Calcium-Dependent Fast Depolarizing Afterpotentials in Vasopressin Neurons in the Rat Supraoptic Nucleus

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INTRODUCTION

The secretion of neurohormones into the circulation largely depends on the pattern of neuronal activity of the synthesizing neurons. The neurohypophyseal hormones vasopressin (VP) and oxytocin (OT) are synthesized in magnocellular cells (MNCs) within the supraoptic nuclei (SON) and these two neuron types show distinct firing patterns when physiological demands for their hormones are high. Preceding each milk ejection in lactating rats, the firing pattern in OT neurons typically described in MNCs. The present study was conducted to investigate the properties of these firing patterns is therefore an important part of the response of MNCs during their hormonal demands.

METHODS

Animals and slice preparation

Brain slices containing the SON were prepared from random cycling, virgin female adult rats (180–210 g body wt; Sprague-Dawley) by 10.220.33.2 on June 21, 2017 http://jn.physiology.org/ Downloaded from

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Dawley, Harlan Laboratories, Indianapolis, IN). The rats were deeply anesthetized with sodium pentobarbital (50 mg/kg ip) and perfused through the heart with a sucrose solution (an artificial cerebrospinal fluid (ACSF) solution (see following text) in which NaCl was replaced by an equiosmolar amount of sucrose). The brains were removed and sliced in the coronal plane at a thickness of 250 μm in the ice-cold sucrose solution. Slices were maintained in ACSF, which was bubbled continuously with 95% O2-5% CO2, containing (in mM) 124 NaCl, 3 KCl, 2.0 CaCl2, 1.3 MgCl2, 1.24 NaH2PO4, 25 NaHCO3, 0.2 ascorbic acid, and 10 d-glucose (pH 7.4). Slices were stored at room temperature prior to recording. Animal procedures were performed under protocols approved by the Institutional Animal Care and Use Committee at University of Tennessee.

**Electrophysiology**

Whole cell patch-clamp recordings were obtained with an Axon 200B amplifier (Axon Instruments, Foster City, CA). Traces were acquired digitally at 20 kHz and filtered at 5 kHz with a Digidata 1320A (Axon Instruments) in conjunction with pClamp 9 software (Axon Instruments). Axograph 4.9 (Axon Instruments) was used to analyze the recordings. The current-voltage relationships were analyzed using Igor Pro Carbon 4.07 (WaveMetrics, Lake Oswego, OR). Averaged data are presented as the means ± SE, where n is the number of cells. Statistically significant difference between means was set to P < 0.05, using paired Student’s t-test, unless otherwise stated.

For analyzing the tail currents, K+- and Cs-glucuronate pipette solutions were used. The K-glucuronate pipette solution consisted of (in mM) 135 K-glucuronate, 2 MgCl2, 10 HEPES, 10 phosphocreatine, 10 myo-inositol (no phosphate), 0.1 EGTA, 0.4 GTP (Na), and 2 ATP (Mg). The pipette solutions were adjusted to a pH of 7.3 with KOH. In some experiments, bis-(o-aminophenox)-N,N,N’,N’-tetraacetic acid (BAPTA, 10 mM) was added to this pipette solution. The Cs-glucuronate pipette solution consisted of (in mM) 100 CsOH, 100 K-gluconic acid, 10 HEPES, 2 MgCl2; 2 ATP (Mg); 0.4 GTP (Na), 10 phosphocreatine, 10 myo-inositol (no phosphate), and 0.1 EGTA. The pipette solutions were adjusted to a pH of 7.3 with HCl. Phosphocreatine was included because “rundown” of Ca2+ conductances. To create a Na+-deficient solution, NaCl was replaced by an equiosmolar amount of sucrose. To create a Cl–-deficient solution, NaCl was replaced by an equiosmolar amount of Na-thiocyanate. All the current-clamp recordings were conducted with the K-glucuronate pipette solution. In some cases, the following compounds were added to the ACSF and perfused through the recording chamber; CdCl2 (400 μM), ifenunalic acid (10 μM), ruthenium red (1–10 μM), capsazepine (10 μM), SKF 96365 (10–100 μM; Tocris, Ellisville, MO), and U-50488 (10 μM; Tocris). All chemicals were purchased from Sigma unless otherwise stated. All extracellular media were saturated with 95% O2-5% CO2, with a pH of 7.3–7.4, had an osmolality of 290–300 mosM/kg H2O, and were warmed to 33°C during the recording.

**Immunocytochemistry**

After recording, the slices were fixed with 4% paraformaldehyde and 0.2% picric acid in phosphate-buffered saline (PBS) at 4°C for at least overnight and processed for double-immunofluorescence labeling. The anti-VP-neurophysin antisera is a rabbit polyclonal provided by A. Robinson and was used at a 1:20,000 dilution. The anti-OT-neurophysin antibody (PS36) is a mouse monoclonal antibody provided by H. Gainer (National Institutes of Health) and was used at a 1:500 dilution. All antibodies and other labeling reagents were dissolved in PBS containing 0.5% Triton X-100. The slices were incubated 48–72 h at 4°C followed by the incubation in a cocktail of secondary antibodies and 7-amino-4-methylcoumarin-3-acetic acid (avidin-AMCA; Vector Labs, Burlingame, CA) 4–6 h at room temperature. The secondary antibodies used were fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit (Vector Labs) and Alexa Fluor 594-conjugated goat anti-mouse IgG (Invitrogen, Eugene, OR). Avidin-AMCA was used to visualize the recorded cells. Neurons were considered as either OT or VP types only if positive staining of one antibody was complemented by a negative reaction with the other (Fig. 1).

**RESULTS**

Cs+-sensitive and -resistant DAPs are both present in MNCs

Prominent DAPs were observed following a train of action potentials in a subpopulation of MNCs. Among those cells expressing DAPs, repetitive single action potentials evoked by intracellular current injections (20 × 5 ms depolarizing pulses, 100–250 pA, 20 Hz) generated a DAP after the AHPs (Fig. 2A). The AHP generated with this protocol appeared to be mostly the medium AHP (mAHP) from its decay time course (~500 ms). The current underlying the mAHP is mediated by the small-conductance Ca2+-activated K+ channels (SK channels) in MNCs because it is blocked by bee venom apamin, a known blocker of SK channels (Armstrong et al. 1994; Bourque and Brown 1987; Greffrath et al. 1998; Kirkpatrick and Bourque 1996; Teruyama and Armstrong 2005a). As expected, bath application of apamin (100 nM) strongly suppressed the

![Patched Neuron](http://jn.physiology.org/)

![VP-NP ir-Neurons](http://jn.physiology.org/)

![OT-NP ir-Neurons](http://jn.physiology.org/)

**FIG. 1.** Immunocytochemical identification of cell types in magnocellular cells (MNCs) from supraoptic nucleus (SON). The patched neuron was filled with biocytin and visualized by 7-amin-4-methylcoumarin-3-acetic acid (AMCA)-conjugated avidin (arrowhead, left). The tissue was also labeled for vasopressin (VP) and oxytocin (OT) neurophysins (NP) by immunofluorescence using fluorescein isothiocyanate and Alexa Fluor 594-conjugated secondary antibodies, respectively. The recorded cell was immunoreactive to VP-NP (arrowhead, middle) but not to OT-VP (*, right).

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mAHP, enhanced the DAP, and shifted its peak to the left (Fig. 2B). It has been known that Cs⁺ blocks the DAP in MNCs (Ghamari-Langroudi and Bourque 1998). However, in the presence of apamin, bath application of Cs⁺ (5 mM) blocked only a slower part of the DAP and not the peak (Fig. 2C). Inhibition of the slow part of the DAP with Cs⁺, in turn, revealed the presence of the slow AHP (sAHP) described previously in MNCs (Ghamari-Langroudi and Bourque 2004; Greffrath et al. 1998; Teruyama and Armstrong 2005a). The time course of the faster part of the DAPs could not be observed easily with this protocol unless the mAHP and the slower part of the DAPs were both suppressed. Because the time course of the Cs⁺-resistant DAP is faster than the Cs⁺-sensitive DAP, we refer to them as fast DAP (fDAP) and slow DAP (sDAP), respectively. The superimposed images from Fig. 2, A–C (Fig. 2D) illustrate the presence of multiple, temporally overlapping afterpotentials in the MNC. It is clear that time courses of the mAHP and sAHP overlap considerably with those of the fDAP and sDAP, respectively.

The fDAP showed strong activity dependence. More detailed observation in the expanded portion of the trace during repetitive spike activation (Fig. 2E) revealed that the onset of the fDAP was seen after the first action potential and its amplitude continued to increase with each subsequent action potential until a plateau was reached after 12 spikes. The time course of the mAHP was fitted with a single-exponential function with a time constant of decay of 283.0 ± 12.7 ms (n = 29) for a pulse duration of 50 ms at a holding potential of −60 mV.

Because the fDAP showed strong activity dependence, the dependence of the IfDAP on the duration of depolarizing steps was evaluated in voltage clamp. These steps would allow a progressive increase in [Ca²⁺]. This protocol was chosen over mimicking spikes in current clamp because it better isolated the fDAP from the sAHP, which increases with spike number during a train, because it provides a better space clamp than transient depolarizations that would be heavily filtered, and because the activity dependence in current clamp is heavily dependent on spike frequency. The amplitude of IfDAP increased with the duration of the stimulus, and the maximum amplitude IfDAP reached with the pulse duration of 150 ms (Fig. 3A; n = 5). The relationship between peak IfDAP amplitude and pulse duration was fitted with a single-exponential function with a time constant of ~100 ms (n = 4; Fig. 3B).

Under our recording conditions, fDAPs were seen in the vast majority of VP neurons. Only 1 in 69 immunolabeled VP neurons failed to express fDAP. In contrast, only 13 of 65 (20%) immunolabeled OT neurons expressed the fDAP. To study the fDAP and its underlying current, we analyzed the recordings exclusively from the immunolabeled VP neurons. To isolate the fDAP, experiments were conducted in the presence of apamin (100 nM) and Cs⁺ (5 mM) unless otherwise stated.

**Ca²⁺ dependence of the fDAP**

Although the mechanisms underlying the DAP in MNCs are controversial, previous studies agree they are Ca²⁺ dependent (Andrew and Dudek 1984b; Ghamari-Langroudi and Bourque 1998; Li and Hatton 1997a). Ca²⁺ influx appeared to be an
The amplitude of the peak current and pulse duration could be fitted with a single-exponential function. The amplitude reached a maximum value at 150 ms of duration (n = 4).

Lowering extracellular Ca\(^{2+}\) concentration reversibly inhibited the fDAP (Fig. 4A; n = 6). A similar effect on I_{fDAP} was observed when Ca\(^{2+}\) channels were blocked by bath application of 400 μM Cd\(^{2+}\) (Fig. 4B; n = 5). These results indicate that the fDAP is also Ca\(^{2+}\) dependent.

To distinguish between I_{fDAP} as a Ca\(^{2+}\)-activated current versus a Ca\(^{2+}\) current per se, recordings were made with high intracellular buffering of Ca\(^{2+}\). If the underlying current is a Ca\(^{2+}\) current, the I_{fDAP} should be observed despite strongly buffering Ca\(^{2+}\) with intracellular BAPTA (10 mM). As illustrated in Fig. 4, C and D, the I_{fDAP} was never observed in the recordings with an intracellular solution containing BAPTA (n = 9). These results showed that the I_{fDAP} is probably not a voltage-gated Ca\(^{2+}\) current but rather is a Ca\(^{2+}\)-activated current.

Na\(^{+}\) influx through TTX-sensitive channel is not required for the fDAP production

Because the application of TTX reduced I_{fDAP} in MNCs in the study of Li and Hatton (1997b), the possibility that the fDAP is a result of TTX-sensitive Na\(^{+}\) channels was evaluated. A train of action potentials was evoked by a 200 ms stimulus pulse (0.2 nA) to generate a fDAP in the presence of apamin (100 nM) and Cs\(^{+}\) (5 mM). Subsequent bath application of TTX (0.5 μM) blocked sodium spikes and a presumptive persistent Na\(^{+}\) current (not shown), but the fDAP evoked by calcium spikes remained constant (Fig. 5; n = 8). Therefore the activation of TTX-sensitive sodium channels is not required for generation of the fDAP in VP neurons.

Current-voltage relationship of the I_{fDAP}

The current-voltage relationship of the I_{fDAP} was examined at various holding potentials under voltage clamp. In this experiment, activation of I_{fDAP} was conducted in VP neurons filled with Cs\(^{+}\)-gluconate pipette solution. The tail current thought to underlie the fDAP was evoked by a 50 ms depolarizing voltage step to 0 mV from the holding potential of −60 mV, then returned to different membrane potentials (Fig. 6A). To isolate this Ca\(^{2+}\)-dependent inward current from the influence of other voltage-dependent currents, traces taken without the conditioning pulse (used to produce Ca\(^{2+}\) influx) were subtracted from the traces obtained from those with test pulses (Fig. 6, A and B). The I-V relationship of the I_{fDAP} was characterized by a relatively linear relation between −90 and −50 mV and pronounced outward rectification above −50 mV (Fig. 6C). The outward rectification suggested that the channel is permeable to Cs\(^{+}\) and is probably regulated by membrane voltage as well as Ca\(^{2+}\), although a more thorough characterization of voltage dependence would require a more extended

![Image](https://via.placeholder.com/150)
Because a mixed cation current was suggested as the ionic basis of the current, we tested Na⁺ dependence of the current underlying fDAP. These recordings were conducted with Cs⁺, and TTX (100 nM, 5 mM, and 500 nM, respectively) in the bathing solution. Despite these treatments, a transient outward current opposing the fDAP appeared at potentials above −50 mV (indicated in Fig. 8A), indicating the presence of a probable K⁺ current (which was blocked by the internal Cs⁺ in the previous experiment). However, the amplitudes obtained for plotting the I-V relationship were taken from the point (indicated in arrowheads) where the outward current subsided, therefore minimizing contamination, and in control solution, produced an I-V curve similar to that when Cs⁺ was used intracellularly to block other K⁺ currents in the experiments testing for Na⁺ dependence (Fig. 7). Raising the extracellular concentration of K⁺ significantly increased amplitudes of the fDAP (n = 6; P < 0.05) and shifted its reversal potential in the depolarizing direction. Thus K⁺ also contributes to the amplitude of the fDAP.

Ca²⁺-activated Cl⁻ currents have been reported to mediate depolarizing afterpotentials in other cell types, such as dorsal root ganglion, spinal cord, and autonomic neurons (reviewed in Hartzell et al. 2005). In VP neurons filled with our K-glucconate pipette solution, ECl⁻ would be approximately −63 mV. Opening of Ca²⁺-activated Cl⁻ channels could contribute to depolarization when the membrane potential is more positive than

**Ionic dependence of the current underlying fDAP**

Because a mixed cation current was suggested as the ionic basis of the current, we tested Na⁺ permeability by lowering [NaCl]₀ to 27 mM in VP neurons filled with Cs⁺-gluconate-filled pipettes. The Nernst equation indicated that this treatment would shift Eₐₙ with negative by 46 mV and E_Cl with positive by 87 mV. As shown in Fig. 7, reduction of the [NaCl]₀ in the bathing solution resulted in significant reduction in the amplitude of fDAP at potentials between −90 and −50 mV (n = 5; P < 0.05). This suggests the involvement of Na⁺ in generation of fDAP.

To test whether fDAP is also carried by K⁺ ions, the effect of raising extracellular K⁺ concentration to 10 mM from control of 2.5 mM was examined in VP neurons filled with K-glucconate pipette solution. This treatment would shift E_K⁺ positive by 37 mV (Nernst equation). To minimize the effect of voltage-dependent K⁺ conductances, TEA and 4-AP (10 mM each) were added along with apamin, Cs⁺, and TTX (100 nM, 5 mM, and 500 nM, respectively) in the bathing solution. Despite these treatments, a transient outward current opposing the fDAP appeared at potentials above −50 mV (indicated in Fig. 8A), indicating the presence of a probable K⁺ current (which was blocked by the internal Cs⁺ in the previous experiment). However, the amplitudes obtained for plotting the I-V relationship were taken from the point (indicated in arrowheads) where the outward current subsided, therefore minimizing contamination, and in control solution, produced an I-V curve similar to that when Cs⁺ was used intracellularly to block other K⁺ currents in the experiments testing for Na⁺ dependence (Fig. 7). Raising the extracellular concentration of K⁺ significantly increased amplitudes of the fDAP (n = 6; P < 0.05) and shifted its reversal potential in the depolarizing direction. Thus K⁺ also contributes to the amplitude of the fDAP.
FIG. 8. Effect of raising [K+]o on I_DAP. A: I_DAP at potentials between −90 and −40 mV was obtained by the subtraction method described in Fig. 7 at 2 different concentrations of extracellular K+. Recordings with K+ gluconate pipette solution. B: I-V relationship of the I_DAP in normal and high [K+]o (100 mM) significantly increased the amplitude of I_DAP from −70 to −40 mV (n = 6; P < 0.05), but the rectification at −50 and −40 mV was still apparent. The recordings were conducted in the presence of CsCl (5 mM), 4-AP (10 mM), TEA (10 mM), apamin (100 nM), and TTX (500 nM). Arrowheads, points where the amplitude of the tail currents was measured.

**DISCUSSION**

**Supraoptic VP neurons generate a fast DAP**

The present study demonstrated that essentially all VP neurons in the SON generate a Ca2+-dependent fDAP following a train of action potentials, whereas only minority of OT neurons expressed this afterpotential. Many studies have attempted to elucidate the mechanisms underlying DAPs in MNCs with disparate results. It has been suggested that discrepancies between labs may arise from the multiple mechanisms underlying the expression of DAPs in MNCs (Ghamari-Langroudi and Bourque 2004; Brown et al. 1999). However, as seen in Fig. 10E, bath application of U50488 did not affect the amplitude fDAP (n = 3). Thus like external Cs+, k-receptor activation probably targets the sDAP, but not the fDAP, in VP neurons.
1987; Smith and Armstrong 1993) or TEA (Greffrath et al. 1998), but they were blocked by external Cs+ (Ghamari-Langroudi and Bourque 1998).

The present study did not suggest the involvement of the Ca2+-dependent reduction of a resting K+ conductance in the fDAP. Moreover, neither TTX nor TEA inhibited the fDAP. In fact, TEA enhanced the fDAP (data not shown), possibly because of increased Ca2+ influx due to prolonged spike width. In addition, the extracellular application of Cs+ did not block the fDAP. These facts imply that the fDAP has not been clearly examined in MNCs before.

Ionic nature of fDAP and possible channels mediating the generation of fDAP in VP neurons

Our results indicate that I_{fDAP} is generated by Ca2+-dependent ion channels that are permeable to Na+ and K+, but not to Cl-. When the I-V relationship of the I_{fDAP} was studied, the amplitude of I_{fDAP} increased with hyperpolarization, and a pronounced outward rectification was observed at potential above -50 mV. This rectification was still observed when the cells were filled with a Cs+-glucanate pipette solution that should inhibit the majority of K+ conductances. Despite eliminating these conductances, however, the I_{fDAP} was still present as was its rectification at depolarized potentials. Although a more thorough examination is required before making a conclusion, these findings indicate that the conductance that underlies the I_{fDAP} is probably permeable to intracellular Cs+, and the I_{fDAP} is not only Ca2+-dependent but also voltage-dependent. To our knowledge, the only ion channel classes known to meet these criteria are Ca2+-activated nonselective cation (CAN) channels.

It has been shown that DAPs and plateau potentials can result from the activation of CAN channels in MNCs (Ghamari-Langroudi and Bourque 2002) as well as in other cell types of mammalian cells. These include neuroblastoma (Yellen 1982), sensory neurons (Razani-Boroujerdi and Partridge 1993), hippocampal CA1 pyramidal neurons (Fraser and MacVicar 1996), prefrontal cortex neurons (Haj-Dahmane and Andrade 1997), dorsal horn neurons (Morisset and Nagy 1999), neocortical cells (Schiller 2004), subthalamic neurons (Zhu et al. 2004, 2005), and olfactory interneurons. Blanes cells (Pressler and Strowbridge 2006). In addition, the plateau potentials and bursting activity mediated by the CAN have been studied to a great extent in invertebrate cells (Hung and Magoski 2007; Kramer and Zucker 1985; Lupinsky and Magoski 2006; Partridge and Swardnalla 1987; Swardnalla and Lux 1985; Zhang et al. 1995). More importantly, FFA has been reported to effectively block DAPs and/or burst firing in MNCs (Ghamari-Langroudi and Bourque 2002), dorsal horn neurons (Morisset and Nagy 1999), neocortical cells (Schiller 2004), subthalamic neurons (Zhu et al. 2004, 2005), and olfactory interneurons (Pressler and Strowbridge 2006). These facts strongly imply the involvement of CAN channels in generation of fDAP in VP neurons. However, it must be noted that the time course of the fDAP in VP neurons is relatively shorter (several hundreds of milliseconds) compared with some other CAN current-mediated phenomena that last several seconds (Pressler and Strowbridge 2006; Schiller 2004), and, as stated herein, shorter than the sDAP in SON neurons. The time course of the fDAP may also be masked by the onset of the sAHP.

TRPM4 and TRPM5, two closely related members of the TRPM channels are unique among the TRP channel family (Clapham 2007; Montell 2005; Nilius et al. 2007; Venkatchalam and Montell 2007). Unlike other TRP channels that are either Ca2+-permeable or even highly Ca2+-selective channels, TRPM4 and TRPM5 have no detectable permeability to Ca2+ or Cl- (Hofmann et al. 2003; Launay et al. 2002; Nilius et al. 2003, 2005). Moreover, gating of TRPM4 and TRPM5 is not only regulated by Ca2+ but also by transmembrane voltage (Hofmann et al. 2003; Launay et al. 2002; Nilius et al. 2003, 2005). Interestingly, it has been reported that TRPM4 and TRPM5 can be blocked by FFA (Ullrich et al. 2005). All these properties fit well with the results from the present study, thus suggesting the involvement of the TRPM4/5 in expression of the fDAP in VP. However, such a conclusion should be reserved until the development of a more selective antagonist of TRPM4/5 because FFA also inhibits a variety of ion chan-
nels in a range of tissues including the voltage-gated Na\(^+\) (Lee et al. 2003) and K\(^-\) (Lee and Wang 1999) channels and Ca\(^{2+}\)-activated chloride currents (Kim et al. 2003). FFA also causes a transient release of intracellular Ca\(^{2+}\)-release from stores (Partridge and Valenzuela 1999). Moreover, the molecular identification and the biophysical properties of these channels must be elucidated by RT-PCR or a comparable technique, and single channel recordings, respectively.

VP neurons in SON are directly osmosensitive, and this osmosensitivity is mediated by stretch-inhibited cation channels (Oliet and Bourque 1993). A recent study showed that SON neurons express an N-terminal splice variant of the TRP vanilloid type-1 (TRPV1) channel but not full-length TRPV1 (Sharif Naeini et al. 2006). In that study, the SON neurons in TRPV1 knockout mice could not generate increases in membrane conductance in response to hyperosmotic stimulation (Sharif Naeini et al. 2006). Thus the N-terminal splice variant of the TRPV1 has been suggested as a functional stretch-inhibited cation channel (Sharif Naeini et al. 2006). In addition, other studies have indicated that TRPV4 channel may contribute to the detection of osmotic signals (Liedtke et al. 2000; Strotmann et al. 2000) and to the osmotic control of VP release (Liedtke and Friedman 2003; Mizuno et al. 2003). The inorganic polyacationic dye, ruthenium red (RuR), appears to be one of the most selective blockers of currents through the TRPV channels (Tominaga et al. 1998) as all TRPV channels are reportedly blocked by RuR (Watanabe et al. 2003). Moreover, the increase in membrane conductance provoked by hyperosmolality was significantly attenuated in the presence of RuR in MNCs of mice (Sharif Naeini et al. 2006). In the present study, an application of RuR did not affect the fDAP in VP neurons. This strongly suggests that the currents underlying fDAP are not mediated by TRPV channels and the \(I_{\text{dAP}}\) probably plays little role in the osmosensitive activation of VP neurons.

Possible functional role of the fDAP in MNCs

Although the precise physiological functions of the fDAP are unknown at this point, the fact that essentially all VP neurons, whereas only a minority of OT neurons, possess this property implicates the involvement of fDAP in the generation of the specific firing pattern observed in VP neurons. In MNCs, Ca\(^{2+}\) influx through high-voltage-gated channels typically evoked by spikes, but not sub-threshold events, is required to generate a plateau potential on which phasic bursts ride (Andrew and Dudek 1984a). Therefore VP neurons require the ability to depolarize to negative potentials to the voltage range where bursts may occur through the activation of the current underlying the sDAP. Thus the fDAP may serve to bootstrap the sDAP, but this would clearly depend on the nature of the interaction between the fDAP and the medium AHP.

The DAPs and phasic bursting activity can be observed in brain slice preparations when synaptic activity has been blocked (Andrew 1987; Bourque and Renaud 1984; Hatton 1982) and even somewhat in dissociated cells (Oliet and Bourque 1992). Therefore phasic bursting in vivo is clearly triggered from synaptic inputs because excitatory amino acid receptor antagonists prevent bursts (Brown et al. 2004; Nissen et al. 1994). Therefore phasic bursts in MNCs are not intrinsically regenerative in vivo. This discrepancy may be due to the steep voltage sensitivity of the sDAP. Phasic firing in vitro is observed only within a relatively narrow range of membrane potential (−48 to −55 mV). Slightly more depolarized potentials result in continuous firing, whereas a slightly hyperpolarized potentials result in slow irregular firing or no firing at all (Inenaga et al. 1993). In contrast, most VP neurons in vivo exhibit a slow irregular discharge in absence of stimulation (Wakerley et al. 1978), suggesting the resting potential in vivo is below the range of sDAP activation. This probably prevents excessive firing that would result in inappropriate hormone secretion at rest. The fDAP may also be needed for the initiation of bursting to occur appropriately in response to strong excitatory synaptic inputs from osmosensitive areas (Denton et al. 1996; McKinley et al. 1996; Richard and Bourque 1995) in response to osmotic challenge. Interestingly, it has been shown that N-methyl-d-aspartate (NMDA) induced burst via activation of CAN current in subthalamic neurons (Zhu et al. 2004) and in neocortical cells (Schiller 2004). In addition, Ca\(^{2+}\) influx responsible for activation of CAN current was mediated by both NMDA-receptor channels and voltage-gated calcium channels and to lesser extent internal calcium stores (Schiller 2004). These reports further support the notion in the synaptic activation of the fDAP in VP neurons. Indeed, NMDA receptor activation in SON neurons also contributes to rhythmic burst firing (Hu and Bourque 1992). Therefore we suggest that the Ca\(^{2+}\)-activated fDAP plays a role in the instigation of phasic firing, the latter relying in turn on the subsequent activation of the sDAP.

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