Variance Analysis of Current Fluctuations of Adenosine- and Baclofen-Activated GIRK Channels in Dissociated Neocortical Pyramidal Cells

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INTRODUCTION

A large number of neurotransmitters including adenosine, GABA (via GABA_B receptors), serotonin, dopamine, norepinephrine, acetylcholine, opioids, and somatostatin modulate neuronal excitability by activating an inwardly rectifying K^+ current via a membrane-delimited, G protein-mediated mechanism (Dascal 1997; Yamada et al. 1998). The notion that G protein-activated, inwardly rectifying K^+ (GIRK) channels represent a common target of many neurotransmitter systems is strongly supported by the following observations. 1) On the whole cell level, GIRK currents display almost identical electrophysiological and pharmacological properties, irrespective of the transmitter used to evoke them. 2) The combined application of transmitters at saturating concentrations produces subadditive and often occlusive GIRK current responses (Andrade et al. 1986; Dascal 1997; McCormick and Williamson 1989; Sodickson and Bean 1998; Yamada et al. 1998). However, despite the large body of evidence suggesting a common mechanism of GIRK current generation, it is still unclear whether this notion also holds on the single channel level. Data on elementary properties of GIRK channels in native CNS neurons are sparse, and the findings published so far do not yield a uniform picture. To our knowledge, elementary properties of adenosine- and baclofen-activated GIRK channels in native CNS neurons have not been reported yet. Here, we employed variance analysis of adenosine- and baclofen-induced current fluctuations to determine and compare properties of the underlying K^+ channels.

METHODS

As described in detail elsewhere (Alzheimer et al. 1993), a combined enzymatic/mechanic dissociation procedure was employed to prepare acutely isolated pyramidal cell somata from the sensorimotor cortex of anesthetized rats that were 2–3 weeks old. The recording chamber was mounted on the stage of an inverted microscope equipped with Hoffman modulation optics. Current signals from visually identified pyramidal cell somata recorded in whole-cell voltage-clamp mode were sampled at 20 kHz and filtered at 5 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 CaCl_2, 2 MgCl_2, 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 CaCl_2, 2 MgCl_2, 5 NaHEPES, 5 HEPES, and 10 d-glucose (pH 7.4). Patch pipettes were coated with Sylgard in the tapered region and filled with (in mM) 135 K-glucuronate, 5 HEPES, 3 MgCl_2, 5 EGTA, 2 Na_2ATP, and 2 NaGTP (pH 7.25, adjusted with KOH). Electrode resistance in the whole cell configuration was 5–15 MΩ before series resistance compensation (70–75%). Voltage readings were corrected for liquid junction potential. If not noted otherwise, holding potential (V_H) was –80 mV. Cell capacitance determined by means of the built-in whole cell capacitance compensation circuit of the amplifier was used to estimate cell surface area assuming a specific membrane capacitance of 1 μFcm^-2. Adenosine and baclofen were applied at increasing concentrations by superfusing the extracellular solution containing the transmitter used to evoke them. Figure 1, A and B shows the rapid and dose-dependent induction of GIRK...
currents by two different concentrations of the GABA_B receptor agonist, baclofen (10 and 100 μM), in an acutely isolated rat pyramidal neuron. Consistent with previous findings from other preparations (Dascal 1997; Sodickson and Bean 1998), GIRK current responses to baclofen as well as those to adenosine were inwardly rectifying and completely suppressed by Ba^{2+} (200 μM, not shown). Dose-response relationships of the two agonists were obtained by relating normalized GIRK current to log agonist concentration. From the curves fitted to the data points, we obtained EC_{50} values for baclofen and adenosine of 25.6 μM and 2.0 μM, respectively, (Fig. 1, C and D). For noise analysis of agonist-evoked current fluctuations, we used stretches of current recordings obtained at increasing agonist concentrations (Fig. 2, A and B). We then calculated the current variance (pA^2) at each agonist concentration and plotted it as a function of the mean GIRK current observed at the same concentration (Fig. 2, C and D). To isolate membrane fluctuations associated with GIRK channel activity from background noise, the current variance of control recordings was subtracted from the current variance determined during agonist applications. The data points were fit to an equation of the form

\[ \sigma^2 = i - I/I_{\text{Max}} \]

where \( \sigma^2 \) is the variance of the current, \( i \) the unitary current amplitude, \( I \) the whole cell current amplitude, and \( N \) the number of available channels (cf. Traynelis and Jaramillo 1998). The continuous solid line through the filled circles is the fit with Eq. 1, giving estimated values for \( i \) and \( N \) of 1.48 pA and 572 channels, respectively, for baclofen, and of 1.26 pA and 584 channels, respectively, for adenosine. To obtain an estimate of GIRK channel density, \( N \) was related to cell surface area and density was expressed as channels μm^{-2}. Single-channel conducance (\( \gamma \)) was determined using the relationship

\[ \gamma = \frac{i}{V - E_K} \]

where \( V \) is the holding potential, and \( E_K \) the equilibrium potential of K^+ under our recording conditions (-24 mV).

The histograms of Fig. 3 depicting the mean single channel conductance and the mean density of GIRK channels evoked by baclofen and adenosine demonstrate that the two agonists appear to activate GIRK channels of identical unitary conductance and density. Average normalized channel density for adenosine-activated GIRK channels was 0.49 ± 0.07 channels μm^{-2} (n = 8), and 0.46 ± 0.097 channels μm^{-2} for baclofen-activated GIRK channels (n = 6). At the [K^+]/([K^+]) gradient of 60/151 mM used in our study, adenosine-induced GIRK channels had an average single-channel conducance of

![Image](http://jn.physiology.org/)

**FIG. 1.** G protein-activated inwardly rectifying K^+ (GIRK) current responses evoked by baclofen and adenosine in acutely isolated pyramidal neurons from rat neocortex. A: morphology of acutely isolated cell soma with patch pipet attached. B: reversible and dose-dependent induction of GIRK current by baclofen. Because of the recording condition ([K^+]_o: 60 mM, V_H: -70 mV), GIRK currents were inward. C, D: dose-response relationship of GIRK currents evoked by baclofen (n = 11) and adenosine (n = 12). Currents were normalized to the maximum response. Dose response curves were fitted to the data points using an equation of the form \( I = I_{\text{Max}}/(1 + (EC_{50}/[A])^n) \), where \( I_{\text{Max}} \) is the maximum current evoked by the agonist, \([A]\) the agonist concentration, EC_{50} the agonist concentration yielding a half-maximal current response, and \( n \) the Hill coefficient. Estimated EC_{50} values for baclofen was 25.6 μM (95% confidence interval 21.5–30.4 μM, Hill slope 0.98) and for adenosine 2.0 μM (0.26–14.5 μM, Hill slope 0.69).
25.0 ± 0.89 pS (n = 8), which was not significantly different from the single-channel conductance of baclofen-induced GIRK channels (25.5 ± 1.57 pS, n = 6). Assuming that the unitary conductance of inwardly rectifying K⁺ channels increases approximately proportional with the square root of [K⁺]₀ (Sakmann and Trube 1984), the GIRK channels studied here should display conductances between 5 and 6 pS at a physiological [K⁺]₀ of 3 mM.

DISCUSSION

This study is the first to report elementary properties of GIRK channels mediating the hyperpolarizing effect of adenosine and baclofen in native CNS neurons. In our hands, both transmitters activated K⁺ channels of virtually identical single-channel conductance, which was estimated as 5–6 pS under physiological conditions. The simplest explanation of this finding would be that both transmitters engage the same type of channel. Since N of Eq. 1 indicates the total number of channels that can potentially contribute to the whole cell current response, it should reflect the pool of channels available for maximum transmitter response. Given the fact that GIRK channels recruited by the two agonists are expressed at equal densities, it appears reasonable to assume that both agonists access the same pool of GIRK channels (or the same pool of G proteins the βγ-subunits of which then activate the GIRK channels). This notion is consistent with previous findings showing subadditive or often occlusive effects when saturating concentrations of adenosine, baclofen or other agonists known to target GIRK channels were applied simultaneously (Andrade et al. 1986; Sodickson and Bean 1998).

Whereas our data indicate that adenosine and baclofen recruit electrophysiologically uniform channels, this does not necessarily imply that the channels are identical in terms of molecular structure. GIRK channels are tetrameric proteins of the Kir3.0 (GIRK) subfamily which comprises four cloned subunits (Kir3.1–3.4) in mammals (Isomoto et al. 1997). In the pyramidal cell layers of rat neocortex, Kir3.1–3.3 subunits are abundantly expressed, suggesting that the GIRK channels of these neurons are assembled from these subunits (Karschin et
Coexpression of adenosine A1 receptors with different combinations of Kir3.0 subunits in Xenopus oocytes demonstrated effective coupling only when A1 receptors were expressed in the presence of Kir3.1 combined with one additional subunit, whereas other Kir3.0 assemblies yielded negligible current responses to A1 receptor activation (Pfaff and Karschin 1997). Single-channel recordings showed that heterologously expressed Kir3.1/3.2 channel multimers displayed a unitary conductance compatible with the one estimated here for adenosine- and baclofen-activated GIRK channels (Lesage et al. 1995; Spauschus et al. 1996). Based on these observations and the localization of Kir3.0 subunits noted above, we propose that the GIRK current responses described here were mediated by heterotetramers of Kir3.1/3.2 and/or Kir3.1/3.3 subunits. Although our data do not allow us to resolve the stoichiometry of adenosine- and baclofen-activated GIRK channels in neocortical pyramidal cells, they strongly suggest that the two transmitters share a common transduction mechanism on the single-channel level.

How do our findings relate to the properties of GIRK channels reported from other CNS regions? Whereas we are not aware of single-channel data on adenosine-activated GIRK channels, Premukar and Gage (1994) reported that, in cultured hippocampal neurons, baclofen induced a much larger, 67 pS K+ channel (in symmetric K+ solution). However, because that baclofen effect was observed in cell-attached recordings with the agonist in the bath (requiring a diffusible second messenger), the K+ channel is unlikely to belong to the GIRK channel subfamily, which is typically activated in a membrane-delimited fashion (Yamada et al. 1998). Noise analysis of slow IPSCs showed that synaptically released GABA acting on GABAA receptors activates a K+ channel, the conductance of which is within the range reported here for baclofen-induced GIRK channels (De Koninck and Mody 1997). Unlike these channels, however, the K+ channels mediating slow IPSCs were nonrectifying or slightly outwardly rectifying suggesting that they might represent a distinct channel type (De Koninck and Mody 1997). Penington et al. (1993) reported inwardly rectifying K+ channels with multiple subconductance levels up to 120 pS that were activated by serotonin in dorsal raphe neurons in a membrane-delimited fashion, whereas two other groups showed that, in hippocampal pyramidal neurons, serotonin induces small-conductance GIRK channels resembling those described here (Oh et al. 1995; Van Dongen et al. 1988). GIRK channels of similar small conductance were also observed in rat locus coeruleus neurons during application of somatostatin, metenkephalin, and noradrenaline (Arima et al. 1998; Grigg et al. 1996). These latter findings, now strengthened by our data on adenosine- and baclofen-activated GIRK channels, lend support to the view that a variety of neurotransmitters converges onto an electrophysiologically uniform, small conductance type of K+ channel. The above discrepancies might be explained by GABA acting on more than one type of K+ channel, or by the fact that gating properties of GIRK channels are influenced by the patch-clamp configuration used to study them.

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