 1 to 5. When cell counts were uncertain due to a low density, the infection medium carried no less than 10^5 viruses per ml [6]. Precautions were observed to prevent accidental exposure of personnel to VV and to inactivate all virus before disposal.

Whole-cell patch clamp recordings were performed as described [7]. Prior to recording, the infected cells on coverslips were washed 3 times with recording solution (135 mM NaCl, 6 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes, 5 mM glucose, pH 7.4). Intracellular pipette solutions consisted of 140(120) mM KCl, 2(2) mM $MgCl_2$, 1(5) mM EGTA, 5(10) Hepes, 1(1) mM $ATPNa_2$, 0.1(0) mM cAMP, pH 7.3 (internal solution for neurons in brackets). Capacitive transient cancellation and series resistance compensation of whole-cell currents was performed on-line using the clamps' features. Digital leak subtraction was used in some cases during the analysis. Results shown were verified in at least three cells of each type.

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3. RESULTS

Whole-cell recordings in primary cells were performed 24–72 h after infection with recombinant VV:H4 virus. Voltage-clamp records indicated that the expressed H4 gene products were succesfully assembled into functional A-type K^+ outward currents. The VV:H4 infected cardiac fibroblast (Fig. 1a,d), the ventricular myocyte (Fig. 1b,e), and the atrial myocyte (Fig. 1c,d) displayed transient outward currents that activated at potentials more positive than −40 mV (Fig. 2a). Non-infected or VV wild-type infected myocytes ($n = 45$) showed only inward (Na^+ channel) currents. In general, the characteristics of the Shaker K^+ currents expressed in rat cardiac cells agree closely with measurements on native fly muscle cells [8] and embryonic myotubes [9,10] as well as H4 RNA injected oocytes [11]. Both the macroscopic current activation τ_{act} and inactivation time constants τ_{inact}, were described by a double exponential function of voltage (Fig. 2b) and were indistinguishable among the majority of cell types. Recovery from steady-state inactivation (Fig. 2a) occurred with the half-inactivation point at -40.2 ± 2.1 mV (mean ± SE; $n = 5$). The induced currents were highly selective for K^+ ions as indicated by the reversal of the tail currents and were completely blocked by 5 mM 4-aminopyridine in all cell types. The efficiency of VV:H4 expression was higher (ca 80%) for cardiac fibroblasts than for heart muscle cells, neurons and glia

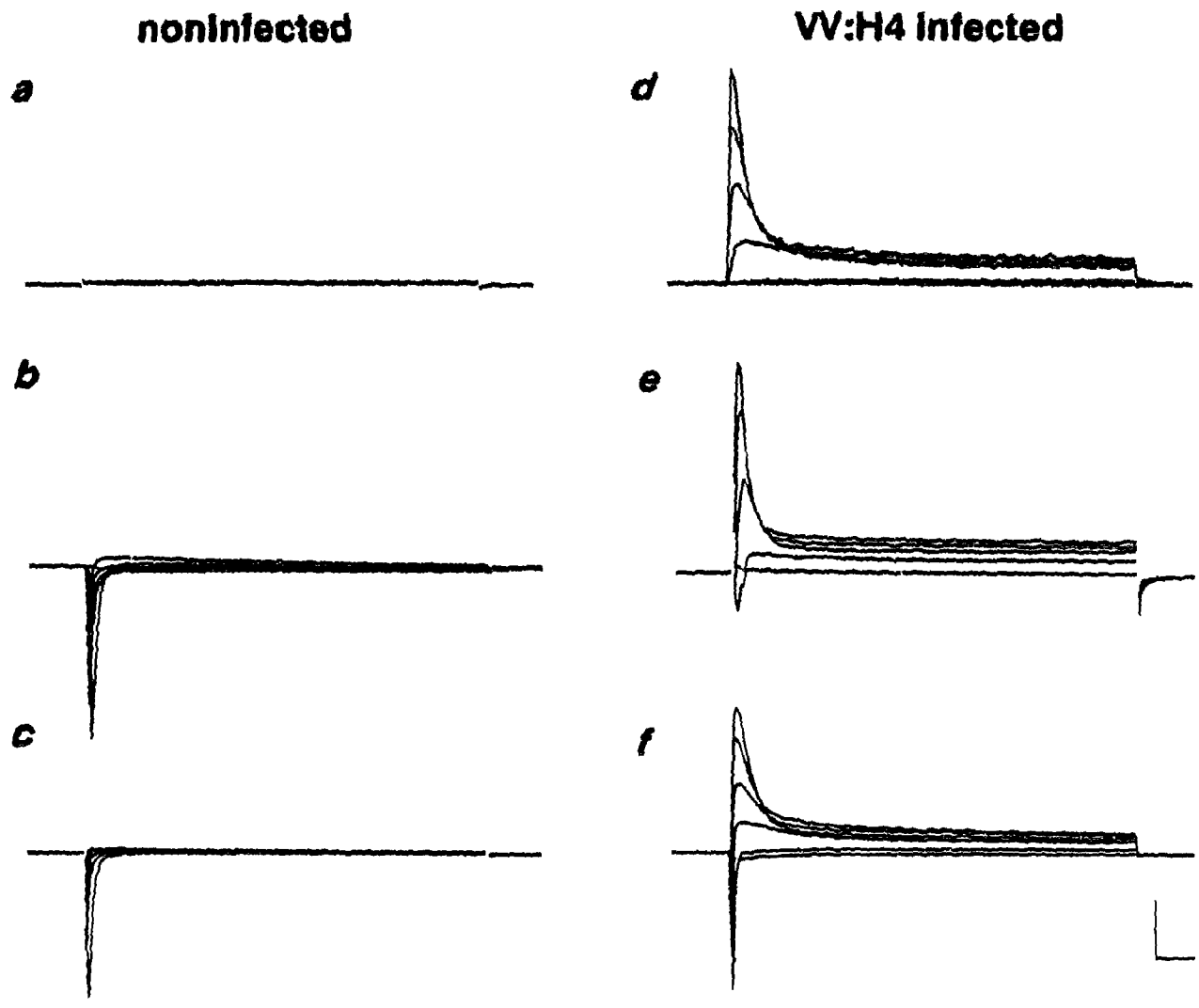


Fig. 1 Membrane currents of a noninfected control rat cardiac fibroblast (a), ventricular myocyte (b), and atrial myocyte (c), and one cell of each type 24 h after VV H4 infection (d-f) Recordings during 180 ms voltage steps between -50 mV and +50 mV from holding potentials of -90 mV Scale bars = 1 nA, 20 ms

(ca 50%). Because the induced current amplitudes were similar, these differences presumably arise from a varying susceptibility to VV, rather than from differing viral replication rates. We calculated densities of expressed H4 channels up to 2–3 channels/μm^2 of cell membrane (see Table I).

We have also expressed H4 currents in rat SCG and hippocampal neurons that endogenously display transient A-type K^+ channels. Unlike the H4 currents, these intrinsic currents were completely inactivated at holding potentials of -60 mV. Together with appropriate pharmacological suppression, the VV:H4 expressed currents could be unequivocally extracted from the current records in most neurons (Fig. 3a). The activation kinetics for infected neurons resembled those for infected myocytes and were faster than in a noninfected neuron (Fig. 3b). The waveforms of the macroscopic currents were variable; however, in some neurons it was impossible to obtain subtraction currents that fitted the waveforms of the endogenous A-type currents (Fig. 3c), suggesting that hetero-oligomers of endogenous and H4 chains had formed.

Current-clamp recordings in VV:H4 infected myocytes revealed action potentials where the early downstroke phase during the first 5 ms was several-fold faster than for noninfected control cells (Fig. 4a,b). The spikes in the infected myocytes resembled those of a noninfected neuron in shape (Fig. 4b). The fast inactivation was typically followed by a slower decay (τ = 25–30 ms) at voltages more negative than -40 mV; this was several times slower than the cell's passive membrane time constants (6-8 ms).

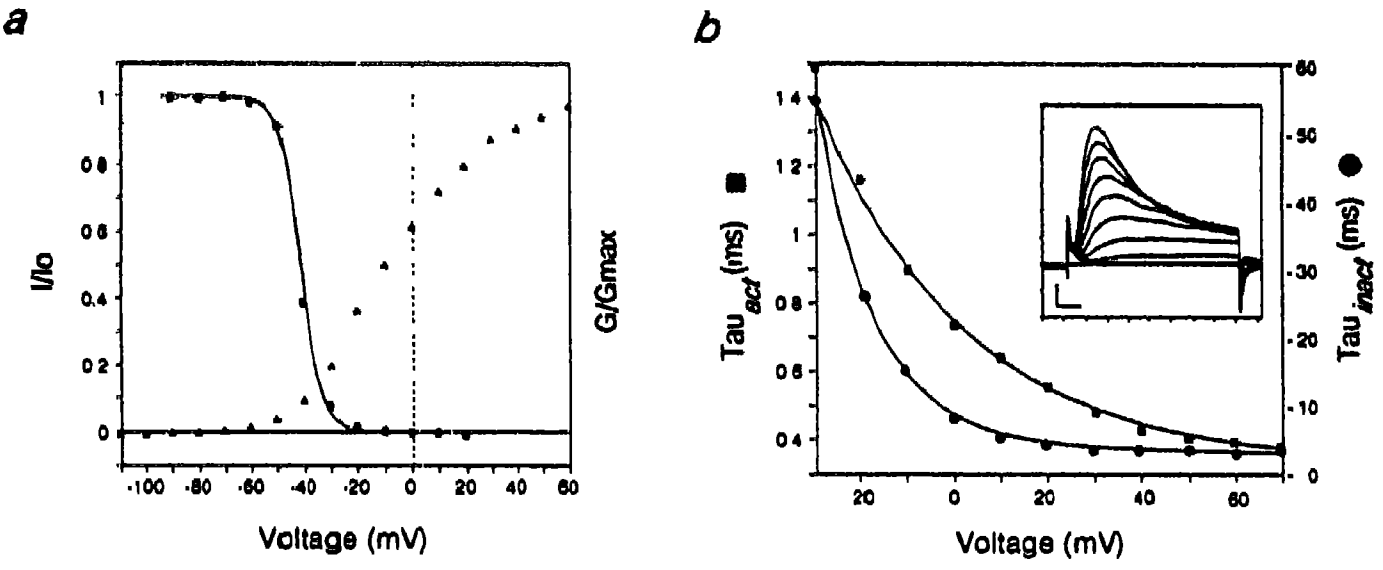


Fig. 2. (a) Steady-state current inactivation I/I_o (●) and the relative peak conductance G/G_{max} (▲) for a VV:H4 infected cardiac fibroblast. The ratio I/I_o is derived as the peak current response to a voltage step from −120 mV to +30 mV (I_o) versus currents induced from voltage steps to +30 mV from various holding potentials (I). The curve, the best data fit to a Boltzman distribution, has a slope of 3.9 mV change for every e-fold change and a midpoint of −41 mV. G was calculated as I/V_m-V_K, assuming V_K = −80 mV and G_{max} at +60 mV. (b) Time constants of macroscopic current activation (τ_{fast1}, ■) and inactivation (τ_{inact}, ▼) in a VV H4 infected cardiac fibroblast plotted versus membrane voltage, and fitted by a double exponential function. The insert shows, at fast time resolution, the cells' responses to a family of voltage steps between −40 and +40 mV. Scale bars = 1 nA, 2 ms.

4. DISCUSSION

The data demonstrate the VV-mediated transient expression of voltage-gated K^+ channels in primary cardiac and neuronal cells. The VV expressed Shaker H4 gene products assembled into functional K^+ channels, producing records that resembled *Drosophila* muscle A or myotube A1 currents. The voltage-dependent K^+ channels encoded by Shaker and its mammalian homologs are probably tetramers [12]. There is evidence that distinct subunits can form hetero-oligomeric channels in *Drosophila* larval muscle [13]. In addition, heteropolymerization of different rat brain subunits and the formation of *Drosophila*/rat brain K^+ channel hybrids has been reported for RNA-injected oocytes [14–16]. Neurons in our experiments have endogenous A-type K^+ channels with turnover rates that could be less than one day, therefore the new waveforms recorded after H4 infection in these cells (Fig. 3) may represent neuronal/H4 hetero-oligomers.

The altered action potential waveform in VV:H4 infected cardiac myocytes deserves special mention, because the controlled alteration and/or complete reconstitution of electrical excitability is one goal for expression of voltage-gated ion channels with VV vectors. Cardiac myocytes, which have a low density of endogenous K^+ currents, represent an appropriate target to demonstrate modification of inherent excitability. By introducing the fast H4 currents, we were successful in producing neuron-like action potential waveforms in heart muscle cells.

For the expression of the H4 channels in primary cells using recombinant VV, we calculated a maximal yield of 10^4 channel molecules in the outer plasma membrane

TABLE I

VV induced expression of *Drosophila* Shaker H4 K^+ channels in primary cell cultures[a]

Host cell	Peak current (nA)	Membrane area (μm^2)	Current density ($\mu A/cm^2$)	Channels total	Density/μm^2
Atria cells	3.7 ± 2.1 (19)	5 ± 0.7×10³	74	4625	0.9
Ventricular cells	4.0 ± 1.3 (3)	4.6 ± 0.3×10³	88	5060	1.1
Cardiac fibroblasts	3.3 ± 0.9 (12)	10.8 ± 2.1×10³	30	4060	0.4
SCG neurons	2.8 ± 1.7 (5)	4.5 ± 1.1×10³	62	3460	0.8
Hippocampal neurons	5.7 ± 1.4 (7)	3.2 ± 0.7×10³	178	7150	2.3
Brain astroglia	7.6 ± 2.8 (8)	10.5 ± 2.3×10³	73	9500	0.9

[a]The cell membrane surface area was determined from the infected cells' capacity assuming a specific membrane capacity of 1 $\mu F/cm^2$. The channel density was estimated based on a single channeld conductance of 16 pS and a channel open probability of 0.5. Given values are mean ± SD (n)

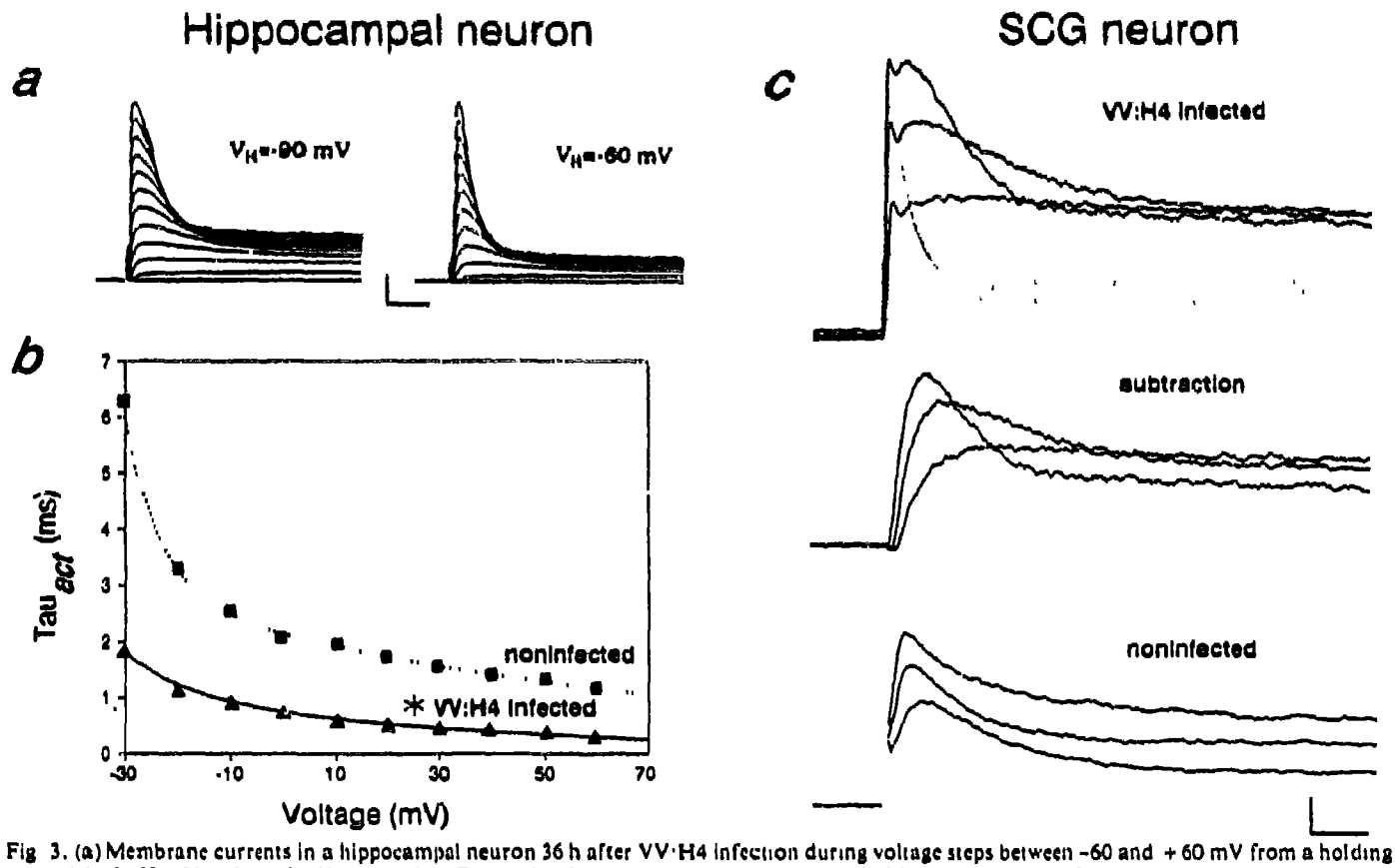
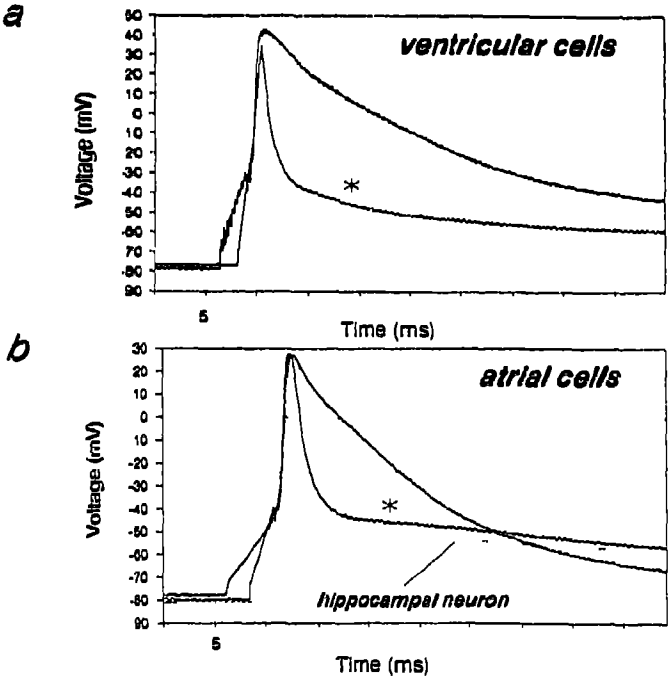


Fig 3. (a) Membrane currents in a hippocampal neuron 36 h after VV·H4 infection during voltage steps between -60 and +60 mV from a holding potential of -90 mV (left) and -60 mV (right). The bath solution contained additional 0.2 μM TTX and 5 mM $CoCl_2$ Scale bars = 1 nA, 20 ms. (b) Activation time constants (τ_{act}) of outward currents in a noninfected (■) and VV H4 infected hippocampal neuron (▲) fitted by a double exponential function. Inserts at fast time scale show the current responses of one cell each (infected cell*) Scale bars 1 nA, 2 ms (c) VV H4 induced and endogenous transient A-type K^+ currents in a SCG neuron during voltage steps to +20, +40, +60 mV from a holding potential of -90 mV Top and bottom recordings have been subtracted by responses obtained from a holding potential of -30 mV, 0 2 μM TTX and 5 mM $CoCl_2$ added to the bath solution The subtraction currents are the difference between the solid and dotted traces at the top, which represent currents from a VV H4 infected SCG neuron (solid lines), and normalized pure H4 currents isolated from a cerebral astrocyte (dotted lines) Scale bars = 250 pA, 20 ms



of a cell. This value is comparable to the density of H4 channels on the plasma membrane recently expressed in an insect cell line using a recombinant baculovirus [17]. By analogy with the expression of mouse nACh receptors, a comparable amount of membrane proteins could be located internally [18]. The principal advantages of VV vectors are the broad host cell range and high efficiency of infection in primary cells as well as cell lines. Thus, vaccinia may prove useful for expression of membrane proteins requiring cell-specific posttranslational processing, association with cell-specific subunits, or coupling to endogenous second messenger pathways. Many ion channels express properties that change with developmental stage, with phylum and species, or with different organs in an organism. It will now be possible to ask whether such functional dif

Fig 4 Action potentials from noninfected and VV·H4 infected (marked with a*) ventricular (a) and atrial myocytes (b) recorded in current-clamp mode (c) The action potential of the infected atrial cell resembles that of a noninfected hippocampal neuron (dotted line)

ferences are encoded in the primary amino-acid sequence or require a particular cellular environment.

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