α₂-Adrenergic Receptors Modify Dendritic Spike Generation Via HCN Channels in the Prefrontal Cortex

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Barth AM, Vizi ES, Zelles T, Lendvai B. α₂-Adrenergic receptors modify dendritic spike generation via HCN channels in the prefrontal cortex. J Neurophysiol 99: 394–401, 2008. First published November 14, 2007; doi:10.1152/jn.00943.2007. Although dendritic spikes are generally thought to be restricted to the distal apical dendrite, we know very little about the possible modulatory mechanisms that set the spatial limits of dendritic spikes. Our experiments demonstrated that high-frequency trains of backpropagating action potentials avoid filtering in the apical dendrite and initiated all-or-none dendritic Ca$^{2+}$ transients associated with dendritic spikes in layer 5 pyramidal neurons of the prefrontal cortex. The block of hyperpolarization-activated currents ($I_h$) by ZD7288 could shift the frequency threshold and decreased the number of action potentials required to produce the all-or-none Ca$^{2+}$ transient. Activation of α₂-adrenergic receptors could also shift the frequency domain of spike induction to lower frequencies. Our data suggest that noradrenergic activity in the prefrontal cortex influences dendritic $I_h$ and extends the zone of dendritic spikes in the apical dendrite via α₂-adrenergic receptors. This mechanism might be one cellular correlate of the α₂-receptor-mediated actions on working memory.

INTRODUCTION

Neuropsychological studies suggest that the prefrontal cortex (PFC) has control function on working memory and episodic long-term memory in humans (Ranganath et al. 2003). Plastic processes of dendrites are shaped by the interaction of backpropagating action potentials (bAPs), synaptic events, and local spikes in dendrites (Haussler et al. 2000). Distant synapses of pyramidal neurons are difficult to reach by bAPs because of their distance- and activity-dependent attenuation (Golding et al. 2002; Helmsen et al. 1999; Spruston et al. 1995; Svoboda et al. 1999). Dendritic spikes recently gained special attention because they can control distal signal integration (Lisman and Spruston 2005). High-intensity distal synaptic stimulation could induce dendritic spikes in apical, basal, and oblique dendrites of CA1 pyramidal neurons (Golding and Spruston 1998; Golding et al. 2002; Kampa and Stuart 2006; Losonczy and Magee 2006), in neocortical pyramidal neurons (Polsky et al. 2004), and in granule cells of olfactory bulb (Zelles et al. 2006). Dendritic spikes can also be produced by high-frequency bAP trains in apical and basal dendrites (Kampa and Stuart 2006; Larkum et al. 1999a,b; Perez-Garcia et al. 2006). Ca$^{2+}$ transients, which appear as all-or-none responses at particular sites of the dendrite with a threshold, can be considered as dendritic spikes (Stuart et al. 1997). Dendritic spikes, generated by synaptic activation and dendritic current injection, exist in PFC layer 5 neurons (Seamans et al. 1997).

Although high-amplitude Ca$^{2+}$ responses have been observed during high-frequency bursts in pyramidal neurons of the PFC (Gulledge and Stuart 2003) and the somatosensory cortex (Larkum et al. 1999a; Perez-Garcia et al. 2006), very little is known about the transitions between frequency profiles of Ca$^{2+}$ responses along the dendrite. This information is essential, however, when studying the modulation of evoked Ca$^{2+}$ transients in the dendrite following receptor activation. Here, we aim to explore the distance-dependent scaling of Ca$^{2+}$ dynamics along the entire length of the apical dendrite at various bAP frequencies in PFC layer 5 pyramidal neurons. Recent studies have established a model that α₂A-adrenergic receptors and hyperpolarization-activated current ($I_h$) interact at the level of dendritic spines to enhance working memory (Arnsten 2007; Wang et al. 2007). The distribution of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, which mediate $I_h$, was found to be skewed to a preferentially distal localization in subicular and in cortical pyramidal neurons (Lorincz et al. 2002; Notomi and Shigemoto 2004). $I_h$ was recently shown to interact with dendritic spike initiation in layer 5 pyramidal neurons of the somatosensory cortex (Berger et al. 2003; Kole et al. 2007). α₂-Adrenergic receptors were detected on apical dendrites of PFC pyramidal neurons (Aoki et al. 1998; Wang et al. 2007). In this report, we uncovered an $I_h$-mediated mechanism by which α₂-adrenergic receptors can shift the frequency profile of bAP-evoked Ca$^{2+}$ responses along the apical dendrite in the PFC.

METHODS

Slice preparation

All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and all efforts were made to minimize animal suffering, to reduce the number of animals used. Postnatal day (P) 18 to P23 male Wistar rats were killed by decapitation and slices (300 μm) containing the prefrontal cortex were prepared using a vibratome (Vibratome 3000 Plus Sectioning System, Vibratome, St. Louis, MO). Brain slices were placed in artificial cerebrospinal fluid containing (in mM) 127 NaCl, 25 NaHCO$_3$, 2.5 n-glucose, 2.5 KCl, 2 CaCl$_2$, 1 MgCl$_2$, and 1.25 NaH$_2$PO$_4$ (all from Sigma), and incubated for 30 min at 32°C. Slices were left at room temperature for ≥45 min before use.

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Electrophysiology

Layer 5 pyramidal neurons in the PFC were visualized using video infrared-differential interference contrast. Patch pipettes were pulled from borosilicate glass (1.2-mm OD; Harvard Instruments, Tübingen, Germany). For whole cell recordings 2–4 MΩ electrodes were filled with 125 mM K-glucurate, 20 mM KCl, 10 mM HEPES, 10 mM di-Tris-salt phosphocreatine, 0.3 mM Na-GTP, 4 mM Mg-ATP, 10 mM NaCl (all from Sigma), and 100 μM Oregon Green BAPTA-1 (from Molecular Probes). ZD7288 [4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino)pyridinium chloride] was purchased from Tocris. Current- and voltage-clamp recordings were made with an Axopatch 1D amplifier (Axon Instruments, Foster City, CA). Cells with an initial resting membrane potential that was more negative than −60 mV were accepted. During voltage-clamp recordings series resistance (typically 8–15 MΩ) was monitored but not compensated. Cells in which the series resistance increased by 15% were discarded. Leak current was not subtracted. Signals were sampled at 10 kHz with a Digidata 1322A interface (Axon Instruments) and low-pass filtered at 1 kHz (four-pole low-pass Bessel). Data acquisition and analysis were performed using pClamp8 (Axon Instruments). In current-clamp experiments in most cases, 5 bAPs were evoked by somatic current injections (five stimuli, 1,800 pA, 2–4 ms) to induce dendritic Ca2+ responses. Spike half-width did not change significantly during the experiment. All experiments were done at 32–34°C.

Two-photon laser scanning imaging

Imaging was performed using a custom-made two-photon laser scanning system consisting of a modified confocal microscope (Olympus FluoView, Munich, Germany) and a titanium–sapphire laser (Millenia/Tsunami, Spectra Physics, Stratford, CT) described previously (Rosza et al. 2004). Briefly, fluorescent indicators were excited at 810-nm wavelength. Detection was performed in both epi- and a transfocus fluorescence mode using external photomultiplier tubes (R3896, Hamamatsu, Hamburg, Germany). To minimize photodamage, the intensity of the excitation laser light was always maintained at the minimum required to attain sufficient signal-to-noise ratio. High time-resolution fluorescence measurements were obtained in line-scan mode (2-ms maximal temporal resolution) after zooming onto a dendritic section. Data recording was started 40 min following break-in. At the end of each experiment, a series of images across the depth of the volume encompassing the imaged neuron were taken. Image data were analyzed off-line using a custom-made program written in Matlab.

Calculations

Fluorescence traces are expressed as relative fluorescence changes [ΔF/ΔF₀ = (F − F₀)/F₀], where F₀ is the background-corrected mean prestimulus fluorescence and F is the background-corrected fluorescence. The amplitude of the Ca2+ transient was determined by the averaging of 15 data points around the largest ΔF/ΔF₀ value of the transient. In some cases frequency dependence was calculated as the amplitude difference between the smallest and the largest frequency bAP-evoked Ca2+ transients. Recordings from dendritic sites were separated into groups according to their distance from the soma. Because of the variation in critical frequencies of control, data of treatment cases were normalized to the critical frequency of the same cell under control condition and expressed as 10-Hz differences compared with the critical frequency of the control experiment.

RESULTS

Scaling of bAP-evoked Ca2+ transients in apical dendrites of PFC pyramidal neurons

To map the dendritic scaling of Ca2+ responses evoked by bAPs we applied trains of 5 APs at different frequencies and imaged the apical dendrite of PFC layer 5 pyramidal neurons at various distances from the soma (Fig. 1A). We applied a range of frequencies (10–100 Hz) to reveal the transitions between frequency profiles of different sites. At the most proximal sites (0–50 μm), Ca2+ transients showed weak frequency dependence (difference between the lowest- and the highest-frequency bAP train in ΔF/ΔF₀: 26%, n = 7). Moving distally along the apical dendrite, the amplitude of the evoked Ca2+ transients gradually increased as a function of bAP frequency (Fig. 1B). At far distal sites, Ca2+ responses had a binary outcome: low-frequency, subcritical stimuli caused no response but after passing a critical frequency (CF) of the bAP train, a stable-amplitude Ca2+ transient was detected (Fig. 1, B–D). The difference between the smallest and largest transients was maximal and stable at these distal responses (Fig. 1D). This all-or-none feature of distal Ca2+ transients and the existence of the threshold stimulation correspond well with the characteristics of dendritic regenerative events, i.e., dendritic spikes (Golding and Spruston 1998; Larkum et al. 1999a). Although the CF for one cell was stable during the control experiments, we observed a considerable variation of the CF across different cells (mean CF: 71 Hz, range: 40–110 Hz, n = 49, Fig. 1E). The large variability of the CF in the PFC may reflect a physiologically active mechanism that sets the actual threshold of dendritic spike generation in the dendrite. Ca2+ transients associated with dendritic spikes were also recorded in adjacent dendrite/spine pairs (Fig. 1F). We found strict correlation between amplitudes of supracritical Ca2+ responses measured in dendrites and spines (r = 0.74), suggesting that dendritic spikes reliably invade all spines in distal dendrites.

bAP attenuation and dendritic spike initiation

Imaging dendritic sites at high spatial resolution, we could reliably explore the transitions between different frequency profiles. Corroborating the findings of earlier experiments (Berger et al. 2003; Larkum et al. 1999a; Perez-Garcia et al. 2006; Stuart et al. 1997), at low stimulation frequencies the amplitude of the bAP-evoked Ca2+ transients declined with distance from the soma (n = 45; Fig. 2A). However, supracritical Ca2+ transients associated with dendritic spikes showed a substantially different scaling: the plot of amplitudes over distance exhibited a maximum in the apical dendrite (Fig. 2A). Different morphologies of layer 5 pyramidal neurons may influence the site of the dendritic spike generation (Spruston et al. 1995; Waters et al. 2003). Plotting the soma distance of the main bifurcation along the apical dendrite for each cell revealed that the supracritical maximum greatly overlapped the bifurcation sites (maximum: 415 ± 21 μm, bifurcation: 431 ± 42 μm; means ± SE) (Fig. 2, A and D).

At low stimulation frequencies the evoked Ca2+ transients not only declined with distance but they completely extinguished at different distances from the soma. The distance of extinction sites depended on the bAP frequency. These extinction sites of low-frequency bAP trains were determined from the trendlines of Ca2+ transient amplitudes. Our data showed that extinction (at subcritical bAP frequencies) occurred in a relatively narrow section in the apical dendrite in layer 5 neurons. The mean length of this section designated by the extinctions at different subcritical frequencies was 62 ± 16 μm (n = 6; Fig. 2, B and C). The mean location of extinction was
at 612 ± 58 µm from the soma. How are the mean extinction site of low-frequency trains and the peak of supracritical Ca\textsuperscript{2+} transients related to the localization of the main bifurcation? To address this we mapped distances of extinction sites and distances of the peaks of supracritical transients as a function of bifurcation distance (Fig. 2C). Extinction of bAP-evoked responses was closer to the soma than the main bifurcation in three cases, whereas extinction fell after the bifurcation in seven other cases of subcritical responses (Fig. 2C). This further corroborated the view that even low-frequency bAP trains can pass the bifurcation in 70% of the cells. The maximum of the supracritical Ca\textsuperscript{2+} responses along the dendrite appeared after the bifurcation in half of the cells, whereas in the other half the supracritical maximum was localized before the bifurcation (Fig. 2C), suggesting that the zone of dendritic spikes, at least as it is suggested from the associated Ca\textsuperscript{2+} transients, did not strictly depend on the exact localization of the bifurcation in spite of the clear overlap.

\textit{I}_h shifts the threshold of dendritic spikes

After the characterization of Ca\textsuperscript{2+} responses associated with distal dendritic spikes, we investigated the possible modulation of frequency profiles of evoked Ca\textsuperscript{2+} transients. We applied ZD7288 to remove \textit{I}_h and studied the generation of dendritic Ca\textsuperscript{2+} transients. In accordance with earlier reports (Berger et al. 2001), application of ZD7288 (50 µM, 8-min perfusion) caused hyperpolarization at the soma (−6.7 ± 0.7 mV, \(n = 6\); data not shown), confirming its action on \textit{I}_h in our experiments. Then, we determined the frequency profile of bAP-evoked Ca\textsuperscript{2+} responses at distal dendritic sites (350–720 µm; \(n = 7\) cells). Black circles indicate the supracritical responses. E: distribution of the CF for all cells (\(n = 49\), 40–110 Hz). F: dendritic spikes invade spines. Top: simultaneous line-scan imaging from a dendrite and adjacent spine (collapsed 3-dimensional 2-photon image stack) 430 µm from the soma. Scale bar: 1 µm. Bottom: bAP-evoked Ca\textsuperscript{2+} transients at supracritical (SpC, 90 Hz) and subcritical (SbC, 20 Hz) frequencies in dendrites (black) and adjacent spines (gray).
ZD7288 decreased the frequency threshold of dendritic spikes: low-frequency bAP trains, which normally fail to evoke the large-amplitude, all-or-none Ca\(^{2+}\) transients, became able to induce suprathreshold responses (Fig. 3, Au and Ab). This was particularly evident for the 50-µM dose of ZD7288; the CF decreased from the control 81.8 ± 3.8 to 45.5 ± 4.1 Hz by ZD7288 (means ± SE, n = 11; P < 0.001). ZD7288 (20 µM) produced a smaller (18.3 Hz) but significant shift in CF (n = 6; P < 0.05; Fig. 3Ba). After lowering the concentration of ZD7288 to 10 µM, we observed a minimal (10-Hz) shift for lower stimulating bAP frequencies (n = 7; P < 0.05; Fig. 3Ba). The effect was the most visible at frequencies that were just subcritical in control but became supracritical in the presence of the ZD7288 (CF\(_{ZD} - CF_{Ctrl}\)). The amplitude increase by 50 µM ZD7288 was highly significant in this group (n = 11; P < 0.001; Fig. 3Bb). In addition, removal of \(I_h\) also increased the amplitude of the Ca\(^{2+}\) transient associated with dendritic spikes at supracritical frequencies (P = 0.01; Fig. 3Bb), indicating that the block of \(I_h\) could also amplify dendritic spikes at suprathreshold bAP frequencies. To ensure that the elapsed time between control and treatment periods did not influence the frequency dependence of dendritic spike generation, we applied the same protocol (10–100 Hz) at three consecutive time points with intervals identical to the ZD experiments (10 min). In contrast to the effect of ZD, the CF did not change significantly under control conditions (P > 0.2, n = 4; Fig. 3C). At proximal sites, 50 µM ZD7288 enhanced Ca\(^{2+}\) transients (Fig. 3D), suggesting that forward propagation of dendritic spikes may also be enhanced under the block of HCN channels.

Then, we analyzed the effect of shorter trains of bAPs. Under control conditions, two bAPs induced small Ca\(^{2+}\) responses in distal dendrites, but in the presence of ZD7288 they consistently induced large Ca\(^{2+}\) responses (Fig. 3Eb). Under the block of \(I_h\), even a single bAP became sufficient to produce large Ca\(^{2+}\) responses; this never occurred in control (n = 3; Fig. 3Ea). Thus the removal of \(I_h\) by ZD7288 facilitated the initiation of dendritic spikes by reducing the threshold number of bAPs. We also note that Ca\(^{2+}\) levels in dendrites elevated slowly during the first bAP at control 5-bAP stimulations (Fig. 3Ec). When \(I_h\) was removed by ZD7288, the rising phase of Ca\(^{2+}\) transients had two components (Fig. 3Ec). The slower component (3.6 ± 0.6% change/ms) was followed by a faster component (6.9 ± 1.7%/ms, n = 4; Fig. 3Ec). The rapid kinetics under ZD perfusion is likely attributable to dendritic spikes, which are turned on during the backpropagation of the third bAP in a 50-Hz train. In addition, we observed irregular, spikeletike events in the soma electrical recording in four cells (Fig. 3, Ea–Ec). These spikelets may result from enhanced propagation of dendritic spikes to the soma under the block of
for all-or-none Ca\textsuperscript{2+} responses with various frequencies. Similarly to the I\textsubscript{h} activation is linked to downstream elevation of 3\',5\'-cyclic adenosine (cAMP) level and subsequent intracellular pathways (Ludwig et al. 1998). Thus neurotransmitters, known to act by changing cAMP levels, are expected to influence I\textsubscript{h} and, consequently, the initiation of dendritic spikes. PFC layer 5 pyramidal neurons express \( \alpha_2 \)-adrenergic receptors (Aoki et al. 1998; Wang et al. 2007). To address the possibility that activation of \( \alpha_2 \)-adrenergic receptors can shift the frequency domain of dendritic spike initiation, we applied clonidine, a specific agonist of \( \alpha_2 \)-adrenergic receptors, at 100 \( \mu \)M concentrations for 9 min and recorded Ca\textsuperscript{2+} responses evoked by bAPs with various frequencies. Similarly to the I\textsubscript{h} block, clonidine enhanced Ca\textsuperscript{2+} transients by lowering the threshold for all-or-none Ca\textsuperscript{2+} transients evoked by dendritic spikes (Fig. 4, A and B). The frequency shift to the left was smaller than that produced by ZD7288 at 50 \( \mu \)M. Nevertheless, at stimulatory frequencies of 10 Hz less than the CF of control, the clonidine-induced increase in the amplitude of the evoked Ca\textsuperscript{2+} response was marked and highly significant (\( P = 0.006; \) Fig. 4, A and B). Next, we studied whether the effect of clonidine was indeed mediated by specific \( \alpha_2 \)-adrenergic receptors. Application of 100 \( \mu \)M RX 821002, a specific antagonist of \( \alpha_2 \)-adrenergic receptors, inhibited the stimulatory effect of clonidine (\( n = 3, P < 0.05; \) Fig. 4C). The similarities between clonidine and ZD7288-mediated action (hyperpolarization at the soma, shift of CF) suggested the potential role of I\textsubscript{h} in the action of clonidine. To investigate this interaction, we applied clonidine in the presence of ZD7288. Clonidine was not able to change the frequency profile in the presence of 50 \( \mu \)M ZD7288 (Fig. 4, D and E). In contrast, applying a lower concentration of ZD7288 (20 \( \mu \)M), which itself produces a small shift in frequency, the effect of clonidine was additive and produced a further 12-Hz shift in the CF (Fig. 4E). The assumption that clonidine acted through the inhibition of I\textsubscript{h} was further supported by voltage-clamp experiments using hyperpolarizing pulses of 30 ms at 50 \( \mu \)M clonidine.
DISCUSSION

In this report, we examined whether local \( \text{Ca}^{2+} \) responses associated with dendritic spikes, initiated by bAP bursts, could interfere with \( I_h \) and \( \alpha_2 \)-adrenergic receptor function in layer 5 pyramidal neurons of the PFC. We explored the transitions between frequency profiles along the apical dendrite from graded-type to all-or-none \( \text{Ca}^{2+} \) responses. The existence of the CF and the all-or-none \( \text{Ca}^{2+} \) transients at far distal sites indicated that the suprathreshold \( \text{Ca}^{2+} \) signals we observed in this study were indeed induced by dendritic spikes. The scaling of suprathreshold \( \text{Ca}^{2+} \) signals had a maximum in the middle part of the apical dendrite. For layer 5 neurons, it has been raised that the generation of dendritic \( \text{Ca}^{2+} \) spikes is located near the main bifurcation (Helmchen et al. 1999). Corroborating this, in layer 2/3 pyramidal neurons, extralinear \( \text{Ca}^{2+} \) influxes and dendritic regenerative potentials also localize to the main bifurcation (Waters et al. 2003). We have shown that suprathreshold bAP trains could initiate dendritic spikes both before and after the bifurcations. Our data suggest that the initiation zone of dendritic spikes is located at the area of the main bifurcation.

\( I_h \) is a modulatory factor for the initiation of dendritic spikes

In our study, the CF substantially varied among layer 5 neurons of the PFC (40–100 Hz), indicating a naturally active cellular mechanism, which sets the CF for a given neuron. One possible candidate for shifting the CF is the modulation of HCN channels by neurotransmitter receptors. The assumption that \( I_h \) can influence dendritic spike initiation comes from structural and functional data showing higher \( I_h \) density at distal sites than at proximal sites of the apical dendrite (Berger et al. 2001; Kole et al. 2006; Lorincz et al. 2002; Wang et al. 2007). In addition, the vast majority of the pyramidal cells in the prelimbic and infralimbic cortex showed detectable levels of HCN1 mRNA (Day et al. 2005). HCN1 channels require the least amount of hyperpolarization to open among HCN channel subtypes, and weakly modulated by cAMP (Santoro et al. 1998). HCN2 channels need stronger hyperpolarizations, but are strongly modulated by cAMP (Ludwig et al. 1998). Formation of heteromultimers between HCN1 and HCN2 subunits may endow channels with a greater sensitivity to cAMP modulation (Chen et al. 2001). Furthermore, the cAMP-sensitive HCN2 and HCN3 isoforms are also detected in the PFC (Day et al. 2005; Franze et al. 2000). Weak staining for HCN3 and HCN4 was found in the somatosensory cortex. It has been shown earlier that the block of HCN channels by ZD7288 reduces the CF of spike generation in dendritic patch recording of layer 5 pyramidal neurons of the somatosensory cortex (Berger et al. 2003; Kole et al. 2007). In addition, our data suggest that the forward propagation and the amplitude of \( \text{Ca}^{2+} \) transients associated with dendritic spikes were also facilitated when \( I_h \) was blocked. Ultimately, in accordance with findings of other laboratories (Berger et al. 2003; Kole et al. 2007), our \( \text{Ca}^{2+} \) imaging data suggest that dendritic spikes originating in far distal sites have relatively high probability of reaching the soma when the distal HCN channels are blocked. Our results revealed that the removal of \( I_h \) can induce large \( \text{Ca}^{2+} \) transients associated with dendritic spikes at low-frequency stimulation.
Adrenergic inputs can modify Ca\(^{2+}\) signaling associated with dendritic spikes

The consequence of shifting the frequency domain of dendritic spike initiation via \(I_h\) might be the adaptation to the changing extracellular transmitter levels that reflect network activity. How can this mechanism be regulated physiologically? Increases in cAMP levels facilitate \(I_h\) by direct binding of the cyclic nucleotide to the channel (Ludwig et al. 1998; Pape 1996). This feature raises the possibility that receptor coupling of the cyclic nucleotide to the channel (Ludwig et al. 1998; Pape 1996) is the major mechanism by which noradrenaline can improve working memory. In addition, the lower threshold and facilitated propagation of dendritic spike initiation may result in an expansion of the frequency profile of dendritic spike generation through the PFC (Wang et al. 2007), we propose that shifting the frequency domain of dendritic spike initiation may result in an expansion of the frequency profile of dendritic spike generation through the PFC (Wang et al. 2007).

**References**


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