Interplay Between Activation of GIRK Current and Deactivation of $I_h$
Modifies Temporal Integration of Excitatory Input in CA1 Pyramidal Cells

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Takigawa, Tomoko and Christian Alzheimer. Interplay between activation of GIRK current and deactivation of $I_h$ modifies temporal integration of excitatory input in CA1 pyramidal cells. J Neurophysiol 89: 2238–2244, 2003. First published November 27, 2002; 10.1152/jn.00957.2002. Trains of brief iontophoretic glutamate pulses were delivered onto the apical dendrites of CA1 pyramidal cells at variable frequencies (3–100 Hz) to examine how the activation of a G protein–activated, inwardly rectifying K+ (GIRK) conductance alters the postsynaptic processing of repetitive excitatory input. Application of the GIRK channel agonist baclofen (20 μM) reduced the amplitude of individual glutamate-evoked postsynaptic potentials (GPSPs) and attenuated summation of GPSPs so that higher stimulus intensities were required to fire the cell. Notably, GIRK channel activation not only decreased GPSPs, but also suppressed the subsequent afterhyperpolarization (AHP), which arises from a transient deactivation of the hyperpolarization-activated cation conductances. Voltage-clamp recordings ruled out a direct modulatory action of baclofen on $I_h$. GIRK channel activation alone accounts for AHP suppression, firstly because, with smaller GPSP amplitudes, fewer $I_h$ channels are deactivated, resulting in a diminished AHP, and secondly because, owing to its progressive increase in the hyperpolarizing direction, the GIRK conductance shunts a large portion of the remaining AHP. We provide experimental evidence that the suppression of the $I_h$-dependent AHP by GIRK channel activation bears particular significance on the processing of repetitive excitatory inputs at frequencies at which the deactivation kinetics of $I_h$ exert a prominent depressing effect. In functional terms, activation of GIRK current not only produces a time-independent mitigation of incoming excitatory input, which results directly from the opening of an instantaneous K+ conductance, but might also cause a time-dependent redistribution of synaptic weight within a stimulus train, which we link to an interplay with the deactivation of $I_h$.

INTRODUCTION

At excitatory synapses of the CNS, the transfer of presynaptic action potentials to the postsynaptic neuron can be profoundly influenced by both presynaptic and postsynaptic mechanisms. For example, repetitive firing of presynaptic fibers has been shown to evoke a sequence of either gradually increasing or decreasing excitatory postsynaptic potentials (EPSPs) in the target cell, which is usually referred to as facilitation or depression. Although axons from a single presynaptic neuron might produce facilitating and depressing postsynaptic responses in different cells (Markram et al. 1998; Reyes et al. 1998), demonstrating the target cell specificity of these phenomena, synaptic facilitation and depression have been largely attributed to the presynaptic site, reflecting an increase or decrease, respectively, in the probability of transmitter release (Thomson 2000). In addition to such presynaptically mediated actions, the passive and, more so, the active electrical properties endow the postsynaptic neuron with enormous computational power to control and modify the integration of EPSPs in its somatodendritic compartments. With the advent of patch-clamp recordings from dendrites of various types of CNS neurons, it became evident over the past several years that dendrites were not only capable of generating Na+ and Ca2+ spikes, but that they also possessed the complete repertoire of voltage-dependent ion currents that are activated in the subthreshold range and thus bear particular significance on the integration of phasic and tonic input (Schwindt and Crill 1997; reviewed in Magee 2000; Reyes 2001). Underscoring the functional significance of these currents for the processing of synaptic signals, most of them are present at higher densities in the dendrites than in the soma when investigated in pyramidal neurons of the neocortex and hippocampus. This holds for the transient K+ (A-type) current (Hoffman et al. 1997), for the low voltage–activates (T-type) Ca2+ current (Christie et al. 1996), and for the hyperpolarization-activated inward rectifier current ($I_h$) (Magee 1998). We have recently demonstrated that a similar somatodendritic gradient of channel density also exists for G protein–activated, inwardly rectifying K+ (GIRK) channels (Takigawa and Alzheimer 1999). These channels are recruited by a large spectrum of neurotransmitters and modulators, including serotonin, noradrenaline, GABA (acting via GABA_B receptors), acetylcholine, adenosine, somatostatin, and opioid peptides (Dascal 1997; Yamada et al. 1998). Here we used the GABA_B agonist baclofen to investigate the effects of GIRK current activation on the temporal processing of EPSPs that were elicited by trains with variable interstimulus intervals. Since GIRK channel agonists also exert presynaptic effects on transmitter release, which are apparently not related to GIRK channel activation (Lüscher et al. 1997), we used very short iontophoretic glutamate pulses delivered to the apical dendrite of CA1 hippocampal pyramidal cells to isolate the postsynaptic GIRK current-mediated effect. We report here that GIRK channel activation has two different effects on the EPSP-like voltage deviations: one results from a shunting inhibition of EPSPs due to the opening of GIRK channels; the

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other results from an indirect modulatory effect of the GIRK conductance on an afterhyperpolarization (AHP)-like undershoot of the membrane potential that is mediated by \( I_h \) (Pape 1996; Storm 1989). Whereas the former equally affects all EPSPs in a train, the latter redistributes the synaptic weight between the incoming excitatory inputs.

METHODS

Using standard procedures, transverse hippocampal slices, 300 \( \mu \text{m} \) thick, were prepared from the brain of Wistar rats (2–3 wk old), which were deeply anesthetized with a ketamine–xylazine solution (1 ml/kg K-113, RBI/Sigma, Deisenhofen, Germany) prior to decapitation. All experiments were carried out according to the guidelines and with the approval of the Animal Care Committee at the University of Munich. After dissection, slices were incubated in warmed (35°C) artificial cerebrospinal fluid (ACSF) for 25 min and then maintained at room temperature (21–24°C) in the same solution. ACSF was constantly gassed with 95% O₂–5% CO₂ and had the following composition (in mM): 125 NaCl, 3 KCl, 2 CaCl₂, 2 MgCl₂, 1.25 NaH₂PO₄, 25 NaHCO₃, and 10 d-glucose (pH 7.4). For electrophysiological measurements, individual slices were transferred to the recording chamber that was mounted on the stage of an upright microscope (Olympus BX50WI). Dodt infrared gradient contrast in conjunction with a contrast-enhanced CCD camera (Hamamatsu) served to identify somata and dendritic processes of pyramidal cells in the hippocampal CA1 region. During experiments, slices were kept submerged in ACSF that was constantly exchanged by means of a gravity-driven superfusion system (flow rate 2–3 ml/min).

EPSP-like waveforms were evoked using short iontophoretic pulses of Na-glutamate (250 mM), which was locally applied onto the apical dendrite of CA1 pyramidal neurons (100–150 \( \mu \text{m} \) from the soma) by means of a new iontophoretic device with fast capacity compensation (MVCS-02C, npi, Tamm, Germany). In most experiments, iontophoretic pulses 0.5- to 1-ms long were delivered as trains of four stimuli at 3–100 Hz. Illustrated membrane potential responses to glutamate are averages of four consecutive sweeps. To functionally isolate the recorded neuron from synaptic input arising in neighboring neurons coactivated by glutamate pulses, experiments were conducted in the presence of TTX (1 \( \mu \text{M} \)) unless otherwise stated. The N-methyl-D-aspartate (NMDA) component of EPSPs was suppressed with \( \text{N}^\text{(-)-2-amino-5-phosphonopentanoic acid (NAPAP)} \) (20 \( \mu \text{M} \)) to eliminate nonlinearities due to NMDA receptor activation. Spontaneous inhibitory postsynaptic potentials (IPSPs) were abolished with bicuculline (10 \( \mu \text{M} \)). Using the same pharmacological cocktail, EPSP-like waveforms were also evoked by trains of triangular current pulses that were delivered through the recording pipette. Electrophysiological signals obtained in the whole-cell configuration of the patchclamp technique were recorded, amplified, and analyzed with the use of an Axopatch 200B amplifier (Axon Instruments) in conjunction with a Digidata 1200 interface and pClamp 6 software (Axon Instruments). All recordings were made at room temperature. Recording pipettes were filled with (in nM) 130 KMeSO₄, 10 KCl, 2 MgCl₂, 10 EGTA, 10 HEPES, 2 Na₂-ATP, and 2 Na-GTP (pH 7.25–7.30) and had a resistance of about 5 MΩ. In the whole cell configuration, series resistance was about 12 MΩ, which was, in voltage-clamp experiments, compensated by 75–85%. Voltage readings were corrected for experimentally determined liquid junction potentials (10 mV). TTX was purchased from Alomone Labs (Jerusalem, Israel), all other substances were from Sigma (Deisenhofen, Germany).

Data are presented as means ± SE. Statistical analysis (t-test, significance set at \( P < 0.05 \)) was performed with the use of Origin (4.1) software.

RESULTS

Effects of GIRK conductance on summation of excitatory inputs

After pharmacological suppression of GABA\(_A\) and NMDA receptors, very brief iontophoretic pulses of glutamate delivered onto the apical dendrite of CA1 pyramidal cells elicited pure AMPA receptor–mediated EPSP-like voltage deviations, which we refer to as glutamate-evoked postsynaptic potentials (GPSPs). To examine the effect of GIRK channel activation on the summation of GPSPs, we applied four identical pulses of glutamate at a membrane potential of −80 mV and varied the interstimulus interval between 300 and 10 ms (\( n = 4 \)). As illustrated in Fig. 1, the progressively shorter intervals between the glutamate pulses led to an increasing summation of GPSPs, which eventually reached the threshold for the firing of an action potential (Fig. 1A–D, left). When we repeated this stimulation protocol in the presence of the GIRK channel activator baclofen (20 \( \mu \text{M} \)), we observed a substantial attenuation of individual GPSPs (Fig. 1, A and B, middle), which consequently failed to sum up to the firing threshold (Fig. 1, C and D, middle). This inhibitory effect of baclofen was fully reversible in control solution (data not shown), as demonstrated previously (Takigawa and Alzheimer 2002). That this effect of baclofen was indeed mediated by the activation of GIRK channels was demonstrated by the reversal of the drug-induced inhibition of GPSPs by Ba\(^{2+}\), which was applied at a concentration (200 \( \mu \text{M} \)) that suppresses selectively inwardly rectifying K\(^+\) channels. As during the application of baclofen, DC injection through the recording pipette served to hold the membrane potential at −80 mV, thereby maintaining equal driving forces for GPSPs. Without DC injection, the neurons had a resting membrane potential (RMP) of −70.7 ± 2.0 mV under control conditions and −77.3 ± 2.6 mV in baclofen (\( n = 15 \)). It is noteworthy that Ba\(^{2+}\) not only reversed the attenuation of GPSPs by baclofen, but actually made GPSPs larger than under control conditions (Fig. 1A–C, right), so that the suprathreshold stimulation now elicited a doublet of action potentials (Fig. 1D, right). Based on previous work from our laboratory (Takigawa and Alzheimer 2002), we attribute this apparently overshooting response to Ba\(^{2+}\) to the fact that the cation not only inhibits GIRK channels, but also constitutive inward rectifier K\(^+\) channels, which exert a tonic control over GPSPs.

Effects of GIRK conductance on AHP

Although it appears that the predominant effect of baclofen is a reduction of the size of GPSPs so that a stronger input is required to fire the neuron, closer examination revealed a second effect, namely the suppression of the AHP-like undershoot that typically followed sub- and suprathreshold GPSPs (Fig. 1, C and D, arrows). The suppression of AHP was also observed, when the membrane potential was depolarized by DC injection to its control value during baclofen application (Fig. 1D, inset of middle panel), ruling out that this effect was a peculiarity of more negative membrane potentials. To elucidate the ionic mechanism underlying the inhibition of AHPs by baclofen, we performed experiments in the presence of TTX (1 \( \mu \text{M} \)), which excludes any interference by slow IPSPs. As illustrated in Fig. 2A, baclofen continued to abrogate AHPs in
a TTX-containing bath solution, indicating that this effect is intrinsic to the postsynaptic neuron. Under our recording conditions, the inhibitory action of baclofen was completed within 15 min. The reversibility of this effect has been shown in a previous study from our laboratory (Takigawa and Alzheimer 2002). Because the GPSPs did not depolarize the membrane potential into a voltage range in which appreciable Ca$^{2+}$ influx through voltage-dependent Ca$^{2+}$ channels would be expected, it seems unlikely that the AHP is predominantly mediated by a Ca$^{2+}$-activated K$^+$ conductance. Rather, the AHP might result from a depolarization-induced deactivation of a standing $I_h$, which gives rise to a transient outward current. In support of this notion, we found that ZD7288 (20 μM), a selective inhibitor of $I_h$ (Gasparini and DiFrancesco 1997; Harris and Constanti 1995), not only enhanced the size of GPSPs, but also completely suppressed the subsequent AHP (Fig. 2B). The effect of ZD7288 was completed within <20 min. Consistent with previous work in brain slices, the action of ZD7288 was not reversible during wash out (Gasparini and DiFrancesco 1997; Harris and Constanti 1995). In this as well as in some of the subsequent experiments we evoked relatively large GPSPs from hyperpolarized membrane potentials to promote deactivation of $I_h$ and thus enhance AHP amplitude. Under this recording condition the modulation of AHP by GIRK channel activation and its functional implications should become particularly prominent.

**FIG. 1.** Effects of GIRK channel activation on trains of excitatory inputs. $A$–$D$: repetitive iontophoretic glutamate pulses to the apical dendrite of CA1 pyramidal cells delivered in the presence of the GABA$_A$ receptor antagonist bicuculline (10 μM) and the N-methyl-d-aspartate receptor antagonist, APV (20 μM), served to mimic trains of excitatory inputs arriving at frequencies between 3 and 100 Hz. The GIRK channel agonist, baclofen (20 μM), reduced the size of individual glutamate-evoked post synaptic potentials (GPSPs, $A$ and $B$), attenuated the summation of GPSPs ($C$), and abrogated action potential firing by a stimulus that was suprathreshold under control conditions ($D$). Note that baclofen also suppressed the afterhyperpolarization (AHP) that followed GPSPs as indicated by arrows in $C$ and $D$. The effects of baclofen on GPSPs and AHPs were restored when Ba$^{2+}$ (200 μM) was added to the baclofen-containing solution ($A$–$D$, right). The "overshooting" responses to Ba$^{2+}$ are most likely attributable to an additional inhibition of constitutive inward rectifier K$^+$ channels. Throughout the experiment (all data from same neuron), the membrane potential was manually held at −80 mV by DC injection as appropriate. Inset in $D$ illustrates effect of baclofen (red trace) on synaptic responses evoked at −70 mV, which is close to the cell’s resting membrane potential (−68 mV). Arrow indicates loss of AHP during baclofen application.

**FIG. 2.** Baclofen reduces $I_h$-dependent AHP. $A$: to quantify the effect of baclofen (20 μM) on AHPs, GPSPs were evoked in the absence and presence of the GIRK channel agonist at different time points of drug application, and the reduction of AHPs was normalized to its peak amplitude under control conditions (arrow). Note that the inhibitory action of baclofen was complete within 15 min. Dashed line indicates membrane potential (−80 mV), which was taken as reference for measurement of AHP amplitudes. Inset: data from $n = 21$ recordings, recorded at a membrane potential of −80 mV ($n = 13$) or −100 mV ($n = 8$). Data were lumped together, since the relative AHP reduction did not differ significantly between the 2 groups. $B$: the $I_h$ inhibitor ZD7288 (20 μM) consistently enhanced GPSP amplitude and completely abrogated AHPs ($n = 4$, neurons were held at −110 mV). Effect of ZD7288 was complete within <20 min of drug washin. **$P < 0.01$.**
Although we used iontophoretic application of brief glutamate pulses in the presence of TTX to isolate the effects of baclofen on the somatodendritic processing of EPSPs, the interpretation of our results might be possibly confounded by effects of baclofen and glutamate on presynaptic glutamate release and/or on the desensitization properties of postsynaptic glutamate receptors. To demonstrate that the reduction of AHPs in baclofen resulted indeed from an interplay between baclofen and glutamate, we performed a series of experiments in which we used trains of triangular current injections through the whole cell pipette to mimic trains of incoming excitatory input. As shown in Fig. 3A, the two characteristic effects of baclofen, namely reduction of depolarizing inputs and decline of subsequent undershoot, were fully reproduced under these recording conditions \( (n = 6) \). To determine to what extent the baclofen-induced reduction of GPSP amplitude is responsible for the decrease in AHP, we enhanced, in the continued presence of baclofen, the strength of the iontophoretic glutamate pulse until we obtained GPSP waveforms that closely matched those of control. As illustrated in Fig. 3B, this protocol restored only partially the size of the control AHP, indicating that, in baclofen, the decreased amplitude of the preceding depolarization is not the sole cause of the AHP suppression. This experiment still leaves open the question of whether baclofen directly affec
ts \( I_h \) or whether the activation of the GIRK conductance is sufficient to account for a concomitant reduction of AHPs. We used two experimental paradigms to address this issue. In the first set of experiments, we examined whether the baclofen-induced suppression of AHPs is sensitive to the GIRK channel blocker \( \text{Ba}^{2+} \), which does not inhibit \( I_h \) in the micromolar range. If added to a baclofen-containing bath solution, \( \text{Ba}^{2+} \) indeed produced a complete reversal of the decrease in AHP amplitude (Fig. 3C and D), suggesting that baclofen does not exert an independent effect on \( I_h \). Vice versa, preincubation with \( \text{Ba}^{2+} \) abrogated any effect of baclofen on GPSP amplitude and subsequent AHP (Fig. 3E).

It might be still argued, however, that, owing to the stronger depolarization attained by GPSPs in \( \text{Ba}^{2+} \), more \( I_h \) is deactivated, thereby giving rise to a larger AHP. To dispel any concerns regarding the ionic mechanism of AHP suppression, we performed, as the second set of experiments, voltage-clamp recordings and examined the action of baclofen on the \( I-V \) relationship of CA1 pyramidal cells in the presence of \( \text{Ba}^{2+} \) \( (200 \mu M, n = 4) \). Figure 4A depicts the current responses to a hyperpolarizing voltage step from \(-80 \) to \(-150 \) mV. \( \text{Ba}^{2+} \) reduced the apparently instantaneous inward rectification that is attributed to constitutive inward rectifier \( K^+ \) current, but did not affect the gradually developing inward current that results from the slow activation of \( I_h \). If examined during inhibition of GIRK channels, i.e., in the presence of \( \text{Ba}^{2+} \), baclofen did not alter the hyperpolarizing current response (Fig. 4A) nor the neuronal \( I-V \) relationship (Fig. 4B), indicating that the suppression of AHPs in current-clamp experiments is secondary to the activation of the GIRK conductance.

**Effects of GIRK conductance on depressing response to repetitive excitatory input**

What is the functional significance of the decrease of AHPs that accompanies the opening of GIRK channels? We speculated that this effect should be particularly important for repetitive excitatory inputs whose interstimulus intervals allow for substantial deactivation of \( I_h \) during the train. To mimic this situation, we applied a 14-Hz train of four identical glutamate pulses. Owing to the gradual deactivation of \( I_h \) and the resulting buildup of the AHP, this protocol gave rise to a sequence of progressively declining GPSPs (Fig. 5A, black trace). In the presence of baclofen, the amplitude of GPSPs was reduced as expected for a \( K^+ \) channel activator (Fig. 5A, red trace). It is noteworthy, however, that a second, time-dependent effect occurred: the extent of inhibition of GPSPs within a train became substantially smaller from stimulus to stimulus. This
The effect of baclofen was also observed if we used brief triangular somatic current injections in lieu of iontophoretic glutamate pulses to mimic trains of depolarizing inputs. Again, baclofen not only reduced each depolarizing voltage deviation, but also equalized the response (Fig. 5B, n = 4). To quantify the intriguing action of baclofen, we compared the ratio between peak amplitudes of the first or the second GPSP and the last (fourth) GPSP (Fig. 5C) and calculated the relative reduction by baclofen for each GPSP in a train (Fig. 5D). These data indicate that, for later GPSPs, the inhibitory potency of baclofen becomes significantly smaller compared with the first GPSP. In other words, baclofen transformed a strongly depressing response into a mildly depressing one.

The pivotal role of the I\textsubscript{h}-associated AHP for the depressing response was corroborated in the experiments depicted in Figs. 6 and 7. First, we used different stimulus intensities to evoke GPSPs of various size. As shown in Fig. 6, a stepwise increase of the iontophoretic current from 200 to 300 nA not only increased the amplitude of the GPSPs but also introduced a time-dependent effect, so that a response that displayed no time-dependent change in amplitude at 200 nA was transformed into a depressing response at higher stimulus intensities. The gradual enhancement of AHPs with each increase in stimulus strength argues strongly in favor of I\textsubscript{h} deactivation being the underlying ionic mechanism of this depressing response. In fact, inhibition of I\textsubscript{h} with ZD7288 (20 μM) completely abrogated the progressive depression of GPSPs in this stimulus paradigm (Fig. 7A, n = 3). Under this condition, baclofen was no longer capable of altering the relative weight of GPSPs within a stimulus train but exerted an equally inhibitory action on all GPSPs (Fig. 7B, n = 4).

**DISCUSSION**

Using brief iontophoretic glutamate pulses (in lieu of afferent stimulation), we found that activation of GIRK channels exerts two different effects on the postsynaptic processing of repetitive excitatory input. First, the opening of GIRK channels produces an increase in membrane conductance, which shunts the excitatory synaptic current in part, thereby reducing the time-dependent change in amplitude at 200 nA was transformed into a depressing response at higher stimulus intensities. The gradual enhancement of AHPs with each increase in stimulus strength argues strongly in favor of I\textsubscript{h} deactivation being the underlying ionic mechanism of this depressing response. In fact, inhibition of I\textsubscript{h} with ZD7288 (20 μM) completely abrogated the progressive depression of GPSPs in this stimulus paradigm (Fig. 7A, n = 3). Under this condition, baclofen was no longer capable of altering the relative weight of GPSPs within a stimulus train but exerted an equally inhibitory action on all GPSPs (Fig. 7B, n = 4).

**FIG. 4.** Baclofen does not alter I\textsubscript{h}. A: current response to a hyperpolarizing voltage step from −80 to −150 mV. Ba\textsuperscript{2+} (200 μM) reduced a time-independent component of hyperpolarizing inward rectification mediated by inward rectifier K\textsuperscript{+} currents. If applied in the presence of Ba\textsuperscript{2+}, baclofen (20 μM) failed to affect the slow inward relaxation generated by the gradual activation of I\textsubscript{h}. B: the I-V relationship was constructed by plotting the amplitude of the current response measured at the end of the step as a function of the command voltage.

**FIG. 5.** Effect of baclofen on depressing response. A: four glutamate pulses at 14 Hz evoked a depressing response. This time-dependent characteristic was significantly attenuated in the presence of baclofen (20 μM). Again, baclofen also caused a substantial reduction of the AHP following the stimulus train (arrow). Dashed line indicates −90 mV. B: injection of brief triangular current pulses through recording pipette served to mimic depressing response (membrane potential ∼−90 mV). Arrow indicates decline of AHP during baclofen application (red trace). Inset: ratio between amplitude of first and fourth depolarization under control conditions (black column) and in baclofen (red column) (n = 4). C: to quantify the action of baclofen on the depressing response evoked by glutamate pulses, we calculated the ratio between the amplitude of the first and the fourth GPSP (left columns) or between the second and the fourth GPSP (right columns) in the absence (black) or presence (red) of baclofen (n = 4). D: columns indicate relative reduction by baclofen for the 4 consecutive GPSPs (n = 4). *P < 0.05, **P < 0.01.
Because the activation and deactivation kinetics of membrane potential back to its original value, thereby terminating when glutamate pulses were delivered at frequencies of 10 Hz. This trajectory displays a transient undershoot in the hyperpolarizing direction resulting from the nonlinear properties of the GIRK current and the deactivation of \( I_h \). This notion was corroborated by findings from substantia nigra neurons (Watts et al. 1996; but see Jiang et al. 1993).

Under control conditions, incoming excitatory signals deactivate a standing \( I_h \) by virtue of their depolarizing action on the membrane potential, producing an effectively outward current during the input. On repolarization, the membrane potential trajectory displays a transient undershoot in the hyperpolarizing direction, before the slow activation of \( I_h \) brings the membrane potential back to its original value, thereby terminating the AHP (Maccacferri et al. 1993; Pape 1996; Spain et al. 1987).

Because the activation and deactivation kinetics of \( I_h \) are much slower than those of GIRK current, which are almost instantaneous, activation of the latter will not per se introduce a time-dependent component in the processing of synaptic input trains.

In elegant studies of pyramidal cells of rat hippocampus and neocortex, Magee (1999) and Williams and Stuart (2000) demonstrated that the kinetics of \( I_h \), in particular of dendritic \( I_h \), are site independent of EPSP time course and temporal summation. Here we show that slow deactivation of \( I_h \) not only prevents summation of EPSPs (cf. Magee 1999; Williams and Stuart 2000) but might also account for a depressing response when glutamate pulses were delivered at frequencies of 10–14 Hz. This depressing response and the subsequent AHP were largely attenuated by baclofen. Owing to the shunting inhibition of the baclofen-induced GIRK conductance, the first GPSP in a train produced less membrane depolarization, which, in turn, reduced the amount of deactivation of \( I_h \). With less \( I_h \) deactivating during the train, less effective outward current becomes available to dampen the subsequent GPSPs. In addition, the progressive increase in membrane conductance in the hyperpolarizing direction resulting from the nonlinear properties of the GIRK current provides an effective mechanism to further mitigate the AHP.

The following observations link the apparently time-dependent effect of GIRK current activation on temporal processing to its (indirect) effect on the \( I_h \)-associated AHP. 1) The depressing response and the subsequent AHP were abrogated in the presence of the \( I_h \) inhibitor ZD7288. 2) After suppression of \( I_h \), baclofen failed to exert a time-dependent effect on repetitive excitatory input. 3) Inhibition of GIRK channels by Ba\(^{2+}\) reversed the baclofen-induced decrease of AHPs. 4) In voltage clamp experiments, baclofen failed to influence \( I_h \) during suppression of GIRK channels, consistent with previous findings from substantia nigra neurons (Watts et al. 1996; but see Jiang et al. 1993).

Depending on RMP and input frequency, time-dependent \( (I_h) \) and time-independent hyperpolarizing inward rectification (GIRK current) might have profound influence on the temporal weighing of repetitive excitatory input. Notably, both types of inward rectifiers are targets of a broad variety of neurotransmitters and -modulators, which use changes in intracellular Ca\(^{2+}\) and a membrane-delimited G protein–dependent pathway to control the gating of \( I_h \) and GIRK current, respectively (Andrade et al. 1986; Pedarzani and Storm 1995). From this, a scenario emerges by which the processing of incoming signals occurs in a highly dynamic fashion so that the upregulation of a single conductance, as demonstrated here for GIRK current, is sufficient to substantially alter the computational properties of the neuron. The present experiments do not allow us to infer at which location along the somatodendritic axis the GIRK channel activation has the strongest effect. It is well established, however, that the density of both GIRK channels and H channels is severalfold higher in the apical dendrite than in the soma (see INTRODUCTION). It is hence easily conceivable that both channels are expressed in close proximity to each other, allowing for an intimate biophysical interaction. It is not known yet whether the two channel types are colocalized on dendrites or dendritic processes such as spines, where their interplay would have the most immediate impact on incoming

**FIG. 6.** Increase of stimulus intensity induces depressing response. A train of four iontophoretic glutamate pulses (14 Hz) was delivered at stimulus intensities of 200, 250, and 300 nA. Note the gradual transformation of a regular into a depressing response, which coincides with the gradual increase in AHP amplitude (arrow). Dashed line indicates \(-90 \text{ mV}.\) Inset: data from \( n = 4 \) experiments. Like-colored columns indicate ratio between first and fourth GPSPs for the 3 recording conditions. **\( P < 0.01.\)**

**FIG. 7.** Inhibition of \( I_h \) abrogates depressing response and time-dependent action of baclofen. A: application of ZD7288 (20 \( \mu \text{M} \)) enhanced GPSP amplitudes and abrogated the depressing response to this 10-Hz stimulation. Note the concomitant inhibition of the AHP. B: during inhibition of \( I_h \), baclofen (20 \( \mu \text{M} \)) produced an equal reduction of all 4 GPSPs. Dashed line indicates \(-110 \text{ mV}.\) Recordings in A and B are from the same neuron.
excitatory input. Although the subcellular (co)localization of GIRK and H channels on dendrites remains to be determined, we would predict that the dendrites are the site where signal processing is most sensitive to the interaction between GIRK current and \( I_h \).

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