Small-conductance Ca\(^{2+}\)-activated K\(^+\) channels modulate action potential-induced Ca\(^{2+}\) transients in hippocampal neurons

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Abstract

Tonini R, Ferraro T, Sampedro-Castañeda M, Cavaccini A, Stocker M, Richards CD, Pedarzani P. Small-conductance Ca\(^{2+}\)-activated K\(^+\) (SK) channels modulate action potential-induced Ca\(^{2+}\) transients in hippocampal neurons. J Neurophysiol 109: 1514–1524, 2013. First published December 19, 2012; doi:10.1152/jn.00346.2012.—In hippocampal pyramidal neurons, voltage-gated Ca\(^{2+}\) channels open in response to action potentials. This results in elevations in the intracellular concentration of Ca\(^{2+}\) that are maximal in the proximal apical dendrites and decrease rapidly with distance from the soma. The control of these action potential-evoked Ca\(^{2+}\) elevations is critical for the regulation of hippocampal neuronal activity. As part of Ca\(^{2+}\) signaling microdomains, small-conductance Ca\(^{2+}\)-activated K\(^+\) (SK) channels have been shown to modulate the amplitude and duration of intracellular Ca\(^{2+}\) signals by feedback regulation of synaptically activated Ca\(^{2+}\) sources in small distal dendrites and dendritic spines, thus affecting synaptic plasticity in the hippocampus. In this study, we investigated the effect of the activation of SK channels on Ca\(^{2+}\) transients specifically induced by action potentials in the proximal processes of hippocampal pyramidal neurons. Our results, obtained by using selective SK channel blockers and enhancers, show that SK channels act in a feedback loop, in which their activation by Ca\(^{2+}\) entering mainly through L-type voltage-gated Ca\(^{2+}\) channels leads to a reduction in the subsequent dendritic influx of Ca\(^{2+}\). This underscores a new role of SK channels in the proximal apical dendrite of hippocampal pyramidal neurons.

SK channel; afterhyperpolarization; apamin; pyramidal neuron; calcium imaging

The afterhyperpolarizing current \(I_{\text{AHP}}\) is mediated by apamin-sensitive, small-conductance Ca\(^{2+}\)-activated K\(^+\) (SK) channels that are voltage independent and activated by increases in intracellular Ca\(^{2+}\), thereby linking intracellular Ca\(^{2+}\) elevations to changes in the membrane potential in a variety of neurons (reviewed by Adelman et al. 2012; Pedarzani and Stocker 2008). The constitutive binding of calmodulin to SK channels is responsible for their high sensitivity to Ca\(^{2+}\) (Xia et al. 1998). However, the sources of Ca\(^{2+}\) leading to the activation of SK channels vary in different types of neurons (reviewed by Pedarzani and Stocker 2008; Stocker 2004). SK channels are part of Ca\(^{2+}\) microdomains (Fakler and Adelman 2008; Lujan et al. 2009; Marrion and Tavalin 1998; Oliver et al. 2000) created by the functional coupling of Ca\(^{2+}\)-permeable channels and Ca\(^{2+}\)-sensitive channels, and may serve diverse roles depending on their subcellular localization. In the soma of CA1 neurons Ca\(^{2+}\) channels and small-conductance Ca\(^{2+}\)-activated K\(^+\) channels are found within 50–150 nm of each other (Marrion and Tavalin 1998).

Synaptic NMDA receptors and SK channels are functionally coupled in the dendritic spines of hippocampal, amygdala, and striatal neurons (Bloodgood and Sabatini 2007; Faber et al. 2005; Higley and Sabatini 2010; Lujan et al. 2009; Ngo-Anh et al. 2005). The activation of SK channels in dendritic spines limits the influx of Ca\(^{2+}\) through NMDA receptors and decreases glutamatergic excitatory postsynaptic potentials (Bloodgood and Sabatini 2007; Faber et al. 2005; Ngo-Anh et al. 2005). Moreover, in distal apical dendrites of hippocampal neurons SK channel activation controls the duration of glutamate-induced Ca\(^{2+}\) plateau potentials (Cai et al. 2004). Thus SK channels are part of a negative feedback loop that limits Ca\(^{2+}\) influx through those Ca\(^{2+}\) sources that initially activated them, shaping the amplitude and duration of synthetically evoked Ca\(^{2+}\) transients and modulating glutamatergic synaptic responses.

The regulation by SK channels of Ca\(^{2+}\) sources that are not dependent on synaptic activation, however, has not been explored so far. This may be of particular relevance in the proximal apical dendrite of hippocampal neurons, where SK channels have also been localized (Lin et al. 2008; Lujan et al. 2009; Sailer et al. 2002) and elevations of intracellular Ca\(^{2+}\) induced by the activation of voltage-gated Ca\(^{2+}\) channels by somatic action potentials (APs), which backpropagate to the dendrites, are largest (Callaway and Ross 1995; Christie et al. 1995; Jaffe et al. 1992; Regehr et al. 1989; Regehr and Tank 1994; Spruston et al. 1995). The proximal dendritic compartment is different from the distal compartment in CA1 neurons also because it receives more effective GABAergic innervation (Papp et al. 2001), while most glutamatergic excitatory inputs converge on the distal portion. Indeed, proximal and distal compartments of apical dendrites have different synaptic plasticity thresholds, which may also reflect a different contribution of voltage-gated Ca\(^{2+}\) channels to plasticity induction mechanisms (Parvez et al. 2010).

In view of the feedback regulation of synthetically evoked Ca\(^{2+}\) entry by SK channels shown in distal dendrites and spines, the present study addresses the question as to whether SK channels can modulate AP-induced Ca\(^{2+}\) transients in the proximal apical dendrites of hippocampal pyramidal neurons. We demonstrate that pharmacological modulation of SK channel activity regulates the amplitude and duration of AP-induced intracellular Ca\(^{2+}\) elevations mainly triggered by L-type volt-
age-gated Ca\(^{2+}\) channels in the proximal neurites of hippocampal neurons.

**METHODS**

**Chemicals.** Tetrodotoxin (TTX) was obtained from Alomone Laboratories (Jerusalem, Israel); apamin from Laxotan (Rosans, France); DL-AP5, NBQX, picrotoxin, and 1-ethyl-2-benzimidazolone (1-EBIO) from Tocris Cookson (Bristol, UK) or Ascent Scientific (Weston-super-Mare, UK); and tetrathylationummonium (TEA), Na\(_2\)-ATP, Na\(_2\)-GTP, and 8-(4-chlorophenylthio)adenosine 3',5'-cyclic monophosphate (CPT-CAMP) from Sigma-Aldrich (Poole, UK); all other salts and chemicals were obtained from Fluka (Sigma-Aldrich).

**Cell culture.** Rats were handled in accordance with the UK Home Office Animal Procedures Act (1986), and protocols were reviewed and approved by the University College London Animal Ethical Committee. Primary hippocampal neurons were cultured from 0- to 1-day old rats (Goslin and Banker 1991) according to a modified protocol. Briefly, after dissection, the hippocampi were treated with 2.5% trypsin (Invitrogen) and mechanically dissociated with a flame-polished Pasteur pipette. Cells were plated onto poly-d-lysine-coated (0.1 mg/ml) glass or plastic (Nalgene) coverslips at a density of 35,000 cells/cm\(^2\) (for electrophysiology and imaging recordings) or 21,000 cells/cm\(^2\) (for immunofluorescence staining) in minimum essential medium (Invitrogen, Paisley, UK) supplemented with 10% horse serum, 1 mM pyruvic acid, and 0.59% glucose. After 4–14 h, the medium was substituted with Neurobasal medium supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), 0.59% glucose, and B27 supplement (Invitrogen). Neurons were kept at 5% CO\(_2\), 37°C, and 95% humidity for a variable number of days before the experiments.

**Electrophysiology.** For recordings, cover slips were mounted in a custom-built recording chamber placed on the stage of a Nikon custom-built recording chamber placed on the stage of a Nikon Electric microscope. Cells were continuously superfused by a 2.5 ml/min flow of extracellular solution containing (in mM) 140 NaCl, 3.5 KCl, 10 HEPES, 20 glucose, 2.5 CaCl\(_2\), and 1.5 MgCl\(_2\) (pH 7.4). Cells were cultured on a 15–25 MΩ recording chamber placed on the stage of a Nikon microscope equipped with a Nikon ×60 NA 1.0 water-immersion objective. A Millennia V pump laser coupled to a mode-locked Ti:sapphire infrared laser (Tsunami, Spectra Physics) was used for fluorescence excitation, tuned to 2 mM. For most experiments the laser power at the sample was 3 mM, but for those cells with a weak dye loading a higher power (up to 7 mW) was used to obtain a clear image. The fluorescence emission was collected with an external photomultiplier detector and was not descanned.

Since two-dimensional scans are too slow for accurate determination of the time course and amplitude of the calcium transients, line scans were used. These consisted of successive sweeps at 6-ms intervals across a single line in the field of view. Images were collected with a Lasersharp software (Bio-Rad) and analyzed with ImageJ (National Institutes of Health) and Origin 7.0 (MicroCal Software). For each recording, background fluorescence was determined from a cell-free area of size comparable to that of the line scan image. After the averaged background signal was subtracted, fluorescence values were taken 300 ms before the triggering of APs and averaged to measure the basal fluorescence (F\(_{basal}\)). The amplitude of the fluorescence transients at the recording sites was expressed as the fractional change in basal fluorescence, (F\(_{max}\)–F\(_{basal}\))/F\(_{basal}\) (ΔF/F, which is approximately proportional to the changes in intracellular Ca\(^{2+}\) (Maravall et al. 2000). F\(_{basal}\) did not change by more than two times the standard deviation of F\(_{basal}\) measured under control conditions over the course of the experiment.

** Statistical analysis.** Data were analyzed with Prism (GraphPad Software, La Jolla, CA), Student’s t-test, paired or unpaired as appropriate, or the nonparametric Mann-Whitney test was used for statistical comparisons between two groups (α = 0.05). For comparisons between more than two groups one-way ANOVA or one-way repeated-measures ANOVA followed by the Bonferroni post hoc test was used. All values are expressed as means ± SE.

**Immunofluorescence.** Hippocampal neurons were fixed in phosphate saline buffer (PBS; 10 mM sodium phosphate, 130 mM NaCl, pH 7.2) containing 4% paraformaldehyde and 4% sucrose for 10 min at ambient temperature, rinsed twice in PBS, and permeabilized in 0.5% Triton X-100 for 15 min, followed by two more washes in PBS. After a 1-h incubation in 2% H\(_2\)O\(_2\) to block the activity of the endogenous peroxidase, immunodetection was performed with the tyramide signal amplification method (Invitrogen). In short, the fixed, permeabilized, and peroxidase-treated neurons were incubated overnight at 4°C with the affinity-purified anti-NSK2 antibody (0.25 μg/ml; see also Cingolani et al. 2002) diluted in blocking buffer. Controls were performed in parallel either by omitting the purified anti-NSK2 or by...
using the anti-NSK2 preadsorbed to a SK2 fusion protein (TRX-NSK2, 20 μg/ml). After repeated washes with PBS to remove the unbound primary antibodies, a 1-h incubation with the HRP-conjugated anti-rabbit secondary antibody (1:200) in blocking buffer was performed. After the cultures were washed with PBS, the tyramide-Alexa Fluor 488 reagent was added and incubated in the dark for 5 min. Slides were washed, mounted with ProLong Antifade (Invitrogen), and examined with a fluorescence microscope (Axioskop, Zeiss). Pictures were taken with a MicroPublisher camera (QImaging).

RESULTS

Apamin-sensitive \( I_{\text{AHP}} \) in cultured hippocampal neurons. Whole cell recordings were performed on morphologically identified hippocampal pyramidal neurons in primary culture. After 10–18 DIV, neurons showed a mean resting potential of \(-59 \pm 1\) mV \((n = 38)\); 100- to 200-ms-long somatic depolarizations to +10 mV from a holding potential of +50 mV, delivered in the presence of 0.5 μM TTX and 1 mM TEA, activated voltage-gated Ca\(^{2+}\) currents, followed by an outward current. The observed currents decayed with either a time constant (\(\tau\)) of 222 ± 17 ms when elicited by a 100-ms-long depolarization (\(n = 8\)), or a \(\tau\) of 485 ± 46 ms for 200-ms-long depolarizations (\(n = 17\)). The mean amplitude of the outward current was 83 ± 20 pA (\(n = 8\)) in response to 100-ms-long depolarizing pulses and 140 ± 23 pA (\(n = 17\)) after 200-ms-long pulses.

\(d\)-Tubocurarine (dTC, 100 μM), which blocks SK channels in a reversible manner, inhibited the outward current (Fig. 1, A and B). Similarly, the specific SK channel blocker apamin (5 nM) produced a strong suppression of the outward current (Fig. 1, A and B), demonstrating that it is mediated by SK channels.

To further validate the molecular identity of the outward current and complete its pharmacological characterization, we tested the SK channel enhancer 1-EBIO (250 μM) (Pedarzani et al. 2001), which increased the \( I_{\text{AHP}} \) peak amplitude (Fig. 1, C and E). A similar increase in \( I_{\text{AHP}} \) amplitude was observed with the more specific and potent SK channel enhancer 6,7-dichloro-1H-indole-2,3-dione 3-oxime (NS309) at 5 μM (Fig. 1, D and F) (Pedarzani et al. 2005). In addition, both 1-EBIO (Fig. 1, C and E) and NS309 (Fig. 1, D and F) prolonged the decay \(\tau\) of the \( I_{\text{AHP}} \).

Taken together, these results demonstrate that the \( I_{\text{AHP}} \) is expressed in cultured primary hippocampal neurons and has properties similar to the SK-mediated \( I_{\text{AHP}} \) recorded in pyramidal neurons from acute hippocampal slices (Sailer et al. 2002; Stocker et al. 1999).

SK2 channel expression in hippocampal neurons. Evidence obtained in pharmacological and biochemical studies, and work performed on genetically modified animals, point to the SK2 (KCa2.2) subunit as a main contributor to the formation of the SK channels mediating the \( I_{\text{AHP}} \) in hippocampal neurons (Bond et al. 2004; Sailer et al. 2002; Stocker et al. 1999).

Therefore, the distribution of SK2 channel subunits in postnatal hippocampal neurons was investigated with a specific antibody (anti-NSK2) raised against the NH2-terminal region of the SK2 protein (Cingolani et al. 2002). Clear SK2 immunostaining was observed in the soma of the neurons from DIV 2 to DIV 13 (Fig. 2, B–E). The staining increased progressively from DIV 2. Maximal expression was seen at DIV 10–13 (Fig. 2E). For this reason, neurons between DIV 10 and DIV 18 were used for electrophysiological and imaging experiments.

SK2 immunoreactivity was visible in the soma of the hippocampal pyramidal neurons and in the proximal and distal portions of neurites (Fig. 2E). Preadsorption of the anti-NSK2 antibodies (Fig. 2F at DIV 13) and omission of the primary antibody (data not shown) resulted in the lack of fluorescent staining at all developmental stages, confirming the specificity of the detected signal.

Imaging and modulation of Ca\(^{2+}\) transients elicited by action potentials backpropagating to proximal dendrite of hippocampal neurons. In hippocampal pyramidal neurons there is plentiful evidence that SK channels can be activated by Ca\(^{2+}\) entering...
through voltage-gated Ca\(^{2+}\) channels opening during trains of APs (Cai et al. 2007; Empson and Jefferys 2001; Fernandez de Sevilla et al. 2006; Kaczorowski et al. 2007; Kramar et al. 2004; Oh et al. 2000; Shah et al. 2006; Stocker et al. 1999; but see Gu et al. 2005). This opens the possibility that the activation of SK channels regulates local, voltage-gated Ca\(^{2+}\) channel-mediated calcium signals.

Simultaneous two-photon Ca\(^{2+}\) imaging and whole cell current-clamp recordings allow the testing of this hypothesis. Recent measurements of Ca\(^{2+}\) transients evoked by single APs in small secondary to quaternary dendritic branches of CA1 pyramidal neurons demonstrated that the calcium-sensitive fluorophore fluo-4, with a $K_d$ ranging from 340 nM (34°C) to 800 nM (24°C) (Yasuda et al. 2004) and its large dynamic range, is the dye of choice when used at low concentrations (Sabatini et al. 2002). A general concern with Ca\(^{2+}\)-sensitive processes is the potential interference exerted by Ca\(^{2+}\)-sensitive dyes because of their concentration and binding properties. In the case of SK channels activated by Ca\(^{2+}\) entering the cell via voltage-gated Ca\(^{2+}\) channels, the dye might act as an exogenous Ca\(^{2+}\) buffer that captures the incoming Ca\(^{2+}\), thereby reducing the activation of the SK channels.

The amplitude and the time course of decay of the $I_{AHP}$ in the absence and presence of fluo-4 were first tested in a subset of voltage-clamp experiments. Fluo-4 at 20 $\mu$M did not significantly alter the amplitude (control: 140 ± 23 pA, $n$ = 17; fluo-4: 98 ± 13 pA, $n$ = 14) and the time constant of decay (control: 458 ± 46 ms, $n$ = 17; fluo-4: 574 ± 50 ms, $n$ = 14) of the $I_{AHP}$ elicited by a 200-ms-long pulse ($P$ = 0.1).

Consequently, this concentration of fluo-4 was used to study the effect of SK channels on the dynamics of intracellular free Ca\(^{2+}\) concentration in response to APs in the proximal dendrite. A typical filled pyramidal cell with two neuronal processes (p1, p2) in the field of vision is shown in Fig. 3A. For visualization, cells were subjected to a prolonged depolarization at the end of the experiment to increase the intracellular Ca\(^{2+}\) concentration. This was necessary because under resting

![Fig. 2. Expression of SK2 in cultured postnatal hippocampal neurons at different stages of in vitro development. A: immunohistochemistry performed with a specific anti-NSK2 antibody at 1 day in culture (DIV 1) shows a fluorescence signal indistinguishable from the background signal. Inset: bright field picture of the stained neurons. B–D: by DIV 2 SK2 can be clearly detected (B), and its expression increases at DIV 4 (C) and DIV 6 (D). E: distribution of the SK2 subunit at DIV 13, showing a clear somato-dendritic localization of the channel. F: nonspecific staining with preadsorbed antibodies (+Trx-NSK2) shows a very weak signal in soma but not in neurites. Inset: bright field picture of the stained neurons. Scale bars: A–D, 20 $\mu$m (shown in B); E and F, 10 $\mu$m (shown in E); insets, 20 $\mu$m in A and 10 $\mu$m in F.](Image)

![Fig. 3. Ca\(^{2+}\) transients induced by action potentials (APs) backpropagating to the proximal processes of hippocampal neurons. A: image of a cultured hippocampal neuron filled with fluo-4 (20 $\mu$M) and stimulated with a prolonged depolarization showing the Ca\(^{2+}\) accumulation within the cell. Two neuronal processes (p1 and p2) are indicated by arrows. Scale bar: 10 $\mu$m. B: short (10 ms) somatic depolarizing current injections elicited 2 APs (top) at 20 Hz. Line scans (middle) were recorded from the 2 processes (p1 and p2), at the position indicated by the dashed line in A. Line scans show an increase in fluorescence as a consequence of intracellular Ca\(^{2+}\) elevation. Scale bar: 250 ms. The corresponding relative changes in fluorescence (bottom) show a faster transient for the thinner process (p1) simply due to the different surface-to-volume ratio. For display purposes transients were digitally filtered off-line (adjacent-averaging routine, smoothing factor $n$ = 10, Origin 7). C: effect of K\(^{+}\) channel blocker tetraethylammonium (TEA, 10 mM) on repolarization of the APs that induced the fluorescence transients displayed in D. Black, APs before TEA application (control); red, APs in the presence of TEA; blue, APs after washout of TEA. D: line scans recorded under control conditions, during application of 10 mM TEA, and after washout (top) and corresponding relative changes in fluorescence (bottom). Scale bar: 250 ms.](Image)
conditions the low level of fluo-4 was too dim to reliably detect neuronal processes. APs were elicited by somatic current
injections of 10-ms-long depolarizing pulses at 20 Hz (Fig. 3B, top), a firing frequency in the range observed in CA1 place 
cells when the animals are in proximity of their place field center (Dragoi and Buzsáki 2006; O’Keefe and Dostrovsky 1971).
The two APs induced an increase in fluorescence, indicative of Ca\(^{2+}\) entry, in the proximal neurites of the cell (<50 \(\mu\m) from soma; Fig. 3B). The fluorescence signal was collected by line scans along the dashed line in Fig. 3A with a 
two-photon imaging system with a photomultiplier as a detector to measure the dynamics of intracellular free Ca\(^{2+}\) concentra-
tion (Fig. 3B, middle). The time course of decay of the evoked fluorescence transients (Fig. 3B, bottom) showed cell-
to-cell variability with an average \(\tau\) of 443 ± 34 ms (range: 221–987 ms in 28 processes of 25 cells). To test whether 
increasing the AP duration (Fig. 3C) saturates the fluo-4 fluorescence signal elicited by two APs at 20 Hz, the K\(^{+}\) channel blocker TEA was applied by bath perfusion. TEA at 10 
mM reversibly prolonged the duration of the APs (Fig. 3C) and doubled the amplitude of the fluorescence transients (198 ± 46\%, \(n = 4\), Mann-Whitney test, \(P < 0.04\); Fig. 3D). TEA also prolonged the decay times of the transients. The prolongation 
was quite variable, and in the presence of TEA one cell displayed a fluorescence transient that developed into a plateau 
and did not decay back to baseline values over the duration of the recording. In the cells where the fluorescence transient 
retained or returned to baseline, the decay time of the transients was increased \(\tau_{\text{Control}}: 316–674 \text{ ms} (n = 4); \tau_{\text{TEA}}: 464–4,800 \text{ ms} (n = 3)\). 
The changes in the amplitude and duration of the Ca\(^{2+}\) transients in the proximal dendrite of hippocampal pyramidal 
neurons are comparable with the effect of TEA on the AP-induced Ca\(^{2+}\) transients in the dendrites of neocortical 
neurons (Markram et al. 1995). The effect of TEA on AP duration (Fig. 3C) and the amplitude and time course of the fluorescence transients was reversible (Fig. 3D). The reversibility of the effect of TEA 
demonstrates the stability of the signals observed under our recording and imaging conditions.

**SK channels regulate action potential-induced Ca\(^{2+}\) influx.** TEA at 10 mM blocks several voltage-gated K\(^{+}\) channels, 
including members of the K\(_{\text{v}1.1}\), K\(_{\text{v}2.1}\), K\(_{\text{v}3.3}\), and K\(_{\text{v}7}\) families, voltage- and Ca\(^{2+}\)-activated large-conductance K\(^{+}\) (BK) chan-
nels (Coetzee et al. 1999), and, to some extent, SK channels (reviewed in Pedarzani and Stocker 2008). Consequently, 
the effect observed in the presence of TEA on the Ca\(^{2+}\) transient (Fig. 3, C and D) is due to the block of different types of K\(^{+}\) 
currents that contribute to the AP repolarization and afterhyper-polarization phases in hippocampal pyramidal neurons. SK 
channels have been shown to terminate glutamate-evoked Ca\(^{2+}\) plateau potentials in distal apical dendrites (Cai et al. 
2004) and regulate the Ca\(^{2+}\) influx through NMDA receptors through a negative feedback mechanism in 
spines of hippocampal pyramidal neurons (Bloodgood and Sabatini 2007; Ngo-Anh et al. 2005). This makes SK channels good 
candidates to regulate AP-induced Ca\(^{2+}\) signals in proximal dendritic regions. To investigate whether and to what extent 
SK channels specifically contribute to the regulation of Ca\(^{2+}\) transients in the proximal dendrites of hippocampal neurons, 
SK channels were inhibited by apamin, a selective inhibitor. Apamin increased the amplitude of the fluorescence transient 
(Fig. 4, A and B) elicited by two APs at 20 Hz in the proximal dendrite to 115 ± 4\% (\(n = 5\), \(P < 0.05\); Fig. 4C). Apamin also 
slowed the decay of the fluorescence transients by 18 ± 5\% (Fig. 4, B and D; control: \(\tau = 298 ± 11 \text{ ms}\), apamin: \(\tau = 353 ± 18 \text{ ms}\). 
\(n = 5\), \(P < 0.05\)). As a result of the increase in amplitude and 
prolongation of \(\tau\), the amount of Ca\(^{2+}\) entering the cell, charac-
terized by the area under the curve of the fluorescence transient, 
was significantly increased in the presence of apamin (Fig. 4, B and 
E; \(138 ± 13\%\); \(n = 5\), \(P < 0.05\)).

To test whether the observed effects were a consequence of apamin acting specifically on Ca\(^{2+}\) influx triggered by APs 
rather than caused by the direct action of the depolarizing current injection, we measured the impact of the SK channel 
inhibitor after TTX application. TTX strongly attenuated the fluorescent transients (Fig. 4, F–H; \(n = 4\), confirming that, 
under our experimental conditions, Ca\(^{2+}\) signals in the proximal dendrite mainly arise from the backpropagation of APs. 
In the presence of TTX, apamin failed to increase Ca\(^{2+}\) influx (Fig. 4, F–H; \(F_{5,2} = 27\), \(P = 0.001\); TTX vs. control \(P < 0.05\); 
TTX+apamin vs. control \(P < 0.05\); TTX+apamin vs. TTX \(P > 0.05\); 1-way repeated-measures ANOVA with Bonferroni 
post hoc test). This indicates that AP-induced activation of calcium channels is necessary for the SK-mediated modulation of 
Ca\(^{2+}\) signals.

To corroborate the result obtained with apamin, we tested the effect of a structurally unrelated small organic SK channel 
blocker, dTC, on the Ca\(^{2+}\) transients in the proximal dendrite of hippocampal pyramidal neurons. Although less specific 
than apamin, dTC has the advantage that it blocks SK channels in a reversible manner. Application of 100 \(\mu\M dTC led to an 
increase in the fluorescence transient amplitude to 113 ± 3\% (Fig. 5, A and B), similar to the change observed with apamin. 
The effect of dTC on the amplitude of the fluorescence transients was reversed in five of six cells (Fig. 5, A and B). The 
reversibility of the dTC effect on the amplitude of the Ca\(^{2+}\) transients rules out the possibility that the observed increase 
might be a consequence of dye loading. Blocking SK channels with dTC also resulted in an increase of the area of the 
fluorescence transients to 120 ± 4\% (Fig. 5, A and C) and in a prolongation of the time course of decay, which is reflected 
by an increase of \(\tau\) by 20 ± 5\%.

Several SK channel enhancers have recently been characterized (reviewed in Pedarzani and Stocker 2008) and act by 
increasing the apparent Ca\(^{2+}\) sensitivity of SK channels (Pedarzani et al. 2001). If the increase in the Ca\(^{2+}\) transients 
observed upon application of apamin and dTC is due to the inhibition of SK channels acting as negative feedback regulators 
of Ca\(^{2+}\) influx triggered by APs, then enhancement of SK channel activity should lead to a reduction in the Ca\(^{2+}\) trans-
tsients. To test this hypothesis the SK channel enhancer NS309 was used (Pedarzani et al. 2005). NS309 (5 \(\mu\M) reduced both 
the amplitude of the fluorescence transients by 25 ± 5\% (Fig. 5, D and E) and their area by 30 ± 6\% (Fig. 5, D and F) in all 
cells tested (\(n = 6\)). In the presence of NS309 the time constant 
of decay of the fluorescence transients was also shortened by 
21 ± 6\% (\(n = 6\), Fig. 5D). The overall reduction of the Ca\(^{2+}\) 
transients observed in the presence of NS309 and the observed 
overall opposite effect observed with SK channel blockers further 
support the hypothesis that SK channels regulate Ca\(^{2+}\) trans-
tsients elicited by APs in the proximal dendrite of hippocampal 
pyramidal neurons by a negative feedback mechanism.
If SK channels, activated by Ca\textsuperscript{2+} entering through voltage-gated Ca\textsuperscript{2+} channels, modulate the Ca\textsuperscript{2+} transients generated by APs, then increasing the number of APs should lead to an enhanced Ca\textsuperscript{2+} influx, and therefore a stronger SK channel recruitment and a greater effect on the Ca\textsuperscript{2+} transients. The fluorescence transients measured in the proximal dendrites of pyramidal neurons in response to four APs were 21\% larger than those observed in response to two APs at 20 Hz (4 APs: Fig. 6, A and B, \(\Delta F/F = 1.72 \pm 0.11, n = 18\); 2 APs: Figs. 3–5, \(\Delta F/F = 1.42 \pm 0.12, n = 28\); \(P = 0.02\), Mann-Whitney test). The inhibition of SK channels by dTC (100 \mu M) resulted in an increase of amplitude of the fluorescence transients to 130 \pm 8\% (Fig. 6, A–C) and of area to 144 \pm 9\% (Fig. 6, B and E; \(n = 4\), \(P < 0.05\)). Additionally, dTC caused a prolongation of the fluorescence transients by 21 \% (Fig. 6D; \(n = 4\), \(P < 0.05\)). The relative increases in amplitude and area of the fluorescence transients were significantly larger than those observed with two APs after application of dTC (compare Fig. 5, B and C, with Fig. 6, C and E; \(P < 0.05\)). To test whether the relative increase in the amplitude of Ca\textsuperscript{2+} transients observed in response to four APs in the presence of dTC was limited by dye saturation, we applied 10 mM TEA in the presence of dTC. As expected (see also Fig. 3, C and D), TEA substantially increased the influx of Ca\textsuperscript{2+} by prolonging the duration of the APs (Fig. 6A, bottom), thereby causing a further large increase of the fluorescence transients (Fig. 6B; amplitude 149 \pm 15\%, area 213 \pm 22\%; \(n = 3\)). The effect of TEA in this context confirms that the increase of the fluorescence transients observed after dTC application was not limited by fluo-4 saturation following four APs at 20 Hz.

In the proximal dendrites of CA1 neurons, different subtypes of voltage-gated Ca\textsuperscript{2+} channels are activated by the backpropagation of APs and contribute to local Ca\textsuperscript{2+} elevations, with a predominant role played by Ca\textsubscript{1,1} (L type) channels (Christie et al. 1995). Moreover, L-type Ca\textsuperscript{2+} channels have been shown to be physically colocalized and selectively coupled to the activation of small-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels in somatic patches from acutely dissociated CA1 pyramidal neurons (Marion and Tavalin 1998). L-type Ca\textsuperscript{2+} channels were shown to be the main contributors to the activation of the SK-mediated I\textsubscript{AMP} also in whole cell recordings from CA1 neurons, with a reduction of I\textsubscript{AMP} by \(-38\%\) upon application of the L-type Ca\textsuperscript{2+} channel blocker nifedipine (Bosurgi and Pedarzani 2006). We therefore tested whether the regulatory effect of SK channels on AP-induced Ca\textsuperscript{2+} transients is triggered by Ca\textsuperscript{2+} influx through L-type Ca\textsuperscript{2+} channels. First the contribution of L-type Ca\textsuperscript{2+} channels to the Ca\textsuperscript{2+} transients in the proximal dendrite was assessed. In all cells tested, application of the L-type channel blocker nifedipine at 10 \mu M markedly decreased the amplitude and the area of the fluorescence transients induced by four APs at 20 Hz (Fig. 7; \(n = 5\)). As expected, \(\tau\), the fluorescence transients was not affected by nifedipine (Fig. 7A; \(\tau\text{control} = 414 \pm 39\) ms, \(\tau\text{Nifedipine} = 423 \pm 64\) ms; \(n = 5\)), because \(\tau\) is mainly determined by Ca\textsuperscript{2+} extrusion. The subsequent application of dTC in the presence of nifedipine did not increase the amplitude of the fluorescence transients significantly (Fig. 7; \(F_{4,2} = 61, P < 0.05\)).

![Figure 4](http://jn.physiology.org/content/vol167/issue2/)

**Fig. 4.** Effect of apamin on Ca\textsuperscript{2+} transients recorded from proximal neuronal processes. A: line scans showing fluorescence changes triggered by 2 APs at 20 Hz in the proximal process of a hippocampal pyramidal neuron in the absence (Control) and in the presence of the SK channel blocker apamin (5 nM). B: relative change in fluorescence obtained from line scans in A. Transients were fitted with monoeponential functions, and time constant of decay \(\tau\) is indicated. Right: fluorescence transients are superimposed to show the increase in amplitude and decay. C: a mean increase of 115 \pm 4\% (\(n = 5\), \(* P < 0.05\)) in the amplitude of the relative change in fluorescence was observed in the presence of apamin. \(D\): mean \(\tau\) before \((\tau = 208 \pm 11\) ms) and after \((\tau = 353 \pm 18\) ms; \(n = 5\)) application of apamin. \(* P < 0.05\). E: relative increase in area under the fluorescence transients in the absence (Control) and presence of apamin (138 \pm 13\%; \(n = 5\), \(* P < 0.05\)). C–E: symbols represent individual cells. Bar diagrams show means \pm SE. F: effect of Na\textsuperscript{+} channel blocker TTX (1 \mu M) on the APs that induced the fluorescence transient displayed in G. Black, APs before TTX application (Control); gray, membrane depolarization in the presence of TTX; dashed, membrane depolarization in the presence of TTX and apamin. G: fluorescence transients elicited by 2 depolarizing pulses under control conditions (i.e., in the presence of 2 APs, see F; black trace) and in the presence of TTX (gray) and TTX + apamin (black). H: summary bar diagram showing a mean decrease to 22 \pm 8\% (\(n = 4\)) in amplitude of the relative change in fluorescence in the presence of TTX. Apamin, when applied in the presence of TTX, did not cause any increase in the fluorescence transients \((F_{2,7} = 27, \*\* P < 0.001); \text{TTX vs. control} P < 0.05; \text{TTX + apamin vs. control} P < 0.05; \text{TTX + apamin vs. TTX} P > 0.05; 1-way repeated-measures ANOVA with Bonferroni post hoc test).
Fig. 5. Modulation of AP-induced Ca\textsuperscript{2+} transients by dTC and NS309. A: representative fluorescence transients elicited by 2 APs at 20 Hz in the proximal dendrite, showing an increase in amplitude and area under the curve caused by the SK channel blocker dTC (100 μM). This effect was partially reversed (Wash). B: increase of mean relative amplitude of fluorescence transients in the presence of dTC to 113 ± 3% of control (n = 13; \(*P < 0.01\)). The effect of dTC on the amplitude of the fluorescence transients was reversed in 5 of 6 cells (Wash, 107 ± 5% of control; n = 5; \(*P < 0.05\)). Only cells with a current amplitude >90% after washout were analyzed and included. C: normalized area under the curve of the fluorescence transients after dTC application increased to 120 ± 4% of the control value (n = 13; *P < 0.01). D: SK channel enhancer NS309 (5 μM) caused a reduction of the fluorescence transient under the same recording conditions. E: reduction by 25 ± 5% (n = 6; \(*P < 0.05\)) of the mean relative amplitude of fluorescence transients in the presence of NS309. F: with respect to control, normalized area under the curve of the fluorescence transients was reduced by 30 ± 6% (n = 6; \(*P < 0.05\)) after application of NS309 (5 μM). Symbols in B, C, E, and F represent individual cells. Bar diagrams show means ± SE.

0.0001; nifedipine vs. control 69.5 ± 1.2%, \(P < 0.05\); nifedipine+dTC vs. control 77.9 ± 1%, \(P < 0.05\); nifedipine vs. nifedipine+dTC, \(P > 0.05\); 1-way repeated-measures ANOVA with Bonferroni post hoc test), and similar results were obtained for the area of the Ca\textsuperscript{2+} transients (\(F_{4,2} = 18.47, P < 0.001\); nifedipine vs. control 81 ± 2%, \(P < 0.01\); nifedipine+dTC vs. control 85.2 ± 4.1%, \(P < 0.01\); nifedipine vs. nifedipine+dTC, \(P > 0.05\); 1-way repeated-measures ANOVA with Bonferroni post hoc test). Similarly, no significant difference for the time constant of decay of the fluorescence transients was observed when comparing controls, nifedipine alone, and the dTC-nifedipine coaplication (Fig. 7A; \(F_{4,2} = 0.14, P = 0.9\); 1-way repeated-measures ANOVA). When applied to neurons in the presence of nifedipine, the effect of dTC on both the amplitude and the duration of the fluorescence transients was therefore strongly attenuated compared with the results obtained in the absence of the L-type Ca\textsuperscript{2+} channel blocker (Fig. 6, B–D, and Fig. 7A). We performed additional experiments by first applying dTC, followed by the addition of nifedipine. When applied in the presence of dTC, nifedipine reduced the amplitude (\(F_{14,2} = 22, P < 0.0001\); dTC vs. control 123 ± 5%, \(P < 0.01\); dTC+nifedipine vs. control 78 ± 7%, \(P < 0.05\); dTC vs.

Fig. 6. Effects of dTC and TEA on Ca\textsuperscript{2+} transients induced by a train of 4 APs. A: line scans (top) and APs (20 Hz; bottom) triggered by somatic current injections in control and after application of dTC (100 μM) and further addition of TEA (10 mM). Scale bar: 500 ms. B: blocking of SK channels by dTC resulted in an increase of the relative fluorescence signal in the proximal dendritic process. This effect was further enhanced by TEA application. C: increase of mean relative amplitude of fluorescence transients in presence of dTC to 130 ± 8% of control (n = 4; \(*P < 0.05\)). D: mean \(\tau\), obtained from monoeXponential fits to the fluorescent transients, increased from \(\tau = 486 ± 99\) ms before to \(\tau = 593 ± 123\) ms after application of dTC (n = 4; \(*P < 0.05\)). E: application of dTC resulted in a mean increase in area under the curve of the fluorescence transients to 144 ± 9% (n = 4; \(*P < 0.05\)) with respect to control. Symbols in C–E represent individual cells. Bar diagrams show means ± SE.

Fig. 7. Inhibition of L-type voltage-gated Ca\textsuperscript{2+} channels prevents the dTC-mediated effect on Ca\textsuperscript{2+} transients induced by a train of 4 APs. A: application of L-type Ca\textsuperscript{2+} channel blocker nifedipine (Nif, 10 μM) significantly decreased the fluorescent transient induced by a train of 4 APs. Subsequent coapplication of dTC (100 μM) caused only a small, nonsignificant increase in the fluorescent transients (Nif+dTC). B: decrease in mean amplitude of relative changes in fluorescence to 69.5 ± 1.2% (n = 5) in the presence of Nif with respect to control. When dTC was subsequently applied in the presence of Nif, amplitude of the fluorescence transients was not significantly changed (\(F_{4,2} = 6.1, ***P < 0.0001\); Nif vs. control \(P < 0.05\); Nif+dTC vs. control \(P < 0.05\); Nif vs. Nif+dTC \(P > 0.05\); 1-way repeated-measures ANOVA with Bonferroni post hoc test). Symbols represent individual cells. Bar diagram shows means ± SE.
dTC+nifedipine, \( P < 0.001 \); 1-way ANOVA with Bonferroni post hoc test) and area (\( F_{1,45} = 21, P < 0.0001 \); dTC vs. control \( 133 \pm 7 \%, P < 0.01 \); dTC+nifedipine vs. control \( 76 \pm 6 \%, P > 0.05 \); dTC vs. dTC+nifedipine, \( P < 0.001 \); 1-way ANOVA with Bonferroni post hoc test) of the Ca\(^{2+}\) transients to values similar or below the control values. These results suggest that the activation of SK channels by APs is at least in part due to the activation of L-type Ca\(^{2+}\) channels and in turn regulates Ca\(^{2+}\) influx in the proximal dendrite.

**DISCUSSION**

The dynamic response of pyramidal neurons in the hippocampus is modulated by Ca\(^{2+}\) transients that result from influx through voltage-gated Ca\(^{2+}\) channels. In particular, Ca\(^{2+}\) elevations triggered by backpropagating APs show a maximal amplitude in the proximal dendrites and decrease rapidly with distance from the soma (Callaway and Ross 1995; Christie et al. 1995; Jaffe et al. 1992; Regehr et al. 1989; Regehr and Tank 1994; Spruston et al. 1995). In this study, we have investigated how the activation of SK channels affects AP-induced changes in intracellular Ca\(^{2+}\) levels in proximal processes of hippocampal pyramidal neurons. We have found that in this cellular compartment SK channels limit the amplitude and duration of AP-induced Ca\(^{2+}\) transients.

SK channels have been shown to modulate the amplitude and duration of intracellular Ca\(^{2+}\) signals by feedback regulation of the relevant Ca\(^{2+}\) sources in different dendritic compartments, thus affecting dendritic signal integration and synaptic plasticity. In organotypic hippocampal cultures, SK channels are responsible for the repolarization of local dendritic plateau potentials triggered by focal glutamate application to distal apical dendrites of CA1 pyramidal neurons (Cai et al. 2004). In acute hippocampal slices, synaptic stimulation activates glutamate receptors, leading to the activation of SK channels located on the spine heads, which in turn reduces Ca\(^{2+}\) influx through the NMDA receptors (Bloodgood and Sabatini 2007; Lujan et al. 2009; Ngo-Anh et al. 2005).

Both types of feedback regulation were shown to occur in response to synaptic inputs and on dendritic compartments receiving primarily glutamatergic excitatory synaptic inputs. The physiological role of the second- and higher-order dendrites, where the excitatory inputs predominate, is fundamentally different from that of the proximal apical dendrite, which mainly receives inhibitory synaptic inputs from GABAergic interneurons (Papp et al. 2001). In the proximal dendrite our results show feedback regulation of the amplitude and duration of AP-induced Ca\(^{2+}\) transients by SK channels. This modulation of transient Ca\(^{2+}\) elevations by SK channels could affect the communication between the soma and the distal apical dendritic tree at the single-cell level and shift the balance between excitation and inhibition at the network level.

Given their high sensitivity to Ca\(^{2+}\) (EC\(_{50}\) \( \approx 300 \) nM; Xia et al. 1998) and relatively fast time constant of activation (\( \approx 5 \) ms at saturating Ca\(^{2+}\) concentrations; Pedarzani et al. 2001; Xia et al. 1998), SK channels are well suited to take part in a feedback loop to regulate Ca\(^{2+}\) influx in proximal apical dendrites of CA1 neurons, where a single AP leads to Ca\(^{2+}\) elevations on the order of \( \approx 300 \) nM lasting 70–90 ms, while higher and longer-lasting free Ca\(^{2+}\) concentrations are reached in response to trains of APs (Helmchen et al. 1996; Maravall et al. 2000).

Our results suggest that SK channels act in a negative feedback loop by reducing Ca\(^{2+}\) influx through the Ca\(^{2+}\) channels that activate them after APs. This role of SK channels is supported by the effects of specific SK channel blockers (apamin, dTC) and enhancers (NS309) on the magnitude of Ca\(^{2+}\) transients. The presence of apamin or dTC boosted the Ca\(^{2+}\) influx induced by a train of two APs. Consistent with a negative feedback mechanism, application of the SK channel enhancer NS309 resulted in a reduction of the Ca\(^{2+}\) transient.

The regulatory effect of SK channels was particularly evident when a train of four APs was used to elicit a larger Ca\(^{2+}\) influx, leading to a stronger recruitment of SK channels. The increases in the amplitude of Ca\(^{2+}\) transients following application of SK channel blockers were larger compared with the stimulation with two APs and consistently observed in every cell tested.

Ca\(^{2+}\) buffering by Ca\(^{2+}\)-sensitive dyes could in principle mimic the effect of SK channel inhibition. However, this is unlikely in our case because we used a low concentration of Ca\(^{2+}\)-sensitive dye and did not observe significant changes in the baseline fluorescence over the course of our experiments. If anything, the added buffer capacity would result in gradual decrease in the Ca\(^{2+}\) transients (Helmchen et al. 1996; Maravall et al. 2000) and lead to an underestimation of the effects of SK channel inhibitors on the amplitude of Ca\(^{2+}\) transients. The validity of the increase of the Ca\(^{2+}\) transients induced by SK blockers is further supported by the reversibility achieved on washout of dTC.

In CA1 pyramidal neurons, APs induce Ca\(^{2+}\) transients that are largest in the proximal dendrites (Callaway and Ross 1995; Christie et al. 1995; Spruston et al. 1995), where they are mediated by different subtypes of high-voltage-activated Ca\(^{2+}\) channels (Bloodgood and Sabatini 2007; Christie et al. 1995, 1996). In particular, L-type Ca\(^{2+}\) channels are highly expressed in the somato-dendritic compartment of pyramidal cells in sections (Leitch et al. 2009; Tippens et al. 2008; Westenbroek et al. 1990) and primary cultures (Pravettoni et al. 2000). Additionally, experiments on the specific high-voltage-activated Ca\(^{2+}\) channel subtypes coupled to the activation of the SK-mediated \( I_{\text{AHP}} \) in hippocampal pyramidal neurons have revealed that L-type Ca\(^{2+}\) channels are important contributors to the activation of \( I_{\text{AHP}} \), which was reduced by \( \approx 48\% \) by the L-type Ca\(^{2+}\) channel blocker nifedipine (Bosurgi and Pedarzani 2006). Application of nifedipine showed a contribution of \( \approx 30\% \) by L-type Ca\(^{2+}\) channels to the total AP-induced Ca\(^{2+}\) elevation in the proximal dendrites of cultured hippocampal pyramidal neurons (Fig. 7). This is in line with a previous report on the relative contributions of different voltage-gated Ca\(^{2+}\) channel subtypes to spike-induced Ca\(^{2+}\) influx in hippocampal pyramidal neurons in brain slices (Christie et al. 1995). L-type Ca\(^{2+}\) channel inhibition prevents the increase in the amplitude of the Ca\(^{2+}\) transients by SK channel blockers (Fig. 7) or reverses it back to control values or below when nifedipine is applied after dTC. This is evidence that L-type Ca\(^{2+}\) channels are implicated in the AP-induced Ca\(^{2+}\) influx leading to SK channel activation in the proximal dendrite of hippocampal pyramidal neurons. We cannot, however, exclude the contribution of other Ca\(^{2+}\) channel subtypes (see also Jones and Stuart 2012).

How do SK channels regulate transient Ca\(^{2+}\) elevations triggered by APs in dendrites? Voltage-gated Ca\(^{2+}\) channels open during the repolarizing phase of APs. While inhibition of BK and voltage-dependent K\(^+\) channels by TEA leads to broader APs and...
increased Ca\(^{2+}\) influx (Fig. 3, C and D), SK channel inhibition does not affect the duration of somatic APs (Fig. 6A). However, we cannot exclude the possibility that SK channels might contribute to shaping the waveform of dendritic APs, which have a lower amplitude and a longer duration in CA1 dendrites (Johnston et al. 2000; Spruston et al. 1995). While the waveform of the somatic AP is not directly affected by SK channel activation, the SK channel effect on AP-induced Ca\(^{2+}\) entry in the proximal dendrite might result from the functional interaction of these channels with other dendritic conductances. Thus two K\(^{-}\) currents, I\(_{A}\) and I\(_{D}\), are expressed in CA1 dendrites (Golding et al. 1999; Hoffman et al. 1997) and are inactivated at depolarized potentials. The voltage-dependent inactivation properties of A-type K\(^{-}\) channels enable modest levels of membrane depolarization to decrease the size of the available A channel population and likewise increase dendritic AP amplitude and duration (Hoffman et al. 1997). By hyperpolarizing the membrane potential, SK channels could affect the availability and activation state of these conductances in AP trains, with SK channel inhibition and corresponding membrane depolarization favoring the inactivation of I\(_{A}\) and I\(_{D}\). Upon inhibition of I\(_{A}\) and I\(_{D}\), large-amplitude, backpropagating APs have been shown to activate dendritic Ca\(^{2+}\) channels or favor the dendritic initiation of Ca\(^{2+}\)-dependent potentials, resulting in a massive influx of Ca\(^{2+}\) into the dendrites (Golding et al. 1999; Hoffman et al. 1997; Magee and Carruth 1999). This supports the possibility of a potential interaction between SK channels and A- and/or D-type K\(^{-}\) channels that may underlie the increase in Ca\(^{2+}\) influx observed upon SK channel inhibitions in our recordings. The relatively small, albeit significant, effect exerted by SK channel inhibition on Ca\(^{2+}\) influx in proximal hippocampal pyramidal cell dendrites might well match the gradient of A-type K\(^{-}\) channel density, with fewer channels in the proximal compared with the distal dendritic compartment.

A second potential mechanism for the SK-mediated enhancement of Ca\(^{2+}\) transients in the proximal dendritic compartment is linked to the coexistence of L-type Ca\(^{2+}\) channels with different gating behaviors in neurons, which are thought to give rise to distinct intracellular calcium signals in response to neuronal activity (Forti and Pietrobon 1993; Kavalali and Plummer 1994; Koschak et al. 2007). Thus, in addition to cardiac-like L-type channels, hippocampal neurons display L-type Ca\(^{2+}\) channels with anomalous gating properties, characterized by long channel reopenings after repolarization following strong depolarizations, such as bursts or trains of APs (Kavalali and Plummer 1994; Schjott and Plummer 2000). One potential mechanism by which SK channels might modulate Ca\(^{2+}\) influx would therefore be by reducing the activity of L-type Ca\(^{2+}\) channels in their “anomalous gating” phase. This hypothesis is supported by our results showing that strong depolarization caused by APs is essential to generate the SK-mediated feedback on Ca\(^{2+}\) influx, because this was absent in response to pure electrotonic spread when Na\(^{+}\) channels were blocked by TTX (Fig. 4, F–H).

In addition to anomalous gating properties, L-type Ca\(^{2+}\) channels are subject to various mechanisms of channel inactivation that contribute to the control of Ca\(^{2+}\) entry during ongoing neuronal electrical activity. These include Ca\(^{2+}\)-dependent inactivation and fast and slow voltage-dependent inactivation (Budde et al. 2002). The inactivation kinetics of L-type Ca\(^{2+}\) channels are generally described as slow, but they vary in different cell types, possibly because of molecular diversity of the channels (splice variants of the pore-forming subunit; interaction with other Ca\(^{2+}\) channel subunits and modulatory proteins) (Budde et al. 2002). The inactivation profile of L-type Ca\(^{2+}\) channels in hippocampal pyramidal neurons has not been specifically characterized. We cannot exclude that SK channels could interfere in some indirect manner with the inactivation process of Ca\(^{2+}\) channels in proximal dendritic processes, contributing to the increase in Ca\(^{2+}\) influx we have observed on SK channel inhibition.

The pharmacological manipulation of SK channel activity not only increased or decreased the amplitude but also affected the area under the curve of the Ca\(^{2+}\) transients elicited by APs in the proximal dendrite of hippocampal pyramidal neurons (Figs. 4–6). SK channel inhibition led also to a significant prolongation of the Ca\(^{2+}\) transients (Figs. 4–6). Interestingly, the L-type Ca\(^{2+}\) channel blocker nifedipine prevented the effect of SK channel inhibitors on the time constant of decay of Ca\(^{2+}\) transients (Fig. 7). The time course of decay of AP-induced Ca\(^{2+}\) transients in dendrites directly reflects the rate of Ca\(^{2+}\) clearance (Scheuss et al. 2006). Sarco(end)plasmic reticulum Ca\(^{2+}\)-ATPases (Mainen et al. 1999; Majewksa et al. 2000; Sabatini et al. 2002), plasma membrane Ca\(^{2+}\)-ATPases, and Na\(^{+}\)/Ca\(^{2+}\) exchangers (Lorincz et al. 2007; Scheuss et al. 2006) are responsible for the Ca\(^{2+}\) clearance from the cytosol of dendrites and spines in CA1 pyramidal neurons. Notably, both plasma membrane Ca\(^{2+}\)-ATPases and Na\(^{+}\)/Ca\(^{2+}\) exchangers are expressed in the dendrites of primary hippocampal neurons (Kiedrowski 2004; Kip et al. 2006). The increased duration of Ca\(^{2+}\) signals might simply reflect the longer time needed to clear the augmented Ca\(^{2+}\) after SK channel inhibition (Regehr and Tank 1992). Alternatively, SK channels might affect extrusion mechanisms in different ways. In the thalamus, for example, SK2 channels and sarco(end)plasmic reticulum Ca\(^{2+}\)-ATPases compete for available Ca\(^{2+}\) and shape Ca\(^{2+}\) transients in an interactive manner (Cueni et al. 2008). Another possibility is that the larger Ca\(^{2+}\) accumulations due to SK channel inhibition attenuated Ca\(^{2+}\) extrusion by plasma membrane Ca\(^{2+}\) ATPases and Na\(^{+}\)/Ca\(^{2+}\) exchangers, whose function is reduced in a Ca\(^{2+}\)-dependent manner (Scheuss et al. 2006), thereby leading to the observed prolongation of the duration of Ca\(^{2+}\) signals.

Our results demonstrate for the first time that the activity of SK channels can regulate the duration of Ca\(^{2+}\) transient decays in the proximal dendrite of hippocampal neurons. This may affect temporal summation of Ca\(^{2+}\) signals, potentially leading to changes in spike timing-dependent plasticity (Caporale and Dan 2008), as we have recently shown in another brain region, the striatum (Nazzaro et al. 2012). Here SK channels take part in the regulation of Ca\(^{2+}\)-dependent release of endocannabinoids and plasticity, through a functional coupling with L-type voltage-gated Ca\(^{2+}\) channels activated by trains of APs (Nazzaro et al. 2012). SK-mediated modulation of intracellular Ca\(^{2+}\) dynamics may similarly be relevant for the activation of Ca\(^{2+}\)-dependent signaling cascades to induce different forms of plasticity also in the hippocampal region (Cummings et al. 1996).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: R.T., T.F., M.S.-C., A.C., C.D.R., and P.P. performed experiments; R.T., T.F., M.S.-C., A.C., and C.D.R. analyzed data; R.T., T.F., M.S., C.D.R., and P.P. interpreted results of experiments; R.T., T.F., and M.S. prepared figures; R.T., T.F., M.S.-C., M.S., C.D.R., and P.P. edited and revised manuscript; R.T., M.S., C.D.R., and P.P. approved final version of manuscript; M.S., C.D.R., and P.P. conceived and designed research; P.P. drafted manuscript.

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