Properties of a T-Type Ca\(^{2+}\) Channel–Activated Slow Afterhyperpolarization in Thalamic Paraventricular Nucleus and Other Thalamic Midline Neurons

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Zhang L, Renaud LP, Kolaj M. Properties of a T-type \(\text{Ca}^{2+}\) channel–activated slow afterhyperpolarization in thalamic paraventricular nucleus and other thalamic midline neurons. J Neurophysiol 101: 2741–2750, 2009. First published March 25, 2009; doi:10.1152/jn.91183.2008. Burst firing mediated by a low-threshold spike (LTS) is the hallmark of many thalamic neurons. However, postburst afterhyperpolarizations (AHPs) are relatively uncommon in thalamic neurons. We now report data from patch-clamp recordings in rat brain slice preparations that reveal an LTS-induced slow AHP (sAHP) in thalamic paraventricular (PVT) and other midline neurons, but not in ventrobasal or reticular thalamic neurons. The LTS-induced sAHP lasts 8.9 ± 0.4 s and has a novel pharmacology, with resistance to tetrodotoxin and cadmium and reduction by Ni\(^{2+}\) or nominally zero extracellular calcium concentration, which also attenuate both the LTS and sAHP. The sAHP is inhibited by 10 mM intracellular EGTA or by equimolar replacement of extracellular \(\text{Ca}^{2+}\) with Sr\(^{2+}\), consistent with select activation of LVA T-type \(\text{Ca}^{2+}\) channels and subsequent \(\text{Ca}^{2+}\) influx. In control media, the sAHP reverses near \(E_K\), shifting to −78 mV in 10.1 mM \([\text{K}^+]_o\), and is reduced by Ba\(^{2+}\) or tetraethylammonium. Although these data are consistent with opening of \(\text{Ca}^{2+}\)-activated \(K^+\) channels, this AHP lacks sensitivity to specific \(\text{Ca}^{2+}\)-activated \(K^+\) channel blockers apamin, iberiotoxin, charybdotoxin, and UCL-2077. The LTS-induced sAHP is suppressed by a \(\beta\)-adrenergic agonist isoproterenol, a serotonin 5-HT\(_1\), receptor agonist 5-CT, a neuropeptide orexin-A, and by stimulation of the \(\alpha_2\)-stimulating protein kinase A pathway with 8-Br-cAMP and forskolin. The data suggest that PVT and certain midline thalamic neurons possess an LTS-induced sAHP that is pharmacologically distinct and may be important for information transfer in thalamic–limbic circuitry during states of attentiveness and motivation.

INTRODUCTION

In the thalamus, the principal gateway through which information pertaining to various sensory modalities reaches higher cognitive centers, neurons signal to their postsynaptic targets via two distinct modes of action potential generation: tonic and burst firing. In vivo data obtained from neurons in the specific relay nuclei recorded over the sleep–wake cycle reveal that tonic firing prevails during waking, whereas rhythmic or burst firing is generally associated with slow-wave sleep (McCormick and Bal 1997; Steriade and Timofeev 2003). Detection of burst firing observed during wakefulness has led to the suggestion that thalamic neurons may use this mechanism to enhance their ability to activate neocortical circuits during perceptual and attentional neural processing (Sherman 2001; Swadlow and Gusev 2001). Burst firing is based on a voltage-gated \(\text{Ca}^{2+}\) conductance that operates via low voltage–activated (LVA) T-type \(\text{Ca}^{2+}\) channels. In thalamic neurons, LVA \(\text{Ca}^{2+}\) channels underlie burst firing patterns during the sleep–wake cycle (McCormick and Bal 1997; Steriade and Timofeev 2003), in the absence of epilepsy (Tsakiridou et al. 1995), and in nociceptive signal processing (Kim et al. 2003). Inactivation of these T-type \(\text{Ca}^{2+}\) channels occurs normally during depolarization and is removed by membrane hyperpolarization, setting the condition for their activation by appropriate depolarizing stimuli, resulting in the generation of a low-threshold \(\text{Ca}^{2+}\) spike (LTS) that may trigger a burst of sodium-dependent action potentials. During this burst of activity, the associated \(\text{Ca}^{2+}\) ion influx through either high-voltage–activated (HVA) or LVA \(\text{Ca}^{2+}\) channels leads to the activation of different \(K^+\) conductances that contribute to membrane afterhyperpolarizations (AHPs). AHPs provide neurons with an important intrinsic means of controlling their excitability and discharge patterns over variable time periods. AHPs are typically subdivided into three phases: fast (fAHP), medium (mAHP), and slow (sAHP), with the fAHPs and mAHPs each having unique molecular and pharmacological profiles (reviewed in Faber and Sah 2003). Slow AHPs may hyperpolarize neurons for intervals ranging from several hundred milliseconds to several seconds and are subject to regulation by neurotransmitters (Nicoll 1988). In addition, through their ability to achieve membrane hyperpolarization sufficient for deinactivation of T-type \(\text{Ca}^{2+}\) channels, sAHPs may provide conditions suitable for a subsequent LTS and burst firing. Indeed, prolonged hyperpolarizations consistent with activation of slow \(K^+\) conductances precede spindle oscillations and may facilitate the transition from tonic to burst firing in thalamic reticular nuclei neurons (Fuentes Elite 2004). Although sAHPs are widely observed within the CNS, they remain a topic of extensive investigation since the molecular identity of the channel(s) underlying sAHPs remains undefined (cf. Faber and Sah 2003; Vogalis et al. 2003). Interestingly, sAHPs are less commonly reported in thalamus and there is relatively little information on their association with T-type \(\text{Ca}^{2+}\) channels, a prominent feature of many thalamic neurons. Of note, McCormick and Prince (1988) initially reported that unusually large norepinephrine-sensitive LTS-induced sAHPs were an intrinsic property that contributed to burst firing observed in vitro in guinea pig midline para-tectal thalamic nucleus neurons. More recently, Hu and Mooney (2005) reported that a tetrodotoxin (TTX)- and apamin-resistant sAHP triggered by LVA \(\text{Ca}^{2+}\) channels was present in a subpopulation of rat ventral medial geniculate nucleus neurons recorded in brain explant preparations. The latter observation in particular implies that activation of a low-threshold \(\text{Ca}^{2+}\) conductance alone is sufficient to induce sAHP inlemniscal thalamic relay neurons.

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Our recent observations indicate a prevalence of low-threshold Ca\(^{2+}\) channel–mediated responses, including the LTS, in rat thalamic paraventricular nucleus (PVT) and other midline thalamic neurons (cf. Richter et al. 2005, 2006). The intralaminar and midline thalamic, different from the sensory thalamus, is deemed to convey information relevant to arousal, awareness, and motivated behaviors (Sewards and Sewards 2003; Van der Werf et al. 2002). We were prompted to explore the possible association of the LTS in these neurons with sAHPs. We now report that a subpopulation of neurons in PVT and other midline thalamic nuclei, but not thalamic reticular or ventrobasal nuclei, exhibit a TTX-resistant K\(^+\)–mediated sAHP triggered by activation of LVA Ca\(^{2+}\) channels. This sAHP displays sensitivity to various neurotransmitter molecules and has pharmacology not previously described in thalamus. A portion of these results has been briefly reported (Zhang et al. 2006).

**METHODS**

Experiments performed on Wistar rats (21–35 days of age) used protocols that conformed to the Canadian Council for Animal Care and were approved by the Ottawa Health Research Institute Animal Care Committee on the ethical use of animals in research. Animals were housed in pairs in a temperature-controlled environment under 12-h light/dark conditions and were killed by guillotine at 4–6 h after lights on (subjective quiet period). The brain was quickly removed in oxygenated (95% O\(_2\)–5% CO\(_2\)) and cooled (4°C) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 127 NaCl, 3.1 KCl, 1.3 MgCl\(_2\), 2.4 CaCl\(_2\), 26 NaHCO\(_3\), and 10 glucose (pH 7.3; osmolality 300–310 mOsm). Brain slices 350–400 microns in thickness were cut in the coronal plane with a Vibratome 3000 Plus Sectioning System (Vibratome, St. Louis, MO), incubated with borosilicate thin-walled micropipettes filled, unless otherwise stated, with (in mM): 140 K\(^{3+}\)MeSO\(_4\), 8 NaCl, 2 MgCl\(_2\), 10 HEPES, 0.1 BAPTA, 2 Mg-ATP, and 0.4 Na-GTP (pH 7.3; osmolality ~290 mOsm; resistance 3–7 MΩ). Lucifer yellow (2 mM) or neurobiotin (2%) was included in the pipette solution to facilitate identification of cell location and morphology. To verify that dialysis of the intracellular compartment during whole cell recordings was not a contributing factor to the LTS-induced sAHP we also performed control experiments using perforated patch recordings where the internal solution contained (in mM) 130 K-gluconate, 10 KCl, 10 NaCl, 1 MgCl\(_2\), 10 HEPES, 1 EGTA, 2 Mg-ATP, 0.3 Na-GTP, and amphoterin C 250 μg/ml plus granudicin 50 μg/ml (pH 7.3; osmolality ~295 mOsm). Access resistance <15 MΩ (∼30 MΩ for perforated-patch recordings) was considered acceptable. Whole cell current-clamp and voltage-clamp recordings (sampling frequency 5 kHz) were obtained using an Axopatch 1D amplifier (MDS, Sunnyvale, CA); data were filtered at 2 kHz, continuously monitored, and stored on disk. Clampex software (pClamp 9; MDS) and a Digidata 1200B interface were used to generate current and voltage commands and to store data. Off-line analyses were performed using Clampfit version 9 (MDS). Initial input conductances were determined from the linear slope (between −50 to −70 mV) of current–voltage (I–V; V\(_{hold} = −50\) mV; 600-ms pulse duration) relationships collected at the beginning of each recording. A voltage step (600-ms duration) from −50 to −100 mV was used to measure the amplitude of time-dependent inward rectification (I\(_{hi}\) the difference between steady-state and transient current responses) and the amplitude of the T-type calcium current (I\(_{T}\); cf. Perez-Reyes 2003; Richter et al. 2005). Low-threshold spikes were activated (at a frequency of 0.0166 Hz) following return to baseline from a 600-ms hyperpolarizing current pulse of −80 to −100 pA, triggered from resting membrane potential. This process, called deinactivation, is due to recovery of T-type calcium channels from inactivation (Perez-Reyes 2003). sAHP amplitude was measured as the voltage difference between the membrane potential before the hyperpolarizing pulse and the peak of the sAHP. Data were normalized to control responses before application of modulators and are presented as means ± SE. The occasional drift in resting membrane potential was adjusted by manual DC current injection to control (before treatment) values to achieve the same level for sAHP activation. Length of the LTS was measured after the baseline was adjusted to the membrane potential that existed before applying a hyperpolarizing pulse. Membrane voltages were corrected for liquid junction potential, measured at +16 mV for these solutions. Statistical comparisons between control and experimental values (P < 0.05 and better) were determined using both the paired or unpaired Student’s t-test and Pearson correlation (SigmaPlot 10, SigmaStat 3). Drugs and reagents were purchased from Sigma Chemical (St. Louis, MO). TTX was obtained from Alomone Labs (Jerusalem, Israel). Dr. David Benton (University College London, UK) provided a generous supply of UCL-2077 [3-(triphenylmethylaminomethyl)pyridine].

**RESULTS**

This investigation is based on data obtained from a total of 185 PVT neurons. Where indicated for comparison, additional recordings were obtained from a sample of neurons in other midline (n = 11), ventrobasal (n = 16), and reticular thalamic nuclei (n = 9; see following text, also Fig. 5). Intracellular labeling allowed for neuronal localization and definition of basic morphological properties. Most PVT neurons displayed oblong or multipolar somata measuring 12–30 microns along their long axes, from which arose three to seven main dendrites that extended for several hundred microns in different directions (Fig. 1D). All cells examined displayed state-dependent properties with tonic firing elicited in response to depolarizing current steps from resting membrane potential and low-threshold spikes (LTS) crowned with sodium-dependent action potentials (APs) when depolarized from a hyperpolarizing membrane potential of sufficient duration to deinactivate T-type calcium channels (Fig. 1A). In the majority of PVT neurons, each of these current-induced events was followed by a prolonged slow membrane afterhyperpolarization, referred to here as the sAHP. However, the sAHPs following these two modes of induction exhibited distinctly different properties. The amplitude of the sAHP following membrane depolarization-induced tonic firing, which would involve the opening of HVA calcium channels, was relatively shallow but clearly increased in magnitude in proportion to the number of APs triggered by the pulse, reaching an amplitude of about 6 mV (gray symbols in Fig. 1B; R = 0.98; P < 0.001) and a duration of around 5 s when six APs were generated (Fig. 1, A–C). By contrast, sAHPs that appeared after a hyperpolarizing-pulse–elicited LTS were consistently larger in amplitude, with a mean amplitude of −8.9 ± 0.5 mV (range −4 to −20 mV; n = 51; P < 0.001) that was clearly not dependent on how many APs could be counted on the crest of the LTS (black symbols in Fig. 1B; R = 0.08; P = 0.948). In addition, this sAHP was significantly longer in duration (range 2 to 24 s; Fig. 1C).

PVT neurons in this preparation displayed heterogeneity in the events following an LTS. In our sample of 185 cells, 82% displayed sAHP following the LTS, with the majority (67%; n = 124) of these cells exhibiting a single sAHP (Fig. 1D). A
subpopulation (15%; \( n = 27 \)) of cells displayed recurrent oscillatory bursts of LTS-sAHP combinations (mean number of bursts = 4.9 ± 0.6) following the initial sAHP (Fig. 1D$_2$). Of the remaining cells (18%; \( n = 34 \)) 22 lacked a discernable sAHP after the LTS (Fig. 1D$_3$). The other 12 cells featured a prominent (8.6 ± 1.1 mV) and long-lasting (15.3 ± 3 s) afterdepolarization (ADP) following the LTS (not illustrated) and were not considered further here.

The intrinsic membrane properties among PVT cells with and without LTS-associated sAHPs are listed in Table 1. Whereas the mean resting membrane potential was essentially similar across groups, cells lacking LTS-associated sAHPs had a significantly lower resting membrane conductance (1.33 ± 0.09 vs. 1.77 ± 0.06 nS; \( P < 0.001 \)). Among the two groups that demonstrated sAHPs, cells with recurrent LTS-sAHP bursts (oscillations) had a significantly larger \( I_L \) (−50.9 ± 7.2 vs. −22.3 ± 1.8 pA; \( P < 0.001 \)) and \( I_T \) (−809 ± 45 vs. −623 ± 21 pA; \( P < 0.001 \)) than cells with a single LTS-sAHP. Cells with recurrent bursts and larger \( I_L \) also had larger sAHP amplitudes (Table 1). Whereas the morphology of the neurons expressing these various types of LTS-associated sAHPs was not appreciably different (Fig. 1D), the present analysis was specifically focused on that population of PVT neurons displaying a single LTS-sAHP.

Properties of the LTS-associated sAHP

Under the present recording conditions, the LTS-associated sAHP described earlier exhibited a time-dependent increase both in amplitude and duration over the course of the initial 5 min after establishing the whole cell recording mode (Fig. 1E). To quantify this change, the LTS-associated sAHP was evoked every 60 s and normalized relative to the first sAHP recorded immediately after membrane rupture. This resembles a progressive increase in the amplitude of the sAHP noted in an earlier study in hippocampal CA1 neurons (Zhang et al. 1995) and did not appear to be due to a problem with our recording technique. We found no correlation between the increase in sAHP amplitude during the first 5 min of recordings and relatively small changes in LTS amplitudes (0.97 ± 0.01% of control) or membrane resistance (0.99 ± 0.01% of control). sAHPs remained relatively stable over the course of 20 min after the initial “runup.” Furthermore, LTS-associated sAHPs obtained with the perforated-patch technique (71%; 10/14 cells) had essentially the same properties as those obtained with whole cell recordings (amplitude −8.3 ± 1.2 mV, \( P = 0.324 \); duration 6.6 ± 0.6 s, \( P = 0.349 \)).

FIG. 1. The low-threshold spike (LTS)-induced slow AHP (sAHP) in paraventricular thalamic nucleus (PVT) neurons. A: superimposed current-clamp traces from the same PVT neuron. In gray, membrane depolarization from rest induces a tonic burst of action potentials (APs) followed by a modest sAHP (below the dotted line). In black, repolarization from a 600-ms membrane-hyperpolarization pulse triggers a typical LTS crowned with a single Na$^+$ spike, followed by a prominent sAHP. B: plot of sAHP amplitudes vs. number of evoked APs contrasts a modest spike-dependent increase in sAHP amplitudes subsequent to tonic firing and activation of high-voltage–activated (HVA) Ca$^{2+}$ channels (gray; \( n = 7–10 \) cells per point) vs. the sAHP amplitude following the LTS; the magnitude of the latter is independent of the number of evoked APs (black; LTS, \( n = 6–30 \) cells per point; *** \( P < 0.001 \), when comparing LTS vs. tonic firing with a single Na$^+$ spike). C: summary histograms contrast the duration of the sAHP following a depolarization-induced tonic firing of 6 APs (gray bar) vs. the afterhyperpolarization (AHP) duration following LTS crowned with 1–3 APs (black bar, ** \( P < 0.01 \)). D: typical current-clamp traces (top) and cell morphology (bottom) from 3 different PVT neurons, representative of different types of events following the LTS. The trace in D$_1$ portrays a typical LTS-induced sAHP lasting several seconds and observed in 124/185 PVT neurons. The trace in D$_2$ was the histogram pattern recorded in 27 PVT neurons where the LTS-sAHP was followed by additional oscillations (range from 2 to 16). The trace in D$_3$ represents 34 PVT neurons where the LTS lacked an ensuing AHP. E: summary (means ± SE) charts a normalized increase in the amplitude of the LTS-induced sAHP from 13 PVT neurons dialyzed with standard internal solution containing 0.1 mM BAPTA. Control was the response at time 0, taken shortly after breaking through the membrane. Inset: typical current-clamp traces obtained at time 0 min, after an initial “runup” of about 5 min, and subsequent stabilization at 15 min. Also note the stability in LTS and hyperpolarizing pulse amplitudes. The LTS was evoked once every minute with a hyperpolarizing pulse of 600 ms (−100 pA).
TABLE 1. Intrinsic properties of PVT neurons with and without LTS-induced sAHPs

<table>
<thead>
<tr>
<th>Property</th>
<th>n</th>
<th>RMP, mV</th>
<th>Conductance, nS</th>
<th>I_Ca, pA</th>
<th>I_Ca, pA</th>
<th>Test MP, mV</th>
<th>AHP, mV</th>
</tr>
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<tbody>
<tr>
<td>Slow AHP</td>
<td>124</td>
<td>71.3 ± 0.9</td>
<td>1.79 ± 0.06</td>
<td>-623 ± 21</td>
<td>-223 ± 1.8</td>
<td>-71.7 ± 0.2</td>
<td>-9.5 ± 0.3</td>
</tr>
<tr>
<td>Oscillations</td>
<td>27</td>
<td>72.5 ± 1.7</td>
<td>1.68 ± 0.16</td>
<td>-809 ± 45**</td>
<td>-509 ± 7.2***</td>
<td>-71.9 ± 0.4</td>
<td>-13.4 ± 0.6***</td>
</tr>
<tr>
<td>No AHP</td>
<td>34</td>
<td>68.9 ± 1.6</td>
<td>1.33 ± 0.09**</td>
<td>-498 ± 39</td>
<td>-161 ± 2.9</td>
<td>-72.8 ± 0.6</td>
<td>n.a.</td>
</tr>
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Values are means ± SE. **P < 0.01; ***P < 0.001; n.a., not applicable.

The lack of an obvious relationship between the number of sodium spikes induced by the LTS and the magnitude of the subsequent sAHP suggested its possible independence from activation of AP-mediated sodium channels. Indeed, observations in seven PVT neurons revealed that the amplitude of the LTS-associated sAHP was not significantly attenuated after the application of TTX (1 μM; P > 0.05; Fig. 2A) to block voltage-dependent sodium channels. Because membrane depolarization during the LTS may attain levels sufficient to activate HVA calcium channels, we further examined the LST-evoked sAHP in the presence of calcium (100 μM; n = 6), a nonselective blocker of HVA calcium channels. Although this treatment abolished the sAHP evoked by a brief strong membrane depolarization that would activate HVA calcium channels (Fig. 2B), the LTS-associated sAHP was not significantly attenuated in this same population of PVT neurons (Fig. 2C; n = 6; P = 0.105). Under voltage clamp, we confirmed that the amplitude of the T-type calcium current was also not attenuated in the presence of 100 μM calcium (100 ± 2%; n = 4; Fig. 2C, inset), a further indication that the LTS-induced sAHP did not involve HVA calcium channels. Further tests with selective HVA calcium channel blockers revealed no significant effect on the LTS-associated sAHP in the presence of omega-agatoxin TK (100 nM, n = 2), omega-conotoxin GVIA (400 nM, n = 2), or nifedipine (3 μM, n = 4). However, in the presence of nickel (Ni²⁺, 500 μM), known to block some neuronal T-type calcium channels (Perez-Reyes 2003), the LTS amplitude was reduced to 2.5 ± 1.2% (n = 6; P < 0.001), at which point the sAHP was completely eliminated (Fig. 2D). Furthermore, NNC 55–3096, a mibefradil analog that is a more selective blocker of HVA calcium channels (Huang et al. 2004), significantly reduced T-current amplitude to 46.4 ± 2.9% (Fig. 2C; P < 0.01; n = 3).

Calcium dependence of the LTS-associated sAHP

Since the LTS is mediated through opening of T-type calcium channels, we tested the hypothesis that the LTS-associated TTX-resistant sAHP is calcium dependent. Consistent with this postulate, on switching from control ACSF (containing TTX) to ACSF with nominally zero calcium we noted a reversible, progressive, and eventual elimination of the LTS-associated sAHP concordant with a decrease in the LTS to 14 ± 6% of control (Fig. 3A; n = 4; P < 0.001), with no change in input resistance (98 ± 1% of control). Data obtained from another four PVT neurons using an internal solution containing the calcium chelating agent EGTA (10 mM) revealed a gradual decrease in both the amplitude and duration of the LTS-associated sAHP but without significantly affecting the LTS amplitude (98 ± 2% of control; Fig. 3B) or input resistance (96 ± 2%). To determine whether the sAHP is mediated by Ca²⁺ ions passing through open T-type calcium channels or is merely dependent on membrane depolarization during the LTS we replaced extracellular Ca²⁺ with strontium (Sr²⁺, 2.4 mM), which supports the LTS but not Ca²⁺-dependent processes (cf. Broberger and McCormick 2005). As illustrated in Fig. 3C, equimolar replacement of external CaCl₂ with SrCl₂ eliminated the sAHP (Fig. 3C; n = 3) without any change in LTS amplitude (97 ± 1% of control) or input resistance (99 ± 1% of control), suggesting that the sAHP is indeed mediated by intracellular Ca²⁺ due to opening of T-type Ca²⁺ channels, therefore justifying a change in the term “LTS-associated” to “LTS-induced.”

K⁺ channels with a distinct pharmacology mediate the LTS-induced sAHP

We next examined channel types underlying the sAHP. In control ACSF, the amplitude of the LTS-induced sAHP was controlled with the resting membrane potential and demonstrated potential reversal around −108 mV (Fig. 4A; n = 3), approximating the calculated value of −98 mV for potassium ions in this preparation. In ACSF containing 10.1 mM [K⁺], the LTS-induced sAHP reversal potential shifted to −78 mV, a shift of about 30 mV that is in agreement with the value calculated using the Nernst equation. The LTS-induced sAHP was significantly reduced in the presence of two nonspecific K⁺ channel blockers, barium (2 mM; Fig. 4, B and E) and tetraethylammonium (TEA, 10 mM; Fig. 4E). Both barium and TEA caused an anticipated increase in input resistance (barium: 191 ± 36%; P < 0.05; TEA: 113 ± 4%; P < 0.05). The LTS was not changed under TEA (94 ± 4%; P > 0.05) and significantly increased with barium (145 ± 7%; P < 0.001). These data support the suggestion that the LTS-induced sAHP is mediated through activation of Ca²⁺-activated K⁺ channels. We next tested the LTS-induced sAHP for sensitivity to known blockers of Ca²⁺-activated K⁺ channels (see Faber and Sah 2003; Vogalis et al. 2003). Whereas other thalamic neurons, including neurons from reticular nucleus (Bal and McCormick 1993) and periganglionic nucleus (Kim and McCormick 1998), possess LTS-induced apamin-sensitive AHPs, apamin had no influence on the LTS-induced sAHPs in PVT neurons (Fig. 4, C and E). Surprisingly, UCL-2077 (10 μM), a compound recently reported to be a potent sAHP blocker in hippocampal neurons (Shah et al. 2006), failed to modify the LTS-induced sAHP. However, UCL-2077 significantly reduced the HVA calcium-channel–activated sAHP in the same population of PVT neurons (P < 0.001; Fig. 4, D and E), suggesting that the LTS-induced sAHP is a novel type of sAHP. Furthermore, as summarized in Fig. 4E, sAHP amplitudes were not influenced by exogenous application of apamin (1 μM), ibotenic acid (IBT, 100 nM), or charybdotoxin (CHT, 200 nM). None of these agents altered either resting membrane potential, input resistance, or LTS amplitude. However, we did note that this sAHP was enhanced in the presence of extracellular cesium (3 mM; 119 ± 6%; P < 0.05; n = 5; Fig. 4F), a known blocker.
a unique T-type Ca\textsuperscript{2+}-dependent rise in intracellular Ca\textsuperscript{2+} that lasts for several seconds. This prompted us to examine whether the LTS-induced sAHP might also be a feature that is unique to midline thalamic neurons. Indeed, all neurons sampled in the midline central medial (n = 3), intermediodorsal (n = 3), centrolateral (n = 2), and mediodorsal nuclei (n = 3; as defined in the atlas of Paxinos and Watson 1998) displayed a similar LTS-induced sAHP with properties similar to those described earlier (Fig. 5A). Interestingly, no similar LTS-associated sAHPs were detected in any recordings obtained from 16 neurons located in various lateral and ventrobasal thalamic nuclei (Fig. 5B). By contrast, in the thalamic reticular nucleus, we observed that the LTS was followed by a medium AHP (269 ± 35 ms; n = 9; Fig. 5C), which was reduced in the presence of 1 μM apamin (from −9.5 ± 1.6 to −2.7 ± 2.5 mV; P < 0.05; n = 4), consistent with earlier reports (cf. Avanzini et al. 1989).

**LTS-induced sAHPs are sensitive to neurotransmitters**

Initial studies of sAHPs evoked by depolarizing current pulses in hippocampal pyramidal neurons noted their sensitivity to activation of various catecholaminergic receptors (i.e., β-adrenoceptors, histamine) and to cyclic adenosine monophosphate (cAMP; e.g., Haas and Konnerth 1983; Madison and Nicoll 1982). The dorsal thalamus and PVT in particular receive a rich catecholaminergic innervation, including noradrenergic fibers that arise mainly from the locus coeruleus (Lindvall et al. 1974) and serotoninergic fibers from the raphe nuclei (Peschanski and Besson 1984; Vertes et al. 1999). PVT and adjacent midline regions also display a modest to high expression for both β-adrenoergic (Wanaka et al. 1989) and serotonin (5-HT) receptors, in particular 5-HT\textsubscript{1} subtypes (Gustafson et al. 1996). In guinea pig brain slice preparations, McCormick and Prince (1988) previously noted the sensitivity (reduction) to norepinephrine of sAHPs recorded in midline thalamic parataenial neurons. Recently, Goaillard and Vincent (2002) reported the sensitivity of postburst sAHPs recorded in rat midline thalamic neurons to a 5-HT\textsubscript{1} receptor agonist. We therefore evaluated the neurotransmitter sensitivity of the LTS-induced sAHP described in the present analysis. We first noted that a β-adrenoceptor agonist isoproterenol (1 μM for 3–6 min) significantly decreased the LTS-induced sAHP (n = 3; Fig. 6, A and D). Isoproterenol also induced a membrane depolarization (5.7 ± 0.9 mV), but there was no significant change in amplitude of the LTS (96 ± 3%). We also observed that bath-applied 5-HT (10 μM; 4–7 min; n = 4) or 5-CT (10 nM; 6–9 min; n = 4), a specific 5-HT\textsubscript{1} receptor agonist, significantly attenuated the LTS-induced sAHP (Fig. 6, B and D). Furthermore, both 5-HT and 5-CT also induced membrane depolarization (7.7 ± 1.5 vs. 6.3 ± 0.9 mV, respectively), but without any significant change in amplitude of the LTS (98 ± 5 vs.100 ± 1%, respectively). In addition to catecholamines, the PVT is unique among midline thalamic nuclei by virtue of a dense innervation from the arousal- and appetite-promoting orexin (hypocretin-) synthesizing neurons located in the lateral hypothalamus (Kirouac et al. 2005; Nambu et al. 1999; Peyron et al. 1998). Exogenous applications of the orexin-1 peptide induced membrane depolarization in PVT neurons, an action medi-
orexin A induced a reduction in the sAHP (136 ± 6%; P < 0.05; n = 3) and 5-CT (124 ± 9%; P < 0.05; n = 4), suggesting an association between the sAHP and LTS duration.

LTS-induced sAHPs are sensitive to cAMP

A common characteristic of sAHPs is their sensitivity to cAMP. It has been shown in neurons in several locations, including thalamus, that activation of protein kinase A by cAMP leads to a reduction in the sAHP that follows activation of high-voltage Ca^2+ channels (Goaillard and Vincent 2002; Nicoll 1988). We therefore examined how cAMP might influence the sAHP following selective activation of low-voltage Ca^2+ channels in PVT neurons. We observed that bath application of the nonhydrolyzable cAMP analog 8-Br-cAMP significantly reduced the LTS-induced sAHP amplitude to 67 ± 4% (Fig. 7, A and D) and was associated with membrane depolarization of 6.5 ± 1.1 mV (n = 6). Application of forskolin (10 μM), a cell-permeable adenyl cyclase activator, also reduced the LTS-induced sAHP amplitude to 49 ± 12% (Fig. 7, B and D; n = 4) and, similar to 8-Br-cAMP, in association with membrane depolarization of 5.8 ± 0.9 mV. In some preparations, forskolin has been reported to have non-specific, non-cAMP-mediated effects that include inhibition of Ca^2+ (Boutjdir et al. 1990) and K^+ channels (Herness et al. 1997). However, bath-applied 1,9-deoxyforskolin (10 μM), a biologically inactive analog of forskolin that does not stimulate
adenyl cyclase, failed to mimic the actions of forskolin (Fig. 7, C and D; n/H11005 H1104), a confirmation of the cAMP sensitivity of this LTS-induced sAHP in PVT neurons.

The majority of treatments described earlier (Figs. 6 and 7; n/H11005 H11026) resulted in both a reduction in the LTS-induced sAHPs and a significant membrane depolarization, raising the possibility that the sAHP decrease was a consequence of membrane depolarization. However, there was no correlation between the decrease in LTS-induced sAHP amplitude and either membrane depolarization or any of the treatments. The forskolin, TTX, and X5285 treatments were then applied to the same neurons under the same conditions to confirm the results.

FIG. 4. Pharmacology of the LTS-activated sAHP in PVT neurons. A: assessment of the sAHP reversal potential. LTS was evoked from different membrane potentials (100-pA 600-ms-duration hyperpolarizing pulse at membrane potentials from −49- to −75- and 100-pA 100-ms-duration depolarizing pulse at membrane potentials below −75 mV) and the sAHP amplitude was plotted against membrane potential (black symbols). The extended linear regression yielded a reversal of about −108 mV. After increase in extracellular potassium concentration ([K/H11001] o) to 10.1 mM, the extrapolated reversal point shifted to about −78 mV (gray symbols). B and C: representative current-clamp traces from 2 different PVT neurons illustrate control LTS-activated sAHP (gray traces) and reduction after bath-applied barium (Ba/H11001, 2 mM; black trace, B) or no change in the presence of apamin (1 μM; black trace, C). D: representative current-clamp trace from the same PVT neuron illustrates that UCL-2077 (10 μM) only reduced sAHP (black trace) that followed activation of HVA calcium channels. E: summary histograms reflect the effects of various agents on sAHP amplitude (gray bars), normalized to control responses before their application. Number of cells tested is indicated below. The sAHP was evoked every 60 s. The HVA calcium channel evoked sAHP (black bar) was activated by a 100-μs-long 1-nA depolarizing pulse. TTX was present throughout all experiments. Concentration and length of application: tetraethylammonium (TEA) 10 mM for 4 –7 min; Ba/H11001 2 mM for 6–9 min; apamin 1 μM for 5 min; ibotenic acid (IBT) 100 nM for 5 min; charybdotoxin (CHT) 200 nM for 5 min; UCL-2077 (UCL) 10 μM for 5 min; Cs/H11001 3 mM for 6 min; *P < 0.05; **P < 0.001.

FIG. 5. Events associated with LTS induction depend on location in thalamus. A: sample trace with a typical LTS-induced sAHP and morphology representative of neurons in midline thalamic paraventricular, central medial, intermediodorsal, centrolateral, and mediodorsal nuclei. B: by contrast, a trace representative of all 16 cells recorded in ventrobasal thalamus illustrates lack of any LTS-induced sAHP. C: trace from a neuron in the reticular nucleus to illustrate that the initial LTS is followed by a medium AHP (see inset) and several oscillatory bursts. Scale bar = 25 microns.

adrennergic, failed to mimic the actions of forskolin (Fig. 7, C and D; n = 4), a confirmation of the cAMP sensitivity of this LTS-induced sAHP in PVT neurons.

The majority of treatments described earlier (Figs. 6 and 7; n = 26) resulted in both a reduction in the LTS-induced sAHPs and a significant membrane depolarization, raising the possibility that the sAHP decrease was a consequence of membrane depolarization. However, there was no correlation between the decrease in LTS-induced sAHP amplitude and either mem-
DISCUSSION

Few investigations have focused on sAHPs that follow activation of LVA Ca$^{2+}$ channels without interference from HVA Ca$^{2+}$ channels. The present report describes some features of a novel sAHP following activation of LVA Ca$^{2+}$ channels in a majority of neurons in the PVT and several neighboring midline thalamic nuclei. The resistance of this sAHP to TTX and cadmium and its dependence on extracellular calcium and T-type Ca$^{2+}$ currents strongly imply that Ca$^{2+}$ entry during the LTS has a key role in its induction. This sAHP was unaffected by apamin, a specific blocker of SK type Ca$^{2+}$-activated K$^{+}$ channels that have been noted to attenuate LTS-activated mAHPs in thalamic perigeniculate (Kim and McCormick 1998) or reticular nucleus neurons (Avanzini et al. 1989; Bal and McCormick 1993). Of note, under the present experimental conditions, none of the neurons sampled in ventrobasal and reticular thalamic nuclei displayed a similar sAHP. Interestingly, in their earlier investigation in guinea pig midline thalamic paratenial nucleus, McCormick and Prince (1988) observed cells with unusually long sAHPs, prompting the suggestion that such LTS-triggered sAHPs could be a distinct feature of neurons in midline thalamic nuclei. A recent report of an LTS-activated sAHP with a similar apamin- and TTX insensitivity noted in a subpopulation oflemniscal neurons in the rat medial geniculate body (Hu and Mooney 2005) suggests that an LTS-induced sAHP with the properties described here has a select distribution within different areas of the thalamus.

PVT is composed of neurons with diverse afferent and efferent connectivity (see Seward and Seward 2003; Van der Werf et al. 2002) and thus is likely to contain neurons that express some heterogeneity in their membrane properties. Indeed, whereas the majority of PVT neurons displayed a single hyperpolarization-activated LTS-sAHP patterns following the initial sAHP (Fig. 1D). In these neurons the initial sAHP featured a significantly larger amplitude, quite possibly a result of their significantly larger $I_{\text{A}}$ and $I_{\text{K}}$ conductances (Table 1). Both of these conductances are known to contribute to oscillatory behaviors (cf. McCormick and Bal 1997). Because these cells displayed properties that differed from those of the majority, we opted to remove them from the current sAHP analyses.

The molecular identity of the channel(s) underlying sAHPs remains undefined. Within the midline thalamus, the PVT is reported to have a moderate to high expression of both SK-type and BK-type of Ca$^{2+}$-activated K$^{+}$ channels (Sausbier et al. 2006; Stocker and Pedarzani 2000). Whereas both of these could be associated with T-type Ca$^{2+}$ channels (Cueni et al. 2008; Smith et al. 2002), the sAHP described here was insensitive to apamin and charybdotoxin, specific blockers of SK and BK Ca$^{2+}$-activated K$^{+}$ channels, respectively. Furthermore, UCL-2077, recently described as a potent and selective sAHP blocker in hippocampal neurons (Shah et al. 2006), failed to modify LTS-induced sAHP, yet in the same neurons significantly reduced sAHPs evoked by stimulation of HVA calcium channels. Thus although our data suggest that channels mediating the LTS-induced sAHPs in this area of the thalamus are unique and different from those responsible for sAHPs in hippocampus, the current lack of specific blockers hinders characterization of the identity of the channel(s) responsible for the LTS-induced sAHP described here in midline thalamus.

The observation that a single LTS can induce a large and prolonged sAHP contrasts sharply with the relatively small and graded sAHP that follows a tonic burst of APs. Might this suggest that the K$^{+}$ channels underlying the LTS-induced sAHP are located close to T-type Ca$^{2+}$ channels? Our analysis of the spatial distribution of LVA Ca$^{2+}$-channel–evoked Ca$^{2+}$ transients in PVT neurons indicates that the magnitude of these transients is significantly greater in proximal dendrites (Richter et al. 2006). Thus the channels responsible for the LTS-induced sAHP may well be present on proximal dendrites, as proposed for the sAHP channels in basal dendrites of CA1 pyramidal neurons (cf. Bekkers 2000; Sah and Bekkers 1996). The fact that the LTS-induced sAHP reported in the present study is effectively blocked by a high concentration of EGTA (Fig. 3), similar to the situation between T-type calcium and SK channels in thalamic reticular nucleus (Cueni et al. 2008), suggests that sAHP channels and T-type Ca$^{2+}$ channels could be somewhere between 100 and 400 nm apart (Fakler and Adelman 2008; Naraghi and Neher 1997), thus increasing the probability of indirect coupling. In PVT and other midline thalamic nuclei, LVA Ca$^{2+}$ channels are coupled with long Ca$^{2+}$ signals that appear to be, at least partly, connected to Ca$^{2+}$-induced Ca$^{2+}$ release (CICR; Richter et al. 2005). Interestingly, both the LTS-induced sAHP and the LVA Ca$^{2+}$ channel–evoked Ca$^{2+}$ transient signal share similar time courses, leading us to hy-
pothesize that the sAHP could be gated and/or modulated through CICR. Involvement of CICR in modulating the sAHP has been shown in guinea pig vagal neurons (Sah and McLachlan 1991) and in hippocampal CA1 and CA3 pyramidal neurons (Borde et al. 2000; Tanabe et al. 1998; Van de Vrede et al. 2007). Furthermore, Foehring and colleagues demonstrated that the activation of an apamin-insensitive slow AHP requires an elevation of Ca2+ signals in the cytoplasm rather than at the membrane (Abel et al. 2004), consistent with a role for some cytoplasmatic intermediary between Ca2+ and K+ channels. Recently it has been reported that the diffusible calcium sensor hippocalcin is a key intermediate between Ca2+ and slow AHP channels in hippocampal pyramidal cells (Tzingounis et al. 2007). Moreover, it has been reported that the connection between T-type Ca2+ and SK channels is modulated by activity of the sarcodendoplasmatic reticulum Ca2+ pump in dendrites of thalamic reticular nucleus neurons (Cueni et al. 2008). Thus we hypothesize that T-type channels, Ca2+ stores, and sAHP potassium channels are colocalized, possibly forming specialized Ca2+ signaling complexes. Further experiments may clarify the role of CICR and a possible diffusible factor in the activation of LTS-induced sAHPs in PVT and other midline thalamic neurons.

In thalamic reticular nucleus neurons, activation of T-type Ca2+ channels may not only activate the SK-type of K+ channels (Cueni et al. 2008) but also trigger a depolarizing afterpotential (DAP) that is mediated by opening of nonselектив cationic conductances (Bal and McCormick 1993). We observed a large DAP following the LTS in a small number of PVT neurons, possibly indicative of LTS induction of competing conductances that can result in diverse patterns of inward and outward currents in different thalamic neurons. Activation of a nonselектив cationic conductance would induce an inward current, mostly through Na+ influx, that would likely oppose the current underlying the sAHP. Should both DAP and sAHP type of conductances be present in the same PVT neuron, their activation could create a competitive mechanism that may contribute to the duration of the LTS and the number of Na+ spikes generated on its crest. Indeed, the LTS-induced sAHP was increased in the presence of cesium, a blocker of the hyperpolarization-activated instantaneous rectifier Ih, suggesting that an Ih current does counteract that which underlies the LTS-induced sAHP. The degree of such interactions is likely to vary among neurons, given that PVT is a collection of somewhat heterogeneous neurons in terms of both their morphology and their electrical properties (cf. Fig. 1D; Table 1; see also Heilbronner and Flügge 2005). Variable expression of an Ih-type conductance may contribute to cell types with recurrent oscillations represented by the cell illustrated in Fig. 1D2.

In all neurons in which the sAHP is expressed, its phosphorylation plays a critical regulatory role. Modulation by activation of the cAMP/protein kinase A pathway seems to be a primary function for many neurotransmitters (Faber and Sah 2003; Nicoll 1988; Vogalis et al. 2003). Not surprisingly, our data confirm this function as activation of β-adrenergic, 5-HT7, and orexin receptors resulted in a significant reduction of the LTS-induced sAHP. Recently, an apamin-insensitive tonic firing-activated sAHP (thus involving HVA Ca2+ channels) was described in neurons located in some midline and intralaminar thalamic nuclei (Goaillard and Vincent 2002). Although PVT neurons were not mentioned in that analysis, the AHP following depolarizing current pulses that evoked >20 Na+ spikes was strongly inhibited by cAMP, thus similar to the LTS-induced sAHP in the present study. Whether activation of different types of calcium channels (HVA vs. LVA) can couple to the same pool of Ca2+-activated K+ channels in PVT neurons remains to be defined.

In many CNS neurons, the amplitude of the sAHP that follows a depolarizing current pulse strongly depends on the number of evoked sodium spikes during tonic firing and underlies spike-frequency adaptation that progressively slows their discharge frequency and the ultimate cessation of action potentials (Faber and Sah 2003). However, the function of the LTS-evoked sAHP described here is unclear at present. This sAHP appears to be novel and prevails among PVT and certain midline thalamic neurons, supporting the notion that LVA Ca2+ channels link to different intracellular signaling pathways in different thalamic locations. At this point one might speculate how this property relates to functions specifically associated with midline thalamus. The LTS and LTS-activated sAHPs may support oscillatory burst firing patterns and allow midline thalamic neurons to signal more effectively to target neurons (e.g., in the accumbens, amygdala, and prefrontal cortex) during arousal and wakefulness (Swadlow and Gusev 2001). The sAHP may function as a pull-back mechanism that ensures the deinactivation of T-type channels, thereby setting the background for another “wake-up call” from the thalamus (Sherman 2001). Since a subpopulation of PVT neurons displayed a limited number of recurrent oscillatory bursts following the LTS, we can only speculate that the sAHP can serve as both a facilitator and a limitation factor of oscillatory burst firing patterns. Regardless of mechanism, these observations support the notion that certain midline thalamic neurons and medial geniculate nucleus possess a novel class of Ca2+-activated K+ channel that is pharmacologically distinct from other known sAHPs, which is not evident among neurons sampled in the ventrobasal or reticular thalamic nuclei, and may be important for information transfer to limbic forebrain regions during states of attentiveness and motivation.

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