Tonic Activation of GABA<sub>B</sub> Receptors Reduces Release Probability at Inhibitory Connections in the Cerebellar Glomerulus

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Mapelli L, Rossi P, Nieus T, D’Angelo E. Tonic activation of GABA<sub>B</sub> receptors reduces release probability at inhibitory connections in the cerebellar glomerulus. J Neurophysiol 101: 3089–3099, 2009. First published April 1, 2009; doi:10.1152/jn.91190.2008. In the cerebellum, granule cells are inhibited by Golgi cells through GABAergic synapses generating complex responses involving both phasic neurotransmitter release and the establishment of ambient γ-aminobutyric acid (GABA) levels. Although at this synapse the mechanisms of postsynaptic integration have been clarified to a considerable extent, the mechanisms of neurotransmitter release remain largely unknown. Here we have investigated the quantal properties of release during repetitive neurotransmission, revealing that tonic GABA<sub>B</sub> receptor activation by ambient GABA regulates release probability. Blocking GABA<sub>B</sub> receptors with CGP55845 enhanced the first inhibitory postsynaptic current (IPSC) and short-term depression in a train while reducing trial-to-trial variability and failures. The changes caused by CGP55845 were similar to those caused by increasing extracellular Ca<sup>2+</sup> concentration, in agreement with a presynaptic GABA<sub>B</sub> receptor modulation of release probability. However, the slow tail following IPSC peak demonstrated a remarkable temporal summation and was not modified by CGP55845 or extracellular Ca<sup>2+</sup> increase. This result shows that tonic activation of presynaptic GABA<sub>B</sub> receptors by ambient GABA selectively regulates the onset of inhibition bearing potential consequences for the dynamic regulation of signal transmission through the mossy fiber–granule cell pathway of the cerebellum.

INTRODUCTION

GABAergic synapses are endowed with a variety of γ-aminobutyric acid type A (GABA<sub>A</sub>) receptor subtypes determining, in association with spillover and tonic GABA levels, the intensity and kinetics of inhibitory neurotransmission (Cherubini and Conti 2001; Farrant and Nusser 2005; Glykys and Mody 2007). GABA<sub>B</sub> receptors, in turn, can limit GABA release (Chen and Yung 2005; Deisz et al. 1997; Fearon et al. 2003; Jensen et al. 1999; Kaneda and Kita 2005; Morishita and Sastry 1995; Mouginot et al. 1998; Tran and Szabo 2002) through a presynaptic mechanism. It has been proposed that, at central synapses, this mechanism could operate by reducing presynaptic calcium influx and synaptic vesicle recovery (Dittman and Regehr 1996; Sakaba and Neher 2003; Takahashi et al. 1998), thereby regulating the dynamics of repetitive neurotransmission (Hefft et al. 2002). However, the impact of this change is likely to depend on the specific property of synapses, including their presynaptic dynamics, the GABA<sub>A</sub> receptor subtypes, the amount of neurotransmitter spillover, and the tonic GABA level.

Along the mossy fiber pathway of cerebellum, GABAergic synapses are formed between Golgi cells and granule cells inside the glomeruli. In response to stimulation, inhibition arises rapidly with millisecond precision before slowly degrading over about 100 ms (in vivo: Eccles et al. 1967; in vitro: Maffei et al. 2002). The Golgi cells are spontaneously active at low frequency (Forti et al. 2006; Solinas et al. 2008a,b) and respond to punctuate stimulation with brief spike bursts (Vos et al. 1999). The granule cells are activated by the mossy fibers (D’Angelo et al. 1993, 1995, 1999, 2001; Nieus et al. 2006; Rossi et al. 2006; Sola et al. 2004) and their activity is limited by the feedforward and feedback inhibitory loops formed by Golgi cells. In granule cells, GABA<sub>B</sub> subunit-containing GABA<sub>A</sub> receptors (Brickley et al. 1999; Farrant and Nusser 2005) generate fast and slow inhibitory postsynaptic current (IPSC) components in response to direct release onto postsynaptic densities and spillover from neighboring contacts (Hamann et al. 2002; Rossi and Hamann 1998; Rossi et al. 2003). In addition, ambient GABA at submicromolar concentration activates high-affinity GABA<sub>B</sub> receptors generating tonic inhibition. This latter has been shown to regulate the input–output relationship of the granule cell (Brickley et al. 1996) and has been proposed to control the mossy fiber–granule cell gain (Mitchell and Silver 2003). However, it is unknown whether GABA<sub>B</sub> receptors, which are present in Golgi cells (Kulik et al. 2002) and have affinities in the same GABA concentration range (Galvez et al. 2000), could also be activated presynaptically and regulate neurotransmitter release. This potential regulation, given the multiple types and different activation modes of postsynaptic receptors, is expected to exert complex effects on repetitive Golgi cell–granule cell neurotransmission.

Herein we report a novel mechanism for tonic GABA levels in the cerebellar glomerulus. In juvenile rats, GABA<sub>B</sub> receptors exerted a tonic depression on release probability at the Golgi cell–granule cell synapse. The main consequence was that of reducing the first response without substantially altering the total inhibitory charge in a train. It is therefore proposed that ambient GABA<sub>B</sub>, by acting through presynaptic GABA<sub>B</sub> receptors, selectively regulates the sharpness of the onset of inhibition in granule cells. The potential consequences for signal transmission along the mossy fiber pathway are discussed.

METHODS

Slice preparation and solutions

Patch-clamp recordings in acute cerebellar slices were performed as previously reported (Armano et al. 2000; D’Angelo et al. 1993, 1995,
transients induced by 10-mV hyperpolarizing voltage steps from a holding potential of −60 mV. The cerebellar granule cell has a compact structure and behaves like a single electrotonic compartment (Cathala et al. 2003; D’Angelo et al. 1993, 1995; Silver et al. 1996). Accordingly, the transients were reliably fitted with a monoeponential function, yielding membrane capacitance \( C_m = 4.3 \pm 0.1 \) pF (\( n = 29 \)), membrane resistance \( R_m = 2.7 \pm 0.3 \) \( \Omega \) (\( n = 29 \)), and series resistance \( R_s = 17.3 \pm 1.1 \) M\( \Omega \) (\( n = 29 \)). The −3-dB cell + electrode cut-off frequency was \( f_{3-dB} = (2\pi R_m C_m)^{-1} = 2.4 \pm 0.1 \) kHz (\( n = 29 \)). Accepted deviations of these parameters in current transients recorded over the time windows used for statistical analysis were <10%.

Data are reported as means ± mean squared error (MSE) and, unless otherwise indicated, statistical comparisons are done using Student’s t-test.

Identification of minimal IPSCs

At several central synapses, identification of minimal stimulation requires adopting indirect statistical methods like establishing a certain percentage of successful responses to stimulation, often preventing establishing an accurate estimate of release failure rate and release probability. The glomerular synapses in the cerebellum, by being composed of a very few (just two to four; Hámori and Somogyi 1983; Harvey and Napper 1991) contacts per granule cell, proves convenient in overcoming these difficulties. At the excitatory connection between mossy fibers and granule cells, the intensity of stimulation can be finely tuned by “counting” the discrete levels of the response amplitude and selecting the minimal one (Saviane and Silver 2006a,b; Sola et al. 2004). At the inhibitory connections investigated here, minimal eIPSCs were similarly obtained by finely tuning the stimulation intensity. In addition, minimal eIPSC amplitude was systematically compared with that of sIPSCs, which are due to the activation of single Golgi cell contacts. Therefore a post hoc criterion could be applied, in that minimal eIPSCs had to be statistically indistinguishable from sIPSCs; otherwise, the recording was not further considered for quantal analysis. An additional advantage was that of identifying stimulation failures from the absence of the slow spillover-dependent “indirect component,” thereby improving the confidence in release probability estimates and allowing application of the “failure method” (see Eq. 3 in the following text). Finally, the limited number of synaptic contacts and the favorable electrotonic properties of recordings allow establishing a reliable estimate of quantum properties from mIPSCs.

Binomial release statistics

The quantal parameters of release were obtained by applying to minimal eIPSCs three statistical methods corresponding to Eq. 1, Eq. 2, and Eq. 3 to improve the reliability of parameter estimation. These methods derive from binomial statistics and are only schematically described here, since they do not significantly differ from previous applications (for a comprehensive treatment see Clements 2003; Clements and Silver 2000; McLachlan 1978; Sola et al. 2004).

The quantal theory states that the mean number of quanta released at each impulse (\( m \), mean quantum content) depends on the number of releasing sites (\( n \)) and on the probability (\( p \)) that each quantum (\( q \), quantum size) is released, so that eIPSC variance (\( S^2 = SD^2 \)) and mean amplitude (\( M = mp \)) are related through a parabolic function and eIPSC variability depends on the number of released quanta. The contribution of intrinsic quantum variability can be accounted for by identifying intrasite (type I) and intersite (type II) sources. Intrasite variability (\( \sigma_{V,I} \)) reflects fluctuation in the number of open channels at single sites (\( s_{V,I} \)) and asynchrony in quantal delay at the eIPSC peak (\( \sigma_{V,op} \)). Intersite variability (\( \sigma_{V,J} \)) reflects differences among postsynaptic densities. Thus the total quantum variance at the eIPSC peak can be expressed as \( \sigma_{V,J}^2 = \sigma_{V,I}^2 + \sigma_{V,op}^2 + (\sigma_{V,ass} + \sigma_{V,J}^2) + \sigma_{V,J}^2 \). The variability of mIPSCs (\( \sigma_{V,J} \)) includes intrasite and intersite quantum variability, \( \sigma_{V,J}^2 = \sigma_{V,ass}^2 + \sigma_{V,J}^2 \). Since \( \sigma_{V,ass} \) can be assumed to be similar to \( \sigma_{V,I} \) (for details see
Clements 2003), we equally divided the two terms as $cv_{I-ss}^2/H_{11015}^2$ and $cv_{II}^2/H_{11005}^2$. The limits of this assumption were assessed by calculating the error introduced by setting $cv_{I-ss}^2$ at its extreme values (either $cv_{I-ss}^2/H_{11005}^0$ or $cv_{I-ss}^2/H_{11005}^0 cv_{q}^2$). The term $cv_{I-qd}$ was obtained by measuring the difference in variance associated with stimulus-aligned eIPSCs compared with onset-aligned quantal eIPSCs in low Ca$^{2+}/H_{11001}$ solutions (as proposed by Clements and Silver 2000). Thus $cv_{I}^2/H_{20850}^2/M_n(1atsu)$

$$S^2 = q_pM(1 + cv_{I}^2) - M_n^2$$ (1)

The model can also be applied without a knowledge of the whole variance/mean distribution (McLachlan 1978), since the parameters $p$ and $n$ can be calculated from the mean amplitude and coefficient of variation of eIPSCs ($M = mq_p$ and $CV = S/M$, where $S$ is eIPSC SD). In this model $m = np$, $SD^2 = np(1 - p)$, and the probability $p$ is

$${p} = 1 - \frac{M(CV^2)}{q_p(1 + cv_{I}^2)}$$ (2)

An estimate of $p$ could also be obtained from the failure rate ($N_f/N$; $N_f$ represents the failures out of $N$ responses), which does not explicitly depend on previous determinations of quantum properties except that the number of releasing sites needs to be calculated beforehand with Eq. 1 or Eq. 2

$$p = 1 - \left(\frac{N_f}{N}ight)^{1/n}$$ (3)

Although the three methods have different dependencies on experimental measurements and different intrinsic estimation errors (McLachian 1978), they yielded very similar parameter values (see Fig. 4B), thus supporting the reliability of $p$ estimates.

RESULTS

Herein we have investigated Golgi cell–granule cell neurotransmission and its modulation by GABA$\_B$ receptors in acute cerebellar slices (the synaptic organization of inhibi-
tion in the cerebellar glomerulus is summarized in Fig. 1A). Whole cell recordings were performed in the presence of glutamate receptor antagonists to block excitatory synaptic transmission.

The granule cells usually showed spontaneous activity (Fig. 1B) characterized by inhibitory synaptic currents occurring at an average frequency of 4.22 ± 0.81 Hz (n = 25). Most (>98%) of these events disappeared during application of 1 μM TTX, which suppresses spontaneous Golgi cell activity (Forti et al. 2006). Thus these events were most likely to represent unitary spontaneous current (sIPSC) generated at individual Golgi cell–granule cell connections. The rare TTX-insensitive spontaneous events (0.066 ± 0.008 Hz, n = 9, 1.6% of all the spontaneous events) were identified as miniature synaptic currents (mIPSCs). Electrical stimulation of the neuropile (Fig. 1C) evoked inhibitory synaptic currents (eIPSCs). Any synaptically related activities were abolished by 10 μM bicuculline and were therefore fully mediated by GABA_A receptors (n = 5; data not shown). These results confirm the absence of slow GABA_A receptor-mediated responses in granule cell inhibitory currents (Rossi et al. 2006; for different neurons: Dutar and Nicoll 1998; Misgeld et al. 1995; Nicoll 2004).

In the glomerulus, numerous synaptic terminals and granule cell dendrites are enwrapped in a glial sheet that limits neurotransmitter diffusion (Fig. 1A). This arrangement was previously shown to cause evident effects on GABA_A receptor-mediated neurotransmission: whereas sIPSCs and a fast transient component of eIPSCs proved dependent on synaptically related activities were abolished by 10 μM bicuculline and were therefore fully mediated by GABA_A receptors (n = 5; data not shown). These results confirm the absence of slow GABA_A receptor-mediated responses in granule cell inhibitory currents (Rossi et al. 2006; for different neurons: Dutar and Nicoll 1998; Misgeld et al. 1995; Nicoll 2004).

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Evidence for multiquantal evoked release

The glomerular synapse offers a convenient model for assessing the quantal properties of neurotransmission, since it allows easy identification of the eIPSCs generated at unitary synaptic connections, to distinguish transmission from stimulation failures and to directly estimate single quantum properties (see Methods). First, the amplitude of eIPSCs elicited by minimal stimulation was statistically indistinguishable from that of sIPSCs (in the same cells: 13.83 ± 0.95 vs. 15.94 ± 1.45 pA; ratio = 1.12 ± 0.07; n = 16; P = 0.2, paired t-test), consistent with an origin of both sIPSCs and minimal eIPSCs from unitary synaptic connections (Fig. 1C). This condition was assessed systematically: by increasing stimulus intensity, eIPSCs outranged sIPSCs (40.3 ± 4.9 pA, n = 13), indicating recruitment of additional connections (Hámori and Somogyi 1983; Harvey and Napper 1991). In continuation of this work, we will exclusively consider eIPSCs elicited by minimal stimulation and therefore presumably arising from single connections. Second, the occurrence of the indirect component in isolation allowed us to identify release failures at the site under investigation and to exclude potential stimulation failures (Fig. 1D; cf. DiGregorio et al. 2002; Sola et al. 2004). Third, the mIPSCs did not show indents in their rising phase (Fig. 2A), suggesting that they originated from release of single neurotransmitter quanta (e.g., see Cathala et al. 2003), whereas indents could often be observed in the sIPSCs and eIPSCs. The mIPSCs were thus used to estimate the quantum size (q = 8.47 ± 0.94 pA, n = 9) and coefficient of variation (cvq = 0.29 ± 0.12, n = 9).

The quantal nature of GABAergic neurotransmission at the glomerular synapse was further investigated by perfusing solutions containing variable Ca^{2+}/Mg^{2+} proportions (Dittman and Regehr 1996; Dodge Jr and Rahamimoff 1967; Katz and Miledi 1967). In normal extracellular calcium (2 mM Ca^{2+}), the eIPSCs showed pronounced trial-to-trial amplitude fluctuations with CV (0.61 ± 0.09, n = 6), which was significantly larger than cvq (P < 0.01, unpaired t-test, n = 6 and 9), indicating that eIPSCs were generated by release of multiple quanta (Fig. 2B). In low extracellular Ca^{2+} (0.5 mM Ca^{2+}), when release tends to occur at single releasing sites or to fail, eIPSC amplitude (10.58 ± 1.26, n = 6, failures excluded) was statistically indistinguishable from that of mIPSC (P = 0.19, unpaired t-test, n = 6 and 9) (Fig. 2C). The increase of

![Fig. 2](http://jn.physiology.org/DownloadedFrom/10.1152/jn.02062.2008)

**Fig. 2.** Quantal components of the inhibitory response. A: the mIPSCs (10 traces) show amplitude fluctuations. The average mIPSC is shown in gray and replotted in the next 2 panels. B: 2 single minimal eIPSCs show a marked indent in their rising phase. The indent overlaps with the average mIPSC. C: the amplitude of most eIPSCs recorded in 0.5 mM extracellular calcium ion concentration ([Ca^{2+}]) does not differ significantly from that of mIPSCs, although some of them (dashed traces with arrowheads) could be biquantal. The failure rate in this cell was 50%. The mIPSCs in A were obtained from a cell different from that in B and C. The arrows indicate the time of synaptic stimulation.
extracellular Ca\(^{2+}\) from 0.5 to 6 mM progressively increased the eIPSC mean amplitude (Fig. 3A). Moreover, the eIPSC amplitude distribution became broader and its mode moved to the right. Finally, the failures and CV were reduced (Fig. 3B). These observations indicate a mechanism in which GABA is released in multiple quanta during evoked neurotransmission at unitary synaptic connections and release probability is controlled by calcium (Edwards et al. 1990; Takahashi et al. 1998).

Statistical properties of Golgi cell–granule cell neurotransmission

The results shown in Figs. 1–3 suggest that eIPSC variability is caused both by intrinsic quantal variability and by the different number of quanta released from trial to trial. Information on quantal variability was provided by mIPSCs and mono-quantal eIPSCs in low Ca\(^{2+}\). The mIPSCs are generated at all synaptic sites impinging on the granule cell, so that their amplitude fluctuates because of variability originating within single sites (\(cv_{\text{ss}}\)) and across different sites (\(cv_1\)). Since no measures of \(cv_{\text{ss}}\) were available, we considered that \(cv_{\text{ss}}\) and \(cv_1\) equally contributed to the quantal variability \(cv_q\), so that \(cv_{\text{ss}} \approx cv_1 = 1/2cv_q = 0.043\) (Clements 2003; this assumption is tested in the following text). An additional factor that can affect the contribution of quanta to eIPSCs is the time scatter in quantal release observed in Fig. 2A. In mono-quantal eIPSCs, which are presumably generated at single sites of just one of the synapses impinging on the granule cell, the time scatter of evoked quantal release can be measured by comparing peak-aligned to stimulus-aligned eIPSCs (see METHODS). This scatter causes a reduction of the eIPSC peak by 17.5 ± 2.8% (\(n = 6\)) and allows us to estimate the variability of quantum delay (qd), \(cv_{\text{qd}}^2 = 0.03 \pm 0.04\) (\(n = 6\)). This term adds to the intrasite variance, which then becomes \(cv_q^2 = cv_{\text{ss}}^2 + cv_{\text{qd}}^2 \approx 1/2cv_q^2 + cv_{\text{qd}}^2 = 0.073\).

The eIPSC variability was investigated by constructing a variance/mean plot, in which eIPSC mean amplitude was changed by varying extracellular Ca\(^{2+}\) concentration (multiple-probability fluctuation analysis [MPFA]; Clements and Silver 2000; Silver 2003; Silver et al. 1996; Sola et al. 2004).

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all the methods yielded similar $p$ values, thus supporting the reliability of estimates (Fig. 4B).

The impact of the assumption that $c_{v_{1,ss}}^{2} = 1/c_{v_q}^{2}$ (Clements 2003) was evaluated by setting either $c_{v_{1,ss}} = 0$ or $c_{v_{1,ss}} = c_{v_q}$. With Eq. 1, the error introduced by setting $c_{v_{1,ss}} = 0$ was $+1\%$ for $q$ and $-1\%$ for both $p$ and $n$; the error introduced by setting $c_{v_{1,ss}} = c_{v_q}$ was $-7\%$ for $q$ and $<1\%$ for both $p$ and $n$. With Eq. 2, the error introduced by setting $c_{v_{1,ss}} = 0$ was $+15\%$ for $p$ and $-18\%$ for $n$; the error introduced by setting $c_{v_{1,ss}} = c_{v_q}$ was $-2\%$ for $p$ and $2\%$ for $n$. With Eq. 3, the dependence on $c_{v}$ is indirect and passes through the $n$ value estimated with Eq. 2. The error propagated from Eq. 2 to Eq. 3 was $17\%$, by setting $c_{v_{1,ss}} = 0$ and $-1.6\%$ by setting $c_{v_{1,ss}} = c_{v_q}$. Therefore, the assumption about single quantum variability introduced relatively little error on $p$, $n$, and $q$ estimates.

**Short-term plasticity during repetitive stimulation**

Golgi cells usually respond to punctuate stimulation with short high-frequency spike bursts (Rancz et al. 2007; Solinas et al. 2007a,b; Vos et al. 1999). To imitate native Golgi cell patterns, we tested the effect of five-impulse 100-Hz stimulus trains (Fig. 5).

In normal calcium (2 mM), the transient eIPSC component showed a clear depression, whereas the persistent component showed a pronounced buildup. In the transient component, $\text{PPR} = 0.50 \pm 0.09 (n = 8)$ was measured on the first two eIPSCs. In low calcium (0.5 mM), the initial depression turned into facilitation, yielding a PPR = 1.50 ± 0.35 ($n = 5$) on the first two eIPSCs. At both calcium concentrations, the eIPSC tended to similar and constant amplitude in the third and fourth responses. Thus as at other synapses (e.g., the neighboring mossy fiber–granule cell synapse; see Sola et al. 2004), facilitation and depression coexisted but, at normal calcium, depression prevailed.

The five neurons covered in this section are a subpopulation of the larger sample reported in Fig. 4. Consistent with the behavior reported in Fig. 4, release probability computed using Eq. 2 on the first eIPSC in the trains changed from $p = 0.42 \pm 0.06$ in 2 mM calcium to $p = 0.23 \pm 0.05$ in 0.5 mM calcium ($n = 5$). Moreover, the variance/mean points obtained for subsequent eIPSCs during the trains also fell along the parabolic trajectory obtained by changing $\text{Ca}^{2+}$ concentration (Fig. 4A), thus indicating that a progressive reduction in the number of released quanta was the main determinant of eIPSC short-term depression.

**Modulation of inhibitory neurotransmission by GABA_B receptors**

At various GABergic synapses, neurotransmitter release has been reported to undergo negative modulation through GABA_B autoreceptors (Dittman and Regehr 1996; Sakaba and Neher 2003; Takahashi et al. 1998). It is therefore possible that GABA_B receptors, which are expressed in Golgi cells (Kulik et al. 2002), also control GABA release from Golgi cell terminals. To investigate this hypothesis, we perfused the

![Figure 4](http://jn.physiology.org/...)

**FIG. 4.** Variance–mean plot and $p$ estimates. **A:** a variance–mean plot was constructed using minimal eIPSCs taken from 20 granule cells recorded with 1, 2, 4, and 6 mM $\text{Ca}^{2+}$, (open circles are single-cell values, filled circles are average values ± MSE, $n = 5$, corresponding to error introduced by setting $c_{v_{1,ss}} = 0$); the data were fitted with a parabolic function (Eq. 1, gray line; $q_o = 10.71 \pm 1.29$ pA, $n = 4.66 \pm 0.76$, cv = 0.073; $R^2 = 0.87$, $P < 0.05$, paired $t$-test). The points corresponding to 4 subsequent responses in a 100-Hz train (control, filled triangles; CGP55845, open triangles; mean ± MSE, $n = 5$, same cells as in Figs. 6 and 7) fall on the same parabolic trajectory. **B:** release probability estimated with 3 different methods (Eq. 1, open circles; Eq. 2, filled circles; Eq. 3, open triangles) at different values of $[\text{Ca}^{2+}]_o$. The 3 estimates were similar for all $[\text{Ca}^{2+}]_o$ values.

![Figure 5](http://jn.physiology.org/...)

**FIG. 5.** Short-term plasticity during repetitive synaptic stimulation. **A:** the traces show the eIPSCs elicited by short 100-Hz trains (averaging of 20 responses) taken at 0.5 mM $[\text{Ca}^{2+}]_o$ and 2 mM $[\text{Ca}^{2+}]_o$ from the same cell. Whereas short-term facilitation appears at 0.5 mM $[\text{Ca}^{2+}]_o$, the response shows short-term depression of peak amplitude at 2 mM $[\text{Ca}^{2+}]_o$. The inset shows exponential fitting to the decay of an IPSC in the train, which was needed to determine the baseline from which the amplitude of the subsequent IPSC could be measured. **B:** plot of normalized eIPSC peak amplitude (mean ± MSE) in the train for 8 experiments, like those shown in A (0.5 mM $[\text{Ca}^{2+}]_o$, $n = 5$; 2 mM $[\text{Ca}^{2+}]_o$, $n = 8$). The histogram represents the charge transfer during the trains. Despite different short-term dynamics, no relevant difference in charge transfer was observed between 0.5 mM $[\text{Ca}^{2+}]_o$ (white bar) and 2 mM $[\text{Ca}^{2+}]_o$ (black bar) ($P = 0.58$, unpaired $t$-test).
GABA<sub>B</sub> receptor blockage increases release probability

The fact that GABA<sub>B</sub> receptor blockage increased the first eIPSC and decreased PPR suggested that neurotransmitter release was enhanced. To investigate the mechanism of GCP55845 action, the PPR and CV changes were plotted versus the amplitude change (Fig. 6C). In both cases the data showed a significant negative correlation (for PPR, $R^2 = 0.91$, $P < 0.01$, paired r-test; for CV, $R^2 = 0.89$ $P < 0.01$, paired r-test), consistent with an increase in release probability as the major factor responsible for the GCP55845 effect. Consistently, in the $M_2/M_1$ vs. $(CV_2/CV_1)^{-2}$ plot, the experimental points fell above the diagonal, as expected from an increase in quantal release (Fig. 7A). The calculation of $p$ using Eq. 2 yielded an increase from $p = 0.42 \pm 0.08$ to $p = 0.67 \pm 0.09$ ($n = 5$, $P < 0.05$, paired t-test) (Fig. 7B).

The mechanistic relationship between GCP55845 action and a change in release probability was further supported by the similar location, in all these graphs (Figs. 6C and 7, A and B), of points obtained by increasing release probability with an increase in extracellular calcium. To this aim, a comparison is explicitly shown for transition from 0.5 to 2 mM calcium, since this matches the eIPSC amplitude variation observed after CGP perfusion, although qualitatively similar results were also obtained by using a change from 2 to 4 mM calcium (data not shown). The relationship between GCP55845 and calcium changes was further assessed by using variance/mean analysis. When the variance/mean points for subsequent eIPSC in the trains, showing no relevant difference between control (white bar) and GCP55845 (black bar) ($P = 0.88$, paired r-test), were used to fit an exponential trajectory (Eq. 1). This observation further supported the conclusion that the changes occurring with CGP55845 were caused by an increase in release probability (Fig. 7C; cf. e.g., Sola et al. 2004).

Consistent with an increased release probability, mIPSCs recorded in 3 μM TTX (Fig. 7D) significantly increased their frequency ($39.3 \pm 7.9\%$, $n = 6$; unpaired r-test, $P < 0.008$), although their amplitude remained unchanged ($0.6 \pm 2.8\%$, $n = 6$; unpaired r-test, $P = 0.37$).

Frequency dependence of GABA<sub>B</sub> receptor blockage and response buildup during repetitive stimulation

Since the trains used in these experiments were delivered every 10 s, the observed GCP55845 modulation on the first IPSC in the trains occurred at low frequency. However, when IPSC changes were measured in the last IPSC in the trains, almost no modulation was observed. This effect was remini-
cent of that reported at the parent synapse between mossy fibers and granule cells, which is regulated by GABA spillover from the neighboring Golgi cell terminals (Mitchell and Silver 2000b). We therefore reconstructed the frequency dependence of CGP55845 effects by reporting the percentage changes at frequencies between 0.1 and 100 Hz (Fig. 8). It turned out that CGP55845 had a much stronger effect at low frequency than that at high frequency, with a sharp roll-off around 10 Hz. Therefore these results indicate that GABAB receptor modulation is prevalent during low-frequency transmission.

As noted in Figs. 5A and 6A, high-frequency 100-Hz IPSC trains showed a pronounced buildup of the response (cf. Rossi et al. 2003). The effectiveness of this temporal summation was monitored through the charge transferred along the trains. No relevant effects of CGP55845 were observed on the total charge transfer (n = 5, P = 0.66, paired t-test; Fig. 6B). Likewise, the charge transfer was similar at both 0.5 and 2 mM calcium (n = 5, P = 0.58, paired t-test; Fig. 5B). Thus different from IPSC peak amplitude, the transferred charge was almost insensitive to GABAB receptor blockage and calcium changes. This result indicates that GABAB receptor regulation of release probability has the initial transient component of the response to a stimulus train as its specific target.

**DISCUSSION**

In this study we show that, in the juvenile rat cerebellum, GABAergic transmission at the Golgi cell–granule cell synapse is multiquantal. During repetitive stimulation, GABAergic transmission undergoes short-term depression of a fast transient component, whereas a slow protracted component shows a remarkable buildup due to temporal summation (Rossi and Haman 1998; Rossi et al. 2002). These observations conform to a mechanism of synaptic integration, in which α1 receptors determine a transient (direct) response to released GABA, whereas α6 receptors determine slow (indirect) spillover-mediated currents (Cherubini and Conti 2001; Farrant and Nusser 2005; Glykys and Mody 2007). The main finding is that release probability was tonically reduced by GABAB receptor activation. This regulation was typically expressed at low frequency (0.1–10 Hz) but became undetectable at high frequency (100 Hz). During high-frequency trains, GABAB receptor-mediated regulation was evident in the first IPSC but no longer so after a few impulses, either in the transient component or in the slow component. Thus ambient GABA in the glomerulus could act presynaptically on Golgi cell GABAB autoreceptors selectively regulating the onset of inhibition.
Quantal properties of neurotransmission at the Golgi cell–granule cell synapse

The Golgi cell–granule cell synapse conforms to a general model of multiquantal neurotransmission, in which IPSC amplitude fluctuations are mostly generated by the variable number of quantal events occurring at multiple releasing sites (Cherubini and Conti 2001; Edwards et al. 1990). Although at other inhibitory synapses mins could be multiquantal (Auger and Marty 2000; Auger et al. 1998; Glykys and Mody 2007), this is probably not the case at the Golgi cell–granule cell synapse, since mins have fast nonindented rising phases and coincide with the uniquantinal eIPSCs measured in low-calcium solutions. Moreover, with a quantum conductance of 214 pS, a single-channel conductance of 30 pS and an open probability of 0.6 (for a critical review on these parameters see Farrant and Nusser 2005), it can be estimated that around 10 GABAA receptors are in the vicinity of a quantum variances and this quantal release is large compared with the corresponding AMPA conductance of 0.6 nS. The single quantum conductance (214 pS) is relatively large compared with the resting whole cell conductance (~1,000 pS; Arentz et al. 2008; Armano et al. 2000; D’Angelo et al. 1995; Rancz et al. 2007), so that even a single quantum can determine a remarkable inhibitory effect contrasting the depolarization caused by excitatory synapses. The proposed synaptic arrangement and release statistics are similar to those of the neighboring mossy fiber–granule cell synapse (Saviane and Silver 2006a,b; Sola et al. 2004), indicating that the glomerular system can provide an appropriate excitatory/inhibitory balance to the granule cells.

Regulation of release probability by tonic GABAB receptor activation

GABAB receptors blockage increased release probability and, accordingly, increased the first eIPSC and accentuated short-term depression in eIPSC trains. Moreover, mins frequency (but not mins amplitude) also increased. A prediction from such presynaptic mechanism is that $p$ variations and associated IPSC amplitude changes should be larger with low initial release probability. Another prediction is that PPR and CV changes should be negatively correlated with IPSC amplitude variation. Actually, all these correlations proved statistically significant and points in the $[M_2/M_1$ vs. $(CV_1/CV_2)^{-2}]$ plot fell above the unitary diagonal, as expected from an increase in quantal release. The range of responses to GABAB receptor blockage may reflect regulation of ambient GABA levels (Farrant and Nusser 2005; Hamann et al. 2002) or variable efficiency of the transduction cascades involving GABA$\text{B}_\text{h}$ receptors.

The effects of the GABAB$\text{h}$ receptor antagonist CGP55845 were similar to those caused by increasing extracellular Ca$^{2+}$, consistent with the observation that CGP55845 preferentially inhibits presynaptic GABA$\text{B}_\text{h}$ receptors (Yamada et al. 1999; see also Chen and Yung 2005; Fearon et al. 2003; Jensen et al. 1999; Kaneda and Kita 2005; Than and Szabo 2002). Moreover, the action of CGP55845, by occurring in the first response in a train, reveals the blockage of GABA receptors that were tonically activated. The estimated ambient GABA level in the cerebellar glomerulus is $10^{-5}$–$10^{-6}$ M (Farrant and Nusser 2005) and can therefore activate presynaptic GABA receptors, which have EC$_{50}$ in the
same concentration range (Galvez et al. 2000). Given the strong analogy with the presynaptic effects of Ca\textsuperscript{2+}, it is possible that the \(p\) reduction caused by GABA\textsubscript{A} receptors involves G-protein–dependent inhibition of voltage-dependent calcium currents, as reported at other central synapses (Dittman and Regehr 1996; Takahashi et al. 1998). A slowing down of vesicle recycling (Sakaba and Neher 2003) might occur along the short trains used for synaptic stimulation in our recordings, thus explaining the absence of CGP modulation at high-frequency. Finally, it should be noted that the mechanism of presynaptic GABAergic regulation may not be the same at other brain synapses. For instance, at inhibitory synapses of the hippocampus, GABA receptors have been reported to exert their presynaptic effect independent from a change in release probability (Hefft et al. 2002).

In aggregate, these results indicate that GABA\textsubscript{B} receptors in Golgi cells (Kulik et al. 2002) are expressed in presynaptic terminals, where they are tonically activated by ambient GABA and negatively regulate neurotransmitter release. Although a tonic presynaptic inhibition of neurotransmitter release by GABA\textsubscript{B} receptor was also reported at other central synapses (Chen and Yung 2005; Fearon 2003; Jensen et al. 1999; Morishita and Sastry 1995; Mouginot et al. 1998; Porter and Nieves 2004), in the cerebellar glomerulus this mechanism would benefit from restricted neurotransmitter diffusion, which can also favor cross talk between glutamatergic and GABAergic terminals. In particular, metabotropic glutamate receptors can inhibit GABA release from Golgi cell terminals and GABA\textsubscript{B} receptors can inhibit glutamate release from mossy fiber (Mitchell and Silver 2000a,b). Moreover, GABA\textsubscript{B} receptors are also expressed postsynaptically, where they can regulate granule cell input resistance through a modulation of inward rectifier channels (Rossi et al. 2006). The frequency dependence of these processes is also peculiar, in that presynaptic GABA\textsubscript{B} receptor-mediated regulation at both excitatory and inhibitory terminals occurs at low frequency (when only tonic GABA levels are relevant), whereas that of granule cell conductance occurs at high frequency (therefore requiring GABA buildup during repetitive neurotransmission). Finally, it should be noted that ambient GABA levels have been reported to regulate granule cell input resistance (Brickley et al. 1996) and mossy fiber–granule cell gain (Mitchell and Silver 2003) through tonic activation of GABA\textsubscript{A} receptors. Thus presynaptic control by GABA\textsubscript{B} autoreceptors on Golgi cell terminals appears as a part of a more complex system based on ambient GABA levels and spillover suited to control multiple aspects of glomerular signal processing.

**Functional implications and potential impact on granular layer functions**

The rapid activation of IPSC is well suited to explain Golgi cell–granule cell inhibition reported in vivo in the adult cat (see Eccles et al. 1967). Presynaptic GABA\textsubscript{B} receptor-mediated control of release probability can be regarded as a mechanism to regulate the sharpness of the onset of inhibition. This effect is better conceived within the time-window hypothesis (D’Angelo and DeZeeuw 2008; Kistler and DeZeeuw 2003; Mapelli and D’Angelo 2007), which assumes that Golgi cell feedforward inhibition can limit the discharge of connected granule cells within about 5 ms. Low ambient GABA and GABA\textsubscript{B} receptor activation would sharply delimit the time window, during which granule cell spikes have the highest probability of being generated. Moreover, since the ambient GABA level is proportional to Golgi cells’ background activity (Rossi et al. 2003), these same granule cells will also have lower GABA\textsubscript{A} receptor-mediated noise and leakage (Brickley et al. 1996), resulting in higher precision and intensity of their response to mossy fiber activation. This temporally limited and precise discharge would then improve the pattern recognition process that is thought to take place in Purkinje cells (Brunel et al. 2004; Steuber et al. 2007). The impact of these time-filtering mechanisms needs to be further investigated using large-scale detailed network models and multisite and imaging recordings of network activity.

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**References**


