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Resilient RTN Fast Spiking in Kv3.1 Null Mice Suggests Redundancy in the Action Potential Repolarization Mechanism

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Porcello, Darrell M., Chi Shun Ho, Rolf H. Joho, and John R. Huguenard. Resilient RTN fast spiking in Kv3.1 null mice suggests redundancy in the action potential repolarization mechanism. *J Neurophysiol* 87: 1303–1310, 2002; 10.1152/jn.00556.2001. Fast spiking (FS), GABAergic neurons of the reticular thalamic nucleus (RTN) are capable of firing high-frequency trains of brief action potentials, with little adaptation. Studies in recombinant systems have shown that high-voltage-activated K^+ channels containing the Kv3.1 and/or Kv3.2 subunits display biophysical properties that may contribute to the FS phenotype. Given that RTN expresses high levels of Kv3.1, with little or no Kv3.2, we tested whether this subunit was required for the fast action potential repolarization mechanism essential to the FS phenotype. Single- and multiple-action potentials were recorded using whole-cell current clamp in RTN neurons from brain slices of wild-type and Kv3.1-deficient mice. At 23°C, action potentials recorded from homozygous Kv3.1 deficient mice ($Kv3.1^{-/-}$) compared with their wild-type ($Kv3.1^{+/+}$) counterparts had reduced amplitudes (−6%) and fast after-hyperpolarizations (−16%). At 34°C, action potentials in $Kv3.1^{-/-}$ mice had increased duration (21%) due to a reduced rate of repolarization (−30%) when compared with wild-type controls. Action potential trains in $Kv3.1^{-/-}$ were associated with a significantly greater spike decrement and broadening and a diminished firing frequency versus injected current relationship (*F/I*) at 34°C. There was no change in either spike count or maximum instantaneous frequency during low-threshold Ca^{2+} bursts in $Kv3.1^{-/-}$ RTN neurons at either temperature tested. Our findings show that Kv3.1 is not solely responsible for fast spikes or high-frequency firing in RTN neurons. This suggests genetic redundancy in the system, possibly in the form of other Kv3 members, which may suffice to maintain the FS phenotype in RTN neurons in the absence of Kv3.1.

INTRODUCTION

In expression systems, all Kv3 subunits (Kv3.1–Kv3.4) are able to form homomeric channels that may be involved in the repolarization of the action potential due to their remarkably fast activation kinetics and high thresholds for activation (near −10 mV) (Rudy et al. 1999). Rapid action potential repolarization is a recognized feature of fast spiking (FS) neurons which are *1*) capable of firing at higher frequencies, *2*) have narrower spikes, and *3*) show little or no adaptation when compared with regular spiking (RS) neurons (Connors and Gutnick 1990). Of the four Kv3 subunits, only Kv3.1 and Kv3.2 have consistently been associated with FS neuron populations throughout the brain, as seen through in situ hybridization, immunocytochemistry, and RT-PCR techniques (Chow et al. 1999; Du et al. 1996; Lenz et al. 1994; Martina et al. 1998; Massengill et al. 1997; Perney et al. 1992; Sekirnjak et al. 1997; Wang et al. 1998; Weiser et al. 1994, 1995).

The absence of inactivation and fast deactivation, as demonstrated by Kv3.1 and Kv3.2 recombinant channels, may function to limit spike duration and high-frequency firing, respectively (Rudy et al. 1999). In addition to a role in non-adapting, high-frequency firing, high-conductance Kv3.1- and/or Kv3.2-containing channels would also contribute to distinctive spike characteristics such as narrow action potentials and prominent fast after-hyperpolarizations (fAHP), as observed in FS neurons, but not in RS neurons (Baranyi et al. 1993; Cauli et al. 1997; Connors and Gutnick 1990; Erisir et al. 1999; Huettner and Baughman 1988; Kawaguchi and Kubota 1998; Massengill et al. 1997; McCormick et al. 1985). Furthermore, pharmacological blockade of Kv channels with tetraethylammonium (TEA) or 4-aminopyridine (4-AP), each with established, but somewhat nonspecific, inhibitory effects on all recombinant Kv3 channels, alters FS cells so that they resemble RS neurons with an increased AP duration, reduced fAHP, and decreased steady-state firing rates (Du et al. 1996; Erisir et al. 1999; Massengill et al. 1997; Wang and Kaczmarek 1998; Wang et al. 1998; Zhang and McBain 1995). While the above findings have advanced the understanding of Kv3 channels in general, because expression system results cannot always be faithfully translated to neurons, and no subunit specific Kv3 pharmacological antagonists are presently available, the functional contributions of individual Kv3 subunits remain to be addressed.

Knockout mice for Kv3.1 ($Kv3.1^{-/-}$) and Kv3.2 have been generated, but exhibit phenotypes similar to wild-type mice at the whole animal level (Ho et al. 1997; Lau et al. 2000; Sanchez et al. 2000). At the neuronal level, one likely area disrupted in $Kv3.1^{-/-}$ mice is the reticular thalamic nucleus (RTN), a shell-like layer of FS GABAergic interneurons. With only a weak presence of Kv3.2, and high levels of Kv3.1, any function of RTN related to its FS phenotype may be intimately dependent on the Kv3.1 subunit (Perney et al. 1992). Although RTN also has high-expression levels of Kv3.3 mRNA, which forms channels with transient, high-threshold, A-type currents in mammalian cell lines (Weiser et al. 1994), due to inconsis-

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tencies among other expression systems, the contribution of Kv3.3 to the fast repolarization of RTN action potentials is controversial (Rudy et al. 1999).

RTN is the primary source of inhibition within the intrathalamic circuit of the dorsal thalamus and has been shown to be involved in the generation of sleep spindles and slow-wave sleep, as well as the refinement and modulation of sensory transmission (Crabtree et al. 1998; McCormick and Bal 1997; Shen et al. 1998). Altered firing properties of RTN neurons may produce changes in the thalamic oscillatory patterns which depends on such inhibition. A simple enhancement of the inhibition from RTN onto the relay nuclei, or a reduction of intra-RTN inhibition, can push the thalamic circuit into a 3- to 4-Hz oscillatory state reminiscent of generalized absence epilepsy (Huguenard and Prince 1994; Huntsman et al. 1999). This capacity for low-frequency hypersynchrony hinges on a carefully controlled RTN output. Interestingly, Kv3.1$^{-/-}$ mice show a 30–50% decrease in delta power, a marker for low-frequency activity (Joho et al. 1999). Despite this change, Kv3.1$^{-/-}$ mice appear behaviorally unperturbed (Ho et al. 1997; Joho et al. 1999). In this paper we study the firing properties of RTN neurons to further understand these findings.

METHODS

Thalamic slice preparation

C57BL/6 wild-type (wt) and 129Sv × C57BL/6 Kv3.1 knockout mice 30 to 60 days old were anesthetized with 50 mg/kg of sodium pentobarbital and decapitated. Brains were blocked, removed, and immediately transferred to 4°C choline chloride (in mM: 119 $C_5H_{14}NOCl$, 5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 2 $MgSO_4$, 10 glucose, 26 $NaHCO_3$) equilibrated with with 95% O_2-5% CO_2. After being submerged for approximately 2 min, brains were glued to a Petri dish filled with the same choline solution as above and sectioned on a vibratome (TPI, St. Louis, MO) into 200-μm-thick horizontal slices. Slices were bisected and trimmed before being placed into an incubator containing artificial cerebral spinal fluid (ACSF; in mM: 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 2 $MgSO_4$, 10 glucose, 26 $NaHCO_3$) continuously bubbled with 95% O_2-5% CO_2 at 32°C at least 1 h prior to recording. The generation of Kv3.1-deficient mice is described elsewhere (Ho et al. 1997; Joho et al. 1999). C57BL/6 Kv3.1$^{+/+}$ mice were deemed suitable controls for 129Sv × C57BL/6 Kv3.1$^{-/-}$ mice after showing no significant difference compared with 129Sv × C57BL/6 Kv3.1$^{+/-}$ mice in all action potential properties tested for in the present study.

Electrophysiology

In the recording chamber, slices were gently held down by a nylon net and superfused with a constant flow of ACSF (2 ml/min) equilibrated with 95% O_2-5% CO_2. Cells were identified by their shape and location in the slice using an infrared visualization system (Edwards et al. 1989). All patch pipettes were pulled from borosilicate glass (Garner Glass, Claremont, CA) and filled with a potassium gluconate solution (in mM: 120 K-Gluconate, 11 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 11 EGTA, pH = 7.3). Current clamp recordings of thalamic reticular neurons were performed with an Axoclamp-2B amplifier (Axon Instruments, Foster City, CA). Neurons selected for analysis had membrane potentials more negative that −60 mV and input resistances above 100 MΩ.

Data analysis

Voltage signals were filtered at 30 kHz, sampled at 17–42 kHz, and recorded with PCLAMP 6 (Axon Instruments). A liquid junction potential of 10 mV was subtracted from all membrane potentials in this study. Action potentials were evoked by depolarizing the cell with DC current injection to a level just under spike threshold (≈ −50 mV) and then applying small depolarizing currents (50–100 pA). Spike onset was defined as the time of the first of four consecutive samples, each of which had progressively larger first derivatives (dV/dt). In those rare circumstances when these criteria were not met, spike onset was defined as the point at which the differentiated spike waveform crossed a predetermined threshold (30 V/s) that was 3 × baseline root-mean-square (rms) noise. Spike threshold was recorded as the voltage at spike onset. The maximum and minimum values from this smoothed differentiated spike were used for the maximum rates of depolarization and repolarization. Spike amplitude and fAHP were calculated as the voltage difference between spike threshold and maximum or minimum voltage deflections, respectively. Half-width was the duration of the spike measured at one-half its peak amplitude. Averaged spikes were created by selecting an equal number of representative threshold evoked spikes from each cell and aligning their peaks in Metatape v14 (J. R. Huguenard, http://huguenard-lab.stanford.edu/~john/metatape.html). All spike trains were 60 ms in duration and initiated near threshold set by DC current. Phasic firing characteristics were examined in low-threshold spike (LTS) bursts initiated with a long-lasting hyperpolarizing current (−500 to −600 pA, 1 s) from rest, followed by a brief depolarizing current (100 to 200 pA, 200 ms).

RESULTS

Reticular neuron properties

No statistical significant differences in RTN cell passive membrane properties were observed between Kv3.1$^{+/+}$ and Kv3.1$^{-/-}$ animals. At room temperature (23°C), mean resting membrane potential (wt: −75.9 ± 1.7 mV, n = 16 vs. Kv3.1$^{-/-}$: −73.5 ± 2.3 mV, n = 13) and mean input resistance (wt: 273.8 ± 26.8 MΩ, n = 14 vs. Kv3.1$^{-/-}$ 273.5 ± 26.2 MΩ, n = 12) were similar for the two groups.

Single action potential properties

Single action potentials were elicited from neurons (wt: n = 16, Kv3.1$^{-/-}$: n = 13) at room temperature (Fig. 1*A*; summary data provided in Table 1). fAHPs were reduced by almost 16% in Kv3.1$^{-/-}$ animals compared with wild-types (wt: −20.2 ± 1.2 mV vs. Kv3.1$^{-/-}$: −16.9 ± 0.9 mV, $P < 0.05$), and mean amplitude was also decreased (wt: 80.8 ± 1.8 mV vs. Kv3.1$^{-/-}$: 75.7 ± 1.5 mV, $P < 0.05$). However, no significant differences were observed in half-width or maximum rate of repolarization. Other parameters not associated with the repolarization portion of the action potential, including threshold and maximum rate of depolarization, were unaffected (Fig. 1*B*).

Warming slices to near physiological temperatures (34°C, wt: n = 6, Kv3.1$^{-/-}$: n = 4) dramatically shortened RTN action potentials both in amplitude (wt: −24 ± 2.6% vs. Kv3.1$^{-/-}$: −21.9 ± 1.3%) and in duration (half-width, wt: −59.3 ± 2.7% vs. Kv3.1$^{-/-}$: −51.2 ± 2.1%) in both animal groups. Previous studies on the kinetics of action potential have shown that temperature changes predominantly affect the repolarization phase of the spike waveform (Frankenhaeuser and Moore 1963; Thompson et al. 1985). In wild-types, warming from 23 to 34°C increased the maximum rate of spike repolarization by 107.4 ± 23.6% compared with a smaller increase of 39.3 ± 12.2% in depolarization. Under these more

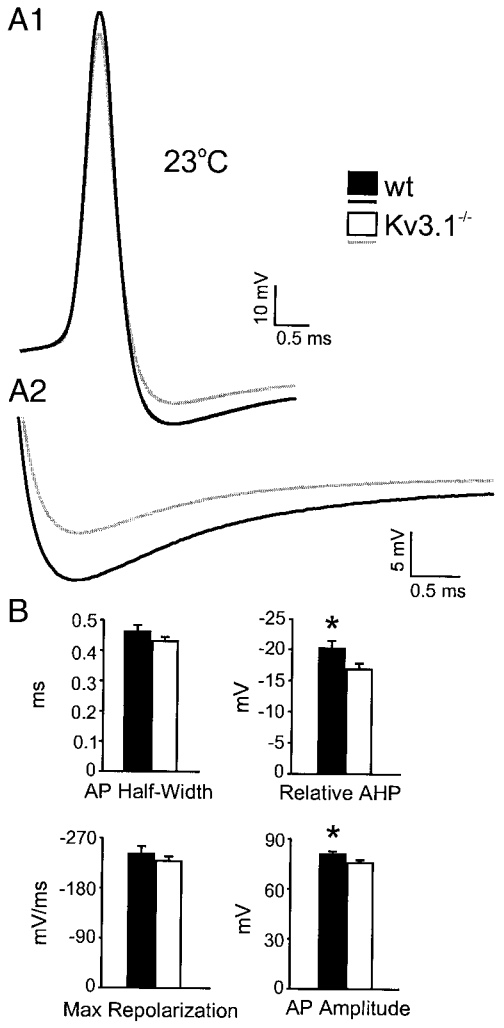

FIG. 1. Room temperature reticular thalamic nucleus (RTN) action potential comparison between wild-type (wt) and Kv3.1$^{-/-}$ animals. *A1*: 2 overlaid spike waveforms, averaged from all wt (black line) and Kv3.1$^{-/-}$ (gray line) neurons. Each spike contained in the 2 average waveform was aligned by peak in the horizontal direction, but not offset in the vertical direction. The only discrepancy seen between the 2 traces is at the peak, and during the afterhyperpolarization, which is emphasized in *A2*. *B*: black bars in the summary histograms refer to wt control animals, while white bars indicate Kv3.1$^{-/-}$ knockout animals. All error bars show SE. For this and all subsequent figures: $*P < 0.05$, $**P < 0.01$, $***P < 0.005$.

physiological conditions (34°C), which presumably enhanced the functioning of the delayed rectifier currents, a significant difference in half-width between wild-types and Kv3.1$^{-/-}$ animals was revealed (Fig. 2*A*).

For single action potentials from cells recorded at 34°C (wt: $n = 8$, Kv3.1$^{-/-}$: $n = 8$) half-widths were 21% longer in Kv3.1$^{-/-}$ than wild-types (Kv3.1$^{-/-}$: 0.23 ± 0.01 ms vs. wt: 0.19 ± 0.01 ms, $P < 0.005$). In addition, maximum rate of repolarization was significantly diminished in Kv3.1$^{-/-}$ neurons at these temperatures (Kv3.1$^{-/-}$: −381.7 ± 18.2 V/s vs. wt: −543.5 ± 49.6 V/s, $P < 0.01$), which is consistent with an impaired repolarization mechanism. Action potential amplitudes and fAHPs were unaffected in Kv3.1$^{-/-}$ neurons under these conditions. As in room temperature recordings, threshold and maximum rate of depolarization were also unchanged in the knockout (Fig. 2*B*).

Repetitive firing characteristics: phasic and tonic firing

Although significant differences were observed in isolated spikes elicited at threshold, the phasic firing of calcium-dependent LTS bursts was unaffected (Fig. 3, *A* and *B*). At room temperature, neither maximum spike count per burst (wt: 19.8 ± 2.4 spikes vs. Kv3.1$^{-/-}$: 16.1 ± 2.1 spikes, $P > 0.05$) nor maximum instantaneous frequency within a burst (wt: 226 ± 14 Hz vs. Kv3.1$^{-/-}$: 255 ± 12 Hz, $P > 0.05$) were different between the two groups (wt: $n = 14$, Kv3.1$^{-/-}$: $n = 9$, Fig. 3*C*). Recordings made at more physiological temperatures (wt: $n = 7$, Kv3.1$^{-/-}$: $n = 13$, Fig. 3*C*) also failed to detect Kv3.1-dependent differences in burst firing. Maximum spike count per burst (wt: 5.7 ± 0.8 spikes vs. Kv3.1$^{-/-}$: 6.9 ± 0.6 spikes, $P > 0.05$), and maximum instantaneous frequency (wt: 516 ± 35 Hz vs. Kv3.1$^{-/-}$: 488 ± 35 Hz, $P > 0.05$) were comparable between wild-type and Kv3.1$^{-/-}$.

Progressive changes in half-width (spike broadening) and amplitude (spike decrement) were observed during tonic spike trains in both wild-type and Kv3.1$^{-/-}$ animals (Fig. 4). At room temperature, even by the second spike of a 100- to 200-Hz train, half-width (compared with the first spike) had increased by 10.8 ± 2.2% in wild-types ($n = 12$) and 14.0 ± 2.3% in Kv3.1$^{-/-}$ ($n = 9$), and amplitude had decreased by 17.6 ± 2.1% in wild-types and 20.6 ± 1.4% in Kv3.1$^{-/-}$. By the tenth spike, both spike broadening (wt: 24.4 ± 2.3% vs. Kv3.1$^{-/-}$: 29.0 ± 4.0%) and spike decrement (wt: −30.0 ± 2.9% vs. Kv3.1$^{-/-}$: −31.5 ± 2.1%) had noticeably risen in each group (Fig. 4, *A* and *B*). However, no difference between the two groups in either comparison was significant ($P > 0.05$, Fig. 4*C*).

As in the previous analysis of single action potentials, significant differences in tonic firing properties emerged at higher temperatures. At 34°C, spikes in a sustained train demonstrated significantly more decrement and broadening in Kv3.1$^{-/-}$ animals than wild-type controls (Fig. 4, *A* and *B*). In 300- to 400-Hz trains recorded at physiological temperatures in wild-type animals, changes in amplitude during the train were less pronounced than at room temperatures ($n = 10$). Comparing the first and the second spike there was only a 9.1 ± 1.4% amplitude reduction. By the twentith spike this reduction had

TABLE 1. *Cell properties and single-action potential characteristics for wt and Kv3.1$^{-/-}$ RTN neurons*

	Wild-Type	Kv3.1$^{-/-}$
Membrane potential, mV	−75.9 ± 1.7	−73.5 ± 2.3
Input resistance, MΩ	273.8 ± 26.8	273.5 ± 26.2
23°C		
AP amplitude, mV	80.8 ± 1.8	75.7 ± 1.5*
AP max rate of depolarization, V/s	350.6 ± 14.6	330.1 ± 10.6
AP max rate of repolarization, V/s	−241.4 ± 13.3	−229.1 ± 7.8
AP half-width, ms	0.46 ± 0.02	0.43 ± 0.01
AP fAHP, mV	−20.2 ± 1.2	−16.9 ± 0.9*
AP threshold, mV	−50.9 ± 3.6	−50.1 ± 3.7
34°C		
AP amplitude, mV	67.4 ± 1.5	61.1 ± 3.0
AP max rate of depolarization, V/s	515.4 ± 25.8	449.5 ± 26.1
AP max rate of repolarization, V/s	−543.5 ± 49.6	−381.7 ± 18.2**
AP half-width, ms	0.19 ± 0.01	0.23 ± 0.01***
AP fAHP, mV	−22.3 ± 1.3	−20.3 ± 1.4
AP threshold, mV	−47.5 ± 1.1	−47.1 ± 1.7

Values are means ± SE. wt, wild-type; RTN, reticular thalamic nucleus; AP, action potential; fAHP, fast afterhyperpolarization. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

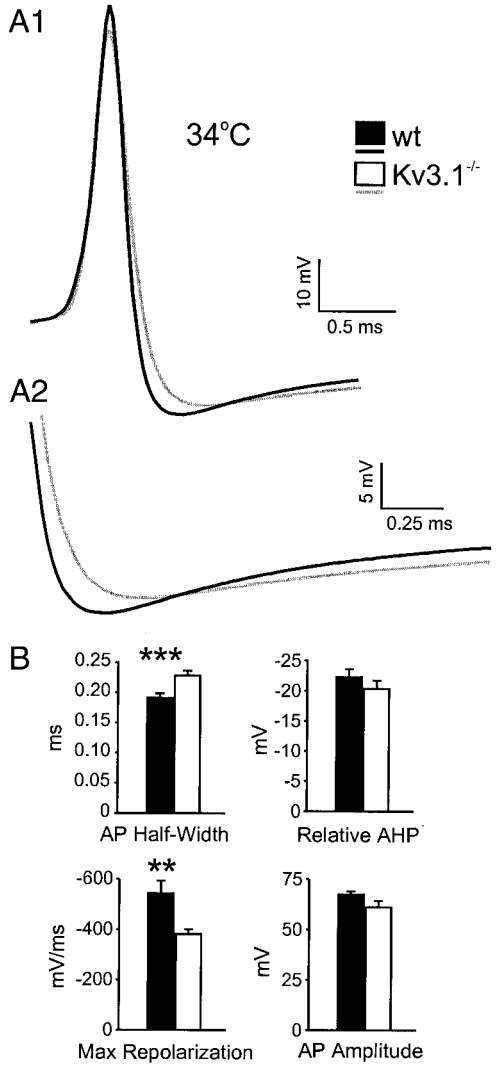

FIG. 2. Near physiological temperature action potential comparison between wt and Kv3.1$^{-/-}$ animals. *A1*: as in Fig. 1, average spike waveforms representing all wt (black line) and Kv3.1$^{-/-}$ (gray line) neurons are overlaid for comparison. Unlike the average waveforms in Fig. 1, the average Kv3.1$^{-/-}$ action potential at 34°C shows a less effective repolarization than the average wt action potential. This is shown in detail in *A2*, which also demonstrates an almost equal fAHP between the two groups, unlike Fig. 1. *B*: black bars in the summary histograms refer to wt animals, while white bars indicate Kv3.1$^{-/-}$ animals. All error bars show SE.

increased to 16.7 ± 3.0%. In Kv3.1$^{-/-}$ neurons, trains of equivalent frequencies ($n = 6$) produced twice as much spike decrement to 20.0 ± 2.3% between the first and second spike ($P < 0.005$) and 34.3 ± 4.4% between the first and twentieth spike ($P < 0.005$, Fig. 4*C*). This trend was also reflected in spike broadening (Fig. 4*C*). Only minor lengthening of half-width was observed in wild-type spike trains between the first and second spike, 4.9 ± 1.8%, and the first and twentieth spike, 10.0 ± 3.2%. As with spike decrement, these values were almost doubled in Kv3.1$^{-/-}$ (1st vs. 2nd: 12.4 ± 2.7% $P < 0.05$, 1st vs. 20th: 21.9 ± 3.2% $P < 0.05$).

Repeated firing characteristics: interspike interval for tonic firing

With the above differences in tonic firing established, we next investigated any possible changes in the firing frequency versus injected current relationship (*F/I*) of sustained spike trains between the two groups. Given that we only found differences in tonic firing properties at near physiological temperatures, we restricted our *F/I* analysis to higher temperature recordings. The *F/I* of a cell was included in the analysis if it possessed a sustained spike train (firing for the full 60 ms) for at least six of the nine injected current levels (250 to 650 pA, increased in 50-pA increments).

Spike trains from wild-type and Kv3.1$^{-/-}$ mice show similar current-dependent increases of the instantaneous frequency, as determined by the first interval (Fig. 5*A*). However, by the end of the trains, there was a clear difference in instantaneous frequency between the two groups (Fig. 5*B*). Using separate two-way analysis of variances (ANOVAs), a significant effect of animal type on the *F/I* relationship was demonstrated for the last interval ($F = 16.654$, $P < 0.0001$) but not the first ($F = 0.0631$, $P = 0.8019$). Furthermore, a post hoc Student-Newman-Keuls revealed that the instantaneous frequency of the last two spikes of Kv3.1$^{-/-}$ trains was significantly less than those of wild-type trains ($P < 0.05$, Fig. 5*B*). Average input resistance for neurons from the two groups was not statistically different.

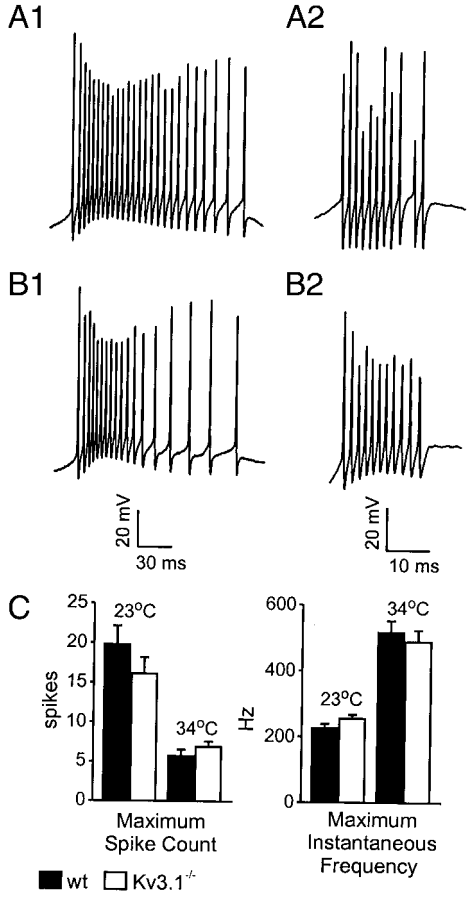

FIG. 3. Burst firing comparison between wt and Kv3.1$^{-/-}$ knockout animals at both room and near physiological temperatures. Representative bursts from 2 wt neurons elicited at (*A1*) 23°C and (*A2*) 34°C. Comparable bursts are also shown for Kv3.1$^{-/-}$ neurons at both temperatures in *B1* (23°C) and *B2* (34°C). Due to sampling deficiencies, some action potential peaks were truncated in the high-temperature recordings shown in *A2* and *B2*. *C*: black bars in the summary histograms refer to wt animals, while white bars indicate Kv3.1$^{-/-}$ animals. All error bars show SE.

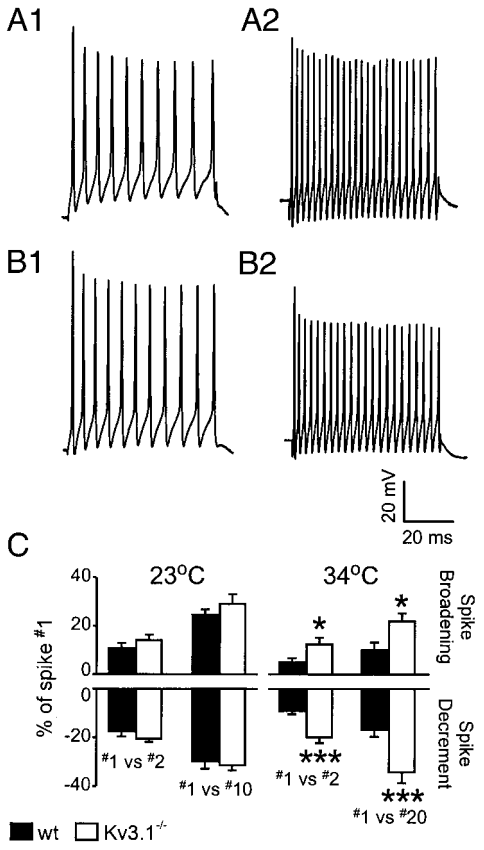

FIG. 4. Tonic firing comparisons between wt and Kv3.1$^{-/-}$ animals at both room and near physiological temperatures. Current pulses (60 ms) were used to elicit spike trains from wt and Kv3.1$^{-/-}$ neurons held near threshold to prevent the contribution of the T-type, low-threshold, calcium current. Spike trains were recorded from neurons from both groups at room temperature (*A1*) wt, (*B1*) Kv3.1$^{-/-}$, and near physiological temperatures (*A2*) wt, (*B2*) Kv3.1$^{-/-}$. *C*: black bars in the summary histograms refer to wt animals, while white bars indicate Kv3.1$^{-/-}$ animals. All error bars show SE. All values were statistically different from 0.

DISCUSSION

In this study we have demonstrated subtle effects on both single action potentials and on the repetitive firing characteristics of RTN neurons in mice lacking the Kv3.1-containing Kv channel. At room temperatures, Kv3.1$^{-/-}$ mice have action potentials with reduced fAHPs and smaller amplitudes, but show no evidence of broadening compared with Kv3.1$^{+/+}$ mice. Only at near physiological temperatures did we begin to see some signs of an incapacitated delayed rectifier. On average, Kv3.1$^{-/-}$ RTN action potentials evoked near or at threshold had larger half-widths and less prominent repolarization. Although these differences were not reflected in the phasic firing mode of RTN neurons, as measured by maximum spike number and instantaneous frequency of LTS bursts, we did observe a disturbance in tonic firing. Kv3.1$^{-/-}$ neurons exhibited spike trains with a greater degree of spike decrement and broadening at physiological temperatures, in addition to a shallower *F/I* relationship, than wild-type controls. While these effects are in agreement with the presumed function of Kv3.1, their magnitude is less than what would be predicted if Kv3.1 was solely responsible for fast action potential repolarization in RTN neurons (see DISCUSSION below).

Single action potential properties

Studies comparing the action potentials of FS versus RS neurons have reported large quantitative differences in fAHPs, half-widths, and maximum rates of repolarization, presumably due to the absence of Kv3.1 and Kv3.2 subunits in RS neurons (Baranyi et al. 1993; Cauli et al. 1997; Erisir et al. 1999; Huettner and Baughman 1988; Kawaguchi and Kubota 1998; Massengill et al. 1997; McCormick et al. 1985). Reviewing several articles yielded rough averages of a 50% smaller maximum rate of repolarization ($n = 4$), 100% longer half-width ($n = 7$), and a 75% smaller fAHP ($n = 4$) for RS neuron action potentials when matched with those recorded from FS neurons (Table 2). More directly, the RS phenotype can be replicated in FS neurons by way of the Kv3 channel antagonists 4-AP and TEA, resulting in similar changes in action potential characteristics (Table 2) (Erisir et al. 1999; Massengill et al. 1997). Although these findings point to a fundamental role for Kv3.1 in fast action potential repolarization, a loss of Kv3.1 in RTN failed to convert the distinctive action potential waveform of FS neurons into one resembling the RS phenotype. At least three possibilities may explain this disparity, as follows: *1*) Kv3.1 has a minor role in AP repolarization in RTN cells; *2*) Kv3.1 normally has a major role in repolarization, but there is functional compensation due to genetic redundancy in the system, such that other native K^+ channels assume a larger

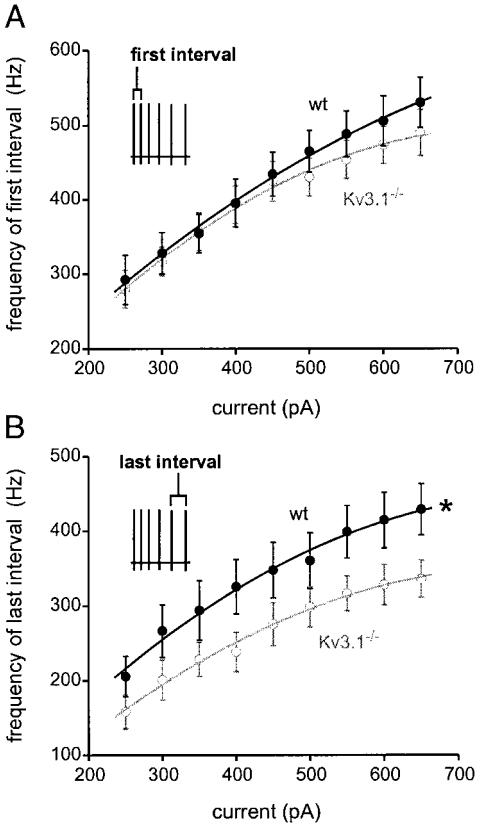

FIG. 5. Early and late frequency comparisons during spike trains elicited at near physiological temperatures. *A*: frequency versus current intensity plot for the first interval (between the first 2 spikes) of spike trains for wt and Kv3.1$^{-/-}$ neurons. Each wt data point represents an average of ≥8 neurons, where each Kv3.1$^{-/-}$ point is an average of 6 neurons. *B*: each wt point is an average of ≥7 neurons, while each Kv3.1$^{-/-}$ point represents an average of ≥5 neurons. All error bars show SE, which is never larger than 14% of the mean.

TABLE 2. *Comparison between RS and FS cells in previous studies*

Study	% Difference in Maximum Rate of Repolarization*	% Difference in Half-Width†	% Difference in fAHP‡	Temperature
McCormick et al. (1985)	70%	150%	NA	35–37°C
Massengill et al. (1997)	NA	37%	108%	Room
Cauli et al. (1997)	NA	80%	38%	Room
Erisir et al. (1999)	73%	183%	99%	Room
Baranyi et al. (1993)	28%	64%	55%	Physiological
Huettner and Baughman (1988)	49%	100%	NA	30°C
Kawaguchi and Kubota (1998)	NA	78%	NA	30°C
Average	55%	99%	75%	
Massengill et al. (1997), 100 μM 4-AP	NA	86%	88%	Room
Erisir et al. (1999), 1 mM TEA	58%	81%	NA	Room
Kv3.1$^{-/-}$	5%	−7%	16%	Room
Kv3.1$^{-/-}$	30%	21%	9%	34°C

RS, regular spiking; FS, fast spiking; fAHP, fast afterhyperpolarization; 4-AP, 4-aminopyridine; TEA, tetraethylammonium; NA, not available in the cited study. * % Difference in maximum rate of repolarization = (FS rate − RS rate)/FS rate. † % Difference in half-width = (RS half-width − FS half-width)/FS half-width. ‡ % Difference in fAHP = (FS fAHP − RS fAHP)/FS AHP.

repolarizing role (Adams and Galvan 1986); or finally, *3*) genetic compensation has occurred in Kv3.1$^{-/-}$ neurons, i.e., increased expression of other Kv subunits in response to the loss of Kv3.1.

Given the potential modifications of Kv3 genes and their products, such as splice variants from the same gene, RNA editing, posttranslational modification, multimeric assembly of channels from distinct principal α and auxiliary β subunits, and phosphorylation, it may be difficult to predict a role for Kv3.1 in RTN based on expression system data alone (Coetzee et al. 1999). While the use of pharmacological agents with known inhibitory effects on recombinant Kv3 channels, such as TEA and 4-AP, can be effective in exploring the contributions of Kv3 subunits in situ, their selectivity remains a concern. Conceivably, TEA and 4-AP could be blocking a whole array of channels, which may contribute in multiple ways to the repolarization of the action potential waveform in FS neurons. This could explain the mild firing deficits in Kv3.1$^{-/-}$ mice shown here, in contrast to the robust effects of TEA and 4-AP in neurons (Table 2). A more specific explanation why action potential disruptions in Kv3.1$^{-/-}$ RTN neurons were smaller in magnitude than the significant broadening caused by a 4-AP application in wt RTN neurons (data not shown) may be the presence of Kv3.3.

The possibility of another TEA/4-AP sensitive Kv channel in Kv3.1$^{-/-}$ RTN neurons, such as Kv3.3, is supported by the finding that FS neurons of the medial nucleus of the trapezoid body (MNTB), which have action potentials susceptible to 1 mM TEA, still retain a TEA-sensitive component in Kv3.1$^{-/-}$ mice (Macica et al. 2000; Wang and Kaczmarek 1998). The MNTB, similar to RTN, contains comparable levels of Kv3.1 and Kv3.3 mRNA, but almost no Kv3.2 or Kv3.4 mRNA (Weiser et al. 1994). If the remaining TEA-sensitive current in MNTB is involved in the repolarization of the action potential, and due to Kv3.3, a similar residual Kv3 current may explain the relatively undisturbed FS phenotype of Kv3.1$^{-/-}$ RTN neurons. This type of genetic redundancy may be adequate for the repolarization of single action potentials at room temperatures, but may begin to fail under the greater demands of higher temperatures, or repetitive firing, as reported here.

Repetitive firing properties

The action potentials of a prolonged train are more likely to succumb to the use-dependent spike decrement and broadening, as readily seen in RS neurons (Cauli et al. 1997), without the fast activating, high-threshold, delayed rectifier channels containing Kv3.1, Kv3.2, or possibly Kv3.3 (Erisir et al. 1999; Kawaguchi and Kubota 1998). In brief 100–200 Hz spike trains at room temperature, we observed no differences in either spike decrement or broadening between wild-type and Kv3.1$^{-/-}$ animals. Defects may have been more pronounced at higher frequencies. MNTB neurons in Kv3.1$^{-/-}$ mice were shown to be significantly impaired at firing frequencies only *above* 200 Hz, compared with wild-type controls (Macica et al. 2000). Wild-type MNTB neurons also exhibit significant spike decrement and broadening above 200 Hz when bathed in 1 mM TEA (Wang and Kaczmarek 1998; Wang et al. 1998). Although the authors did not report which effect is greater (knockout of Kv3.1 or TEA), it is interesting to note that their results in MNTB spike trains are closer in magnitude than analogous ones for single action potentials in RTN neurons. Assuming the mechanisms available to respond to the loss of Kv3.1 are similar in RTN and MNTB, whatever allows RTN neurons to maintain rapid spike repolarization in Kv3.1$^{-/-}$ mice may be inadequate for high-frequency tonic firing at room temperature in MNTB neurons according to results in Macica et al. (2000). Here we show this shortfall may be exacerbated at higher temperatures, where there is significantly more spike broadening and decrement in Kv3.1$^{-/-}$ RTN neurons within the first few spikes of a short train compared with trains recorded at room temperature. Difficulties in maintaining a sustained spike train in Kv3.1$^{-/-}$ are amplified as the train progresses, shown in the significant *F/I* differences late in the train between Kv3.1$^{-/-}$ and wt RTN neurons. Possible explanations for the firing deficits shown here might include greater susceptibility to inactivation as well as slower deactivation rates, smaller unit conductances, and more hyperpolarized activation levels of the Kv channels responsible for action potential repolarization in the absence of Kv3.1 compared with wt Kv channels. For a better explanation of the discrepancies between knockout and pharmacological studies across different brain regions, future studies should match the effects of TEA

or 4-AP at physiological temperatures in wt and knockout systems with established ratios of Kv3.1 and Kv3.3 protein levels.

One intriguing caveat is that burst firing appeared unaffected at both room and near physiological temperatures in Kv3.1$^{-/-}$ mice. It maybe that the total number of spikes contained in a burst (mean = 7) is too low to be affected by the use-dependent defects that were seen in the tonic firing patterns of the knockout. The stability of the burst firing mode might account for the lack of seizures in Kv3.1$^{-/-}$ mice.

The prospects for genetic redundancy

Among Kv subunits, the facilitation of high-frequency firing has been attributed solely to Kv3.1 and Kv3.2 (Rudy et al. 1999). Recombinant channels composed of the Kv3.3 subunit have kinetics comparable to those containing Kv3.1 or Kv3.2, in some, *but not all*, tested expression systems. Kv3.3 homomers exhibit inactivation in *Xenopus* oocytes, but not in CHO or HEK 293 cells (Rudy et al. 1999). Additional work has predicted that channels made from truncated, or oxidized, Kv3.3 subunits would substantially slow this inactivation (Rae and Shepard 2000; Ruppersberg et al. 1991). These anomalies attest to the importance of the native environment in determining these channel features. A homologue of the Kv3.3 subunit has been shown to be responsible for the TEA-sensitive current underlying the narrow action potential in neurons of the electrosensory lobe of an apteronotid weakly electric fish (Rashid et al. 2001). If Kv3.3 is capable of assuming the function of Kv3.1 in the Kv3.1 knockout, at least to the point of preventing catastrophic failure, it may act as a molecular backup system in the many cell types which co-express both transcripts (Martina et al. 1998). Although no obvious differences in whole-brain mRNA levels of Kv3.2, Kv3.3, or Kv3.4 were observed in Kv3.1$^{-/-}$ mice (Ho and Joho 1997), more localized compensatory changes in RTN cannot be ruled out. Recently a Kv3.1/3.3 double knockout has been produced that has far greater behavioral defects than those observed in Kv3.1$^{-/-}$ mice, including ataxia, spontaneous myoclonus, and ethanol hypersensitivity (Espinosa et al. 2001). This new mouse represents a unique opportunity to explore the possibility that Kv3.3 is playing a supportive role for the fast repolarizer in RTN neurons.

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