GABA_B Receptor Modulation of Voltage-Sensitive Calcium Channels in Spines and Dendrites

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Although primarily studied at the cell body, GABA_B receptors (GABA_BRs) are abundant at spines and dendrites of cortical pyramidal neurons, where they are positioned to influence both synaptic and dendritic function. Here, we examine how GABA_BRs modulate calcium (Ca) signals evoked by action potentials (APs) in spines and dendrites of layer 2/3 pyramidal neurons in mouse prefrontal cortex. We first use two-photon microscopy to show that GABA_BRs inhibit AP Ca signals throughout the entire dendritic arbor of these neurons. We then use local pharmacology and GABA uncaging to show that dendritic GABA_BRs also decrease the input resistance, shorten the AP afterdepolarization, and generate inhibitory postsynaptic potentials. However, we find that these electrophysiological effects recorded at the cell body do not correlate with the inhibition of AP Ca signals measured in spines and dendrites. Instead, we use voltage-clamp recordings to show that GABA_BRs directly inhibit several subtypes of voltage-sensitive calcium channels (VSCCs) in both spines and dendrites. Given the importance of VSCC-mediated Ca signals for neuronal function, our results have implications for the functional role of dendritic GABA_BRs in the prefrontal cortex and throughout the brain.

Introduction

GABA is the major inhibitory neurotransmitter in the cortex, activating both ionotropic and metabotropic receptors (Sivilotti and Nistri, 1991). GABA_B receptors (GABA_BRs) are G-protein-coupled receptors that modulate neuronal excitability (Bowerly et al., 2002). GABA_BRs are highly expressed in the prefrontal cortex (PFC) (Margeta-Mitrovic et al., 1999) and regulate higher cognitive function (Mott and Lewis, 1991). They are also therapeutic targets for neuropsychiatric disorders, including schizophrenia and epilepsy (Bettler et al., 2004).

Although GABA_BRs have been primarily studied at the cell body, they are also abundant at spines and dendrites (Kulik et al., 2003), which receive many inhibitory inputs (Beaulieu and Somogyi, 1990). GABA_BRs open potassium (K) channels (Sodickson and Bean, 1996; Lüscher et al., 1997), also found at spines and dendrites (Chen and Johnston, 2005; Kulik et al., 2006). Activating K channels increases membrane conductance and evokes slow IPSPs (sIPSPs) (Newberry and Nicoll, 1984). These effects can suppress postsynaptic responses (Morrisett et al., 1991; Otmakhova and Lisman, 2004) and limit action potential (AP) backpropagation (Buzsáki et al., 1996; Tsubokawa and Ross, 1996; Leung and Peloquin, 2006).

GABA_BRs also inhibit many voltage-sensitive calcium (Ca) channel (VSCC) subtypes (Mintz and Bean, 1993; Pfrieger et al.,

1994; Lambert and Wilson, 1996). VSCCs are the primary source of AP Ca signals in spines (Carter and Sabatini, 2004; Bloodgood and Sabatini, 2007) and dendrites (Magee and Johnston, 1995b; Markram et al., 1995). They also contribute to subthreshold synaptic responses (Magee et al., 1995; Bloodgood and Sabatini, 2007), suprathreshold dendritic spikes (Losonczy and Magee, 2006; Pérez-Garci et al., 2006; Larkum et al., 2007), and the induction of synaptic plasticity (Huang and Malenka, 1993; Zucker, 1999). By inhibiting spine and dendrite VSCCs, GABA_BRs may control many fundamental Ca-dependent neuronal processes.

The apical and basal dendrites of pyramidal neurons receive distinct inputs (Larkman, 1991; Petreanu et al., 2009) and have unique electrophysiological properties (Häusser et al., 2000; Spruston, 2008). In CA1 pyramidal neurons, GABA_BRs inhibit AP Ca signals in apical spines but not nearby dendrites or basal spines and dendrites (Sabatini and Svoboda, 2000). In cortical pyramidal neurons, this type of inhibition may prevent dendritic Ca spikes in the apical tufts (Pérez-Garci et al., 2006; Larkum et al., 2007). In layer 2/3 pyramidal neurons, GABA_BRs are also found in basal spines, where they strongly inhibit NMDA receptor Ca signals (Chalifoux and Carter, 2010). However, the extent to which GABA_BRs modulate AP Ca signals in spines and dendrites of these neurons remains unknown.

Here, we examine GABA_BR modulation in spines and dendrites of layer 2/3 pyramidal neurons in acute mouse PFC slices. We use two-photon microscopy to show widespread inhibition of AP Ca signals throughout the dendritic arbor. Although GABA_BRs also decrease the input resistance, shorten the AP afterdepolarization and generate sIPSPs, these effects do not cause the inhibition of AP Ca signals. Instead, we find direct inhibition of multiple VSCC subtypes in both spines and dendrites. Our results have important implications for the subcellular roles of GABA_BRs in the PFC and throughout the brain.

Received Aug. 31, 2010; revised Jan. 5, 2011; accepted Jan. 12, 2011.

This work was supported by National Institutes of Health (NIH) Grant F30MH087409 (J.R.C.) and The Klingenstein Fund and NIH Grant 1R01MH085974 (A.G.C.). We thank members of the Carter Laboratory for helpful discussions and comments on this manuscript.

The authors declare no competing financial interests.

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DOI:10.1523/JNEUROSCI.4561-10.2011

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Materials and Methods

Preparation. Recordings were made from layer 2/3 pyramidal neurons in the medial PFC of acute slices from male and female, postnatal day 21 (P21) to P28 C57BL/6 mice, as previously described (Chalifoux and Carter, 2010). Mice were anesthetized with a lethal dose of ketamine/xylazine and perfused intracardially with ice-cold external solution containing the following (in mM): 65 sucrose, 75 NaCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 25 glucose, 2.5 KCl, 1 CaCl₂, 5 MgCl₂, 0.4 Na-ascorbate, 3 Na-pyruvate (295–305 mOsm), bubbled with 95% O₂/5% CO₂. Coronal slices (300 μm thick) were cut in ice-cold external solution and transferred to artificial CSF (ACSF) containing the following (in mM): 119 NaCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 21 glucose, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 0.4 Na-ascorbate, 3 Na-pyruvate (295–305 mOsm), bubbled with 95% O₂/5% CO₂. After 30 min in ACSF at 35°C, slices were stored for ~30 min at 24°C, after which experiments were conducted at 32–34°C.

In all experiments, the ACSF contained 10 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide (NBQX), 10 μM 3-((*R*)-2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid [(*R*)-CPP], and 10 μM gabazine to block AMPARs, NMDARs, and GABA_ARs, respectively. In some experiments, GABA_BRs were activated by wash-in of 5 μM baclofen or blocked by wash-in of 2 μM (2*S*)-3-[[1*S*]-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl]-[phenylmethyl]phosphinic acid (CGP-55845). For GABA uncaging experiments, the ACSF contained 10 μM RuBi-GABA. For voltage-clamp experiments, the ACSF also contained 1 μM TTX to block Na channels. In some of these experiments, the ACSF contained 2 mM BaCl₂ to block K_{2P} channels or 3 mM CsCl₂ to block K_{ir} channels. The mixture of VSCC blockers consisted of 10 μM mibefradil, 20 μM nimodipine, 0.3 μM SNX-482, and 1 μM ω-conotoxin-MVIIIC. All chemicals were from Sigma-Aldrich or Tocris, with the exception of SNX-482 (Alomone Labs).

Physiology recordings. Whole-cell recordings were obtained from layer 2/3 pyramidal neurons identified with infrared–differential interference contrast at 200–300 μm from the pial surface. Borosilicate recording pipettes (2–4 MΩ) were filled with one of two internal solutions. Current-clamp recordings used the following (in mM): 135 K-gluconate, 7 KCl, 10 HEPES, 10 Na-phosphocreatine, 4 Mg₂-ATP, 0.4 Na-GTP, 290–295 mOsm, pH 7.35 with KOH. Voltage-clamp recordings used the following (in mM): 135 Cs-gluconate, 10 HEPES, 10 Na-phosphocreatine, 4 Mg₂-ATP, 0.4 Na-GTP, 290–295 mOsm, pH 7.35 with CsOH. Solutions contained 200 μM Fluo-5F to monitor Ca levels and 20 μM Alexa Fluor 594 to image neuronal morphology. Neurons were filled via the patch electrode for at least 15–20 min (for proximal regions; <75 μm) or 25–30 min (for distal regions; >75 μm) before imaging. Dye concentrations were chosen to ensure that Ca signals were in the linear range of the indicators (Carter and Sabatini, 2004).

Recordings were made using a MultiClamp 700B amplifier, filtered at 5 kHz for current-clamp recordings and 2 kHz for voltage-clamp recordings, and sampled at 10 kHz. For current-clamp recordings, APs were triggered with brief (2 ms) current injections (1500–3000 pA) through the recording pipette. Input resistance (*R*_{in}) and series resistance (*R*_s) were calculated using long (300 ms) hyperpolarizing current injections (−30 pA). After wash-in of 5 μM baclofen, to counteract a small somatic hyperpolarization (−3.5 ± 2 mV; *n* = 5 cells), cells were held at −70 mV by injecting a small positive current (<80 pA), which was necessary to use the same current injection for triggering action potentials. In control experiments, we found that 5 μM baclofen inhibited the [Ca]_{bAP} to the same degree in spines and dendrites when the soma was allowed to hyperpolarize (supplemental Fig. S1, available at www.jneurosci.org as supplemental material).

For baclofen puffing, a glass pipette with a 2–4 μm tip was bent using a microforge (Narishige) to allow focal application of baclofen at different depths of the slice without disturbing the whole-cell recording. The puffing pipette was filled with ACSF containing the following (in μM): 10 NBQX, 10 (*R*)-CPP, 10 gabazine, and 15 baclofen and was pressure-ejected (5–15 psi) using a Picospritzer. In separate experiments, tip size and pressure were empirically determined using ACSF with 20 μM Alexa 488 to visualize the

spread of the ejected ACSF (see Fig. 4). The tip of the puffing pipette was placed 5–10 μm from the imaged spine–dendrite pair.

In voltage-clamp recordings, cells were held at −70 mV and VSCCs were opened by steps to +10 mV. The step duration ranged from 3 to 6 ms and was adjusted to produce baseline Ca signals similar to those evoked by action potentials in current-clamp recordings. Spine–dendrite pairs were chosen within 50 μm from the soma to minimize voltage-clamp errors. *R*_{in} and *R*_s were calculated using a 300 ms, 10 mV hyperpolarizing voltage step. For voltage-clamp recordings, data was discarded if the *R*_s changed by >15%.

For VSCC experiments, the selective blocker and then baclofen were sequentially added while recording from the same spine–dendrite pair. In control experiments, ACSF was substituted for the VSCC blocker. Drugs were allowed to wash-in for 5 min before acquiring data. For each recording condition, a total of 10 APs were collected at 0.07 Hz.

To predict the amount of modulation in the presence of blockers of VSCCs, we used the following:

$$\lambda = 100 - \left(\frac{\Delta_{\text{bac}} - \theta \Delta_{\text{VSCC}}}{100 - \Delta_{\text{VSCC}}} \right), \text{ where } (\Delta_{\text{bac}} - \theta \Delta_{\text{VSCC}}) \geq \Delta_r \quad (1)$$

where λ represents percentage of [Ca]_{step} remaining after inhibition by baclofen in the presence of a selective VSCC blocker; Δ_{bac} , percentage of [Ca]_{step} inhibited by baclofen without the VSCC blocker; θ , fraction of the VSCC subtype that is inhibited by baclofen (0–1); Δ_{VSCC} , percentage of [Ca]_{step} eliminated by the VSCC blocker; Δ_r , percentage of [Ca]_{step} contributed by SNX-resistant R-type VSCCs. The numerator, $(\Delta_{\text{bac}} - \theta \Delta_{\text{VSCC}})$, represents the remaining percentage of VSCCs that can be modulated by baclofen. Because we cannot block the SNX-resistant R-type VSCCs, this numerator will never be less than Δ_r . The denominator, $(1 - \Delta_{\text{VSCC}})$, represents the percentage of remaining VSCCs after addition of the selective VSCC blocker. Full block of a channel subtype by baclofen ($\theta = 0.9$ –1) and no modulation of a channel subtype ($\theta = 0$ –0.1) were calculated and plotted as gray boxes in Figure 8.

Two-photon microscopy. Intracellular Ca imaging was performed with a custom microscope, as previously described (Carter and Sabatini, 2004; Carter et al., 2007). For two-photon laser-scanning microscopy (2PLSM), 810 nm light was used to excite Fluo-5F (green) and Alexa Fluor 594 (red), to monitor Ca signals and spine morphology, respectively. Line scans with current injection were interleaved with line scans with no current injection to detect any photobleaching. Reference frame scans were taken between each acquisition to correct for small spatial drift of the preparation over time. Baseline fluorescence was monitored and recordings were discarded if an increase was detected, which would indicate photodamage.

To quantify Ca signals, green and red fluorescence were collected during 500 Hz line scans across a spine–dendrite pair. Ca signals were quantified as changes in green fluorescence to red fluorescence ($\Delta G/R$), normalized to the maximal green fluorescence to red fluorescence (G_{sat}/R), giving $\Delta G/G_{\text{sat}}$. The value of G_{sat}/R was measured after each recording using a thin-walled pipette, containing the internal solution with a saturating concentration of Ca, which was positioned directly above the recorded cell and used at the same recording temperature (32–34°C). To create an internal solution with saturating Ca, 1 M CaCl₂ was added at 1:25 to the internal.

GABA uncaging. The back focal plane of the objective was filled with collimated 473 nm light from a DPSS laser (Laserglow Technologies) using a fiber optic cable (Thorlabs), planoconvex lenses (Edmund Optics), and a dichroic mirror (Chroma). Laser power was modulated by the variable current power supply and pulse duration was controlled with a TTL trigger. A fast shutter (Uniblitz) was placed before the photomultiplier tubes to protect them from the uncaging laser light while imaging. A telescope using two planoconvex lenses was used to focus the collected fluorescence through the shutter aperture.

Uncaging of 10 μM RuBi-GABA was evoked using 10–13 mW laser power for 10 ms on spines <25 μm from the surface of the slice. This power and duration was chosen because it elicited reliable sIPSPs at the

Figure 1. AP Ca signals in all dendritic compartments. **A**, 2PLSM image stack of a layer 2/3 pyramidal neuron, with different dendritic domains labeled. The inset shows a line scan (yellow dashed line) through the spine (S) and dendrite (D). **B**, Top, Current-clamp recording with brief current injection (arrow) to elicit an AP. Middle, A 500 Hz line scan through the spine (S) and dendrite (D) shows a green AP Ca signal ($[Ca]_{bAP}$). Bottom, $[Ca]_{bAP}$ quantified as $\Delta G/G_{sat}$ in spine (red) and dendrite (black). **C**, $[Ca]_{bAP}$ amplitude is calculated over a 20 ms window after the AP (gray box). **C**, Average $[Ca]_{bAP}$ in baseline conditions (red) and after wash-in of the VSCC mixture (black) in spines (left) and dendrites (right). SEM is represented by the shaded regions for baseline (pink) and after wash-in of the VSCC mixture (gray). **D**, $[Ca]_{bAP}$ amplitude versus radial distance from the soma for spines (red) and dendrites (black) in apical (left) and basal (right) dendrites. $[Ca]_{bAP}$ amplitude versus radial distance for spines (red line) and dendrites (black line) was fit with a single exponential.

soma and did not cause photodamage in the spine or dendrite after repeated uncaging (detected by increases in baseline fluorescence). In Figure 5, sIPSPs were measured at the soma as the uncaging point was moved laterally away from the soma. Because of radial branching of the basal dendrites, it was not possible to completely avoid uncaging on dendritic branches. In Figure 6, test trials consisted of an uncaging pulse followed with a 200 ms delay by a somatically evoked AP. These were interleaved with control trials with no uncaging pulse for comparison with baseline. Additional trials with no AP were used to subtract out any light-evoked phosphorescence and to obtain a stable baseline.

Data acquisition and analysis. Image and physiology data were acquired using National Instruments boards and custom software written

in Matlab (The MathWorks). Off-line analysis was performed using custom routines written in Igor Pro (Wavemetrics). Ca signal amplitudes were calculated over a 20 ms window, starting after the end of the stimulus (102–122 ms). sIPSP amplitudes were calculated over a 10 ms window around the peak. Two-photon images were treated with a 1.5 pixel radius Gaussian filter for display purposes. Average traces in Figure 8C were low-pass filtered for display purposes.

Electrophysiological and imaging data are reported in the text as median $\pm$ SE. The SE was calculated as the SD of the medians computed from 10,000 bootstrapped samples from the data. Traces in figures are the arithmetic mean $\pm$ SEM. There were occasionally small differences between the median and mean within a group, which explains some discrepancies between traces in figures and medians in the text and supplemental Table S1 (available at www.jneurosci.org as supplemental material). Summary data in figures is in box plot form, showing the median, interquartile range, 10–90% range (whiskers), and including the data from individual experiments (open circles). Significance was defined as $p < 0.05$ (*) and determined using the nonparametric two-tailed Wilcoxon–Mann–Whitney two-sample rank test or the two-tailed Wilcoxon signed rank test for paired data (when appropriate), neither of which make assumptions about the data distribution. Correlations are shown in scatter plots as straight lines and were calculated using a linear regression. These correlations are reported in figures as Spearman's rank correlation coefficient (ρ), which makes no assumption about distributions, but differed slightly in some cases from the linear regression (see Figs. 3, 4, 6).

Results

We used whole-cell recordings and two-photon imaging to investigate subcellular GABA_BR modulation in layer 2/3 pyramidal neurons of acute slices of mouse PFC (Fig. 1A). We found that somatic APs backpropagated throughout the dendritic arbor and triggered Ca signals ($[Ca]_{bAP}$) in spines and dendrites (Fig. 1B). $[Ca]_{bAP}$ was mostly blocked by a mixture of VSCC blockers, indicating they were attributable to the opening of VSCCs (see Materials and Methods) ($p = 0.008$; $n = 4$ cells/8 spine–dendrite pairs) (Fig. 1C). The amplitude of $[Ca]_{bAP}$ in spines and dendrites decreased exponentially away from the soma, with similar length constants in apical dendrites (177 μ m; $n = 160$) and basal dendrites (180 μ m; $n = 155$) (Fig. 1D). In contrast to previous findings in the main apical dendrite of layer 2/3 pyramidal neurons in somatosensory cortex (Svoboda et al., 1997; Waters et al., 2003), we do not observe a peak dendritic Ca signal at 100 μ m from the soma. Although signals were smaller in the distal dendrites, the ability to record $[Ca]_{bAP}$ throughout the dendrites allowed us to compare GABA_BR modulation in different subcellular compartments.

Apical dendrites

Previous results in CA1 pyramidal neurons indicate that GABA_BRs modulate $[Ca]_{bAP}$ only in apical oblique spines but not dendrites (Sabatini and Svoboda, 2000). We found that bath application of 5 μ M baclofen, a selective activator of GABA_BRs, decreased $[Ca]_{bAP}$ in apical oblique spines ($p = 0.007$; $n = 8/16$) (Fig. 2*A,B*) (radial distance: median, 48 μ m; range, 20–91 μ m) (for full quantification of Ca imaging data, see supplemental Table S1, available at www.jneurosci.org as supplemental material). In contrast, wash-in of ACSF as a vehicle control had no effect on these signals ($p = 0.85$, $n = 4/8$; baclofen vs ACSF, $p = 0.001$). However, baclofen also decreased $[Ca]_{bAP}$ in apical oblique dendrites ($p = 0.0005$), whereas ACSF again had no effect ($p = 0.20$; baclofen vs ACSF, $p = 0.0001$). Modulation was not significantly different between spines and dendrites in these or any of the following experiments. Thus, in contrast to CA1 pyramidal neurons, GABA_BRs inhibit $[Ca]_{bAP}$ in apical oblique spines and dendrites of layer 2/3 pyramidal neurons.

The main apical dendrite emerges from the cell body, projects to layer 1, and also receives excitatory input onto dendritic spines (Larkman, 1991). Similar to the apical obliques, we found that baclofen significantly decreased $[Ca]_{bAP}$ in main apical spines ($p = 0.005$, $n = 7/11$; ACSF, $p = 0.27$, $n = 8/12$; baclofen vs ACSF, $p = 0.02$) and dendrites ($p = 0.001$; ACSF, $p = 0.02$; baclofen vs ACSF, $p = 0.0001$) (Fig. 2*B*; supplemental Fig. S1, available at www.jneurosci.org as supplemental material) (radial distance: median, 73 μ m; range, 17–125 μ m). The small but significant decrease in the dendritic $[Ca]_{bAP}$ during ACSF controls may reflect gradual washout of cellular factors and was not seen in other compartments.

The apical tuft is distal to the bifurcation of the main apical dendrite and receives a distinct complement of synaptic inputs (Petreanu et al., 2009). Previous work indicates that distal GABA_BRs can inhibit the generation of dendritic Ca spikes (Pérez-García et al., 2006), but it is unknown whether the inhibition occurs in spines, dendrites, or both compartments. We found that baclofen significantly reduced $[Ca]_{bAP}$ in apical tuft spines ($p = 0.03$, $n = 7/14$; ACSF, $p = 0.49$, $n = 9/17$; baclofen vs ACSF, $p = 0.03$) and dendrites ($p = 0.0005$; ACSF, $p = 0.49$; baclofen vs ACSF, $p = 0.006$) (Fig. 2*B*; supplemental Fig. S1, available at www.jneurosci.org as supplemental material) (radial distance: median, 128 μ m; range, 83–211 μ m). Together, our results indicate that GABA_BR modulation of $[Ca]_{bAP}$ is widespread across apical spines and dendrites of layer 2/3 pyramidal neurons.

Figure 2. Modulation of AP Ca signals in apical and basal spines and dendrites. *A*, Average $[Ca]_{bAP}$ for apical oblique spines (left) and dendrites (right) in baseline conditions (red) and after wash-in of 5 μ M baclofen (black). *B*, Summary of changes in $[Ca]_{bAP}$ amplitude after wash-in of ACSF (blue) or 5 μ M baclofen (green) in apical oblique, apical main, and apical tuft spines and dendrites. The box plots show median, interquartile range, and 10–90% range. The circles indicate individual experiments. The asterisks indicate significant ($p < 0.05$) difference from 100% or between ACSF and 5 μ M baclofen. *C*, Average $[Ca]_{bAP}$ for proximal (<75 μ m) basal spines (left) and dendrites (right) in baseline conditions (red) and after wash-in of 5 μ M baclofen (black). *D*, Summary of changes in $[Ca]_{bAP}$ amplitude after wash-in of ACSF (blue) or 5 μ M baclofen (green) in proximal and distal basal spines and dendrites. The asterisks indicate significant ($p < 0.05$) difference from 100% or between ACSF and 5 μ M baclofen.

Basal dendrites

The basal dendrites of pyramidal neurons receive the majority of synaptic contacts (Larkman, 1991) and may integrate inputs differently than apical dendrites (Schiller et al., 2000). Previous studies in CA1 pyramidal neurons show that GABA_BRs do not modulate $[Ca]_{bAP}$ in basal spines or dendrites (Sabatini and Svoboda, 2000). We initially recorded $[Ca]_{bAP}$ in basal spines and dendrites proximal to the cell body (radial distance: median, 43 μ m; range, 20–75 μ m). We found that bath application of 5 μ M baclofen significantly decreased $[Ca]_{bAP}$ in proximal basal spines ($p = 0.0002$, $n = 5/10$; ACSF, $p = 0.70$, $n = 7/14$;

baclofen vs ACSF, $p = 0.0001$) and dendrites ($p = 0.0001$; ACSF, $p = 0.06$; baclofen vs ACSF, $p = 0.0002$) (Fig. 2C,D). These results contrast with CA1 pyramidal neurons and indicate that GABA_BR modulation of $[Ca]_{bAP}$ is not restricted to apical compartments.

The distal basal dendrites have different properties of synaptic plasticity and integration compared with proximal regions (Schiller et al., 2000; Gordon et al., 2006; Major et al., 2008). We found that baclofen also decreased $[Ca]_{bAP}$ in distal basal spines ($p = 0.003$, $n = 8/15$; ACSF, $p = 0.77$, $n = 7/14$; baclofen vs ACSF, $p = 0.02$) and dendrites ($p = 0.0005$; ACSF, $p = 0.85$; baclofen vs ACSF, $p = 0.001$) (Fig. 2D; supplemental Fig. S1, available at www.jneurosci.org as supplemental material) (radial distance; median, 130 μm ; range, 99–150 μm). Together, these findings show that GABA_BR activation decreases $[Ca]_{bAP}$ in spines and dendrites across the entire dendritic arbor of layer 2/3 pyramidal neurons.

Modulation of excitability

GABA_BRs often activate inwardly rectifying K channels (Gähwiler and Brown, 1985; Sodickson and Bean, 1996; Lüscher et al., 1997) found in the spines and dendrites of pyramidal neurons (Takigawa and Alzheimer, 1999; Chen and Johnston, 2005; Kulik et al., 2006). Opening these channels can generate a shunting conductance that changes passive membrane properties. We found that bath application of 5 μM baclofen decreased the input resistance (R_{in}) recorded at the soma ($p = 1 \times 10^{-11}$, $n = 41$ cells; ACSF, $p = 0.98$, $n = 34$ cells; baclofen vs ACSF, $p = 1 \times 10^{-10}$) (Fig. 3A,B). Baclofen also decreased the width at 10% amplitude of the AP (width_{10%}), which is a measure of the afterdepolarization (ADP) ($p = 9 \times 10^{-12}$; ACSF, $p = 0.03$; baclofen vs ACSF, $p = 2 \times 10^{-10}$) (Fig. 3A,B). However, baclofen had only a small effect on AP amplitude, which was not significant compared with ACSF controls ($p = 0.03$; ACSF, $p = 0.90$; baclofen vs ACSF, $p = 0.06$) (Fig. 3B). Moreover, baclofen had no effect on AP half-width (width_{50%}) ($p = 0.49$; ACSF, $p = 0.30$; baclofen vs ACSF, $p = 0.76$) (Fig. 3B). The change in ADP positively correlated with the change in R_{in} ($p < 0.01$) (Fig. 3C), suggesting that changes in the AP waveform after GABA_BR activation reflect changes in passive membrane properties (Fernandez and White, 2009).

By changing the somatic R_{in} and ADP, GABA_BRs could influence backpropagation of APs into the dendrites and consequently attenuate $[Ca]_{bAP}$ (Buzsáki et al., 1996; Tsubokawa and Ross, 1996; Leung and Peloquin, 2006). However, we observed no correlation between the change in either spine or dendrite $[Ca]_{bAP}$ and the change in R_{in} after baclofen (spine, $p > 0.05$; dendrite, $p > 0.05$; $n = 80$ spine–dendrite pairs) (Fig. 3D). Similarly, we observed no correlation between the change in spine or dendrite $[Ca]_{bAP}$ and the change in ADP after baclofen (spine, $p > 0.05$; dendrite, $p > 0.05$) (Fig. 3D). These findings suggest that modulation in spines and dendrites is not attributable to changes in either passive membrane properties or AP waveform.

Local modulation

The lack of correlation between changes in $[Ca]_{bAP}$ and either R_{in} or ADP suggests that GABA_BR modulation may occur locally in spines and dendrites. We next tested this possibility by puffing 15 μM baclofen from a pipette placed adjacent ($\Delta x = 5$ –10 μm) to either apical tuft or distal basal dendrites (see Materials and Methods) (Fig. 4A). We found that puffing baclofen continued to decrease $[Ca]_{bAP}$ in apical tuft spines

Figure 3. Modulation of excitability. **A**, Left, Average hyperpolarization in response to a -30 pA current injection for 300 ms in baseline conditions (red) and after wash-in of 5 μM baclofen (black). Right, Average AP elicited by brief current injection in baseline conditions (red) and after wash-in of 5 μM baclofen (black). The ADP was measured by taking the width at 10% amplitude (width_{10%}) (dashed blue line). **B**, Summary of changes in input resistance (R_{in}), AP amplitude (Amp), half-width (width_{50%}), and width_{10%} after wash-in of ACSF (blue) or 5 μM baclofen (green). The asterisks indicate significant ($p < 0.05$) difference from 100% or between ACSF and 5 μM baclofen. **C**, Positive correlation between width_{10%} and R_{in} for individual cells (circles) in baseline conditions (red) and after wash-in of 5 μM baclofen (black). The data were fit with a linear regression (blue line) with a Spearman's correlation coefficient (ρ) of 0.85. **D**, Left, No correlation between the change in $[Ca]_{bAP}$ and the change in R_{in} in either spines (red) or dendrites (black). Right, No correlation between the change in $[Ca]_{bAP}$ and the change in width_{10%} in either spines (red) or dendrites (black).

($p = 0.004$, $n = 5/10$; ACSF, $p = 0.57$, $n = 6/11$; baclofen vs ACSF, $p = 0.0002$) and dendrites ($p = 0.008$; ACSF, $p = 0.04$; baclofen vs ACSF, $p = 0.008$) (Fig. 4B,C) (radial distance; median, 153 μm ; range, 95–200 μm). Similar results were found in distal basal spines ($p = 0.0002$, $n = 10/15$; ACSF, $p = 0.53$, $n = 7/16$; baclofen vs ACSF, $p = 0.04$) and dendrites ($p = 0.0002$; ACSF, $p = 0.98$; baclofen vs ACSF, $p = 0.0001$) (Fig. 4B,C) (radial distance; median, 113 μm , range, 90–142 μm). These results indicate that GABA_BR modulation occurs locally in distal spines and dendrites and is unlikely attributable to failure of AP backpropagation.

In these puffing experiments, we found that local application of baclofen also decreased R_{in} and ADP recorded at the soma (Fig. 4D,E; supplemental Fig. S2, available at www.jneurosci.org as supplemental material). These findings are consistent with the dendritic localization of inwardly rectifying K channels (Takigawa and Alzheimer, 1999; Chen and Johnston, 2005; Kulik et al., 2006). Interestingly, the changes in R_{in} were larger for the apical dendrites (apical, $n = 5$ cells/5 dendrites; basal, $n = 10$ cells/10

dendrites; apical vs basal, $p = 0.004$) (Fig. 4D; supplemental Fig. S2, available at www.jneurosci.org as supplemental material). A similar difference was found for changes in ADP (apical vs basal, $p = 0.04$) (Fig. 4E; supplemental Fig. S2, available at www.jneurosci.org as supplemental material). These findings suggest that the apical dendrites may have more GABA_BRs, more K channels, greater coupling between the two, or greater electrical access to the soma. However, there was again no correlation between the change in $[Ca]_{bAP}$ and the change in either R_{in} (spine, $p > 0.05$; dendrite, $p > 0.05$; $n = 25$ spine–dendrite pairs) (Fig. 4D) or ADP (spine, $p > 0.05$; dendrite, $p > 0.05$; $n = 25$ spine–dendrite pairs) (Fig. 4E). Together, these results suggest a minor role for dendritic GABA_BR-activated K channels in local inhibition of $[Ca]_{bAP}$.

GABA uncaging

In our previous experiments, we examined GABA_BR modulation of $[Ca]_{bAP}$ in response to tonic application of baclofen. We next used GABA uncaging to determine whether GABA_BR modulation occurs in response to a brief exposure of the endogenous neurotransmitter. The compound RuBi-GABA is excited at blue wavelengths and has previously been used to study GABA_AR activation (Rial Verde et al., 2008). When fast neurotransmission was blocked, uncaging of 10 μ M RuBi-GABA with a spot of 473 nm light centered at the soma produced a sIPSP that peaked at 146 ± 13 ms after the stimulus ($n = 5$ cells) (Fig. 5A) (see Materials and Methods). This sIPSP decreased in amplitude with a length constant of 34 μ m as the uncaging spot was moved laterally away from the soma ($n = 4$ cells) (Fig. 5B) and was blocked by wash-in of 2 μ M CGP-55845, a selective GABA_BR antagonist ($p = 0.03$; $n = 5$ cells) (Fig. 5C). In control experiments, we confirmed that bath application of baclofen continued to inhibit $[Ca]_{bAP}$ in spines and dendrites (supplemental Fig. S3, available at www.jneurosci.org as supplemental material), indicating that RuBi-GABA does not interfere with GABA_BR modulation. These findings show that GABA uncaging is a useful approach for studying transient GABA_BR modulation in spines and dendrites.

To examine uncaging-evoked GABA_BR modulation, we centered the laser spot on the imaged spines and dendrites, which we briefly illuminated for 10 ms, and evoked an action potential after a 200 ms delay (see Materials and Methods). We found that GABA uncaging reduced $[Ca]_{bAP}$ in apical oblique

Figure 4. Local modulation of AP Ca signals and excitability. **A**, 2PLSM image stack of a layer 2/3 pyramidal neuron with pressure ejection of Alexa 488 (green) from a bent glass pipette (asterisk) positioned adjacent to a dendritic segment in the apical tuft. **B**, Average $[Ca]_{bAP}$ for the apical tuft (top) and distal basal (bottom) spines (left) and dendrites (right) in baseline conditions (red) and after puffing of 15 μ M baclofen (black). **C**, Summary of changes in $[Ca]_{bAP}$ amplitude after puffing of ACSF (blue) or 15 μ M baclofen (green) in apical tuft and distal basal spines and dendrites. **D**, Top, Summary of changes in R_{in} after puffing of ACSF or 15 μ M baclofen in apical and basal dendrites. Bottom, No correlation between the change in R_{in} and the change in $[Ca]_{bAP}$ after puffing of baclofen in either spines (red) or dendrites (black). **E**, Top, Summary of changes in width_{10%} after puffing of ACSF or 15 μ M baclofen in apical and basal dendrites. The asterisks indicate significant ($p < 0.05$) difference from 100% or between ACSF and 15 μ M baclofen. Bottom, No correlation between the change in $[Ca]_{bAP}$ and the change in width_{10%} after puffing of baclofen in either spines (red) or dendrites (black).

spines ($p = 0.003$; $n = 9/16$) and dendrites ($p = 3 \times 10^{-5}$) (Fig. 6A,B). This effect was prevented by 2 μ M CGP-55845, confirming that it was GABA_BR mediated (spine, $p = 0.11$, $n = 7/13$; ACSF vs CGP, $p = 0.002$; dendrite, $p = 0.31$; ACSF vs CGP, $p = 4 \times 10^{-5}$). Similar results were found in basal spines ($p = 0.03$, $n = 8/12$; CGP, $p = 0.16$, $n = 4/14$; ACSF vs CGP, $p = 0.01$) and dendrites

Figure 5. GABA uncaging. **A**, Left, 2PLSM image stack of a layer 2/3 pyramidal neuron, displaying uncaging locations (asterisks) at the soma and at 10 μm intervals away. Right, Uncaging-evoked sIPSPs evoked at 10 μm intervals away from the soma. sIPSPs are color-coded to corresponding uncaging locations in 2PLSM image. **B**, sIPSP amplitude normalized to the response at the soma, as a function of distance from the soma. The vertical bars are the SEM. Points were fit with a single exponential (blue dashed line). **C**, Average sIPSP evoked by uncaging at the soma in baseline conditions (red) and after wash-in of 2 μM CGP-55845 (black).

($p = 0.001$; CGP, $p = 0.22$; ACSF vs CGP, $p = 0.01$) (Fig. 6*A,B*). These results demonstrate that GABA_BR inhibition of $[\text{Ca}]_{\text{bAP}}$ can occur in response to a short exposure of GABA at spines and dendrites.

Similar to the changes in R_{in} and ADP found in our puffing experiments, we observed larger sIPSPs when uncaging in apical versus basal dendrites (apical, $n = 9$ cells, 16 dendrites; basal, $n = 8$ cells, 12 dendrites; apical vs basal, $p = 0.02$) (Fig. 6*C*; supplemental Fig. S3, available at www.jneurosci.org as supplemental material). These results support the idea that apical GABA_BR have a greater impact on somatic membrane potential. However, we also observed no correlation between the change in $[\text{Ca}]_{\text{bAP}}$ and the amplitude of the sIPSP measured at the soma (spine, $p > 0.05$; dendrite, $p > 0.05$; $n = 28$ spine–dendrite pairs) (Fig. 6*D*). Together, these results further suggest that GABA_BR modulation of K channels plays a limited role in the inhibition of $[\text{Ca}]_{\text{bAP}}$.

Direct modulation

The lack of correlation between K channel activation and changes in $[\text{Ca}]_{\text{bAP}}$ suggest that GABA_BR activation directly modulates VSCCs in spines and dendrites. We next performed voltage-clamp recordings to test this idea, using internal cesium (Cs) to block K channels and external TTX to block Na channels (see Materials and Methods) (Sabatini et al., 2002). Ca signals were elicited by brief steps from -70 to $+10$ mV and measured in apical oblique and proximal basal dendrites (Fig. 7*A*) (see Materials and Methods). These step-evoked Ca signals ($[\text{Ca}]_{\text{step}}$) had similar amplitudes and kinetics to $[\text{Ca}]_{\text{bAP}}$ recorded in current clamp and allowed us to explore VSCC

modulation without confounding effects caused by K channel activation.

In voltage-clamp recordings, we found that wash-in of 5 μM baclofen reduced $[\text{Ca}]_{\text{step}}$ in apical oblique spines ($p = 0.004$, $n = 7/9$; ACSF, $p = 1$, $n = 5/6$; baclofen vs ACSF, $p = 0.004$) and dendrites ($p = 0.002$; ACSF, $p = 1$; baclofen vs ACSF, $p = 0.005$) (Fig. 7*A,B*). Baclofen also inhibited $[\text{Ca}]_{\text{step}}$ in proximal basal spines ($p = 0.002$, $n = 10/10$; ACSF, $p = 0.7$, $n = 18/18$; baclofen vs ACSF, $p = 4 \times 10^{-4}$) and dendrites ($p = 0.002$; ACSF, $p = 0.06$; baclofen vs ACSF, $p = 0.005$) (Fig. 7*A,B*). Internal Cs blocked most of the change in R_{in} after baclofen ($p = 1 \times 10^{-4}$, $n = 17$ cells; ACSF, $p = 1$, $n = 19$ cells; baclofen vs ACSF, $p = 0.02$), and the remaining current was inward, indicating that K channel activation is prevented (Torrecilla et al., 2002) (Fig. 7*C*). Similar results were found in both 3 mM external Cs to block any residual K_{ir} channels (Sodickson and Bean, 1996) and 2 mM external barium (Ba) to block leak K channels ($\text{K}_{2\text{p}}$) (Deng et al., 2009) (supplemental Fig. S4, available at www.jneurosci.org as supplemental material). These findings suggest that GABA_BR may directly inhibit VSCCs to suppress Ca signals in spines and dendrites, without involving the activation of dendritic K channels.

Voltage-sensitive Ca channels

We next examined which VSCCs are present in spines and dendrites, focusing on the basal compartment, which has not previously been examined at this level. We found that wash-in of the VSCC mixture greatly reduced $[\text{Ca}]_{\text{step}}$ in both spines and dendrites (spine, $p = 0.03$; mixture vs ACSF, $p = 0.0004$; dendrite, $p = 0.03$; mixture vs ACSF, $p = 0.0004$; $n = 12/16$) (Fig. 8*A,B*). We attribute the remaining Ca signal to SNX-insensitive R-type channels (Newcomb et al., 1998; Tottene et al., 2000; Sochivko et al., 2002, 2003). Wash-in of 10 μM mibefradil, a T-type VSCC blocker, greatly reduced $[\text{Ca}]_{\text{step}}$ in both spines and dendrites (spine, $p = 0.004$; mibefradil vs ACSF, $p = 0.002$; dendrite, $p = 0.002$; mibefradil vs ACSF, $p = 0.0002$; $n = 9/10$) (Fig. 8*B*; supplemental Fig. S5, available at www.jneurosci.org as supplemental material). Wash-in of 20 μM nimodipine, an L-type VSCC blocker, also decreased $[\text{Ca}]_{\text{step}}$ in both spines and dendrites (spine, $p = 0.02$; nimodipine vs ACSF, $p = 0.046$; dendrite, $p = 0.008$; nimodipine vs ACSF, $p = 0.0004$; $n = 8/8$) (Fig. 8*B*; supplemental Fig. S5, available at www.jneurosci.org as supplemental material). Wash-in of 0.3 μM SNX, an R-type VSCC blocker, again reduced $[\text{Ca}]_{\text{step}}$ in both spines and dendrites (spine, $p = 0.02$; SNX vs ACSF, $p = 0.038$; dendrite, $p = 0.008$; SNX vs ACSF, $p = 0.02$; $n = 9/9$) (Fig. 8*B*; supplemental Fig. S5, available at www.jneurosci.org as supplemental material). We found that mibefradil did not block inhibition by either nimodipine or SNX, indicating that it is selective for T-type VSCCs (supplemental Fig. S5, available at www.jneurosci.org as supplemental material). Finally, wash-in of 1 μM ω -conotoxin-MVIIIC, a P/Q/N-type VSCC blocker, decreased $[\text{Ca}]_{\text{step}}$ in dendrites but

Figure 6. Rapid local modulation of AP Ca signals and membrane potential. **A**, Average $[Ca]_{bAP}$ for apical oblique (top) and proximal basal (bottom) spines (left) and dendrites (right) with no uncaging (red) and 200 ms after uncaging of RuBi-GABA (black). **B**, Summary of changes in $[Ca]_{bAP}$ amplitude with uncaging (green) or uncaging in the presence of $2 \mu M$ CGP-55845 (blue) in apical oblique and proximal basal spines and dendrites. The asterisks indicate significant ($p < 0.05$) difference from 100% or between recording conditions. **C**, Summary of sIPSP amplitude elicited by uncaging (green) or uncaging in the presence of $2 \mu M$ CGP-55845 (blue) in apical and basal dendrites. The asterisks indicate significant ($p < 0.05$) difference from 100% or between uncaging conditions. **D**, No correlation between the uncaging-evoked change in $[Ca]_{bAP}$ and sIPSP amplitude in spines (red) or dendrites (black).

not spines (spine, $p = 0.98$; MVIIC vs ACSF, $p = 0.73$; dendrite, $p = 6 \times 10^{-5}$; MVIIC vs ACSF, $p = 0.002$; $n = 15/15$) (Fig. 8B; supplemental Fig. S5, available at www.jneurosci.org as supplemental material). Thus, a combination of L-, R-, and T-type channels contribute to $[Ca]_{step}$ in both spines and dendrites, whereas N/P/Q-type channels contribute only in the dendrites. These results are similar to other neurons in which a wide variety of VSCCs contribute to Ca signals in spines and dendrites (Carter and Sabatini, 2004; Bloodgood and Sabatini, 2007).

Modulation of VSCC subtypes

We last sought to determine which VSCCs are modulated by GABA_BR activation in proximal spines and dendrites. Previous results using recordings at the cell body indicate that GABA_BRs can couple to a variety of VSCCs (Holz et al., 1986; Scholz and Miller, 1991; Mintz and Bean, 1993; Pfrieger et al., 1994; Guyon and Leresche, 1995; Lambert and Wilson, 1996). However, studies in CA1 pyramidal neurons indicate that these receptors primarily target R-type VSCCs in apical spines (Sabatini and Svoboda, 2000). We found that bath application of $5 \mu M$ baclofen continued to cause inhibition of spines and dendrites in the

Figure 7. Direct modulation of VSCCs. **A**, Average $[Ca]_{step}$ for apical oblique (top) and proximal basal (middle) spines (left) and dendrites (right) in baseline conditions (red) and after wash-in of $5 \mu M$ baclofen (black). Bottom, Schematic of the voltage step used to elicit the $[Ca]_{step}$. **B**, Summary of changes in $[Ca]_{step}$ amplitude after wash-in of ACSF (blue) or $5 \mu M$ baclofen (green) in apical oblique and proximal basal spines and dendrites. The asterisks indicate significant ($p < 0.05$) difference from 100% or between ACSF and $5 \mu M$ baclofen. **C**, Left, Summary of changes in R_{in} after wash-in of ACSF (blue) or $5 \mu M$ baclofen with either Cs-gluconate (green) or K-gluconate (yellow) internal. Right, Change in holding current in these different recording conditions. The asterisks indicate significant ($p < 0.05$) difference from 100%, or between different conditions.

VSCC mixture, suggesting that SNX-insensitive R-type VSCCs are indeed modulated (spine, $p = 0.02$; mixture vs ACSF, $p = 0.003$; dendrite, $p = 0.02$; mixture vs ACSF, $p = 0.0001$; $n = 6/6$) (Fig. 8C,D; supplemental Fig. S6, available at www.jneurosci.org as supplemental material). However, because these channels contribute only a small fraction of the total Ca signal, these results suggest that other channels must also be targeted.

To determine which other VSCCs were affected by GABA_BRs, we used bath application of $5 \mu M$ baclofen in the presence of the different blockers. For comparison, we calculated the range of inhibition expected if a given VSCC was unaffected (0–10% change) or completely blocked (90–100% change) by GABA_BRs (see Materials and Methods) (Eq. 1). We found that inhibition was significantly enhanced in $10 \mu M$ mibefradil compared with ACSF controls (spine, $p = 0.002$; mibefradil vs ACSF, $p = 0.004$; dendrite, $p = 0.002$; mibefradil vs ACSF, $p = 0.01$; $n = 8/10$) (Fig. 8D; supplemental Fig. S6, available at www.jneurosci.org as supplemental material), suggesting that T-type VSCCs are minimally modulated. In contrast, inhibition remained unchanged in either $20 \mu M$ nimodipine (spine, $p = 0.002$; nimodipine vs ACSF, $p = 0.22$; dendrite, $p = 0.002$; nimodipine vs ACSF, $p = 0.48$, $n = 10/10$) (Fig. 8D; supplemental Fig. S6, available at www.jneurosci.org as supplemental material).

Figure 8. Modulation of multiple VSCC subtypes. **A**, Average $[Ca]_{step}$ in baseline conditions (red) and after wash-in of the VSCC mixture (black) in spines (left) and dendrites (right). **B**, Summary of changes in $[Ca]_{step}$ after wash-in of ACSF, mibefradil (Mib), nimodipine (Nim), SNX-482, ω -conotoxin-MVIIC, or the VSCC mixture, in proximal basal spines (blue) and dendrites (green). The asterisks indicate significant ($p < 0.05$) difference from 100%, and the number signs (#) indicate significant ($p < 0.05$) difference from ACSF. **C**, Average $[Ca]_{step}$ in proximal basal spines (left) and dendrites (right) in the presence of the mixture (red) and after wash-in of $5 \mu M$ baclofen (black). **D**, Summary of changes in $[Ca]_{step}$ in proximal basal spines (blue) and dendrites (green) after wash-in of $5 \mu M$ baclofen in the presence of ACSF, mibefradil, nimodipine, SNX-482, ω -conotoxin-MVIIC, or VSCC mixture. The ranges for 90–100% block (top gray box) and 0–10% block (bottom gray box) were calculated using Equation 1 (see Materials and Methods), except for the spine in MVIIC, because MVIIC did not significantly influence the $[Ca]_{step}$ in **B**. All conditions are significant ($p < 0.05$) from 100%, and the asterisks have been omitted for clarity. The number signs (#) indicate significant ($p < 0.05$) difference from ACSF.

jneurosci.org as supplemental material) or $0.3 \mu M$ SNX (spine, $p = 0.004$; SNX vs ACSF, $p = 0.18$; dendrite, $p = 0.004$; SNX vs ACSF, $p = 0.78$, $n = 9/9$) (Fig. 8D; supplemental Fig. S6, available at www.jneurosci.org as supplemental material), suggesting partial modulation of L- and R-type VSCCs. Finally, inhibition was slightly decreased in $1 \mu M$ ω -conotoxin-MVIIC (spine, $p = 0.004$; MVIIC vs ACSF, $p = 0.60$; dendrite, $p = 0.004$; MVIIC vs ACSF, $p = 0.31$, $n = 9/9$) (Fig. 8D; supplemental Fig. S6, available at www.jneurosci.org as supplemental material), suggesting that P/Q/N-type VSCCs are fully modulated. Together, these results

indicate that GABA_BR modulation is more complex than previously thought and targets multiple subtypes of VSCCs in spines and dendrites of layer 2/3 pyramidal neurons.

Discussion

We have shown that GABA_BR modulation of AP Ca signals is widespread throughout the dendritic arbor of layer 2/3 pyramidal neurons in the mouse PFC. Although GABA_BRs activate K channels to increase membrane conductance, change the AP waveform, and generate sIPSPs, we found no correlation between these effects and inhibition of Ca signals. Instead, by using voltage-clamp recordings that block K channels, we found that GABA_BRs directly inhibit a variety of VSCC subtypes in both spines and dendrites. Our results have important implications for how GABA_BRs shape subcellular Ca signals to influence Ca-dependent neuronal function in the PFC and throughout the brain.

Modulation of AP Ca signals is widespread

Modulation via GABA_BRs has been primarily studied using electrophysiological recordings at the cell body. However, anatomical studies indicate that GABA_BRs are abundant in the spines and dendrites of pyramidal neurons (Kulik et al., 2003, 2006). In the apical tuft, dendritic GABA_BRs potentially inhibit Ca spikes mediated by VSCCs (Pérez-García et al., 2006). In basal spines, GABA_BRs inhibit postsynaptic NMDA receptor Ca signals (Chalifoux and Carter, 2010). Here, we found that GABA_BRs also inhibit AP Ca signals in spines and dendrites across the entire dendritic arbor. Our results contrast with CA1 pyramidal neurons, where GABA_BRs only inhibit these Ca signals in apical spines (Sabatini and Svoboda, 2000). The widespread modulation of AP Ca signals in the PFC may reflect the fundamental importance of GABA_BRs in modulating synaptic and dendritic function in these pyramidal neurons.

When using bath application of baclofen, it is difficult to determine whether somatic or dendritic GABA_BRs are responsible for modulating AP Ca signals.

For example, any change in the membrane conductance or AP waveform could limit backpropagation and reduce Ca signals in spines and dendrites. We used two complementary approaches to show that GABA_BR modulation occurs locally in spines and dendrites. First, puffing baclofen tonically activated GABA_BRs and revealed that inhibition occurs at distal locations. Second, uncaging of RuBi-GABA rapidly activated GABA_BRs and confirmed that inhibition also occurs at proximal locations. In both cases, we observed prominent modulation of AP Ca signals in both spines and

their parent dendrites. Our findings indicate that modulation of AP Ca signals occurs locally and is attributable to activation of GABA_BRs found throughout the dendritic arbor.

K channel activation does not explain inhibition

GABA_BRs can influence excitability by activating a variety of K channels (Gähwiler and Brown, 1985; Sodickson and Bean, 1996; Lüscher et al., 1997; Deng et al., 2009). We found that bath application of baclofen decreased R_{in} and shortened the ADP measured at the soma. Similar effects were seen with puffing of baclofen, suggesting they reflect the activation of dendritic GABA_BRs. These effects of K channel activation could reduce backpropagation and indirectly decrease Ca signals in spines and dendrites (Buzsáki et al., 1996; Tsubokawa and Ross, 1996; Leung and Peloquin, 2006). However, we observed no significant correlations between changes in AP Ca signals and changes in either R_{in} or ADP. Moreover, apical puffing caused larger changes in R_{in} and ADP compared with basal puffing, but similar changes in AP Ca signals were found in both compartments. These results suggest that changes in membrane conductance and AP waveform caused by K channel activation cannot explain the inhibition of AP Ca signals.

By activating K channels, GABA_BRs also generate sIPSPs (Newberry and Nicoll, 1984; Andrade et al., 1986; Dutar and Nicoll, 1988). In our bath application experiments, we corrected for a small and tonic hyperpolarization by injecting an inward current at the soma. However, we continued to observe pronounced inhibition of AP Ca signals when cells were allowed to hyperpolarize (supplemental Fig. S1, available at www.jneurosci.org as supplemental material). In our uncaging experiments, we observed a GABA_BR sIPSP that peaked at ~150 ms. Given the spatial resolution of our uncaging, this sIPSP is likely attributable to activation of dendritic GABA_BRs. However, we observed no significant correlation between the change in AP Ca signals and the amplitude of this sIPSP. Moreover, basal uncaging generated smaller sIPSPs compared with apical uncaging, but similar changes in AP Ca signals were found in both compartments. Thus, although a local hyperpolarization occurs during the sIPSP, it may not be sufficient to influence the activation of voltage-sensitive Ca channels in spines and dendrites. In summary, although our results show that dendritic GABA_BRs activate K channels to decrease R_{in} , shorten the ADP, and cause sIPSPs, these effects are not responsible for inhibition of AP Ca signals. Instead, we conclude that GABA_BRs must directly modulate VSCCs to locally inhibit these Ca signals in spines and dendrites.

GABA_BRs modulate multiple VSCC subtypes in spines and dendrites

GABA_BRs can potentially inhibit a variety of VSCCs to influence neuronal function (Holz et al., 1986; Scholz and Miller, 1991; Mintz and Bean, 1993; Pfrieger et al., 1994; Guyon and Leresche, 1995; Lambert and Wilson, 1996). We examined modulation of VSCCs using voltage-clamp recordings, which allowed us to circumvent indirect influences caused by modulation of K channels (Sabatini et al., 2002). As with our current-clamp experiments, we observed prominent inhibition of step-evoked Ca signals in apical and basal spines and dendrites. We determined that these Ca signals are mediated by a variety of VSCCs, as observed in other neurons in different brain regions (Carter and Sabatini, 2004; Bloodgood and Sabatini, 2007). Modulation persisted in the presence of a mixture to block all VSCCs, consistent with modulation of residual SNX-resistant

R-type channels (Sabatini and Svoboda, 2000). However, because these VSCCs contribute a small fraction of the total step-evoked Ca signal, these results also indicate that other VSCCs must also be modulated.

Identifying which VSCCs are modulated by GABA_BRs is complicated by the presence of multiple potential targets. If GABA_BRs completely block a single VSCC, including the appropriate channel blocker should eliminate inhibition. However, if GABA_BRs target many VSCCs, including this blocker should only cause a shift in the amount of inhibition. For example, GABA_BRs normally inhibit ~40% of the total VSCCs in spines, and L-type currents make up ~25% of the total VSCCs in spines. If all of the L-type channels are inhibited by GABA_BRs, we expect that baclofen should have less effect in the presence of nimodipine ($20\% = (40 - (1 * 25)) / (100 - 25)$) (see Materials and Methods) (Eq. 1). In contrast, if none of the L-type channels are inhibited, we expect more modulation [$53\% = (40 - (0 * 25)) / (100 - 25)$]. Finally, if only one-half of the L-type channel population is inhibited, we expect similar modulation to controls [$37\% = (40 - (0.5 * 25)) / (100 - 25)$]. Thus, the shift of inhibition also depends on the extent to which a given population of VSCCs is modulated by GABA_BRs.

With this conceptual framework, our results imply that GABA_BRs modulate multiple VSCC subtypes in spines and dendrites. For example, less inhibition of dendritic Ca signals occurs in the presence of ω -conotoxin-MVIIC, suggesting that N/P/Q-type VSCCs are fully modulated. However, because these channels contribute only ~20% of the dendrite Ca signal and none of the spine Ca signal, other VSCCs must also be targeted. More inhibition of spine and dendrite Ca signals occurs in the presence of mibefradil, suggesting that low-threshold T-type VSCCs are minimally modulated. Again, these results suggest that other VSCCs must also be targeted to account for the ~40% total modulation. Finally, intermediate inhibition of spine and dendrite Ca signals occurs in the presence of nimodipine or SNX, suggesting that a subpopulation of both L- and R-type VSCCs is modulated. Together, our results indicate that GABA_BR modulation of AP Ca signals is more complex than previously thought, with a single class of receptors able to inhibit multiple VSCC subtypes in both spines and dendrites.

Functional consequences

Extensive GABA_BR modulation of VSCCs could greatly impact synaptic transmission, integration, and plasticity. For example, GABA_BRs could attenuate subthreshold EPSPs by inhibiting spine and dendrite VSCCs (Magee and Johnston, 1995a). Alternatively, if these VSCCs participate in local feedback loops with K channels, GABA_BRs could actually enhance both EPSPs and synaptic Ca signals (Bloodgood and Sabatini, 2007). Our results also suggest that GABA_BRs could inhibit VSCC-mediated dendritic spikes throughout the dendritic arbor, as previously demonstrated in the apical tufts of layer 2/3 and layer 5 pyramidal neurons (Pérez-García et al., 2006; Larkum et al., 2007). Finally, because Ca influx via VSCCs is an important intracellular messenger, GABA_BRs could help control synaptic plasticity (Grover and Teyler, 1990; Huang and Malenka, 1993; Zucker, 1999). Given the widespread importance of Ca signals mediated by VSCCs, our results have important implications for synaptic and dendritic function in the PFC and throughout the brain.

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