H_2 histamine receptor-phosphorylation of Kv3.2 modulates interneuron fast spiking

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Histamine-containing neurons of the tuberomammilary nucleus project to the hippocampal formation to innervate H_1 and H_2 receptors on both principal and inhibitory interneurons. Here we show that H_2 receptor activation negatively modulates outward currents through Kv3.2-containing potassium channels by a mechanism involving PKA phosphorylation in inhibitory interneurons. PKA phosphorylation of Kv3.2 lowered the maximum firing frequency of inhibitory neurons, which in turn negatively modulated high-frequency population oscillations recorded in principal cell layers. All these effects were absent in a Kv3.2 knockout mouse. These data reveal a novel pathway for histamine-dependent regulation of high-frequency oscillations within the hippocampal formation.

Histamine-containing neurons of the posterior hypothalamic tuberomammilary nucleus make a widespread projection throughout the mammalian central nervous system, which includes all fields of the hippocampus[1–6]. Activation of this histaminergic system is crucial in the sleep–waking cycle and attentiveness, and is implicated in various behavioral states, including learning and memory[7,8]. Histamine released from axonal varicosities act on two postsynaptic receptors, H_1 and H_2, which are coupled positively to phospholipase C and adenylyl cyclase pathways respectively. In contrast, H_3 receptors are autoreceptors regulating histamine release or heteroreceptors located on axon presynaptic terminals regulating release of other transmitters[4,9]. In rodent hippocampus, both H_1 and H_2 receptors are distributed throughout all subfields[10]. In hippocampal principal cells, activation of H_2 receptors depolarizes pyramidal cells by reducing currents through a calcium-activated potassium current[11–14], and induces mossy fiber- and perforant path-evoked burst firing[15,16], in part by reducing inhibitory neurotransmission[16–18]. The molecular targets downstream of histamine receptor activation and how they modulate inhibitory neuron function are unexplored.

The ability to fire short-duration action potentials at high frequencies is a hallmark property of several subpopulations of local circuit inhibitory interneurons[19]. This firing pattern is determined by the biophysical properties of intrinsic voltage-dependent conductances. However, the molecular identity of these conductances and how they are modulated is only beginning to be understood. In recombinant systems, the potassium channel subunits Kv3.1b and Kv3.2 express currents that activate at very depolarized potentials, show little inactivation and deactivate rapidly compared to other K^+ channel subunits[20–22]. Consequently, Kv3.1b and Kv3.2 keep action potentials brief, permitting high-frequency firing[22–28]. Within the hippocampus, both Kv3.1b and Kv3.2 are expressed predominantly in inhibitory interneurons[21,24,25,29,30–32]. Although they have similar biophysical properties, Kv3.2 differs from Kv3.1b by the presence of a putative protein kinase A (PKA) phosphorylation site[21,31,33] that can be phosphorylated *in situ* by intrinsic PKA[33]. Current through recombinant Kv3.2 (but not Kv3.1) channels is inhibited by PKA phosphorylation[33]. Whether native Kv3.2 channels are similarly a target for PKA modulation and whether this is physiologically relevant remains unknown.

Here we tested the hypothesis that Kv3.2-containing channels on hippocampal interneurons are a target for histamine receptor-dependent PKA modulation. Specifically, we wished to determine whether H_2 receptor-dependent modulation of Kv3.2 channel function influenced fast-spiking properties of interneurons in wild-type and Kv3.2 knockout mice.

Results

Kv3.2 is expressed in inhibitory interneurons

In the hippocampus, Kv3.2 mRNA is expressed primarily in inhibitory interneurons[25,29], but its distribution in specific subpopulations has not been described. Using a Kv3.2-selective antibody[28], we determined Kv3.2 protein expression throughout hippocampal interneuron subpopulations. At the light microscopic level, Kv3.2 protein expression was restricted to the somata, proximal dendrites and axons of interneurons (Fig. 1a and b) located in both CA1/CA3 stratum oriens-alveus and stratum pyramidale and in cells within the hilus and subgranular layer of the dentate gyrus (DG). Double labeling with Kv3.2 and monoclonal antibodies against somatostatin and the Ca^{2+}-binding proteins parvalbumin, calbindin and calretinin identified the expression of Kv3.2 within particular populations of inhibitory interneurons. All parvalbumin-containing inhibitory neurons (100 ± 0%, n = 3 independent experiments) and 32.8 ± 2.3% (n = 3) of somatostatin-positive cells were Kv3.2 positive. In contrast, Kv3.2 was not present in cells labeled with either calbindin or calretinin. This expression pattern is similar but not identical

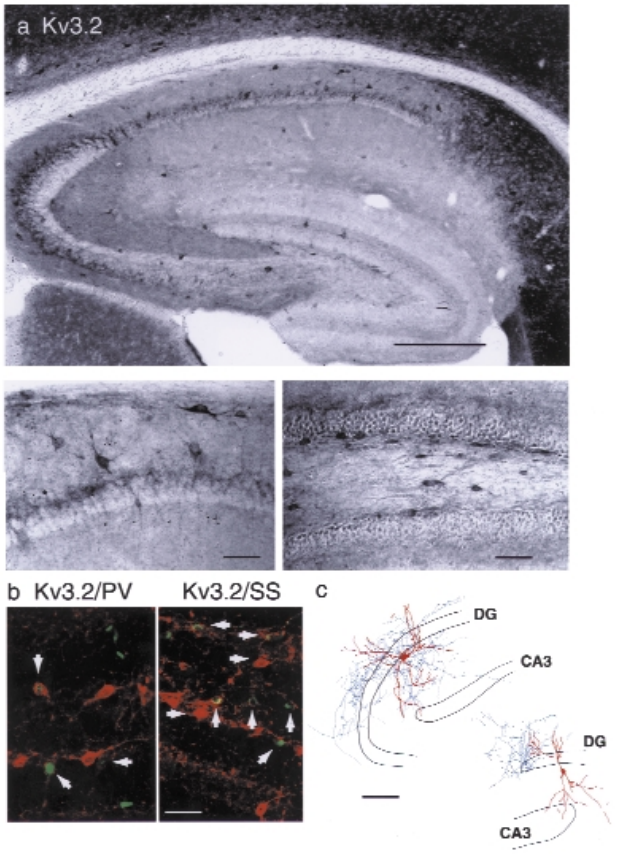


Fig. 1. Kv3.2 is expressed in parvalbumin- and a subpopulation of somatostatin-containing hippocampal interneurons. (**a**) Kv3.2 light immunohistochemistry. Kv3.2 protein is expressed in the somata, proximal dendrites and axons of local inhibitory interneurons distributed throughout all hippocampal subfields. Scale bar, 330 μm. Bottom, high-power magnification of Kv3.2 expression in CA1 (left) and DG/hilus (right). Scale bars, 150 μm. (**b**) Double immunofluorescence showing that Kv3.2 (red) is expressed in both parvalbumin (PV)- and somatostatin (SS)-containing interneurons (green, arrows) in the DG. Scale bar, 120 μm. (**c**). Two representative cells recorded physiologically, labeled with biocytin and drawn by camera lucida. Both cells were located within the dentate gyrus/hilar region and had axons that ramified either within the hilus and the inner molecular layer or throughout the granule cell body layer. Axons are shown in blue, dendrites in red. Scale bar, 200 μm. Both cells showed modulation of Kv3.2 outward currents.

to that of the related subunit Kv3.1b, which is expressed exclusively in parvalbumin interneurons[24,30,34] (Fig. 4d).

cAMP modulates Kv3.2 currents in interneurons

Channels formed by Kv3 subfamily members are thought to carry a major component of the sustained outward current in many hippocampal interneurons[24,25]. However, 4-aminopyridine (4-AP) and tetraethylammonium (TEA) block currents through both recombinant Kv3.1b and Kv3.2 with equal affinity[22], making characterization of Kv3.2-containing channels problematic. The presence of a putative PKA phosphorylation site within Kv3.2 (but not Kv3.1b), however, suggests that elevation of cAMP may inhibit current through native Kv3.2-containing channels, as it does currents through recombinant Kv3.2 (ref. 33). Sustained outward currents were recorded from interneurons located at the subgranular region of the DG or within the hilus, both known populations of parvalbumin- and somatostatin-containing interneurons[19] (Fig. 1c). Outward currents were activated at test potentials positive to –40 mV (V_{hold} = –70 mV, 200 ms duration) and were essentially non-inactivating (Figs. 2 and 3)[7,24,35]. Sustained currents were blocked by TEA (1–2 mM; mean current, 56 ± 5% of control, n = 16; Fig. 2a and d). Application of dibutyryl cAMP (or 8-Br-cAMP, 2 mM) together with IBMX (100 μM) also reduced outward currents in 12 of 18 cells (Fig. 2b and e); the mean current was 74 ± 2% of control (n = 12, p = 0.004).

Innervation of the DG by axons of tuberomammillary nucleus neurons[6], and the dense H_2 receptor distribution within this region[10], prompted us to determine whether H_2 receptor activation modulated Kv3.2 currents by a mechanism involving adenylyl cylase pathways. Application of histamine (10 μM) alone reduced outward currents to 60 ± 4% of control (n = 13, p = 0.0003). The action of histamine was mimicked by the selective H_2 receptor agonist dimaprit (10 μM; 72 ± 5% of control, n = 10, p = 0.0013; Fig. 2c and f) but not by the selective H_1 receptor agonist 6-[2-(4-imidazolyl)ethylamino]-N-(4-trifluoromethylphenyl)heptanecarboxamide (HTMT, 100 μm; 89 ± 16%, n = 8, not significant; data not shown). Reduction of the outward current by either histamine or dimaprit was blocked by the H_2-selective antagonist cimetidine (10 μM; 91 ± 9% of control, n = 7; 110 ± 6%, n = 5, respectively, not significant; Fig. 3c).

The difference currents obtained by subtraction of the current families activated in the presence of TEA, dimaprit or PKA activators from their control possessed similar voltage dependence, activated at very depolarized potentials and were essentially non-inactivating (Fig. 2d–f). The activation voltage for the PKA-sensitive current was often 10–20 mV more negative than the current component blocked by TEA or dimaprit, suggesting the presence of a second PKA-sensitive current component in these cells (Fig. 2e). However, at 0 mV, this current contributed only 6% of the total outward current. The TEA- or dimaprit-sensitive current components possessed similar half-activation properties ($V_{1/2}$ = 16.0 ± 1.2 mV, k = 16.0 ± 1; 18.7 ± 1.6 mV, k = 16.1 ± 1.4, respectively).

Because TEA, H_2 receptor activation or PKA activators failed to completely block outward currents, we next confirmed that these agents acted on the same current component. In cells where TEA (2 mM) reduced the outward current (mean reduction 50 ± 8% of control, n = 16, p = 0.00013), addition of either dimaprit or PKA activators in the continued presence of TEA had no further effect (54 ± 7% of original control, n = 8; 49 ± 2%, n = 8, respectively, Fig. 3a and b). This suggests that TEA removed all the current modulated both by H_2 receptor activation and by direct activation of PKA, presumably by blocking current through Kv3.2-containing channels.

Prior application of the H_2 receptor antagonist cimetidine (100 μM) blocked the action of both dimaprit (91 ± 2%, n = 7, Fig. 3c) and histamine (110 ± 6%, n = 5), confirming that the reduction of K^+ currents by both agents occurred through H_2 receptor activation. In contrast, in cells where cimetidine successfully blocked the action of histamine, PKA activators still reduced outward currents (77 ± 7%, n = 5, p = 0.041), confirming that PKA activators act downstream from H_2 receptor activation.

Preincubation of slices with the PKA inhibitor rp-cAMPs (0.1 μM) prevented the action of dimaprit (103 ± 5% of control, n = 10), confirming that H_2 receptor activation modulated Kv3.2-containing channels by a mechanism involving PKA phosphorylation (Fig. 3d). Furthermore, following reduction

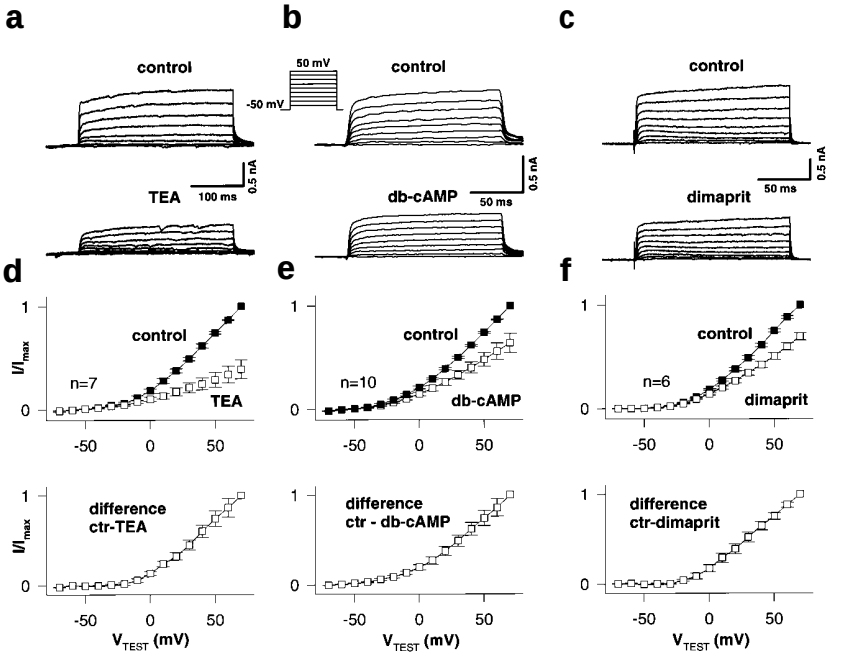
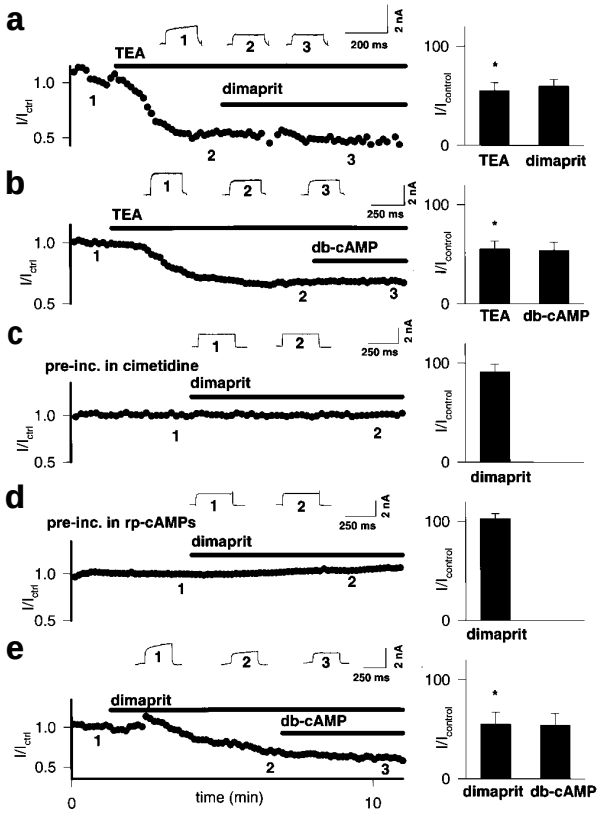


Fig. 2. Sustained outward K^+ currents resembling Kv3.2 currents are modulated by H_2 receptor activation and db-cAMP in interneurons. (**a–c**) Whole-cell sustained outward potassium currents in interneurons were activated by a 300-ms test pulse between –50 mV and +70 mV (10-mV increments, V_{hold} = –70 mV; inset in **a**). The sustained current activated rapidly and was essentially non-inactivating over the duration of the pulse. Outward currents were reduced by TEA (1mM; **a**), PKA activators (2 mM db cAMP or 100 µM IBMX; **b**) or the selective H_2 receptor agonist dimaprit (10 µM; **c**). (**d–f**) Top, averaged normalized *I–V* relationships of outward currents measured in control or in the presence of TEA (**d**; n = 7), db-cAMP/IBMX (**e**; n = 10) or dimaprit (**f**; n = 6). Bottom, averaged difference current, isolated by the digital subtraction of the current in TEA, db-cAMP or dimaprit from control. Both TEA and dimaprit removed a current component that activated at test potentials ~–10 mV. In contrast, the difference current obtained in the presence of PKA activators activated at roughly –30–20 mV.

of outward currents by dimaprit (55 ± 12% of the control), subsequent application of PKA activators in the continued presence of dimaprit failed to further reduce outward currents (99 ± 12% of current in dimaprit, n = 4; Fig. 3e), confirming that PKA activators and H_2 receptor activation act on the same outward K^+ current.



To unequivocally confirm that H_2 receptor activation reduced outward currents by acting on Kv3.2-containing channels, we made recordings from the same population of cells in mice lacking the *Kv3.2* gene. [Lau, D. H. P. *et al.*, *Somat. Motor Res.* 16, 182 (1999).] Dimaprit (mean current 104 ± 6 % of control, n = 9), histamine (mean current 103 ± 3 % of control, n = 7) or PKA activators (mean current 104 ± 5 % of control, n = 9) failed to reduce the sustained outward current (Fig. 4a–c). Current–voltage relationships generated in the presence and absence of dimaprit or PKA activators revealed no effect of either agent at any test potentials. However, in 9 of 11 cells, application of TEA blocked a fraction of current similar to that observed in wild-type neurons (mean current, 63.0 ± 7% of control, P = 0.008; Fig. 4b and c). Although these data demonstrate a lack of H_2 receptor- or cAMP-modifiable current in DG interneurons of Kv3.2 mutants, the block of outward currents by TEA suggested that channels containing Kv3.1b may be formed in the absence

Fig. 3. TEA, H2 agonists and PKA activation modulate the same outward current. In all traces, dot plots show representative experiments taken from the mean data set shown on the right. Sustained outward currents were activated by test pulses to +40 mV (duration 300 ms, 0.125 Hz, V_{hold} –70 mV). (**a**, **b**) Left, following a control period (10 test pulses), the sustained outward current was reduced by TEA (1 mM). Subsequent addition of either dimaprit (**a**) or db-cAMP/IBMX (**b**) had no further effect on the outward current amplitude. Traces shown above dot plots are averaged from 10 records at time points indicated by numbers. Histograms (right) show averaged data from 8 cells for both (**a**) and (**b**). (**c**) The action of dimaprit on the outward current was selectively blocked by prior exposure to the selective H_2 receptor antagonist cimetidine (100 µM). Right, averaged data from 7 cells. (**d**) Similarly, the block of outward currents by dimaprit required activation of adenylyl cyclase. Preincubation of slices for up to 1 hour in the PKA inhibitor rp-cAMP (0.1 mM) prevented the effects of dimaprit (n = 10). (**e**) Reduction of outward currents by dimaprit occluded the reduction of currents by db-cAMP (n = 4). The above data demonstrate that H_2 receptor activation acts via PKA phosphorylation to reduce outward currents in dentate gyrus/hilar interneurons.

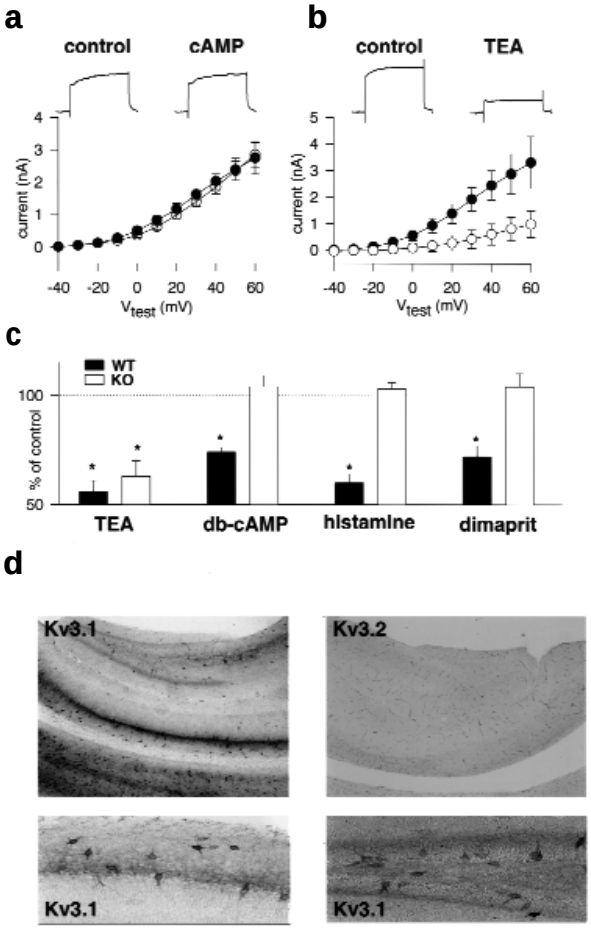


Fig. 4. H_2 agonist and PKA modulation of outward currents are absent in interneurons from Kv3.2 mutant hippocampus, but block by TEA is normal. (**a**) Average *I–V* relationships show no effect of PKA activators (open symbols) at any test potentials compared to control (n = 9). Traces above *I–V* plots show averaged currents (V_{test} = +40 mV). (**b**) In contrast, sustained outward currents in the Kv3.2 mutant interneurons (control, closed symbols) were reduced by TEA (1 mM, open symbols; n = 9). Average *I–V* relationships show that, despite the absence of Kv3.2 subunits, TEA blocked a current component similar to that observed in wild type. (**c**) Mean reduction of outward currents in wild-type (WT) interneurons by TEA (n = 16), db-cAMP (n = 12), histamine (n = 13) or dimaprit (n = 10). Interneurons in the Kv3.2 knockout (KO) mouse showed no effect of db-cAMP (n = 9), histamine (n = 7) or dimaprit (n = 9), but did respond to TEA (n = 7). (**d**) Immunohistochemistry showing that Kv3.1b protein expression persisted (top left) despite the absence of Kv3.2 protein (top right) in the Kv3.2 mutant mouse. Bottom, high-magnification images of Kv3.1 in CA1 (left) and dentate gyrus (right).

of Kv3.2 protein. This was confirmed by Kv3.1b immunohistochemistry (Fig. 4d). Consistent with a functional role for Kv3.1-containing channels in the absence of Kv3.2, no significant reduction in current amplitude (recorded at +50 mV) was observed in Kv3.2 mutant cells (2.2 ± 0.2 nA, n = 26) compared to wild type (2.5 ± 0.2 nA, n = 106, p = 0.41).

Kv3.2 channels set the upper limit of firing frequency

To investigate the physiological role of Kv3.2-containing channels and how H_2 receptor-dependent PKA phosphorylation of Kv3.2 affects action potential properties in hippocampal interneurons, we made recordings under current clamp. First, to determine the upper limit of interneuron firing frequency, we delivered trains (500 ms) of suprathreshold, brief-duration current pulses at increasing frequencies. The pulse-train frequency that failed to elicit a spike on each pulse (Fig. 5a) was termed the cutoff frequency (f_c). In a typical experiment from a DG interneuron, action potential firing was faithfully followed up to 167 Hz (Fig. 5a–c). The mean f_c for all interneuron experiments was 134 ± 13 Hz (n = 21). For comparison, similar data from CA3 pyramidal neurons demonstrated the lower frequency range sustained by principal cells (mean f_c, 60 ± 3 Hz, n = 23; Fig. 5d).

To determine the role of Kv3.2-containing channels in setting the upper frequency of interneuronal firing, we monitored the effects of both the H_2 receptor agonist dimaprit and direct activation of PKA on the f_c of individual neurons in both wild-type and Kv3.2 mutant animals (Fig. 6). In wild-type interneurons, both dimaprit and db-cAMP shifted f_c to a lower frequency; mean f_c was reduced by 26 ± 3% (n = 5, p = 0.004) and 32 ± 11% of the control (n = 7, p = 0.004), respectively (Fig. 6a and b).

H_2 agonists and PKA activators had no effect on the amplitude and 50% duration of the first spike but significantly prolonged the tenth spike (Fig. 6e). The mean amplitudes of the first and tenth action potentials in control conditions were 85.0 ± 6.9 mV and 78.8 ± 8.0 mV (n = 10). Following application of PKA activators, the amplitudes of the first and tenth spikes were 78.8 ± 6.7 mV and 68.0 ± 8.9 mV, respectively. In control conditions, the 50% durations of the first and tenth spikes were 1.4 ± 0.08 and 1.7 ± 0.2 ms, respectively. In the presence of PKA activators, the mean durations of the first and tenth spikes were 1.5 ± 0.1 and 2.5 ± 0.3 ms, respectively (p < 0.01). H_2 agonists or PKA activators did not significantly alter mean input resistance or resting membrane potential of any cell tested.

In the Kv3.2 mutant, interneurons sustained action potential firing frequencies similar to wild type (mean f_c, 115 ± 10 Hz, n = 24 versus 134 ± 13 Hz, n = 21, respectively), although a tendency towards lower values is evident in KO interneurons. However, H_2 receptor activation or PKA activators now failed to modulate firing frequency (96 ± 2%, n = 10; 96 ± 3% of control, n = 11, respectively), and they did not increase the 50% duration of the tenth action potential (102 ± 6%, n = 11 and 106 ± 9% of control, n = 7) in the train as observed in wild type (Fig. 6e). These results suggest that H_2-receptor-dependent PKA phosphorylation selectively modulated the Kv3.2 conductance. The ability of interneurons to fire high frequencies without Kv3.2 protein is presumably due to the presence of Kv3.1b-containing channels, consistent with the immunohistochemical data (Fig. 4d).

Identical data was obtained when action potential firing was induced by single suprathreshold electrotonic pulses (300 ms duration). In these experiments, action potential firing frequency was reduced from 46.3 ± 3.4 Hz in control to 28.7 ± 4.9 Hz by db-cAMP (n = 10, p = 0.017). Similarly PKA modulation of the steady-state firing frequency was absent in recordings from Kv3.2 knockout mice (mean reduction 4 ± 2% of control, n = 10).

Kv3.2-containing interneurons modulate oscillations

In hippocampus, oscillations in which neuronal ensembles fire in a repeated and synchronous manner are present at theta (4–10 Hz), gamma (20–80 Hz) and high (~200 Hz) frequencies[19]. GABAergic interneurons participate in the generation and synchronization of these oscillations[19,36,37,44–47]. Spontaneous high-frequency

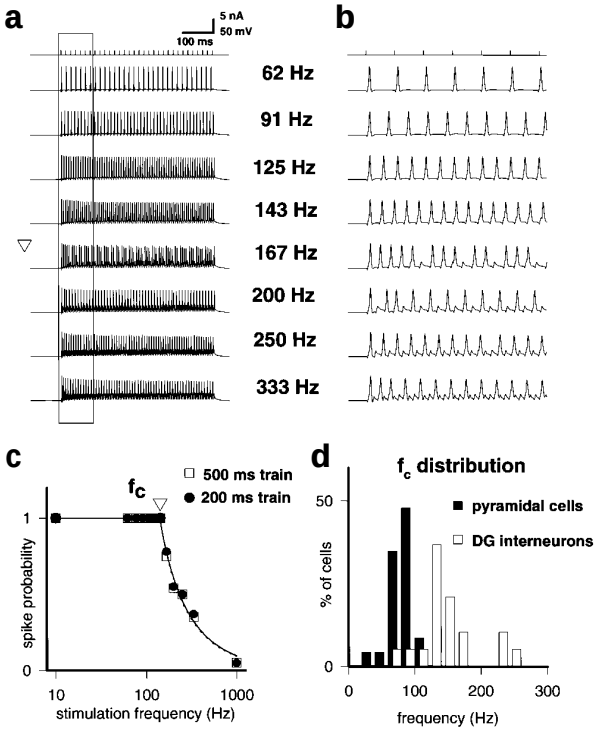
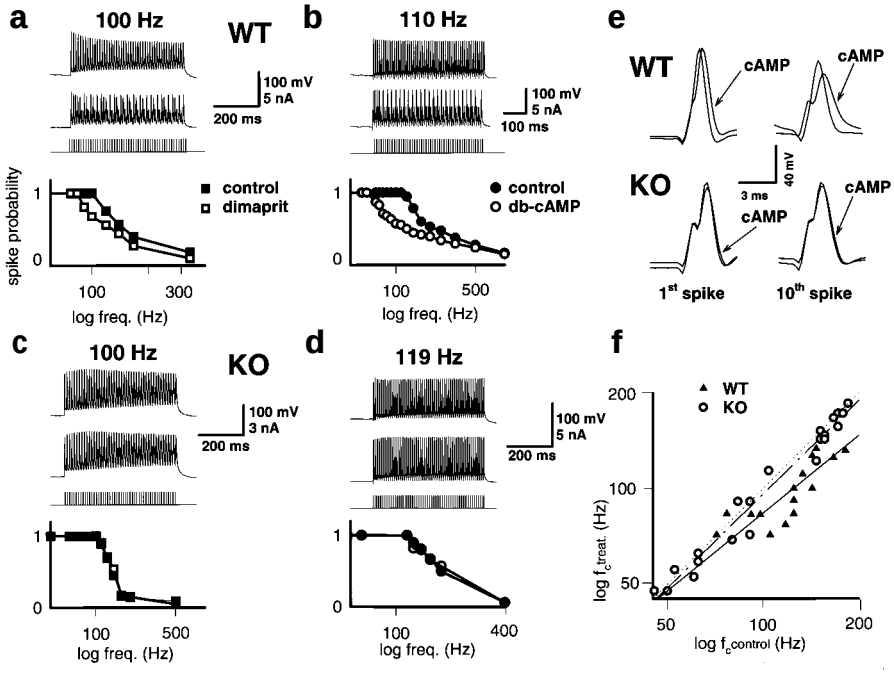


Fig. 5. Dentate gyrus interneurons but not CA3 pyramidal neurons sustain high action-potential firing frequencies. (**a**) Representative recording from a DG interneuron demonstrates that single cells can faithfully sustain action potential firing on every suprathreshold pulse (1 nA, 1.0 ms duration) up to a frequency of 167 Hz (triangle) (**b**) Initial 200ms of the trains of action potentials indicated by boxes in (**a**). (**c**) Plot of stimulation-train frequency against probability of successful spikes from the data in (**a**) allows the extraction of the f_c. At frequencies greater than 167 Hz, the percentage of spikes elicited on each suprathreshold stimuli fell hyperbolically. (**d**) Summary data from 21 DG interneurons and 23 CA3 pyramidal cells. With few exceptions, interneurons can successfully support frequencies of action potential firing (mean f_c =158 ± 23 Hz) twofold higher than those of pyramidal neurons (mean f_c = 60 ± 3 Hz).

oscillations, occurring as a brief series of repetitive population spikes, occur within the pyramidal cell layer of the hippocampus *in vitro*[39].

Because Kv3.2-containing interneurons support high-frequency firing, we determined whether PKA phosphorylation of Kv3.2 modulated spontaneously occurring high-frequency oscillations in the principal cell layers. Extracellular recordings were made from the CA3 pyramidal cell layer of both wild-type and Kv3.2 mutant animals (n = 12, 14, respectively). Conventional glutamatergic synaptic transmission was blocked by inclusion of AMPA and NMDA receptor channel blockers (DNQX and APV, 10 and 25 μM respectively). Under these conditions, spontaneous high-frequency population events[39] were observed (Fig. 7). Analysis of the interevent distribution, autocorrelation function and power spectrum showed oscillation frequencies in the range ~70–300 Hz (Fig. 7a and b). In wild-type animals, application of either the H_2 agonist dimaprit or PKA activators decreased high-frequency oscillations (> 70 Hz), whereas population events occurring at frequencies below 70 Hz were largely unaffected. Analysis of the ratio of the integrals of probability for events over 70 Hz to those under 70 Hz (R) revealed a change in the frequency distribution for both dimaprit (R = 0.38 ± 0.04% in control; 0.26 ± 0.05% after dimaprit, n = 8, p = 0.007) and PKA

Fig. 6. H_2 receptor- and PKA-modulation of interneuron firing frequency is absent in the Kv3.2 knockout mouse. (**a**, **b**) In wild-type (WT) interneurons, both dimaprit (10 μM) and PKA activators reduced the *f*c. (**a**) A representative interneuron showing that under control conditions a train of stimuli delivered at 100 Hz can be followed for the entire 500 ms duration. Following application of dimaprit, action potential firing was observed on ~50% of the suprathreshold stimuli. Data obtained at all stimulus frequencies demonstrates that dimaprit reduced the *f*c (f_c = 77 Hz in dimaprit, 100 Hz in control). (**b**) PKA activators also reduce the maximal sustainable firing frequency in wild-type interneurons (*f*c = 78 Hz in PKA activators, 118 Hz in control). (**c**, **d**) In contrast, in Kv3.2 mutant interneurons, both H_2 agonists and PKA activators had no effect on the maximum sustainable firing frequency. (**e**) Alignment of the first and tenth spikes in the train in wild-type control and in the presence of PKA activators showed prolonged spike duration and reduced peak amplitude in the tenth spike but not in the first. In Kv3.2 knockout (KO) animals, the first and tenth spikes were unaffected by PKA activators. (**f**) Summary plot from both wild-type (triangles) and Kv3.2 mutant (circles) interneurons. Regression lines for the measured f_c for both mutant (dot-dashed) and wild type (solid) demonstrate that modulation of maximal firing frequency by both H_2 agonists and PKA activators is lost in all Kv3.2 mutant cells.



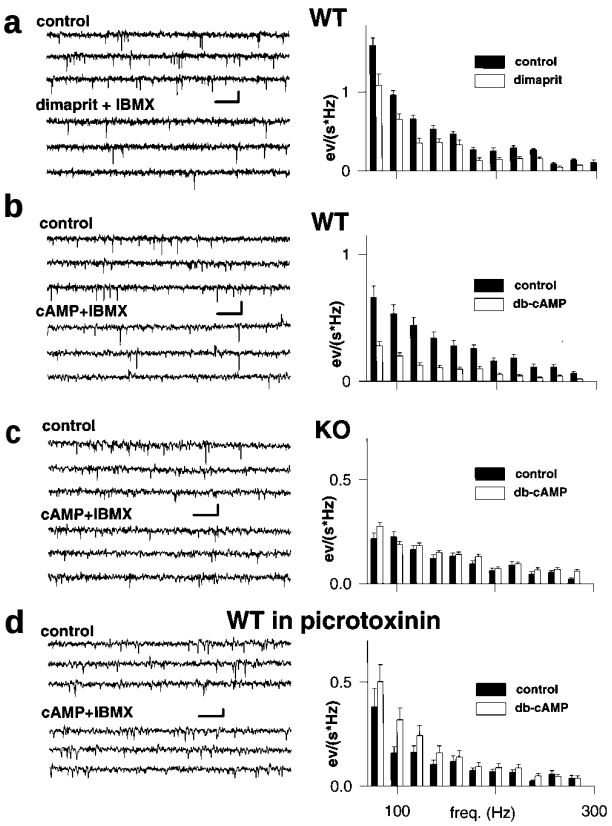


Fig. 7. PKA phosphorylation of Kv3.2 reduces the incidence of high-frequency oscillations in CA3. (**a**, **b**) Representative extracellular recordings within the CA3 pyramidal cell layer of wild-type (WT) animals revealed high-frequency population activity in the presence of glutamatergic receptor blockers. Bottom, the prevalence of high frequency bursts was reduced by either dimaprit (**a**) or PKA activators (**b**). Right, histogram plots of interspike frequency versus probability reveal a reduction of the high-frequency component (> 70 Hz) by dimaprit (n = 8) or PKA activators (n = 12). (**c**) In contrast, PKA activators failed to modulate the occurrence of high-frequency oscillations in the Kv3.2 knockout hippocampus (n = 12). Note that the event probability in mutant animals is significantly lower than that of wild-type animals, indicating a lower occurrence of high-frequency events (> 70 Hz) in mutant mice. (**d**) The modulation of high-frequency oscillations required intact GABAergic inhibitory transmission. Experiments identical to those in (**a**) and (**b**) were repeated in the presence of the GABA receptor antagonist picrotoxinin in wild-type mice. In the presence of picrotoxinin, application of PKA activators had no effect on the high-frequency component of the interevent distribution. Scale bars, 50 μV, 50 ms.

activators (R = 0.35 ± 0.04 in control; 0.17 ± 0.02 in db-cAMP, n = 12, p = 0.002).

Oscillations at frequencies similar to wild type were detected in the Kv3.2 mutant (Fig. 7c). However, the integral of the interevent distribution, which is proportional to the number of oscillatory events per unit time, revealed a lower occurrence of high-frequency events (> 70 Hz; 3.9 ± 0.9 events/s in wild type, n = 12, p = 0.025; 1.7 ± 0.4 events/s in knockout, n = 14) but not oscillations under 70 Hz. In Kv3.2 mutants, population events occurring at all frequencies were now unaffected by PKA activators (R = 0.29 ± 0.04 versus 0.34 ± 0.03, n = 14; Fig 7c).

To confirm that PKA activation modulated spontaneous oscillations by affecting GABAergic transmission, we repeated the experiments in the presence of the GABA receptor antagonists picrotoxinin (50 μM) or bicuculline (10 μM). In wild-type animals, both picrotoxinin and bicuculline reduced the occurrence of high-frequency oscillations (> 70 Hz) compared to control (Fig. 7d). The integral of the interevent distribution was 3.9 ± 0.9 events/second in control (n = 12), 1.7 ± 0.6 events/second with picrotoxinin (n = 9) and 2.1 ± 1.7 events/second with bicuculline (n = 5). In the presence of picrotoxinin, application of db-cAMP did not affect the high-frequency component of the interevent distribution (R = 0.17 ± 0.04 in control; 0.2 ± 0.04 in db-cAMP). Identical data were obtained using bicuculline. These data suggest that GABAergic transmission modulates high-frequency oscillations within the principal cell layer.

Discussion

The axons of histaminergic neurons originating within the posterior hypothalamic tuberomammillary nucleus ramify extensively throughout the hippocampal formation. Ultrastructural studies show that histaminergic varicosities form few synaptic contacts, implying that most neuronal histamine is released by non-synaptic mechanisms with a widespread locus of activity[40]. Thus activation of a small number of tuberomammillary neurons act to simultaneously excite target cells distributed throughout many CNS areas. Consequently, histamine receptor activation participates in a variety of brain functions, including locomotor and exploratory behavior, arousal and the sleep–wake cycle. Within the hippocampus, histamine modulates neuronal function via both H_1 and H_2 receptor activation, the latter most commonly linked to a reduction in Ca^{2+}-activated K^+ conductance in principal neurons, the molecular identity of which is presently unknown. A role for histamine receptor (either H_1 or H_2) modulation of inhibitory interneuron function has never been demonstrated, although a previous study[16] indirectly demonstrated that H_2 receptor enhancement of synaptic excitability seems to arise in part from a reduction of inhibitory neurotransmission. The present demonstration that H_2 receptor function is linked to Kv3.2 function, and that phosphorylation of this K^+ channel alters a fundamental property of inhibitory interneurons, provides a major insight into histamine receptor regulation of hippocampal function.

Kv3.2 protein was expressed in all parvalbumin-containing interneurons[34], a population of cells that also express Kv3.1b (ref. 21), suggesting that, similar to cortical neurons[28], native channels within some hippocampus interneurons may be formed by combination of these two subunits. The expression of Kv3 subunits in inhibitory interneurons and their absence in principal cells has identified these subunits as important determinants of the fast-spiking phenotype[23–27,30]. In the hippocampus, parvalbumin-immunoreactive interneurons and somatostatin-containing cells in the stratum oriens–alveus and hilus have long been recognized as being fast spiking[19]. Our observation that Kv3.2 was expressed in fast-spiking hippocampal interneurons and that H_2 receptor modulation of Kv3.2 influenced the upper limit for fast spiking supports and furthers these initial observations. Although we unequivocally demonstrate a role for Kv3.2 in determining fast spiking, we cannot rule out a role for other potassium currents, including Ca^{2+}-dependent potassium conductances[41]. However, in the present experiments, this conductance would seem to have only a minor role in fast spiking, as high-frequency action potential firing was observed when 5–10 mM BAPTA was included in the recording pipette[27].

The reduction in the upper limit of firing frequency by H_2 receptor-dependent PKA phosphorylation of Kv3.2 was associ-

ated with prolonged action potential duration and decreased maximal spike amplitude, which was most obvious in spikes occurring late in the train. This is consistent with the suggestion that Kv3-containing channels enable sustained high-frequency firing by facilitating the recovery of Na^+ channels (and possibly transient I_A channels) from inactivation, and by minimizing the duration of the afterhyperpolarization[27]. PKA modulation of the action potential waveform was absent in interneurons from mice lacking Kv3.2, suggesting that in wild-type animals this occurred as a direct consequence of the reduction of Kv3.2 currents and not by modulation of Na^+ channels[42].

The high degree of divergence of hippocampal interneurons makes them suitable candidates for controlling the activity of large populations of principal neurons[37,43]. Interneuron activity is phase-locked to sharp waves in both CA1 and CA3 subfields[43]. Consequently they are thought to be involved in maintenance of high-frequency oscillations as constituents of a network in which they are synaptically connected with principal cells[19,38,44–47]. Here we showed that PKA activators reduced, but did not abolish, population events in the frequency range 70–300 Hz, an effect absent in Kv3.2 mutant mice. PKA modulation was blocked in wild-type animals by antagonists of $GABA_A$ receptors, confirming a role for GABAergic transmission in the modulation of spontaneous oscillations. Although spontaneous high-frequency oscillation events were reduced in the Kv3.2 mutant mouse, activity in the frequency range 70–300 Hz was still observed. These results are compatible with a model in which high-frequency oscillations are generated independently and propagated by an ensemble of electrically coupled principal cells[39], but are modulated by Kv3.2-containing interneurons. Such an ensemble would activate a pyramidal cell–interneuron network that would, in turn, enhance the amplitude or frequency of oscillations and increase the size of the coherent field of synchronously firing pyramidal cells involved in the oscillations, consistent with a proposed model[48].

In conclusion, these data demonstrate that Kv3.2 expressed in fast-spiking interneurons is a target for H_2 receptor-dependent PKA modulation. This mechanism for histamine receptor activation would act to trigger PKA phosphorylation of Kv3.2 to tune the firing frequency within specific populations of interneurons, which in turn would directly influence the ability of the hippocampal network to generate coherent high-frequency oscillations.

Methods

Hippocampal slices. Wild-type and Kv3.2 mutant [Lau, D. H. P. *et al*., *Somat. Motor Res.* 16, 182 (1999)] C57/BL6 mice (20–60 days old) were anesthetized by isoflurane volatile inhalation and decapitated according to NIH guidelines. Coronal hippocampal slices (250–300 μm) were prepared in a solution of 130 mM NaCl, 3.5 mM KCl, 24 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 0.5 mM $CaCl_2$, 3.0 mM $MgCl_2$ and 10 mM glucose (95% O_2, 5%CO_2). Slices were superfused with a solution of the same composition, with the addition of 1.5 mM $CaCl_2$ and 1.5 mM $MgCl_2$. When outward potassium currents were studied, all solutions contained the Na^+-channel blocker tetrodotoxin (TTX, 0.3 μM). Glass pipettes of 2–3 MΩ were pulled from thin-walled borosilicate glass (TW150F, WPI, Sarasota, Florida) and filled with 130 mM KGlu, 10 mM HEPES, 5–10 mM BAPTA, 0.2 mM ATP.Na, 0.3 mM GTP.Na, 1.0 mM $MgCl_2$ and 0.5% biocytin. Recordings were made from cells located in the subgranular layer or hilus and subsequently identified based on anatomy as inhibitory interneurons.

The membrane-permeable cAMP-analogs 8-Br-cAMP or db-cAMP and the phosphodiesterase inhibitor iso-butyl-methyl-xanthine (IBMX; termed PKA activators throughout the text) were dissolved immediately before bath application at a concentration of 2 mM and 100 μM, respectively.

Electrophysiology. Patch-clamp and extracellular recordings were made at room temperature using an Axopatch-1D amplifier with the pClamp acquisition software (Axon Instruments, Foster City, California). To analyze spike frequency, we delivered a 500-ms train of fixed-current-amplitude pulses to the cell. The amplitude and duration of the pulse was adjusted (in the ranges of 0.5–2 nA, 0.4–1.0 ms) to determine the smallest pulse required to reliably evoked action potentials on every pulse at a starting frequency of 10 Hz. Pulse trains of increasing frequency were then delivered at 0.1 Hz. The pulse train frequency that failed to elicit a spike on every pulse was termed the cut-off frequency.

Mean values ± s.e.m. are reported throughout the manuscript. The paired Student's *t* test was used to assess statistical significance.

For voltage-clamp experiments, cells were voltage clamped at –70 mV, and outward currents were activated by test potentials up to +60 mV (10–20 mV increments, 100–500 ms duration). To inactivate transient currents, test pulses were preceded by a 50-ms pulse to –50 mV. Recording pipettes contained the calcium chelator BAPTA (5–10 mM) to minimize the contribution of Ca^{2+}-activated outward currents. Series resistances (in the range of 10–25 MΩ) were monitored continuously. Currents were measured at test potentials of +60 mV unless stated otherwise in the text.

For extracellular recordings, electrodes were filled with oxygenated bath solution and positioned within the CA3 stratum pyramidale. All recordings were made in the presence of 6-7dinitroquinoxaline-2,3(1H)-dione (DNQX, 10 μM) and D-2-amino-5-phosphonovaleric acid (D-APV, 25 μM) to block AMPA and NMDA receptor-mediated excitatory synaptic transmission, respectively. Extracellular population spike data were extracted using the Mini Analysis program (Jaejin Software, Leonia, New Jersey).

Immunohistochemistry. Sagittal sections (30 μm, postnatal day 51) were incubated in rabbit anti-Kv3.2 (1:100)[22]. Biotinylated goat anti-rabbit secondary antibody (1:200, Vector Labs, Burlingame, California) was incubated overnight. Cells were visualized using an Elite-ABC kit (Vector Labs). Double immunofluorescence was done using a TSA signal amplification kit (NEN Life Sciences, Boston, Massachusetts). Sections were incubated with polyclonal rabbit anti-Kv3.2 (1:200) and one of four monoclonal antibodies: mouse anti-calbindin (1:200, Sigma, St Louis, Missouri), mouse anti-calretinin (1:50, Chemicon, Temicula, California), mouse anti-parvalbumin (1:1000, Sigma) or mouse anti-somatostatin (1:10, Biomeda, Foster City, California). Secondary antibodies (goat anti-rabbit, biotin conjugated and goat anti-mouse, FITC-conjugated) were diluted 1:200 in blocking buffer and incubated simultaneously overnight.

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