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**Vasopressin induces depolarization and state-dependent firing patterns in rat thalamic paraventricular nucleus neurons in vitro**

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**Zhang, L., P. Doroshenko, X. Y. Cao, N. Irfan, E. Coderre, M. Kolaj, and L. P. Renaud**. Vasopressin induces depolarization and state-dependent firing patterns in rat thalamic paraventricular nucleus neurons in vitro. *Am J Physiol Regul Integr Comp Physiol 290: R1226–R1232, 2006. First published December 8, 2005; doi:10.1152/ajpregu.00770.2005.—The thalamic midline paraventricular nucleus (PVT) is prominently innervated by vasopressin-immunoreactive neurons from the suprachiasmatic nucleus (SCN), site of the brain’s biological clock. Using patch-clamp recordings in slice preparations taken from Wistar rats during the subjective day, we examined 90 PVT neurons for responses to bath-applied AVP (0.5–2 μM; 1–3 min). In current clamp at resting membrane potentials (−65 ± 1 mV), PVT neurons displayed low-threshold spikes (LTSs) and burst firing patterns. In 50% of cells tested, AVP induced a slowly rising, prolonged membrane depolarization and tonic firing, returning to burst firing upon recovery. AVP modulated hyperpolarization-activated LTSs by decreasing the time to the initial sodium spike at the onset of LTS, also increasing the duration of the afterdepolarization. Responses were blockable with a V1a receptor antagonist (Mannering compound). Under voltage clamp, AVP induced a TTX-resistant, slowly rising, and prolonged (~15 min) inward current (<40 pA). Current-voltage relationship (I-V) analyses of the AVP responses revealed a decrease in membrane conductance to 73.1 ± 6.2% of control, with net AVP current reversing at −106 ± 4 mV, and decreased inward rectification at negative potentials. These observations are consistent with an AVP-induced closure of an inwardly rectifying potassium conductance. On the basis of these in vitro observations, we suggest that the SCN vasopressinergic innervation of PVT is excitatory in nature, possibly releasing AVP with circadian rhythmicity and contributing to state-dependent firing patterns in PVT neurons over the sleep-wake cycle.

suprachiasmatic nucleus; midline thalamus; neurohypophyseal peptide; circadian

**METHODS**

**Electrophysiology.** Wistar rats weighing 50–120 g (21 to 35 days old) were used. Experiments conformed to the Canadian Council for Animal Care and Ottawa Health Research Institute guidelines on the ethical use of animals in research. Recordings were made from PVT neurons in brain slices in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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neurons in acutely prepared coronal brain slice preparations using methods described in detail previously (19). Briefly, after decapitation, the brain was quickly removed and immersed in oxygenated (95% O2-5% CO2) cooled (<4°C) artificial cerebrospinal fluid (ACSF) of the following composition (mM): 127 NaCl, 3.1 KCl, 1.3 MgCl2, 2.4 CaCl2, 26 NaHCO3, 10 glucose, pH 7.3, osmolality 300–310 mosmol/kgH2O. Brain slices (400–500 μm) containing anterior through to posterior PVT were cut in the coronal plane with a vibrating blade microtome (Leica VT1000S; Nussloch, Germany) and incubated in gassed ACSF for >1 h at room temperature, then transferred to a submerged recording chamber and superfused (2–4 ml/min) with oxygenated ACSF at 22–24°C. Recordings were obtained from PVT neurons with borosilicate thin-walled micropipettes filled with (mM) 130 Kgluconate, 10 KCl, 2 MgCl2, 10 HEPES, 1 EGTA, 2 Mg-ATP, 0.3 Na-GTP (pH adjusted with NaOH to 7.3). Pipettes had resistances of 3–7 MΩ. Lucifer yellow (2 mM) was included in the pipette solution to facilitate identification of the cell location and to generate a profile of cell morphology. The membrane voltages were corrected for liquid junction potential (~12 mV). Access resistance of <15 MΩ was considered acceptable. Input resistance was determined from the linear slope (between ~50 to ~70 mV) of the current-voltage (I-V) relationship. The inward rectification was measured from I-V plots as the difference between currents obtained at ~110 mV and ~40 mV. Ih was measured as the difference between steady-state current and transient current during a voltage step from ~50 to ~110 mV. In a majority of experiments, the blind-patch technique was used for whole cell current-clamp and voltage-clamp recordings using either a Axopatch 200B or 1D amplifier (Axon Instruments, Foster City, CA). Some experiments were done using infrared videomicroscopy with a fixed-stage microscope equipped with the Dott gradual contrast optics (Luigs and Neumann, Ratigen, Germany), an infrared sensitive charge-coupled device camera (Hamamatsu C2400–77) and EPC10 amplifier (HEKA, Mahone Bay, Canada). Data obtained with either technique were indistinguishable and pooled. Data were filtered at 2 kHz, continuously monitored and stored on disk. Digidata 1200B interface with Clampex software (pClamp 9; Axon Instruments) were used to generate current and voltage commands and to store data.

Off-line analyses were performed using Clampfit version 9 (Axon Instruments). Statistical comparisons between control and experimental values (P ≤ 0.05 and better) were determined using both the paired or unpaired Student t-test and ANOVA (SigmaPlot 8, SigmaStat 3, Systat). Results are expressed as means ± SE.

Drugs were bath applied at the concentrations indicated. Unless stated otherwise, drugs and reagents were purchased from Sigma (St. Louis, MO). AVP, [β-mercapto-β-cyclopentamethylenepropionyl]3, O-Me-Tyr2]-AVP (Manning compound), and DDAVP were obtained from American Peptide (Sunnyvale CA) and TTX from Alomone Labs (Jerusalem, Israel).

Immunocytochemistry. Adult male Wistar rats (200–300 g; Charles River, QC) were deeply anesthetized with pentobarbital sodium (60 mg ip) and perfused through the left ventricle with 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4. Brains were removed and stored in the same fixative overnight at 4°C before being cut into transverse 40-μm-thick sections with a Vibratome. Sections were rinsed 3 × 10 min in Tris buffer containing 0.3% Triton × 100 (TBS-T), blocked in 4% normal goat serum in TBS-T for 1 h at room temperature, and then incubated for 72 hrs at 4°C with a mouse antivasopressin antibody 1:1,000 (kindly provided by Dr Harold Gainer, National Institute of Neurological Disorders and Stroke, National Institutes of Health). After several washes in TBS-T, sections were incubated with a goat anti-mouse antibody conjugated with Alexa 488 1:100 (Invitrogen, Carlsbad, CA) for 1 h at room temperature and then coveredslipped with Prolong antifade (Molecular Probes, Eugene, OR). Sections were viewed with a ×20 objective on a Bio-Rad confocal system.

RESULTS

Vasopressin immunoreactivity. Consistent with earlier reports (6, 36), vasopressin-immunoreactive fibers were observed throughout the rostro-caudal extent of PVT (Fig. 1), with little or no staining detected in other midline thalamic nuclei. In sections from anterior PVT, fibers were notably coarser than those in more posterior PVT where they displayed a finer appearance.

Electrophysiological properties of PVT neurons. Data were obtained from 90 neurons localized to PVT. Neurons visualized after filling with Lucifer yellow displayed oblong or multipolar somata that measured 12–30 μm along their long axes, contained 3 to 7 main dendrites and lacked any specific orientation. Resting membrane potential was −65 ± 1 mV, and input conductance was 1.5 ± 0.1 nS. The majority of PVT neurons displayed modest time-dependent inward rectification (Ih; Fig. 1C) and strong inward rectification (Fig. 1D). All neurons were capable of generating hyperpolarization-activated, low-threshold calcium spikes (LTS; Fig. 1, C and D) topped with TTX-sensitive sodium spikes. Firing patterns typical of PVT neurons included tonic firing when depolarized from a resting level and LTSs crowned with sodium spikes when depolarized from hyperpolarized membrane potentials (Fig. 1C).

Vasopressin V1a-type receptors can influence state-dependent firing patterns. In 27/55 neurons tested while recording in current-clamp mode, bath application of AVP (0.5–2 μM; 1–3 min) was followed by a slowly rising membrane depolarization of 10 ± 1 mV that (depending on the resting membrane potential) could evoke a burst of action potentials (Fig. 2, inset 2; Fig. 3A). The membrane potential slowly returned to the resting level after ~15 min, and the responses were repeatable when tested. Prior application of a V1a receptor antagonist (Manning compound, 1 μM) blocked AVP-induced responses in 3/3 cells. In 3/3 cells responding to AVP, application of a specific V2 type AVP receptor agonist (DDAVP, 3 μM) was without effect. These observations are therefore in agreement with the detection of V1a receptor transcripts in PVT (27).

When cells were sorted according to location in PVT, 12/34 neurons located in anterior PVT (bregma ~ −0.9 to −1.4) and 15/21 in the remainder of PVT (bregma ~ −1.6 to −3.6) were responsive to AVP. Differences in the magnitude of AVP-induced depolarization (anterior ~8.3 ± 1.2 mV vs. remainder ~10.3 ± 2.1 mV) were not significant (P = 0.460).

Many thalamic neurons display state-dependent properties, with tonic firing patterns at depolarized membrane potentials and low-threshold spikes and burst firing patterns at hyperpolarized membrane potential levels (21). Typically, PVT neurons displayed similar properties. As illustrated in Fig. 2, the AVP-induced depolarization was observed to change resting activity from a LTS bursting pattern (inset 1) to a continuous tonic pattern upon depolarization (inset 2), followed by a return to a LTS bursting pattern as the membrane potential recovered (inset 4). During these transitions, changes were also noted in the length of a burst (also termed “afterdepolarization”; see...
Fig. 1. Properties of paraventricular nucleus of thalamus (PVT) and its neurons. 
A1: diagram from rat brain atlas illustrates a coronal section through the anterior PVT. 
A2: section to illustrate vasopressin immunoreactive fibers at this level. 
A3: photomicrograph of a typical Lucifer yellow-stained cell located (asterisk) in anterior PVT. 
B1–3: similar to A for a more posterior section through PVT (see RESULTS for levels). Vasopressin fibers are almost exclusively limited to PVT. 
C: current-clamp traces from a representative PVT neuron (resting membrane potential (RMP) = −64 mV), illustrating tonic firing (top trace) and low-threshold spike (LTS; bottom trace) crowned with sodium spikes. The dotted line indicates time-dependent rectification (Ih; arrow). 
D: Current-voltage relationship (I-V) on the right reflects the mean current responses from 44 PVT neurons to a series of voltage pulses (duration 600 ms, from −110 to −40 mV with 10-mV steps). In the sample on the left, note the strong time-independent inward rectification (●) and large T-type low-threshold calcium current (arrow), both typical for PVT neurons. (A1 and B1 are modified from Ref. 29a.)
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Ref. 21) as well as an increase in the number of sodium spikes crowning the burst (compare Fig. 2, insets 1 vs. 3).

To define more clearly how a LTS-dependent burst firing pattern could be modified (or modulated) by AVP, we took advantage of the ability of thalamic neurons to generate a LTS following a transient hyperpolarization (Fig. 1C). As illustrated in Fig. 3A, the AVP-induced response also included a prolongation in the duration of the LTS and the subsequent afterdepolarization (Fig. 3A, red trace in inset 1), a significant increase in the number of sodium spikes riding on the LTS (Fig. 3A, red traces in inset 2; Fig. 3B) and a significant shortening of the time of appearance of the first sodium spike at the onset of LTS (Fig. 3A, red traces in inset 2; Fig. 3C).

In the experiments that examined the effects of AVP on the hyperpolarization-evoked LTS (Fig. 3, inset 1), we also observed an increase in the amplitude of the hyperpolarizing test pulse, suggesting an increase in membrane resistance. This was verified under voltage-clamp conditions, in which AVP was noted to induce a slowly rising and transient inward current (Fig. 4A). The current amplitude was not affected by TTX (−14.9 ± 1.7 pA; n = 14 vs. −17.3 ± 2.5 pA in the presence of 1 μM TTX; n = 13; P = 0.442), implying that this was a direct action of AVP on a postsynaptic receptor. Detailed analysis of the I-V relationships revealed a significant decrease (P = 0.004) of the resting membrane conductance to 73.1 ± 6.2% of control in the majority (7/10) PVT neurons (Fig. 4B); no change (a parallel shift) in the I-Vs was observed in the other three neurons (101.7 ± 1.9% of control; P = 0.775). The net AVP-induced current reversed at −106 ± 4 mV (n = 7) and showed rectification at negative potentials (Fig. 4C). In the remaining three neurons, the net current did not cross the Vm axis within the tested voltage range of −20 to −110 mV. In addition, the inward rectification [see methods; the ratio (I−110 − I−40)AVP/(I−110 − I−40)control] in cells with the I-V reversal close to the potassium equilibrium potential decreased to 89 ± 7%, but increased to 131 ± 11% in cells displaying a parallel shift in the I-Vs (P = 0.013 for these two groups). In 3/3 PVT neurons tested, barium (1 mM) mimicked the AVP effect by inducing an inward current of −37 ± 12 pA, which reversed at −101 ± 2 mV, and was associated with a decreased AVP-induced inward current (−5 ± 3 pA; P = 0.047), suggesting that the involved conductance is partly barium sensitive. Thus these data suggest for the majority of PVT neurons depolarized by exposure to AVP that activation at their V1a receptors results mainly in the closure of an inwardly rectifying potassium conductance(s), probably via a G protein-coupled inwardly rectifying potassium channel (GIRK).

A hyperpolarization-activated cation current with an inward rectification Ih is variably expressed in PVT neurons, contributing to the voltage “sag” in response to a sustained hyperpolarizing current pulse (arrow in Fig. 1C). In a subset of PVT neurons responding to AVP, Ih (measured at −110 mV) was reduced significantly from 42.7 ± 7.9 pA (control) to 20.8 ± 4.9 pA (n = 6; P = 0.04; Fig. 4B). However, Ih was not changed in those cells where its amplitude was less than 15 pA (10.3 ± 1.9 pA in control; vs. 9.3 ± 1.4 pA under AVP; n = 4; P = 0.39). Comparing the magnitude of their AVP-induced currents in these two cell groups with small and large Ih revealed a nonsignificant trend toward reduced responses in the cells with the smaller Ih (−17 ± 3 pA, n = 4 vs. −19 ± 5 pA, n = 6; P = 0.746) and no correlation between changes in the Ih in and the resting conductance (P = 0.547). As expected, bath application of a specific Ih antagonist ZD7233 (50–100 μM; n = 7) significantly decreased Ih (34.7 ± 12.4% of control; P = 0.003), and the input conductance (82.3 ± 4.1% of control; P = 0.004) but had no effect on the membrane potential (100.4 ± 1.6% of control; P = 0.823). Thus the Ih type conductance does not appear to be the primary target of AVP or to contribute significantly to AVP-induced inward currents in PVT neurons.

DISCUSSION

In the rat thalamus, the AVP-like immunoreactivity is confined to a midline cell group anatomically identified as PVT (Fig. 1, A and B; cf. 7, 36, 40). Located along the anterior and dorsal aspects of the thalamus bordering the third cerebral ventricle, these AVP immunoreactive fibers are derived exclusively from neurons located within the dorsomedial cap of the hypothalamic SCN (14). SCN neurons express diurnal rhythmicity in neuronal activity (measured electrically and by expression of c-Fos protein), in their immunoreactivity and mRNA for AVP, and AVP release into the extracellular space and cerebrospinal fluid (e.g., Refs. 12, 33, and 38). Thus it is possible that this rhythmicity is transmitted synchronically to axonal targets of SCN neurons, in this case, resulting in rhythmic release of AVP in PVT. The few details available on AVP receptors in the thalamus (39) imply the presence of a V1 subtype, which is consistent with their prevalence in most other central nervous system regions. This has also been confirmed functionally in a number of electrophysiological studies (19, 24, 31). As an initial step in characterizing a role for AVP in an extrahypothalamic circadian pathway (SCN → PVT), we...
now demonstrate that exogenous AVP actives postsynaptic V_1 type receptors in PVT neurons, resulting in a TTX-resistant, slowly rising and prolonged membrane depolarization. This implies that SCN vasopressinergic fibers might convey an excitatory input to PVT neurons.

Voltage-clamp analyses revealed that the net AVP-induced current was associated with a reduction in membrane conductance and showed rectification at negative potentials, and a net current reversal around -94 mV, a value that approximated the potassium equilibrium potential. In the presence of barium ions, a significant reduction in the AVP-induced inward currents was noted. An inward rectification was also reduced by AVP. Collectively, these data suggest that AVP acts through modulation of an inwardly rectifying conductance. Since AVP acts via G-protein-coupled receptors (3), it is likely that the vasopressin V_1 type receptors in PVT neurons promote closure of GIRKs. In situ hybridization studies have noted a differential and developmentally regulated expression of inwardly rectifying K^+ channel mRNAs in rat thalamus, particularly of the GIRK3 transcripts (8, 17).

In a subpopulation of PVT neurons, the AVP-induced depolarization occurred without an obvious change in membrane conductance and the net current lacked a reversal. Additionally, an increase in the length of the LTS afterdepolarization (Fig. 3A), believed to reflect an increase in a cationic conductance in thalamocortical neurons (1, 9), suggests that AVP may also activate a cationic-like conductance in PVT. In the case of AVP and certain other peptides (e.g., angiotensin and TRH in neonatal spinal cord), the involvement of more than one conductance has been detected (see Refs. 19, 20, 28). Such heterogeneity of AVP action in various neuronal populations has also been noted in studies on different populations of brainstem motoneurons in...
which the AVP-induced inward currents involved a nonspecific cationic current in facial motoneurons compared with the opening of persistent voltage-dependent sodium channels in hypoglossal motoneurons (reviewed in Ref. 31). The subtleties of these mechanisms will require further analysis.

In PVT, as in other thalamic neurons, an intrinsic state-dependent mechanism exists, whereby neurons display patterns of activity that shift from LTS-based burst firing to tonic firing (see Fig. 1C). Several conductances are known to underlie such oscillatory behavior, notably $I_h$, $I_{can}$, and $I_{h}$ (9), all of which are detected in PVT neurons. Our data indicate that AVP can significantly decrease $I_h$ (Fig. 4B), an action that appears unrelated to any change in membrane potential. The $I_h$ channels are encoded by a family of genes hyperpolarization-activated cation channels 1–4, or HCN1–4, with all 4 subunits detected in PVT (25). Interestingly, peptide-induced modulation of $I_h$ has been reported in other thalamocortical neurons (for a review, see Ref. 29). How $I_h$ is modulated in PVT neurons will require further investigation.

**Perspectives**

The activity of thalamocortical relay neurons is state dependent, a reflection of the interaction between their intrinsic properties and synaptic connections: during wakefulness, specific thalamocortical relay neurons are slightly depolarized and discharge in a single-spike mode; during slow-wake sleep, these neurons are relatively hyperpolarized and display rhythmic burst firing (21). In the event that the vasopressinergic SCN efferent neurons are rhythmically active, and therefore induce a rhythmic release AVP in PVT, our observations suggest that activation of postsynaptic AVP V1-type receptors to reduce potassium conductances and decrease $I_h$ would lead to depolarization and favor single-spike firing pattern over oscillatory and bursting firing patterns, as expected for other thalamocortical neurons in the awake animal. We are also aware that AVP likely coexists with another rapidly acting transmitter, possibly GABA (18) or glutamate (cf. Ref. 13). The dynamics of a peptide release vs. a classical transmitter release at synapses is reported to differ, with the probability of peptide release increasing proportionately to the frequency of action potentials arriving at the synaptic terminals (see Ref. 34). Thus, in the circadian system of the rat, which transmitter predominates at any given target site (e.g., PVT) during the solar day may depend on the level of activity generated within specific SCN efferent pathways (cf. Ref. 6). It will be a challenge to delineate how these mechanisms operate within
this particular circadian pathway to the midline thalamus and how this ultimately relates to behavior.

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