Pore Mutation in a G-Protein-Gated Inwardly Rectifying K\(^{+}\) Channel Subunit Causes Loss of K\(^{+}\)-Dependent Inhibition in weaver hippocampus

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Weaver (wv) mice carry a point mutation in the pore region of a G-protein-gated inwardly rectifying K\(^{+}\) channel subunit (Kir3.2). wvKir3.2 conducts inward currents that may cause the loss of neurons in the cerebellum and substantia nigra. Although Kir3.2 is widely expressed in the CNS, significant morphological or physiological changes have not been reported for other brain areas. We studied the role of wvKir3.2 in hippocampal slices of young [postnatal day (P) 4–18] and adult wv/wv (≥P24) mice, because protein levels of Kir3.1 and Kir3.2 appear to be normal in the first 3 postnatal weeks and only decrease thereafter. In disinhibited slices, the GABA\(_{B}\) receptor agonist R-baclofen reduced burst activity in wv/wv mice but was much more potent in wild-type mice. Mean resting membrane potential, slope input resistance, and membrane time constant of CA3 neurons of adult wv/wv and wild-type mice were indistinguishable. However, R-baclofen or chloroadenosine did not induce K\(^{+}\) currents or any other conductance change in wv/wv mice. Moreover, electrical or chemical stimulation of inhibitory neurons did not evoke slow IPSPs in adult wv/wv mice. Only in a few cells of young wv/wv mice did GABA\(_{B}\) receptor activation by R-baclofen or presynaptic stimulation induce small inward currents, which were likely caused by a Na\(^{+}\) ion influx through wvKir3.2 channels. The data show that the pore mutation in wvKir3.2 channels results in a hippocampal phenotype resembling Kir3.2-deficient mutants, although it is not associated with the occurrence of seizures.

Key words: weaver; hippocampus; R-baclofen; slow IPSPs; Kir3.2; GIRK2; G-protein-activated potassium currents; adenosine; serotonin; GABA\(_{B}\) receptors

Homozygous weaver (wv/wv) mice are characterized by ataxia, hyperactivity, and tremor (Rakic and Sidman, 1973a,b). The neurological defects are associated with loss of cerebellar granule cells and dopaminergic neurons in the substantia nigra within the first 3 postnatal weeks (Rakic and Sidman, 1973b; Goldowitz and Mullen, 1982; Schmidt et al., 1982; Hatten et al., 1984; Rolfer-Tarlov and Graybiel, 1984; Triarhou et al., 1988; Smeyne and Goldowitz, 1989). The weaver defect has been identified as a point mutation in the presumed pore region of a G-protein-gated inwardly rectifying K\(^{+}\) channel subunit (wvKir3.2, previously GIRQ2) (Patil et al., 1995). Functional G-protein-gated channels are formed by co-assembly of different subunits of the Kir3.0 subfamily (for review, see Wickman and Clapham, 1995). They selectively permit K\(^{+}\) ion effluxes near resting membrane potential that hyperpolarize cells. In Xenopus oocytes wvKir3.2 channels cause loss of K\(^{+}\) selectivity, induce a constitutive Na\(^{+}\) conductance in homomultimers and heteromultimers with Kir3.1, and may render the channel G-protein insensitive (Kofuji et al., 1996; Navarro et al., 1996; Silverman et al., 1996; Slesinger et al., 1996, 1997; Surmeier et al., 1996; Tong et al., 1996). Cultured cerebellar granule cells from wv/wv mice display a reduced G-protein-gated current (Kofuji et al., 1996; Slesinger et al., 1996, 1997; Surmeier et al., 1996; Lauritzen et al., 1997), and in some reports they express an anomalous nonselective “leakage” current (Kofuji et al., 1996; Slesinger et al., 1996, 1997) (but see Surmeier et al., 1996).

Because of motor disturbances, previous studies concentrated on cerebellar and midbrain neurons of wv/wv mice. However, Kir3.2 is widely expressed in neurons throughout the mouse brain and particularly in the hippocampus (Kobayashi et al., 1995; Liao et al., 1996; Murer et al., 1997; Wei et al., 1997). Kv3.1–Kv3.2 heteromultimers are most likely the molecular substrate for GABA\(_{B}\) receptor-mediated IPSPs in mouse hippocampus because K\(^{+}\)-dependent inhibition in hippocampal neurons of Kir3.2-deficient mice is absent (Lüscher et al., 1997). In the hippocampus of wv/wv mice, the protein levels of wvKir3.2 appear normal in the first 3 postnatal weeks and decrease subsequently (Liao et al., 1996), but no prominent morphological (Sekigushi et al., 1995; Liao et al., 1996) or behavioral abnormalities have been described. In contrast, Kir3.2-deficient mice exhibit spontaneous seizure activity (Signorini et al., 1997), which has been attributed to an impaired K\(^{+}\)-dependent inhibition (Lüscher et al., 1997). The lack of seizure activity in homozygous wv/wv mice may indicate that slow synaptic inhibition is intact, despite the point mutation in the Kir3.2 pore. Sporadic seizures in heterozygous wv/+ mice (Eisenberg and Messer, 1989) are likely caused by additional genetic factors (Goldowitz and Smeyne, 1995). To establish the effects of the wvKir3.2 mutation in the hippocampus we studied passive membrane properties and ligand-gated K\(^{+}\) currents in slices from young and adult wv/wv mice. We found that K\(^{+}\)-dependent inhibition in the CA3 region of homozygous wv/wv mice is severely impaired despite an apparently normal hip-
Intracellular microelectrodes were filled with 3 M KCl (resistance 60–90 MΩ). MgSO₄ and KH₂PO₄ were replaced by the Cl⁻-weaver of ductance. In a first series of experiments, we examined the ability of nanomolar concentrations of 8-OHDPAT to block fast synaptic transmission through the application of bicuculline (20 μM) extracellular [K⁺] 6.25 mM). R-baclofen reversibly reduced the frequency of spontaneous recurrent burst discharges, but effective concentrations had to be larger than 100 μM. 8-OHDPAT showed the burst marked with ▲ in A1 at a higher sweep speed. In the presence of bicuculline (20 μM) and 4-AP (50 μM; extracellular [K⁺] 3.25 mM), R-baclofen reduced the frequency of spontaneous recurrent burst discharges. A4, B3. Concentration–response relation between R-baclofen and the inhibition of the frequency (expressed as percentage of control) in wv/wv and +/- mice. Individual symbols with bars indicate the mean ± SEM value. Each concentration was tested in five to nine slices from at least five animals. Solid lines represent the best fit of the Hill function (k, Hill coefficient) to data points (bicuculline: wv/wv, k = 0.63, EC₅₀ = 3.7 μM; +/-, k = 1.8, EC₅₀ = 0.043 μM; bicuculline and 4-AP: wv/wv, k = 0.47, EC₅₀ > 100 μM; +/-, k = 1.36, EC₅₀ = 0.35 μM). In the hippocampus of +/- mice, barium (Ba²⁺, 1 mM) abolished the inhibition by R-baclofen. Calibration (for all traces): 1 mV, 2 min. In this and the following figures, horizontal bars indicate the drug application.

**Figure 1.** Inhibition of spontaneous recurrent burst activity in the CA3 pyramidal cell layer of hippocampal slices from wv/wv mice with Baclofen receptor agonist Baclofen. A. Field potential recordings in the presence of bicuculline (20 μM) extracellular [K⁺] 6.25 mM). Baclofen reversibly reduced the frequency of spontaneous recurrent burst discharges, but effective concentrations had to be larger than 100 μM. B. Inhibition of spontaneous recurrent burst discharges in +/- mice. Concentration–response relation between Baclofen and the inhibition of the frequency (expressed as percentage of control) in wv/wv and +/- mice. Individual symbols with bars indicate the mean ± SEM value. Each concentration was tested in five to nine slices from at least five animals. Solid lines represent the best fit of the Hill function (k, Hill coefficient) to data points (bicuculline: wv/wv, k = 0.63, EC₅₀ = 3.7 μM; +/-, k = 1.8, EC₅₀ = 0.043 μM; bicuculline and 4-AP: wv/wv, k = 0.47, EC₅₀ > 100 μM; +/-, k = 1.36, EC₅₀ = 0.35 μM). In the hippocampus of +/- mice, barium (Ba²⁺, 1 mM) abolished the inhibition by Baclofen. Calibration (for all traces): 1 mV, 2 min. In this and the following figures, horizontal bars indicate the drug application.

**MATERIALS AND METHODS**

wv/wv (B6CBA background) and +/- (B6CBA) mice were the offspring of parents initially purchased from Jackson Laboratories (Bar Harbor, ME). All experiments had been approved by the Animal Care and Use Committees responsible for our institutions and conform to National Institutes of Health guidelines. Hippocampal slices were prepared from 2- to 6-week-old mice using techniques described previously from our laboratory (Misgeld et al., 1979). Slices were preincubated and maintained in an oxygenated (95% O₂ and 5% CO₂) solution containing (in mM): NaCl 130, KCl 2.5, CaCl₂ 1.5, MgCl₂ 2.5, HEPES 5, pH 7.4. For experiments with elevated extracellular K⁺, NaCl was lowered to 127 mM and KCl was increased to 5 mM. Recordings were obtained in normal extracellular solution as described previously (Jarolimek and Misgeld, 1993). To evoke synaptic potentials, stimuli (0.1 msec duration) were delivered by bipolar stainless steel electrodes that were placed in the mossy fiber region close to the CA3 cell layer. Ligand-induced currents were recorded at a holding potential of −65 mV in the presence of the AMPA-type glutamate antagonist 6,7-dinitroquinoxaline-2,3-dione (D-NQX; 10 μM), the NMDA-type glutamate antagonist DL-2-amino-5-methyl-5-phosphono-3-pentenoic acid (4-MeAPPA; 2 μM), and the GABA A receptor antagonists picrotoxin (25 μM) and bicuculline (25 μM) to block fast synaptic transmission. Agonists [R-baclofen, chlorodroxolene, serotonin, and R-8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT)] were applied for 3 min. To measure the agonist-induced conductance changes, voltage ramps (from −120 to −60 mV in 4 sec) were used before, at the end of, and 15 min after the drug application. All drugs were from Sigma (Deisenhofen, Germany) except D-NQX (Biotrend, Köln, Germany). R-Baclofen and CGP55845A were kindly provided by Novartis (Basel, Switzerland). All data are expressed as mean ± SEM.

**RESULTS**

Reduced potency of R-baclofen to diminish burst activity in adult weaver mice

Spontaneous recurrent burst activity in disinfibited rat hippocampal slices is suppressed at nanomolar concentrations of the selective GABA A receptor agonist R-baclofen. Because effective concentrations of R-baclofen are considerably lower than reported in other physiological studies, Swartzwelder et al. (1986) suggested that high-affinity GABA A receptors increase K⁺ conductance. In a first series of experiments, we examined the ability of R-baclofen to reduce excitability of CA3 neurons in adult weaver (wv/wv) and wild-type (+/+) mice [postnatal day (P) 24–42]. Bicuculline (20 μM) induced spontaneous recurrent burst discharges in the CA3 region of the hippocampus in +/- and wv/wv mice at elevated extracellular [K⁺] 6.25 mM (Fig. 1A1,2). Nanomolar concentrations of R-baclofen strongly reduced the frequency of spontaneous burst discharges in +/- mice (EC₅₀, 0.043 μM) (Fig. 1A3). In wv/wv mice, much larger concentrations of R-baclofen were required for the inhibition of burst discharges (EC₅₀, 3.7 μM) (Fig. 1A1,4).

Recurrent burst activity is abolished by the AMPA-type glutamate receptor antagonist D-NQX (10 μM; n = 4; data not shown), demonstrating that synaptic excitation is a critical step in the generation of burst activity. The described blockade of recurrent burst activity by R-baclofen could occur as a result of (1) inhibition of Ca²⁺ influx, (2) the transmitter release cascade, or (3) activation of a K⁺ conductance (for review, see Misgeld et al., 1995). Reduction of transmitter release can be counterbalanced by strengthening synaptic transmission through the application of the K⁺ channel blocker 4-aminopyridine (4-AP). 4-AP does not block GABA A receptor-activated K⁺ conductances (Solís and Nicoll, 1992; Jarolimek et al., 1994). In bicuculline (20 μM) and 4-AP (50 μM), R-baclofen reduced the frequency of spontaneously occurring recurrent burst discharges in +/- mice. Effective concentrations (EC₅₀, 0.35 μM) were higher than those necessary to reduce the frequency in the absence of 4-AP (Fig. 1A3 vs 1B2).

Apart from a reduction in the efficacy of R-baclofen on recurrent burst activity, the difference in the effects of R-baclofen on slices from +/- and wv/wv mice not only persisted but was enhanced. R-baclofen even at a high concentration (100 μM) was almost ineffective in wv/wv mice (EC₅₀ > 100 μM) (Fig. 1B1,3). A similar loss of R-baclofen efficacy was attained in slices of +/- mice if Ba²⁺ (1 mM) was applied in the presence of 4-AP and bicuculline (n = 5 slices) (Fig. 1C). Ba²⁺ is known to block GABA A receptors.
receptor-mediated K⁺ conductance increases in hippocampal CA3 neurons (Gähwiler and Brown, 1985; Jarolimek et al., 1994; Sodickson and Bean, 1996). As described for recurrent bursts in bicuculline, burst discharges in bicuculline, 4-AP, and Ba²⁺ were reduced by DNQX (10 μM; n = 5; data not shown). Our findings suggest that the depressant effect of R-baclofen on the excitability of CA3 neurons is reduced in adult wv/wv mice, because R-baclofen fails to activate a K⁺ conductance.

Elimination of activation of postsynaptic K⁺ conductance by neurotransmitters in adult wv/wv mice

The lack of G-protein-gated K⁺ conductances in adult wv/wv mice was supported by studying the effects of R-baclofen, chloroadenosine, and serotonin, which are known to activate K⁺ conductances on holding current in CA3 neurons. We found no significant differences in mean resting membrane potential, input resistance, and passive membrane time constant of CA3 pyramidal cells between wv/wv and +/- mice of the same age (Table 1). The input resistance slopes of CA3 cells were measured with voltage ramps in the range of -120 to -60 mV. As shown in Figure 2, current–voltage relationships were not different for adult wv/wv and +/- mice.

In CA3 neurons from +/- mice, R-baclofen (5 μM) induced a large outward current at a membrane potential of -65 mV in every cell tested (n = 12 cells) (Fig. 3A). The properties of the underlying conductance were determined with voltage ramps.

The R-baclofen-induced current was obtained by subtracting traces recorded in the presence and absence of R-baclofen. As shown in dissociated hippocampal CA3 pyramidal cells (Sodickson and Bean, 1996) the R-baclofen-induced current was caused by the activation of an inwardly rectifying K⁺ conductance (Fig. 3A) (n = 5). This current could be blocked by the selective GABAᵦ receptors antagonist CGP55845A (0.5 μM; n = 3; data not shown) (Jarolimek et al., 1993). In wv/wv mice R-baclofen had no effect on the holding current or the input resistance in all but two cells (n = 20). In one cell R-baclofen induced a small inward current and conductance increase (Fig. 4B), which may reflect a Na⁺ current through wvKir3.2 channels. In the other cell, R-baclofen evoked a small outward current that was most likely caused by an efflux of K⁺ ions (Fig. 4C).

In the mammalian CNS many G-protein-coupled receptors may activate the same K⁺ channels (Nicoll, 1988). Therefore, we measured outward currents induced by the activation of adenosine A1 and serotonin 5-HT1A receptors. The selective A1 receptor agonist chloroadenosine (10 μM) consistently induced large currents in CA3 cells of +/- hippocampi (Fig. 4A) that were caused by a K⁺ conductance increase. However, chloroadenosine failed to induce any current in adult wv/wv mice (Fig. 4C). Large concentrations of serotonin (50 μM) or the selective 5HT1A receptor agonist 8-OHDPAT (10 μM) were necessary to induce small outward currents in +/- mice (27 ± 1 pA; n = 89 cells). In wv/wv mice, serotonin (50 μM) did not induce any current (n = 6) or small inward currents (15 ± 4 pA; n = 3), probably because of the activation of 5HT4 receptors that close K⁺ channels (Andrade and Nicoll, 1987). Thus, various G-protein-coupled receptors that activate K⁺ currents in CA3 pyramidal cells of +/- mice do not activate a current in adult wv/wv mice.

Absence of slow IPSCs in adult wv/wv mice

In the hippocampus, GABA induces a fast Cl⁻ conductance increase mediated via GABAᵦ receptors (fast IPSC) and a slow GABAᵦ receptor-mediated K⁺ conductance increase (slow IPSC) (for review, see Misgeld et al., 1995). Therefore, we tested for the presence of a slow IPSP in wv/wv mice by applying electrical stimulation near the CA3 cell layer. To ensure that we were stimulating inhibitory neurons, we monitored fast IPSPs and slow IPSPs in the presence of glutamate receptor antagonists (Fig. 5A). Despite the presence of large fast IPSPs in wv/wv mice,
Figure 3. R-Baclofen-induced K⁺ conductance increases in CA3 pyramidal cells of +/+ mice but not of adult wv/wv mice. A, In +/+ mice, R-baclofen induced an outward current that was caused by activation of an inwardly rectifying K⁺ conductance. Current–voltage relationship was calculated as the difference between the current responses to voltage ramps before, after, and during the R-baclofen application. The interruption in the top trace marks the point when the voltage ramp was applied. B, In wv/wv mice, R-baclofen failed to induce a current (top trace) or conductance change (bottom traces) that are averages of 3 current responses to 10 mV steps. In this and the following figures currents were recorded in conductance change (ΔG). Current–voltage relationship was measured as the difference between the current responses to voltage ramps before, after, and during the R-baclofen application. The interruption in the top trace marks the point when the voltage ramp was applied. In the presence of AMPA-type (DNQX, 10 μM) and NMDA-type (4-MeAPPA, 2 μM) glutamate receptor antagonists and GABA₅ receptor antagonists (bicuculline, 25 μM; picrotoxin, 25 μM) at a holding potential of ~65 mV.

Figure 4. Ligand-induced outward currents in CA3 pyramidal cells of +/+ mice and inward currents in wv/wv mice. A, In a CA3 pyramidal cell of a +/+ mouse, R-baclofen and the selective adenosine A1 receptor agonist chloroadenosine induced large outward currents. B, In one cell (P30) of a wv/wv mouse, R-baclofen induced an inward current and conductance increase (bottom traces) that are averages of 3 current responses to 10 mV steps. C, Summary graph of ligand-gated currents tested in young and adult CA3 neurons. The amplitude of the current induced by R-baclofen (5 μM) and chloroadenosine (5 μM) and the amplitude of the electrically evoked IPSC are significantly smaller in wv/wv mice. Numbers on top or below the bars indicate the number of CA3 cells in which a current was observed versus the total number of cells recorded. Amplitude of ligand-gated K⁺ currents in young (P14–P18) and adult (≥P24) +/+ mice were similar and therefore were pooled.

no slow IPSP was recorded (n = 6). Moreover, we stimulated inhibitory neurons chemically by 4-AP in the presence of GABA₅ and glutamate antagonists (Segal, 1990; Misgeld et al., 1992; Jarolimek et al., 1994). 4-AP induced large recurrent outward current transients in +/+ mice (n = 8) (Fig. 5B) but not in wv/wv mice (n = 6). In the presence of glutamate and GABA₅ receptor antagonists, we finally applied triplets of stimuli, which we found to be very effective in evoking slow IPSPs (Müller and Misgeld, 1990). This stimulation was strong enough to induce residual inward currents despite the presence of antagonists for fast syn-
aptic transmission (Fig. 6B) (Scanziani et al., 1993). Inward currents were followed by slow outwardly directed IPSCs in control \((n = 14)\) but not in \(vw/vw\) mice \((n = 15)\). Slow IPSCs reversed their polarity near \(-100\) \text{mV} \((n = 8)\) (Fig. 6B) and were blocked by the selective GABA\(_B\) receptor antagonist CGP55845A \((n = 3)\) (Fig. 6D), indicating that they were indeed mediated by GABA\(_B\) receptors. No slow IPSCs were recorded in \(vw/vw\) mice at holding potentials of \(-65\) to \(-95\) \text{mV} \((n = 9)\) (Fig. 6A).

**Ligand-gated, G-protein-mediated currents in young \(vw/vw\) mice**

In hippocampi of P19 and P20 \(vw/vw\) mice, Kir3.1 and Kir3.2 channel proteins are clearly detectable. Protein levels start to decrease at P27 (Liao et al., 1996). Because there was no G-protein-mediated current expressed in P24–P42 \(vw/vw\) mice, we examined ligand-gated G-protein-mediated conductance increases in younger animals. Passive membrane properties of \(vw/vw\) and +/- mice between P14 and P18 were identical. Small developmental differences, however, were observed in mean membrane potential and membrane resistance of CA3 pyramidal cells of young and adult mice (Table 1). In CA3 pyramidal cells of young +/- mice (P14–P18), R-baclofen (5 \text{mM})-induced chlordenosine (10 \text{mM})-induced outward currents and electrically stimulated as well as 4-AP-evoked slow IPSCs showed amplitudes similar to those in adult CA3 cells \((n = 6)\; \text{data not shown}\). In young \(vw/vw\) mice, R-baclofen and chlordenosine induced small outward currents in only a few neurons (Fig. 4C). In one cell R-baclofen induced an inward current. In 4 of 11 CA3 cells, electrical stimulation induced inwardly directed slow IPSCs that could be blocked by CGP55845A \((0.5\ \text{mM}; n = 2)\) (Fig. 6C). In one of those four cells, R-baclofen also induced an inward current, whereas in the other cells R-baclofen did not evoke any current or conductance change. Thus, already at a time when Kir3.1 and Kir3.2 protein expression appears to be normal, K\(^+\)-dependent inhibition is severely impaired. This is even true in very young animals (P4–P9). In CA3 pyramidal cells of +/- mice, R-baclofen (10 \text{mM}) induced small outward currents \((80 \pm 20\ \text{pA})\) in six of seven cells, and slow IPSCs were small \((30\ \text{pA}; n = 3)\) or absent \((n = 3)\). In \(vw/vw\) mice there was no R-baclofen-induced current in seven of eight cells. In the one cell R-baclofen evoked an inward current \((20\ \text{pA})\).

**DISCUSSION**

In hippocampal CA3 pyramidal neurons of \(vw/vw\) mice, the point mutation in \(vw/Kir3.2\) exerts a dominant-negative effect on the expression of G-protein-gated currents. Consequently, slow GABA\(_B\) receptor-mediated IPSCs as well as ligand-induced K\(^+\) currents are absent. Our data show that expression of \(vw/Kir3.2\) channels, like a knockout of the same gene, impairs K\(^+\)-dependent inhibition. In contrast to Kir3.2-deficient mice, \(vw/vw\) mice do not exhibit seizure activity, suggesting that additional factors determine seizure manifestation.

**Inhibition of spontaneous burst activity by GABA\(_B\) receptor activation**

The GABA\(_B\) receptor agonist R-baclofen reduces spontaneous recurrent field burst activity in the disinhibited CA3 region of +/- mice with high efficacy, whereas R-baclofen is less potent in diminishing burst discharges in adult \(vw/vw\) mice and +/- mice exposed to K\(^+\) channel blockers. This finding suggests that R-baclofen does not activate a K\(^+\) conductance in \(vw/vw\) mice. Intracellular recording from CA3 neurons confirmed this hypothesis. Therefore the hippocampal slice prepared from \(vw/vw\) mice helps us to better understand the pharmacological actions of R-baclofen. In control animals GABA\(_B\) receptor activation increases K\(^+\) conductance, diminishes Ca\(^2+\) currents, and inhibits the transmitter release cascade (for review, see Misgeld et al., 1995). For inhibition of Ca\(^2+\) currents or transmitter release, R-baclofen was applied in micromolar concentrations \((EC_{50}, \ 3–7\ \text{mM})\). The concentration that effectively reduced recurrent burst discharges in \(vw/vw\) mice was in the same range. When high concentrations of R-baclofen are applied, the inhibition of Ca\(^2+\) currents and of the release cascade dominates, and the contribution of K\(^+\) conductance becomes small (Hirata et al., 1992; Misgeld et al., 1995). Our data clearly show that nanomolar concentrations of R-baclofen strongly reduce the frequency of recurrent burst discharges in +/- but not in \(vw/vw\) mice, indicating that Kv3.1–Kv3.2 channels contribute to the inhibitory effects of low R-baclofen concentrations in the mouse hippocampus.

Similar to GABA\(_B\) receptors, activation of adenosine receptors induces various effector mechanisms, including a K\(^+\) conductance increase (for review, see Greene and Haas, 1991). Endogenous adenosine exerts a tonic inhibitory tone in the hippocampus that is increased under conditions of metabolic stress, e.g., hypoxia or hypoglycemia or during hyperexcitability (for review, see Greene and Haas, 1991). The lack of activation of K\(^+\) conductance by adenosine in CA3 neurons of adult \(vw/vw\) mice may also impair inhibition by endogenous adenosine.

**Failure of ligands to activate K\(^+\) conductance in young and adult \(vw/vw\) mice**

mRNA for Kir3.1–3.3 as well as Kir3.1 and Kir3.2 protein has been detected at high levels in the hippocampus of +/- mice (Lesage et al., 1994; Kobayashi et al., 1995; Liao et al., 1996; Murer et al., 1997; Wei et al., 1997). In Kir3.2-deficient mice, Kir3.1 expression is strongly reduced (Signorini et al., 1997). A very similar situation can be found in the hippocampus of adult \(vw/vw\) mice in which Kir3.1 and Kir3.2 protein levels are strongly reduced at P27 and absent at P95 (Liao et al., 1996). Kir3.1 and Kir3.2 are thought to be essential components of neuronal G-protein-gated K\(^+\) channels (Duprat et al., 1995; Wischmeyer et al., 1997), and their absence should result in a lack of G-protein-gated currents. Accordingly, in both Kir3.2-deficient mice (Lüscher et al., 1997) and \(vw/vw\) mice (this study), the K\(^+\) conductance increase induced by G-protein-coupled receptors is markedly reduced or absent.

A surprising finding was that in young (P14–P18) \(vw/vw\) mice, which express normal amounts of Kir3.1 and Kir3.2 proteins (Liao et al., 1996), activation of G-protein-coupled receptors did not induce a conductance change. Only small inward or outward currents could be detected in a few cells when GABA\(_B\) receptors were activated. Small inward currents were to be expected from the biophysical properties of \(vw/Kir3.2\) channels. Kir3.2 channels form heterotetramers with Kir3.1 in vivo, and \(vw/Kir3.2\) coexpressed with Kir3.1 result in a phenotype with reduced function and loss of K\(^+\) selectivity (Kofuji et al., 1996; Navarro et al., 1996; Silverman et al., 1996; Slesinger et al., 1996, 1997; Surmeier et al., 1996; Tong et al., 1996). Synaptically released GABA induces small inward currents in a few cells; in most cells, however, we observed no conductance change. The absence of G-protein-gated currents may be attributed to lack of Kir3.1–\(vw/Kir3.2\) protein in the membrane. R-baclofen-evoked outward currents are probably caused by K\(^+\) currents through homomultimeric Kir3.1–3.3 channels, which conduct substantial amounts of currents when coexpressed (Duprat et al., 1995; Wischmeyer et al., 1997).
Our findings show that the expression of G-protein-gated currents is already severely impaired in young \( wv/wv \) mice, followed by the complete downregulation of protein levels in adult \( wv/wv \) mice.

One important difference in the function of the Kir3.0 family is found between Kir3.2-deficient and \( wv/wv \) mice. In the absence of any substantial receptor activation, the resting membrane potential of CA1 pyramidal cells of Kir3.2-deficient mice is more depolarized compared with control animals, suggesting that Kir3.1–Kir3.2 channels contribute to the leak \( K^+ \) current (Lüscher et al., 1997). We found no difference in mean resting membrane potentials and slope resistances between CA3 neurons of cher et al., 1997). We found no difference in mean resting membrane potential and the persistent depolarizations of \( wv/wv \) and +/- mice. The reasons for the discrepancy in the effect of Kir3.2 on resting membrane potential are yet unknown and could be attributable to regional (CA1 vs CA3) or strain (C57BL/6 vs B6CBA) differences. However, the similarity in the passive membrane properties of +/- and \( wv/wv \) mice excludes the existence of a constitutive inward current in CA3 neurons in situ that has been described in oocytes expressing \( wv/Kir3.2 \) as well as in cultured cerebellar granule cells of \( wv/wv \) mice (Kofuji et al., 1996; Silverman et al., 1996; Tong et al., 1996; Slesinger et al., 1997). In hippocampal CA3 neurons, all inward currents were gated by GABA\(_B\) receptor agonists and could be abolished by a selective GABA\(_B\) receptor antagonist.

**Lack of ligand-gated Kir3.0 conductances and hippocampal function**

Apart from severe motor performance disturbances no massive abnormalities have been reported in \( wv/wv \) mice. As far as hippocampal function is concerned, there might be subtle signs that have been overlooked because of the disturbed motor behavior of these animals. In any case, morphological abnormalities are minor in the CA3 area (Sekigushi et al., 1995; Liao et al., 1996), which sets this region apart from the cerebellum and the substantia nigra where massive cell death occurs (for references, see introductory remarks). Considering the apparently normal function and morphology of the \( wv/wv \) hippocampus, it was surprising to observe that \( K^+ \)-dependent inhibition was severely impaired.

Cell death in the cerebellum and substantia nigra of \( wv/wv \) mice is most likely caused by a gain of function of \( wv/Kir3.2 \) (Signorini et al., 1997) that results in inward instead of outward currents at resting membrane potential and the persistent depolarizations of some cells (Kofuji et al., 1996; Silverman et al., 1996; Slesinger et al., 1997). Our findings show that abnormal constitutive inward currents do not exist in the hippocampus. Strong electrical stimulation evoked small GABA\(_B\) receptor-mediated inward currents only in a few cells. Given the late postnatal development of slow IPSPs in the hippocampus (this study) (Gaiarsa et al., 1995) and the downregulation of Kir3.1–Kir3.2 protein in adult \( wv/wv \) mice (Liao et al., 1996), it is unlikely that slow IPSCs of considerable amplitude exist at any time. The small amplitude and the rare occurrence of synaptic inward currents is not sufficient to induce cell death in the hippocampus but may cause small morphological changes.

Kir3.2-deficient mice exhibit spontaneous seizure activity (Signorini et al., 1997), whereas homozygous \( wv/wv \) mice do not. In both models outward currents evoked by agonists for GABA\(_A\), adenosine and 5HT1A receptors are essentially absent. On the other hand, it is rather unlikely that the abnormalities found in the cerebellum and substantia nigra prevent seizure occurrence. Therefore, loss of \( K^+ \)-dependent inhibition may not be sufficient to induce seizures. The reduced membrane potential in the hippocampus of the Kir3.2-deficient mice and the different genetic background (see introductory remarks) are two of several possible factors that could induce seizure activity.

**REFERENCES**


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