Short-Term Desensitization of G-Protein-Activated, Inwardly Rectifying K\(^{+}\) (GIRK) Currents in Pyramidal Neurons of Rat Neocortex

Thomas Sickmann\(^1\) and Christian Alzheimer\(^{1,2}\)

\(^{1}\)Department of Physiology, University of Munich, D-80336 Munich; and \(^{2}\)Department of Physiology, University of Kiel, D-24098 Kiel, Germany

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INTRODUCTION

G-protein-activated inwardly rectifying K\(^{+}\) (GIRK) channels are recruited by an impressively broad spectrum of neurotransmitters and neuromodulators including serotonin, dopamine, norepinephrine, acetylcholine, \(\gamma\)-amino-butyric acid (GABA; acting on GABA\(_B\) receptors), adenosine, opioids, and somatostatin (Dascal 1997; Yamada et al. 1998). This high degree of convergence of various neuroactive substances onto the same effector system makes GIRK channels a central inhibitory mechanism in the control of neuronal excitability. Owing to their gating through a membrane-delimited pathway, activation of GIRK channels occurs rapidly after agonist binding to the respective G-protein-coupled receptor subtype. The time course of activation and deactivation of neuronal GIRK currents during brief agonist pulses have been studied in detail using acutely isolated hippocampal neurons in combination with a rapid drug-application system. This study yielded time constants of activation which ranged between 0.25 and 0.6 s depending on the agonist concentration (Sodickson and Bean 1996). By contrast, data on the GIRK response during longer agonist application (~2–10 min) in CNS neurons are sparse. To our knowledge, short-term desensitization of GIRK currents in the CNS has been studied so far only in locus ceruleus neurons in the context of waning opioid responses (Blanchet and Lüscher 2002; Fiorillo and Williams 1996; Harris and Williams 1991). However, the issue of how much of the initial GIRK current response persists during prolonged agonist exposure is of more general importance because, in addition to opioids, many other GIRK channel activators are released in a prolonged or even tonic rather than in a pulsatile fashion. This holds most prominently for adenosine whose concentration in the extracellular space is sufficient to exert a purinergic inhibitory tonus, but the release patterns of many other GIRK channel activators also predict that they act on neurons for prolonged periods of time.

In the present study, we investigated the kinetics and the putative mechanism(s) of the decline of the current response that occurred within 5 min of drug application. This phenomenon is commonly referred to as short-term desensitization and can be homologous or heterologous. Homologous desensitization describes a form of attenuated responsiveness that is strictly agonist-dependent, turning off only activated receptors. By contrast, during heterologous desensitization the fading of receptor signaling affects several (or all) of the receptors that are coupled to the same down-stream effector system. To separate between these different forms of desensitization, we examined and compared GIRK current responses to adenosine, serotonin, and the GABA\(_B\) receptor agonist, baclofen, using whole cell recordings from acutely isolated rat neocortical pyramidal cells. We report here that most neurons display a biphasic type of short-term desensitization, with time constants for the fast and slow desensitization in the range of 8 and 120 s, respectively. Note that we use the terms “fast” and “slow” (sometimes also called “intermediate”) desensitization to designate two kinetically and presumably mechanistically distinct forms of short-term desensitization. We do not address a form of very slow desensitization occurring during much longer agonist exposure (12–96 h) (Wetherington and Lambert 2002) that is often referred to as receptor downregulation (Bunemann et
al. 1996) and might involve receptor internalization, degradation, and inhibition of receptor synthesis (Bunemann et al. 1999). In our preparation, short-term desensitization of GIRK currents is primarily heterologous and is not attributable to hydrolysis of PIP2 or changes in intrinsic channel gating. Our data suggest that the hydrolysis cycle of G proteins underlies fast GIRK current desensitization. Slow desensitization is unlikely to occur at the level of the receptors or the GIRK channels but might involve an intermediate step in G-protein-dependent signaling.

METHODS

Using an established method of combined enzymatic/mechanic dissociation (Alzheimer 1994), acutely isolated pyramidal cell somata were prepared from the sensorimotor cortex of anesthetized and then decapitated rats 7–19 days old (P7–P19). After preparation, neocortical slices were incubated for 30 min in warmed (30°C) Ringer solution that was allowed to equilibrate with room temperature for another 30 min. Small pieces of slice tissue (1–2 mm²) were then incubated for 90 min at 29°C in standard bath solution (composition see following text) containing 19 U/ml papain. After washing, tissue pieces were maintained in standard bath solution at room temperature. Before each recording session, individual tissue pieces were mechanically dissociated using Pasteur pipettes of increasingly smaller bore diameter. After dissociation, the cell suspension was immediately transferred to the recording chamber, which was mounted on the stage of an inverted microscope equipped with Hoffman modulation optics. All experiments were carried out according to the guidelines and with the approval of the Animal Care Committee at the University of Munich.

Current signals from visually identified pyramidal cell somata recorded in whole cell voltage-clamp mode were sampled at 3–5 kHz and filtered at 1 kHz (-3 dB) using an Axopatch 200 amplifier in conjunction with a TL-1 interface and pClamp 6.0 software (all from Axon Instruments). All recordings were made at room temperature (21–24°C). Standard bath solution was composed of (in mM) 150 NaCl, 3 KCl, 2 CaCl₂, 2 MgCl₂, 10 HEPES, and 10 D-glucose (pH 7.4). After whole cell access was established, GIRK currents were investigated in an extracellular solution containing (in mM) 85 NaCl, 60 KCl, 2 MgCl₂, 2 CaCl₂, 5 Na-HEPES, 5 HEPES, and 10 D-glucose (pH 7.4). Patch pipettes were filled with (in mM) 135 K-glucuronate, 5 HEPES, 5 MgCl₂, 5 EGTA, 2 Na₂-ATP, and 2 Na-GTP (pH 7.25, adjusted with K-OH and Tris, final K⁺ 151). In one set of experiments, we used electrodes filled with nystatin (100 mg/l) to obtain whole cell recordings in the perforated-patch configuration. Electrode resistance in the whole cell configuration was 10–15 MΩ before series resistance compensation (75–80%). Voltage readings were corrected for liquid junction potential. If not noted otherwise, holding potential (Vₗ₀) was −70 mV. A remotely controlled, solenoid-operated Y tube system was used for rapid application of the GIRK channel agonists adenosine (100 μM), serotonin (20 μM), and baclofen (20 μM) to the cell under study. With this system, time constants of GIRK current activation ranged between 0.7 and 1.3 s (Sickmann and Alzheimer 2002).

Substances were purchased from Sigma (Deisenhofen, Germany). Data are presented as means ± SE. Data were statistically analyzed (ANOVA, significance set at P < 0.05), and histograms were constructed with the use of Excel (7.0) and Origin (4.1) software.

RESULTS

Prolonged agonist application induces different types of desensitization.

To increase current flow through GIRK channels, extracellular K⁺ was elevated to 60 mM after whole cell access was established, and GIRK channel activity was recorded as inward current at a holding potential (Vₗ₀) of −70 mV. Consistent with previous reports from our and other laboratories (Alzheimer and ten Bruggencate 1991; Bayliss et al. 1997; Okuhara and Beck 1994; Penington et al. 1993; Sodickson and Bean 1998; Takigawa and Alzheimer 1999), the agonist-induced currents were identified as GIRK currents based on their complete suppression by Ba²⁺ at low concentration (200 μM) and their inward rectification in the hyperpolarizing direction (data not shown). Demonstrating their mediation by GIRK channels, K⁺ currents evoked by adenosine, serotonin, and baclofen were essentially absent in hippocampal neurons from Kir3.2 (GIRK2) knock-out mice (Lüscher et al. 1997), and opioid-induced K⁺ currents were reduced by 80% in locus ceruleus neurons from Kir3.2/3.3 double knock-out mice (Torrecilla et al. 2002). To examine the behavior of GIRK currents during prolonged agonist exposure, we superfused neurons for 5 min with near-saturating concentrations of either baclofen (20 μM), adenosine (100 μM), or serotonin (20 μM). As shown in the current traces of Fig. 1, A–C, the neurons displayed different types of current responses under this condition that ranged from nondesensitizing to mono- and biphasic. To exclude that prolonged agonist application affected other ion currents the activation or inhibition of which might interfere with the current trajectory that we attribute to GIRK current desensitization, we examined the effects of the agonists in a bath solution containing 200 μM Ba²⁺. We have previously shown that, at this concentration, Ba²⁺ completely suppresses GIRK current responses in our preparation (Takigawa and Alzheimer 1999). Because the agonists consistently failed to induce a change in membrane current during the typical 5-min application period, if Ba²⁺ was present in the bathing medium (data not shown, n = 3), we conclude that the current decline in Ba²⁺-free solution truly reflects a fading of the GIRK current response. The closure of GIRK channels during desensitization was also reflected in the decline of membrane conductance, which was determined by 20-mV hyperpolarizing voltage steps. During the peak of the GIRK current response, membrane conductance was enhanced by 2.2 ± 0.2 nS, which went down to 1.2 ± 0.3 nS after 1 min of agonist application (n = 3).

Interestingly, the probability to encounter a specific type of response was largely independent of the agonist used to evoke the GIRK current (Fig. 1D). On average, sustained, i.e., non-desensitizing responses were the least frequent type (baclofen 9.1%, n = 33; adenosine 16.6%, n = 12; serotonin 25%, n = 8), followed by monophasic current decay (baclofen, 18.2%; adenosine, 41.7%; serotonin, 25%), whereas the most frequently observed pattern was characterized by a biphasic current decay (baclofen, 72.7%; adenosine, 41.7%; serotonin, 50%). In the case of baclofen, we also observed biphasic responses that did not reach steady state but appeared to display a third slow decay time constant (24.2%). Because this behavior was absent in GIRK current responses to adenosine and serotonin, we considered this apparently triphasic form of desensitization a peculiarity of the baclofen response rather than a genuine category of GIRK current desensitization. We hence classified baclofen responses with an additional slow decay time constant not as a separate group but included them in the biphasic group.
Figure 1. G-protein-activated inwardly rectifying K\(^+\) (GIRK) currents display different types of short-term desensitization during prolonged agonist application, with biphasic kinetics emerging as predominant pattern. A–C: GIRK current responses to adenosine (100 \(\mu\)M) were recorded as inward currents at \(-70\) mV ([K\(^-\)]/K\(^+\)] = 60/151 mM). Recordings were obtained from 3 different neurons and illustrate a nondesensitizing response (A), a monophasic response (B), and a biphasic response (C). D: histogram summarizes relative occurrence of the 3 types of responses for baclofen (20 \(\mu\)M), serotonin (100 \(\mu\)M), and adenosine (100 \(\mu\)M). III, number of neurons displaying an additional slow component of current decline. For explanation see text.

**Kinetics of desensitization are agonist independent**

The heterogeneity of desensitization between individual neurons was not correlated with their degree of maturation, their apparent morphology, or the agonist tested. Because the biphasic response emerged as the predominant pattern, we focused our further analysis on this type of desensitization. Figure 2, A–C, illustrates representative biphasic responses to the three GIRK channel activators. To better resolve the initial rapid phase of current decay, the area marked by the gray boxes was blown up and depicted (inset below the original trace). The solid lines superimposed on the current traces represent the curves that were fitted to the original data to calculate the time constants of current decay. A comparison of the mean decay time constants of GIRK currents evoked by baclofen \((\tau_{\text{fast}} 7.3 \pm 0.75 \text{ s}, \tau_{\text{slow}} 125.7 \pm 10.0, n = 13)\), adenosine \((\tau_{\text{fast}} 9.8 \pm 1.6 \text{ s}, \tau_{\text{slow}} 116.8 \pm 12.6 \text{ s}, n = 5)\), and serotonin \((\tau_{\text{fast}} 7.8 \pm 1.3 \text{ s}, \tau_{\text{slow}} 118.3 \pm 14.7 \text{ s}, n = 4)\) showed no statistical difference between the agonists (Fig. 2D). We conclude hence that the kinetics of fast and slow desensitization are apparently independent of the agonist used to induce the GIRK current. We arrived at the same conclusion when we analyzed the kinetics of those GIRK currents that declined in a monophasic fashion. Again, there was no significant difference in the decay time constants during application of baclofen \((59.7 \pm 10.8 \text{ s}, n = 6)\), adenosine \((59.5 \pm 12.6 \text{ s}, n = 4)\), and serotonin \((55.5 \pm 1.9 \text{ s}, n = 2)\).

A common concern with standard whole cell recordings is the dialysis of the intracellular milieu that might dilute or remove cytosolic components that are essential for the cellular function under study. To rule out that the decline of GIRK currents during sustained agonist application represents an experimental artifact associated with whole cell perfusion, we performed a series of experiments in which we used the perforated-patch configuration of the whole cell mode. Patch pipettes were filled with the pore-forming antibiotic nystatin, which allows electrical access to the neuron without causing internal dialysis (Horn and Marty 1988). As shown in Fig. 3A, the biphasic kinetics of GIRK current desensitization were preserved in perforated-patch recordings. When compared with the time course of desensitization obtained in the standard whole cell configuration during baclofen application, the time constants of GIRK current decline \((\tau_{\text{fast}} 12.7 \pm 1.2 \text{ s}, \tau_{\text{slow}} 115.5 \pm 27.7 \text{ s}, n = 5)\) were well within the same temporal range, with only the small deceleration of \(\tau_{\text{fast}}\) pointing to a possible effect of cell dialysis (Fig. 3B).

**Desensitization during repetitive activation of GIRK current**

To determine how the kinetics of fast desensitization were affected during repetitive application of GIRK channel activators, we exposed neurons to drug pulses ~20 s long at either fixed (Fig. 4A) or variable drug-free intervals (Fig. 4B). These experiments demonstrated that the rapidly decaying component of the current response is particularly susceptible to repetitive drug application. For example, in the sequence depicted in Fig. 4A, five applications of baclofen completely abrogated the initial peak of the GIRK current and slowed the kinetics of current decay \((n = 3)\). Once developed, the loss of the fast decaying peak current component was relatively long-lived, as drug-free intervals of \(\leq 3\) min failed to restore the complete response (Fig. 4A). To determine the minimal time interval preserving the fast component, we reduced the drug-free gap gradually from 3 min to 15 s. As shown in Fig. 4B, the initial peak was beginning to decline at inter-drug intervals \(\leq 2\) min. The loss of the rapidly decaying component becomes evident if one compares the blown-up time course of desensitization shown in the gray insets, which were taken from the first and the fifth current response. When plotted on a semi-logarithmic scale, the mean decrease of peak current during repetitive application followed a straight line indicating a mono-expo-
nential decay (Fig. 4C). The histogram of Fig. 4D, which summarizes the data from the experiments with variable drug-free intervals (n = 5), shows that, on average, the decline of peak current reached significance at and below the 1-min interval and failed to recover within 3 min of drug washout.

Desensitization has a strong heterologous component

Heterologous desensitization was tested using the following protocol: after application of the test agonist for 1 min and complete drug washout, we superfused the neuron with a different, conditioning agonist for 5 min. After complete recovery from the conditioning agonist, the test agonist was applied again. The ratio between the size of the GIRK current response to the test agonist before and after application of the conditioning agonist is then taken as an indicator of the degree of cross desensitization. As shown in Fig. 5A, the second response to the test agonist adenosine was subjected to pronounced heterologous desensitization, if the cells were exposed to baclofen before the second adenosine application. In the absence of baclofen, application of adenosine alone for 1 min did not affect the second response evoked 3 min later (Fig. 5A, inset), thus excluding that desensitization at the adenosine receptors during this test protocol is responsible for the diminished second response after baclofen. Virtually identical cross-desensitization occurred in the opposite direction, i.e., adenosine onto baclofen, and was also observed for baclofen onto serotonin (Fig. 5B). On average, heterologous desensitization reduced the current response to the test agonist to 20.4 ± 5.01% of control (n = 6, P < 0.01), suggesting that the mechanisms of short-term desensitization are located predominantly downstream of receptor activation.

Desensitization is not associated with reduction of PIP₂

It has recently been demonstrated that phospholipase C (PLC)-mediated hydrolysis of phosphatidylinositol bisphosphate (PIP₂) leads to desensitization of cardiac GIRK current and that the decline of current can be largely abrogated if GIRK current responses were recorded in the presence of the PLC inhibitor U73122 (Kobrinsky et al. 2000). To find out whether PIP₂ hydrolysis is also involved in the desensitization of GIRK currents in our preparation, we recorded current responses to baclofen in the presence of U73122 (1 μM) and compared their size and kinetics to those of control recordings. As shown in Fig. 6A, the PLC inhibitor did not affect the

**FIG. 2.** Kinetics of desensitization are independent of GIRK current agonist. A–C: GIRK current responses to baclofen (20 μM, A), adenosine (100 μM, B), and serotonin (20 μM, C) are depicted on a slow and a fast time scale (inset) to resolve the 2 time constants of current decay. Solid lines superimposed on current traces were obtained by nonlinear fitting. D: histogram summarizes and compares fast and slow time constants of GIRK current decay during application of the 3 drugs.

**FIG. 3.** Biphasic time course of desensitization does not result from cell dialysis. A: representative GIRK current response to baclofen obtained by means of perforated-patch-clamp recording using nystatin as pore-forming agent (see METHODS). Solid line was fitted to original trace to determine the 2 time constants of current decay. B: histogram summarizes and compares kinetics of desensitization to baclofen (20 μM) in normal whole cell recordings and in perforated-patch recordings. * P < 0.05.
typical biphasic desensitization of the GIRK during a 5-min period of baclofen application; it did, however, alter the time constants of the decay of the current. Statistical analysis (Fig. 6B) indicated that U73122 enhanced \( \tau_{fast} \) from 7.3 ± 0.7 s (\( n = 13 \)) to 13.1 ± 3.1 s (\( n = 6 \), \( P < 0.05 \)), whereas \( \tau_{slow} \) was reduced from 125.7 ± 10.0 s (\( n = 13 \)) to 79.2 ± 13.0 s (\( n = 6 \), \( P < 0.05 \)). Owing to the acceleration of slow desensitization, the percentage of current decline attributable to slow desensitization was larger in U73122 (56.7 ± 9.3%, \( n = 6 \)) compared with baclofen alone (38.8 ± 5.3%, \( n = 13 \)), and the overall reduction of the GIRK current during the agonist application appeared more pronounced (baclofen alone 73.3 ± 7.2%, \( n = 13 \)).

**FIG. 4.** Desensitization during repetitive activation of GIRK current. A: baclofen (20 \( \mu M \)) was applied repeatedly for 20-s periods at fixed drug-free intervals. Note gradual loss of fast decaying current component (dotted to dashed line) with repeated application. B: baclofen was applied with increasingly shorter drug-free intervals. Insets: loss of fast desensitizing current component as drug-free interval shrinks. C: relative decrease of peak GIRK current amplitude determined at like-numbered baclofen application of B. Data points represent means ± SE of 5 independent measurements (error bars not shown when smaller than symbol size). D: histogram summarizes data from 5 experiments using the protocol of B. Note that a significant reduction of peak amplitude occurs at drug-free intervals ≤1 min. * \( P < 0.05 \).
lacked fast desensitization (Fig. 7). Evoked in neurons with altered nucleotide levels typically G-protein cycling in desensitization. In fact, GIRK currents with high GDP/low GTP should be indicative of a role of kinetics in a larger population of neurons internally perfused. We reasoned that a substantial alteration of the desensitization to change the GDP/GTP ratio within a given experiment, we were not able to directly replicate the experiments of Chuang et al. (1998) in our preparation. Instead, we elevated GDP in the pipette solution to 1.9 mM and reduced GTP to 100 μM at the same time. Although this protocol did not allow us to change the GDP/GTP ratio within a given experiment, we reasoned that a substantial alteration of the desensitization kinetics in a larger population of neurons internally perfused with high GDP/low GTP should be indicative of a role of G-protein cycling in desensitization. In fact, GIRK currents evoked in neurons with altered nucleotide levels typically lacked fast desensitization (Fig. 7A). Whereas the decay of the GIRK current during sustained baclofen application displayed a fast time constant in 75% of control neurons (n = 33), a rapid component of desensitization was observed in only 16.7% of the neurons that were internally perfused with high GDP/low GTP levels (n = 12, Fig. 7B). Furthermore, the percentage of neurons lacking any form of GIRK current desensitization was more than threefold higher in GDP-enriched neurons (25%, n = 12) than in control neurons (9.1%, n = 33, Fig. 7B). The absence of the initial, fast decaying current component had two consequences for the GIRK current response. First, the peak current in GDP-enriched neurons was significantly smaller (31.6 ± 6.7 pA, n = 7) than in control neurons (85.3 ± 17.0 pA, n = 13, P < 0.05, Fig. 7C). Second, the relative reduction of the GIRK current within the drug application period was significantly smaller in GDP-enriched neurons (47.2 ± 7.7% of peak, n = 7) than in control neurons (73.3 ± 7.2% of peak, n = 13, P < 0.05, Fig. 7D). It is noteworthy that neither the percentage of neurons displaying slow desensitization (control: 52%, high GDP: 50%, Fig. 7B) nor τslow were significantly affected by the altered GDP/GTP relation in the pipette solution (τslow control: 125.7 ± 10.0 s, n = 13; τslow GDP-enriched: 108.5 ± 20.5 s, n = 7, P > 0.05, Fig. 7E). This suggests that the G-protein turnover cycle might contribute to the fast, but not to the slow desensitization of the GIRK current.

**Discussion**

Our data indicate that the majority of neocortical pyramidal cells display a biphasic pattern of current desensitization during sustained application of the GIRK channel activators baclofen, adenosine, and serotonin. The only electrophysiological studies on short-term GIRK current desensitization in native CNS neurons, that we are aware of, were performed on locus ceruleus neurons in brain slices (see Introduction). Presumably owing to the relatively slow onset of drug action in the slice tissue, these studies did not resolve the fast kinetics of current desensitization. Using acutely isolated cells and rapid drug application, we report here that in most pyramidal cells, the slow phase of desensitization (τslow about 120 s) that was

![FIG. 5. Desensitization of GIRK currents has a strong heterologous component. A: application of baclofen (20 μM) was sandwiched between 2 applications of adenosine (100 μM). Note strong reduction of 2nd adenosine response despite complete recovery of preceding baclofen response. Inset: without application of other GIRK agonist in between, 1st and 2nd adenosine response are virtually identical. B: summary of experimental protocol of A using different combinations of agonists as indicated by symbols.](http://jn.physiology.org/doi/10.1152/jn.00370.2001)
port of this notion, we found that short-term desensitization of GIRK currents occurred predominantly in a heterologous fashion because the responsiveness to one agonist was substantially reduced after prior exposure of the cells to another agonist. Previous electrophysiological studies on GIRK currents evoked by opioid receptor activation in locus ceruleus neurons (see preceding text) yielded somewhat conflicting results on the nature of desensitization. While one group reported predominant homologous desensitization (Fiorillo and Williams 1996; Harris and Williams 1991), a recent study found evidence for heterologous effects of opioid receptor activation on current responses to other GIRK channel activators (Blanchet and Lüscher 2002).

Slow desensitization

In principle, interference with any step of the signaling cascade, from agonist binding, G-protein cycling to GIRK channel opening, might diminish the GIRK current in the continued presence of the agonist (for review, see Dascal 1997). To pinpoint the mechanism(s) responsible for the GIRK current desensitization in CNS neurons, we manipulated the individual signaling steps separately and examined how this affected the extent and the kinetics of desensitization. With respect to the mechanism(s) underlying slow desensitization, our data allowed us to exclude a number of possible candidates: Receptor phosphorylation by G-protein-coupled receptor kinases (GRKs) has been proposed to account for slow desensitization of the muscarinic GIRK current in rat atrial cells (Shui et al. 1995). However, the heterologous nature of desensitization in our preparation makes this mechanism unlikely because GRK-dependent receptor phosphorylation has been generally implicated in homologous desensitization (Bunemann et al. 1999). It should be noted, however, that GRKs also act as scavengers of free βγ subunits, which compete with the GIRK channel for the βγ subunits in a phosphorylation-independent fashion. It has been suggested that this additional effect of GRKs might be indeed responsible for a large portion of the slow desensitization attributed to GRK activity (Shui et al. 2002). Because GRKs acting as sinks of free βγ subunits should produce heterologous desensitization, this scheme would be consistent with our data (see following text).

Do second-messenger-activated protein kinases play a role in the slow desensitization of GIRK channels? Several lines of evidence have demonstrated that protein kinase A (PKA) and, to some extent, protein kinase C (PKC) mediate heterologous desensitization through phosphorylation of G-protein receptors (Bunemann et al. 1999). It cannot be ruled out that this process also contributes to the slow desensitization of GIRK currents in pyramidal cells. However, the striking agonist independence of the rate constant of slow desensitization would be difficult to reconcile with the differential effects of adenosine, baclofen, and serotonin on PKA- and PKC-mediated signaling.

On the other end of the signaling cascade, experiments using the nonhydrolyzable GTP analogue GTPγS, which maintains the G protein in a permanently activated state, demonstrated that the current response to the agonists did not display any appreciable desensitization under these conditions (Sickmann and Alzheimer 2002). It thus appears unlikely that an intrinsic alteration of channel gating or a change in channel phosphorylation is predominantly responsible for slow desensitization.
This is in contrast to the behavior of heterologous GIRK channels co-expressed with μ opioid receptors in *Xenopus* oocytes. In that system, slow desensitization was preserved after GTPγS injection and was proposed to result from channel inactivation (Kovoor et al. 1995).

Finally, we did not obtain evidence for an appreciable involvement of PIP_2_ breakdown in slow desensitization as recently observed in native cardiac cells and a heterologous expression system (Kobrinsky et al. 2000). In our hands, however, inhibition by U73122 of PLC failed to abrogate fast or slow desensitization. If anything, the PLC inhibitor altered the kinetics of the two phases, decelerating fast and accelerating slow desensitization. Although we cannot provide positive evidence, our data point, by exclusion, to an intermediate step of the G-protein-dependent signaling pathway being responsible for slow desensitization. In agreement with this notion, Blanchet and Lüscher (2002) recently proposed a model in which competitive inhibition of GIRK channel activation is proposed to underlie the slow desensitization of GIRK current responses to μ opioid receptor activation in locus ceruleus neurons. According to their scheme, free βγ might be gradually reduced by a scavenger such as GRK2 or GRK3 (see preceding text) or, alternatively, sustained receptor activation might recruit a different type of G protein, the dimer composition of which might somehow interfere with GIRK channel activation.

**Fast desensitization**

In addition to slow desensitization, the majority of neurons displayed fast desensitization. This early component of desensitization has considerable functional significance because it accounts for almost half of the total desensitization. The rapidly relaxing component of the GIRK current response proved highly susceptible to repeated brief agonist applications. In our hands, drug-free intervals of ≥1 min produced a significant
loss of the fast decaying GIRK current component. Previous work in atrial cells implicated GIRK channel dephosphorylation in fast desensitization (Kim 1993; Shui et al. 1997). In contrast to those studies, we observed a complete disappearance of fast desensitization when cells were internally perfused with GTPγS (Sickmann and Alzheimer 2002). This suggests that alteration of channel phosphorylation is unlikely to explain the fast desensitization of GIRK currents in pyramidal neurons. We also exclude receptor phosphorylation as the predominant mechanism based on the strong heterologous nature of desensitization and the independence of \( \tau_{\text{fast}} \) from any particular GIRK current agonist.

Instead, we propose that the nucleotide exchange and hydrolysis cycle of G proteins plays an essential role in fast desensitization. Such a mechanism was first advanced by Chuang et al. (1998), who studied GIRK current desensitization in a heterologous expression system. Their hypothesis is that if a sufficiently large number of receptors is activated, the fast decay of the GIRK current results from the initially synchronized release of \( \beta \gamma \) subunits from “primed” G proteins, which have GDP still bound but immediately ready to dissociate on stimulation by activated receptors. The rapidly decaying overshoot of the GIRK current response would thus result from the relaxation of the G-protein cycle to a (lower) steady-state level of \( \beta \gamma \) subunit generation. Lending credence to this hypothesis, we found, as predicted by the model of Chuang et al. (1998), that a reversal of the GTP/GDP ratio in the pipette solution from high GTP/0 GDP to high GDP/low GTP selectively abrogated the fast decaying component of the GIRK current response, thereby reducing significantly the peak amplitude. According to the model, excess GDP acts to reduce or prevent the priming of G proteins. Under this condition, no relaxation to a lower steady-state level of \( \beta \gamma \) subunit release will occur and consequently, fast desensitization, but not slow desensitization, will be absent in the current recording, as observed. The gradual disappearance of the fast decaying current component during repetitive drug application with decreasing drug-free intervals might then result from less and less G proteins being capable of re-entering the primed state during the short washout periods.

How do the concentrations of GTP and GDP used in whole cell recordings relate to normal intracellular concentrations of guanine nucleotides? In experiments with native CNS neurons, GTP concentrations in the pipette solution ranged between 0.3 (Sodickson and Bean 1996) and 3 mM (Lüscher et al. 1997). This is higher than the physiological concentration of GTP that is generally thought to be \( \sim 0.1 \) mM. Because the desensitization kinetics of GIRK currents obtained in whole cell recordings agree well with those obtained in perforated-patch recordings (Fig. 3), excess application of GTP is unlikely to alter the mechanisms underlying short-term desensitization. The normal intracellular concentration of GDP is about one order of magnitude smaller than that of GTP (Reinhardt et al. 2002). This is consistent with the above model attributing fast desensitization to the cycling of G proteins.

**Functional implications of short-term desensitization**

In functional terms, our data indicate that the fast and the slow component of short-term desensitization reduce the initially available GIRK current by \( >70\% \) within 5 min of drug application with both components contributing about equally to the fading response. The efficacy of GIRK current activators to dampen neuronal activity will thus critically depend on their release pattern. Phasic and tonic release would therefore be predicted to exert vastly different actions on the target cells. Owing to the strong short-term desensitization, exposure of neurons to a GIRK channel activator at saturating concentration will leave a powerful but short-lived trace of inhibition in the postsynaptic neuron. Because not only principal cells but also GABAergic interneurons are known to display GIRK current responses, it is worth noting that phasic GIRK channel activation might also produce a transient disinhibition of projection neurons.

When extrapolating our data to the electrical behavior of CNS neurons in a living animal, one should bear in mind that our experiments were performed at room temperature and that the \( Q_{10} \) of the rate constant of fast desensitization is 2.2 as demonstrated in atrial cells (Zang et al. 1993). Taking the substantial acceleration of GIRK current decay under physiological temperature into account, the trajectory of an acutely desensitizing GIRK current response might serve to bridge the window between fast GABAergic synaptic inhibition on the one end of the temporal scale, and tonic inhibition generated, for example, by ambient adenosine or tonic GABA release, on the other end.

Studies in heterologous expression systems have demonstrated that the degree and the kinetics of short-term desensitization of GIRK currents depend on channel subunit composition (Bender et al. 2001) and might be modulated by signaling molecules such as the endogenous regulators of G-protein signaling (Chuang et al. 1998; Jeong and Ikeda 2001). This opens the intriguing possibility that the temporal regulation of GIRK current desensitization might not only be adapted to the momentary functions and needs of CNS neurons but that changes might also occur as these neurons proceed through the various stages of development, maturation and aging.

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**DISCLOSURES**

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**REFERENCES**


