Electrophysiological Evidence for Vasopressin V 1 Receptors on Neonatal Motoneurons, Premotor and Other Ventral Horn Neurons

MURAT OZ, 2 MILOSŁAV KOLAJ, and LEO P. RENAUD 1

1Neurosciences, Ottawa Health Research Institute, University of Ottawa, Ottawa, Ontario K1Y 4E9, Canada; and 2National Institute on Drug Abuse, Intramural Research Program, Baltimore, Maryland 21224

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Oz, Murat, Miloslav Kolaj, and Leo P. Renaud. Electrophysiological evidence for vasopressin V 1 receptors on neonatal motoneurons, premotor and other ventral horn neurons. J Neurophysiol 86: 1202–1210, 2001. Prominent arginine-vasopressin (AVP) binding and AVP V 1 type receptors are expressed early in the developing rat spinal cord. We sought to characterize their influence on neural excitability by using patch-clamp techniques to record AVP-induced responses from a population of motoneurons and interneurons in neonatal (5–18 days) rat spinal cord slices. Data were obtained from 58 thoracolumbar (T 7 –L 5 ) motoneurons and 166 local interneurons. A majority (98%) of neurons responded to bath applied AVP (10 nM to 3 μM) and (Phe 2 , Orn 3 )-vasotocin, a V 1 receptor agonist, but not V 2 or oxytocin receptor agonists. In voltage-clamp, postsynaptic responses in motoneurons were characterized by slowly rising, prolonged (7–10 min) and tetrodotoxin-resistant inward currents associated with a 25% reduction in a membrane potassium conductance that reversed near −100 mV. In interneurons, net AVP-induced inward currents displayed three patterns: decreasing membrane conductance with reversal near −100 mV, i.e., similar to that in motoneurons (24 cells); increasing conductance with reversal near −40 mV (21 cells); small reduction in conductance with no reversal within the current range tested (41 cells). A presynaptic component recorded in most neurons was evident as an increase in the frequency but not amplitude (in motoneurons) of inhibitory and excitatory postsynaptic currents (IPSCs and EPSCs), in large part due to AVP-induced firing in inhibitory (mainly glycinergic) and excitatory (glutamatergic) neurons synapsing on the recorded cells. An increase in frequency but not amplitude of miniature IPSCs and EPSCs also indicated an AVP enhancement of neurotransmitter release from axon terminals of inhibitory and excitatory interneurons. These observations provide support for a broad presynaptic and postsynaptic distribution of AVP V 1 type receptors and indicate that their activation can enhance the excitability of a majority of neurons in neonatal ventral spinal cord.

INTRODUCTION

In mammals, the nonapeptide arginine-vasopressin (AVP) is widely recognized for its hormonal antidiuretic and vasopressor properties when released into the circulation following activation of neurohypophysial-projecting hypothalamic magnocellular neurosecretory neurons. However, the immunocytochemical demonstration of additional hypothalamic and extrahypothalamic AVP-synthesizing parvocellular neurons and axonal pathways in specific independent CNS sites implied that this molecule also served as a possible neurotransmitter in brain (Buijs 1978). Indeed AVP has now been shown to influence a wide but specific range of behavioral, memory, learning and autonomic functions and to have neuromodulatory and neurotransmitter-like actions in studies at the cellular level (reviewed in Urban et al. 1998). Recently a novel form of local intracerebral release of neurohypophysial peptides by exocytosis from somatodendritic membranes has been observed in supraoptic nucleus (Pow and Morris 1989), possibly serving a autocrine or paracrine role to alter firing patterns in the same AVP-synthesizing magnocellular neurons (Gouzenes et al. 1998). Endogenous AVP is also released into the extracellular space (Landgraf 1995) as well as into the cerebrospinal fluid (Pittman et al. 1984; Reppert et al. 1987), thereby dispersing the peptide in a hormonal-like fashion throughout the brain and spinal cord.

The observation that neuronal response to exogenous AVP varies among different brain regions and among specific neurons within a given region is an indication of regional and cell-specific expression of AVP receptors. In neural tissue, data from binding and molecular biological studies indicate that these actions are mediated predominantly by AVP acting at V 1 subtype receptors (Lolait et al. 1995; Tribollet et al. 1997). The spinal cord is one area where AVP binding, presumably a reflection of receptor expression, has been reported to be prominent and to undergo developmental change (Tribollet et al. 1997). In adults, AVP binding in the area of the intermediolateral cell column of the spinal lateral horn coincides with the distribution of vasopressinergic fibers and axon terminals that arise from parvocellular neurons located in the hypothalamus, notably the paraventricular nucleus (Buijs 1978; Hallbeck and Blomqvist 1999). A likely target for AVP’s actions at this specific site is the sympathetic preganglionic neurons whose response to exogenous AVP has already been noted (Kolaj and Renaud 1998; Ma and Dun 1985). This is in accordance with evidence that AVP has a role in central regulation of cardiovascular and renal function (Crowley 1982; Pittman et al. 1982; Riphagen and Pittman 1989). However, the distribution of AVP receptors in the adult and particularly the neonatal spinal cord extends throughout the gray matter of the lateral and ventral spinal horns (Tribollet et al. 1997). In a recent patch-clamp analysis in neonatal tissue (Kolaj and Renaud 1998), we noted that AVP induced membrane depolarization and inward current in a majority of lateral horn neurons.

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neurons. Extending this investigation to thoracolumbar motoneurons and interneurons, we now report evidence for the presence of AVP V₁ subtype receptors on their somatodendritic membranes and also on presynaptic terminals of inhibitory and excitatory interneurons that synapse on motoneurons.

METHODS

Experiments used Sprague-Dawley rats of either sex (5–18 days old) cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. On the day of experiment, animals were anesthetized with methoxyflurane, decapitated, the spinal cord excised after dorsal laminectomy and a 10- to 15-mm section of thoracolumbar spinal cord resected and placed in ice-cold (4°C) artificial cerebrospinal fluid (ACSF). The latter contained in (mM) 127 NaCl, 26 NaHCO₃, 3.1 KCl, 1.2 MgCl₂, 2.4 CaCl₂, and 10 d-glucose (pH 7.35; osmolarity 290–305 mosmol) and was gassed with 95% O₂-5% CO₂. Transverse 350–450 (voltage-clamp mode, recordings were made at a holding potential current-voltage (I-V) able. Corrections of the liquid-junction potentials were performed. K-gluconate was replaced with CsCl. Pipette resistances measured amplification of spontaneous and miniature IPSCs, 100 mM of tetraacetic acid (EGTA), 2 Mg-ATP adjusted to a pH of 7.3 with Tris acid (HEPES), 1 ethyl glycol-bis-β-aminoethyl ether)-N,N,N',N' -tetraacetic acid (EGTA), 2 Mg-ATP adjusted to a pH of 7.3 with Tris buffer. The addition of Lucifer yellow (dipotassium salt 1 mg/ml; Sigma) provided a convenient intracellular label. In recordings requiring amplification of spontaneous and miniature IPSCs, 100 mM of K-gluconate was replaced with CsCl. Pipette resistances measured 3–7 MΩ. A series/access resistance <25 MΩ was considered acceptable. Corrections of the liquid-junction potentials were performed off-line on recorded membrane current and voltage traces. Input resistance of the neurons was determined from the slope of the current-voltage (I-V) relationships at the range of −50 to −80 mV.

Both current- and voltage-clamp recordings were obtained with an Axopatch 200A or Axopatch 1D amplifier (Axon Instruments, Foster City, CA), and data were filtered on-line at 2 kHz. In whole cell voltage-clamp mode, recordings were made at a holding potential (Vh) of −65 mV. Membrane currents and potentials were continuously monitored on an oscilloscope and displayed on a pressure ink pen recorder (Gould, Valley View, OH; Gould 2400S or Gould VS3200) simultaneously. Recordings were also stored on videotape for later, off-line analysis. Digidata 1200 interface and version 7 of pCLAMP software (Axon Instruments) were used on-line to generate current and voltage-clamp commands.

Motoneurons were identified by their all-or-none antidromic responses to ventral rootlet stimulation applied with a concentric bipolar electrode (FHC, Bowdoinham, ME, tip diameter: 25 μm; 1–12 V, duration: 0.02 s) and/or by their morphology and evidence of an axon projecting toward the ventral root. Following the recording, slices were transferred to a fixation medium (4% paraformaldehyde with 0.1 mM phosphate buffer) and stored overnight at 4°C. After clearing for 60 min with dimethyl sulfoxide and fixation on microscope lamels, labeled cells were viewed and measured under epifluorescence. AVP and drugs were dissolved in ACSF at their final concentrations and applied by a computer-controlled, fast local pressure application system (DAD-12; Adams and D’Adamo and Associated Scientific Instruments, Westbury, NY). In some of the experiments, agents were delivered bath application at a perfusion rate of 4–6 ml/min. Arg⁸-vasopressin (AVP), desamin[D-Arg⁸]-vasopressin (DDAVP), [β-mercapto-β,β-cyclopentamethylenepropionyl11,0-O-Me-Tyr³] or Manning compound, (Phe², Orn⁸)-vasotocin, [The⁴, Gly⁷]-oxytocin, were from American Peptide (APC, Sunnyvale, CA). Tetrodotoxin (TTX) was from Alomone Labs (Jerusalem, Israel). Amastatin was from Sigma, St. Louis, MO.

Data were analyzed off-line with Clampfit software of pClamp versions 7 and 8 (Axon Instruments). For statistical evaluation, we used paired, unpaired Student’s t-test, or ANOVA as it is indicated in the text (Origin version 6, Microcal Software, Northampton, MA). Results are presented as means ± SE. For assessment of spontaneous and miniature postsynaptic events, 2–3 min of recordings were sampled at 5 kHz and analyzed for frequency, amplitude, time to peak, and time constant of decay using commercially available Mini Analysis software (Synaptosoft, Leonia, NJ). Spontaneous and miniature events were defined as those recorded in the absence and presence of 1 μM tetrodotoxin (TTX), respectively. Synaptic events were detected with an adjustable threshold, often set at 8–15 pA and maintained at a constant level in a given neuron. The analysis of mIPSCs and mEPSCs was performed with cumulative probability plots. Frequencies of synaptic events were calculated as the reciprocals of interevent intervals. Statistical comparisons of the frequency and/or amplitude of the synaptic currents before and after AVP were made using the Kolmogorov-Smirnov (K-S) test; P < 0.05 was considered significant.

RESULTS

Motoneurons

Data were obtained from 39 cells antidromically identified as motoneurons. Another 19 neurons were classified as motoneurons on the basis of their location within Rexed laminae VIII and IX (Molander et al. 1984), similar electrical properties and morphology. Collectively these cells measured 31.1 ± 1.7 μm in diameter, with a mean resting membrane potential of −73.1 ± 1.2 mV and input resistance of 58.9 ± 5.6 MΩ. A majority (>90%) of cells responded to exogenous applications of AVP and/or V₁ receptor agonists.

AVP INDUCES POSTSYNAPTIC MEMBRANE DEPOLARIZATION AND INWARD CURRENT

In 7/8 neurons tested while recording in current-clamp mode, bath application of AVP (1 μM; 30 s) was followed by a slowly rising (60–90 s to peak) membrane depolarization that reached a plateau of 14.7 ± 3.1 mV, sufficient to trigger a burst of action potentials in three of the seven cells. Responses required several minutes to recover (Fig. 1A). Since washout intervals of 30 min were needed before regaining full recovery, some desensitization seemed likely. In all instances, responses were accompanied by a thickening of the baseline (see following text). Several observations imply that these effects were mediated via V₁ type receptors: application of a V₁ receptor antagonist (Manning compound, 1 μM), while without effect on resting membrane properties could block the AVP-induced responses in 5/5 cells tested (Fig. 1B); similar membrane depolarizations (14.8 ± 2.3 mV) followed applications of a specific V₁ receptor agonist, (Phe², Orn⁸)-vasotocin in 4/4 cells tested (Fig. 1C); and cells lacked any response to application of either a V₂ receptor agonist (DDAVP, 1 μM; 6 cells tested) or an oxytocin receptor agonist [The⁴, Gly⁷]-oxytocin (1 μM; 7 cells tested; Fig. 1D).

The latter would indicate that, at least in neonatal thoracolumbar motoneurons, vasopressin rather than oxytocin is the ligand for the effects observed at this level. It is notable that neither Manning compound (5 cells) nor amastatin, an aminopeptidase inhibitor (10 μM; 3 cells), induced significant changes in
resting membrane properties or inward currents, features that might indicate an endogenous AVP action (cf. Chen and Pittman 1999).

The AVP response was further examined under voltage clamp ($V_H$ = −65 mV) in the presence of 1 μM TTX. All of 12 cells tested responded to AVP 1 μM with a slowly developing inward current (peak of 174 ± 45 pA) and slow recovery over 7–10 min (Fig. 2A). Notable was a lack of baseline thickening in these circumstances (see following text). Responses to AVP were concentration dependent with an EC50 of 0.42 μM (Fig. 2C). Comparison of instantaneous $I$-$V$ plots before and at the peak of the AVP responses revealed net AVP currents (difference between control and peak AVP effect) whose slope indicated a 25% reduction in membrane conductance (from 13 ± 2.2 to 9.7 ± 1.8 nS). In nine of these neurons tested with AVP 1 μM, the net current in ACSF reversed at −65 mV (Fig. 2A). In the remaining three neurons, the $I$-$V$ plots indicated a reduction in the conductance ($17.1 ± 5.2%$); however, no reversal of the net AVP currents was observed at the voltage range tested (−120 to −10 mV).

**FIG. 2.** AVP induces TTX-resistant inward currents. A: voltage-clamp trace from a motoneuron (PN 13; · · ·, holding current, $V_H$ = −65 mV) illustrates the response to AVP as a slowly rising and prolonged inward current. B: current traces on the left in response to a series of voltage pulses delivered before (control) and at the peak of the AVP response provide data for $I$-$V$ plots on the right, constructed from values taken at indicated points. The net AVP-induced current (●) determined by subtraction of these $I$-$V$ values displays reversal −100 mV. C: dose-response relationships illustrate a concentration dependence of the AVP-induced inward current (numbers refer to tested cells). D: plots of net AVP-induced currents from 3 motoneurons. In control artificial cerebrospinal fluid (ACSF) (●), note a decrease in membrane conductance with a reversal potential about −105 mV, approximating the potassium equilibrium potential $E_K$. In ACSF containing 10 mM potassium (●), note the shift in reversal potential toward the Nernstian predicted $E_K$ of −68 mV. The AVP response was blocked by prior application of [β-mercapto-β-cyclopentamethylenepropiony1, O-Me-Tyr2] or Manning compound, a V1-type receptor antagonist. C: recording from a different motoneuron (PN 11 days; $V_H$ = −73 mV) illustrates similar depolarization when exposed to (Phe2, Orn8)-vasotocin, a specific V1 receptor agonist. D: when tested 40 min later, the same cell was unresponsive to applications of either DDAVP, a specific V2 receptor agonist or [Thr4-Gly7]-oxytocin, an oxytocin receptor agonist.

**FIG. 1.** Arginine-vasopressin (AVP) induces prolonged depolarizations through activation of V1-type receptors in motoneurons. A: whole cell current-clamp recording from a spinal motoneuron recorded on day 12 postnatal (PN; resting membrane potential, $V_H$ = −71 mV). The response to a 30-s AVP application (horizontal bar; estimated maximum concentration 1 μM) is a slowly rising, prolonged and reversible membrane depolarization sufficient to initiate a burst of action potentials. B: in the same cell, 25 min later, the AVP response is blocked by prior application of [β-mercapto-β-cyclopentamethylenepropiony1, O-Me-Tyr2] or Manning compound, a V1-type receptor antagonist. C: recording from a different motoneuron (PN 13 days; $V_H$ = −73 mV) illustrates similar depolarization when exposed to (Phe2, Orn8)-vasotocin, a specific V1 receptor agonist. D: when tested 40 min later, the same cell was unresponsive to applications of either DDAVP, a specific V2 receptor agonist or [Thr4-Gly7]-oxytocin, an oxytocin receptor agonist.

**AVP ENHANCES PRESYNAPTIC EXCITATORY AND INHIBITORY INPUTS TO MOTONEURONS.** As noted in the preceding text, AVP-induced responses were invariably accompanied by a TTX-sensitive increase in baseline thickening. The latter was due to a marked increase in the frequency of spontaneous postsynaptic currents (sPSCs). In standard ACSF, tests for a response to AVP 1 μM revealed an increase in sPSCs frequency from 1.3 ± 0.2 to 14.6 ± 2.3 Hz ($P < 0.01$, paired t-test, $n = 7$ cells). The major component of this response was clearly action potential dependent because it was abolished in the presence of TTX. Therefore neurons presynaptic to motoneurons were being activated by AVP. We next applied pharmacological antagonists to clarify whether this reflected input from excitatory or inhibitory sources.

After blockade of ionotropic glutamate receptors with 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[1]quinoxaline-7-sulfonamide (NBQX, 5 μM) and d-2-amino-5-phosphonovaleric acid (APV, 100 μM) is a specific V2 receptor antagonist, responses to AVP were concentration dependent with an EC50 of 0.42 μM (Fig. 2C). Comparison of instantaneous $I$-$V$ plots before and at the peak of the AVP responses revealed net AVP currents (difference between control and peak AVP effect) whose slope indicated a 25% reduction in membrane conductance (from 13 ± 2.2 to 9.7 ± 1.8 nS). In nine of these neurons tested with AVP 1 μM, the net current in ACSF reversed at −65 mV (Fig. 2A). In the remaining three neurons, the $I$-$V$ plots indicated a reduction in the conductance ($17.1 ± 5.2%$); however, no reversal of the net AVP currents was observed at the voltage range tested (−120 to −10 mV).
acid (α-APV, 50 μM), cells were tested for a response to 0.3 μM AVP, a concentration that was ~50% effective in inducing a postsynaptic response. We observed a significant increase in sIPSC frequency, from 0.6 ± 0.3 to 4.9 ± 1.6 Hz (P < 0.05, paired t-test, n = 6 cells; Fig. 3, A and B). In three cells tested, sIPSCs were completely abolished by addition of strychnine (2 μM) and bicuculline (20 μM; Fig. 3A). In three other cells tested, most sIPSCs were abolished with the addition of strychnine at a concentration of 1 μM, suggesting that most sIPSCs were glycine in nature.

We then used a cocktail of strychnine (2 μM) and bicuculline (20 μM) to block glycine and any possible GABA\textsubscript{A} receptors. Addition of AVP significantly increased the frequency of sEPSCs from 0.8 ± 0.7 Hz in control to 11.3 ± 2.8 Hz (P < 0.01, paired t-test, n = 4 cells; Fig. 3, C and D). It is notable that in these motoneurons there was no significant change in amplitude of sIPSCs and sEPSCs (Fig. 3, B and D), implying that these effects were presynaptic in origin, and unlikely to result from a postsynaptic signal transduction-mediated increase in current through ionotropic glutamate receptors. In view of their TTX-sensitivity, the most likely explanation is that both excitatory and inhibitory interneurons projecting to motoneurons contain somatodendritic AVP receptors whose activation can trigger membrane depolarization sufficient to generate action potentials that are subsequently conducted into terminals that synapse on motoneurons.

We next applied TTX to assess a possible contribution of miniature events (mIPSCs) to these AVP-induced presynaptic responses. In media containing NBQX, α-APV, and bicuculline and a pipette solution containing 100 mM CsCl to amplify mIPSCs, we observed that 0.3 μM AVP significantly increased mIPSC frequency (0.4 ± 0.1 Hz in control vs. 1.2 ± 0.2 Hz in AVP, P < 0.05, paired t-test, n = 5) but did not alter amplitudes (Fig. 4, A–E) or kinetics (Fig. 4D, inset). This would imply that AVP could be enhancing transmitter release through an action on receptors located directly on axon terminals of inhibitory interneurons that synapse on motoneurons. By contrast, the analysis for isolated mEPSCs (in the presence of strychnine, 2 μM, and bicuculline, 20 μM) revealed no significant change in frequency (0.7 ± 0.1 Hz in control vs. 0.8 ± 0.2 Hz in AVP, n = 6 cells; Fig. 4F). Although not extensive, this analysis could indicate a preferential distribution of AVP receptors on inhibitory rather than excitatory presynaptic terminals.

**Interneurons**

In view of the substantial enhancement of presynaptic events after AVP, we further investigated AVP’s actions on a population of 166 Rexed laminae VIII and IX cells that failed to demonstrate antidromic activation and/or had a distinct morphology (smaller cell diameter, i.e., 25.1 ± 1.2 μm; P < 0.05 vs. motoneurons; ANOVA). We label these as “interneurons” recognizing that this includes premotor and other unidentified ventral horn cells. As a group, these cells displayed a lower resting membrane potential of (~59.7 ± 1.1 mV; P < 0.05, ANOVA) and higher input resistance (167.1 ± 14.3 MΩ; P < 0.05, ANOVA). Responses to AVP included both postsynaptic and presynaptic components.

**AVP INDUCES POSTSYNAPTIC RESPONSES.** In the presence of TTX, a majority of the tested neurons (86/93) responded to AVP (1 μM) with membrane depolarization (14.9 ± 1.6 mV; n = 4 cells) or a mean inward current of 45.7 ± 8.6 pA (n = 86 cells; Fig. 5A). Responses were dose dependent, with inward currents of 21.5 ± 5.4 and 6.7 ± 2.3 pA in response to AVP at 100 (n = 7 cells) and 10 nM (n = 4 cells), respectively. Although of different magnitude, the time course of these AVP responses were virtually identical to the postsynaptic currents recorded in motoneurons and resembled the time course of the AVP-induced changes in frequency of their spontaneous and miniature postsynaptic currents. When we analyzed voltage-current relationships to delineate net AVP current (as in Fig. 4), the data collectively indicated only a minor decrease in membrane conductance from a control value of 4.8 ± 0.6 to 4.4 ± 0.5 nS at the peak of the AVP response (P > 0.05, paired t-test, n = 86). However, a comparison of the net AVP-induced currents for individual cells revealed three significantly differ-
induced current displayed a slight reduction in membrane conductance. In the remaining 41 cells, the mean net AVP-action was mediated via an increase in a nonselective cationic conductance that decreased in conductance (from 5.0 ± 0.7 to 4.7 ± 0.8 nS; P > 0.05, paired t-test) but did not reverse within the voltage range tested.

**AVP INDUCES PRESYNAPTIC RESPONSES.** In addition to a postsynaptic inward current, all interneurons exposed to AVP in normal ACSF displayed an increase in the frequency and the amplitude of TTX-sensitive spontaneous postsynaptic potentials (e.g., Fig. 6, A and B). In the presence of amino acid receptor antagonists, it was apparent that these were composed of both inhibitory and excitatory events (Fig. 6, C and D), implying that AVP receptors were present on the somata and/or dendrites of both inhibitory and excitatory neurons that synopsis on interneurons. For IPSCs (n = 4 cells), frequency increased from 0.9 ± 0.2 to 11.2 ± 2.8 Hz (P < 0.01) while amplitudes increased from 27.9 ± 4.3 to 48.7 ± 6.1 pA (P < 0.05). For EPSCs (n = 5 cells), frequency increased from 1.7 ± 0.2 to 14.3 ± 3.1 Hz (P < 0.01) and amplitude increased from 16.4 ± 1.1 to 24.7 ± 3.2 pA (P < 0.05).

Further tests implied that mPSCs were a small but definable component of these responses. In ACSF containing TTX, application of 0.3 μM AVP in the presence of strychnine and bicuculline was seen to enhance mEPSC frequency from 0.7 ± 0.2 Hz in control to 2.9 ± 0.4 Hz (P < 0.05; paired t-test, n = 5). Conversely, in the presence of NBQX and d-APV, recordings with pipettes containing 100 mM CsCl revealed a significant increase in mIPSC frequency from 0.4 ± 0.1 to 1.3 ± 0.3 Hz in AVP (P < 0.05; paired t-test, n = 6; Fig. 7F). Notably, one group of 24 neurons demonstrated a net AVP-induced conductance that decreased from a control of 4.7 ± 0.5 to 3.6 ± 0.4 nS (P < 0.05, paired t-test) and reversed close to −100 mV (−101.8 ± 4.1 mV). This value approximated the potassium equilibrium potential under these conditions, suggesting that the action of AVP in these cells was mediated via reduction in conductance for potassium ions. By contrast, in another 21 cells, the slope of the net AVP current reflected an increase in conductance (from 4.5 ± 0.9 to 5.1 ± 1.1 nS; P < 0.05, paired t-test) with current reversal at about −40 mV (−38.4 ± 2.9 mV). The latter suggests that the AVP action was mediated via an increase in a nonselective cationic conductance. In the remaining 41 cells, the mean net AVP-induced current displayed a slight reduction in membrane conductance (from 5.0 ± 0.7 to 4.7 ± 0.8 nS; P > 0.05, paired t-test) but did not reverse within the voltage range tested.

**FIG. 5.** AVP induces TTX-resistant inward current in interneurons. A: in ACSF containing TTX (1 μM), NBQX (5 μM), and d-APV (50 μM), sample of control mIPSCs in sequential current traces from a motoneuron (PN 11 days) recorded with a pipette containing 100 mM CsCl. B: note the increase in miniature IPSC (mIPSC) frequency following application of 0.3 μM AVP. C: cumulative distribution plots illustrate the difference in inter-event intervals of mIPSCs during a control period (---) and after application of AVP (---). D: cumulative distribution plots of mIPSC amplitudes sampled indicate no significant difference between control and after AVP. Inset: no change in kinetics of superimposed averaged traces of 20 consecutive mIPSCs with pipettes containing 100 mM CsCl revealed a significant increase in mIPSC frequency from 0.4 to 14.3 Hz (P < 0.01) in AVP (P < 0.05; paired t-test) but did not reverse within the voltage range tested.

**FIG. 4.** AVP differentially alters TTX-resistant (miniature) synaptic currents. A: in ACSF containing TTX (1 μM), NBQX (5 μM), and d-APV (50 μM), sample of control mIPSCs in sequential current traces from a motoneuron (PN 11 days) recorded with a pipette containing 100 mM CsCl. B: note the increase in miniature IPSC (mIPSC) frequency following application of 0.3 μM AVP. C: cumulative distribution plots illustrate the difference in inter-event intervals of mIPSCs during a control period (---) and after application of AVP (---). D: cumulative distribution plots of mIPSC amplitudes sampled indicate no significant difference between control and after AVP. Inset: no change in kinetics of superimposed averaged traces of 20 consecutive mIPSCs with pipettes containing 100 mM CsCl revealed a significant increase in mIPSC frequency from 0.4 to 14.3 Hz (P < 0.01) in AVP (P < 0.05; paired t-test) but did not reverse within the voltage range tested.
Although AVP induced clear changes in the amplitudes of the spontaneous events (Fig. 6), such was not the case with mPSCs that failed to demonstrate a significant change in amplitude (Fig. 7, A–E). Thus the latter are regarded as “postsynaptic” action potential-independent influences of AVP, presumably to alter transmitter release from axon terminals of both inhibitory and excitatory neurons that synapse on interneurons. On the other hand, most of the AVP changes in frequency and amplitude of sPSCs in these interneurons are TTX sensitive and therefore action potential dependent, indicating that AVP’s effects most likely reflecting the presence of receptors on their somatic and/or dendritic membranes, but not on their terminals. Under resting conditions, it would appear that these receptors are silent.

**Discussion**

This study provides electrophysiological evidence for the existence of AVP V₁ receptors on a majority of thoracolumbar motoneurons and other ventral horn neurons (collectively termed interneurons) in the neonatal rat spinal cord. Responses to bath-applied AVP (10 nM to 3 μM) and V₁ receptor agonists featured a slowly rising and prolonged TTX-resistant inward current. In motoneurons, the net AVP-induced currents were associated with a reduction in a membrane potassium conductance that reversed near −100 mV. By contrast in interneurons, three patterns of AVP-induced inward currents were evident: decreasing membrane conductance with reversal near −100 mV; increasing conductance with reversal near −40 mV; small reduction in conductance with no reversal within the current range tested. A presynaptic component recorded in most neurons was evident as an increase in the frequency, but not amplitude (in motoneurons) of spontaneous IPSCs and EPSCs, attributed to AVP-induced activity in inhibitory (mainly glycnergic) and excitatory (glutamergic) neurons synapsing on the recorded cells. On the basis of AVP-induced TTX-sensitive increases in both IPSC and EPSC spontaneous activity onto motoneurons, it can be concluded that both excitatory and inhibitory premotor neurons must be included among the “interneuron” population that is activated by AVP. An increase in frequency, but not amplitude of mIPSCs and mEPSCs, also indicated another presynaptic action of AVP, i.e., to enhance neurotransmitter release from axon terminals of inhibitory and excitatory interneurons. This further supports the broad distribution of pre- and postsynaptic AVP receptors among ventral horn neurons in this age group. These data supplement earlier electrophysiological evidence for AVP receptors among a majority of lateral horn neurons in the neonatal spinal cord (Kolaj and Renaud 1998; Ma and Dun 1985). In our recent analysis (Kolaj and Renaud 1998), we reported that AVP and V₁ agonists applied to spinal preganglionic neurons and the majority of unidentified lateral horn neurons also induced a prolonged, G-protein-coupled membrane depolarization and TTX-resistant inward current. While net AVP-induced currents in 36% of these lateral horn neurons reversed about −100 mV, reflecting reduction in one or more barium-sensitive potassium conductances, the net AVP-induced current in another 20% of cells reversed about −40 mV, suggestive of an increase in a nonselective cationic conductance. This resembles the data obtained from the “interneuron” population in the current analysis (see following text). These differences aside, the fact that a majority of cells respond to AVP and that [¹²⁵I]vasopressin antagonist binding and AVP V₁ receptor mRNA expression are prominent in these areas of spinal cord (Tribollet et al. 1997), one is left with the impression that AVP receptors are present on most neurons in the lateral and ventral horns in the neonatal rat spinal cord.

Responses identified as “postsynaptic” are deemed to arise from activation of AVP receptors located on the somata and dendrites of both motoneurons and interneurons, and are responsible for the TTX-resistant membrane depolarizations seen in current-clamp recordings and the inward currents seen under...
voltage clamp. Analyses of $I-V$ relationships and net-AVP-induced inward currents indicate that two different conductances are likely to mediate these responses to AVP. In motoneurons, and in a population of interneurons, the linearity and the reversal points for the net AVP-induced currents indicate reduction of voltage-independent potassium conductances that contribute to resting membrane potential, often referred to as “leak” conductances. The features of these conductances, also noted in a population of lateral horn neurons (Kolaj and Renaud 1998), resemble a neurotransmitter-modulated conductance observed in brain-stem motoneurons that has recently been attributed to a family of two-pore domain pH-sensitive potassium channels named TASK-1 (Talley et al. 2000). However the validity of this comparison with channels responsible for AVP-induced inward current in neonatal spinal motoneurons remains to be established. By contrast, the AVP-induced inward currents in another population of interneurons are associated with an increase in membrane conductance that reverses about $240 \text{ mV}$, suggesting opening of nonselective cationic channels. Should AVP receptors couple with both conductances, their competition in the same neuron could explain the “parallel shift” and lack of reversal in net AVP currents noted in a third population of interneurons. Perhaps more importantly is the fact that here, as in lateral horn neurons, these two patterns of AVP-induced conductances reflect mechanisms that can be established early in development. In lateral horn cells, we (Kolaj et al. 1999) have suggested that these conductances may be developmentally regulated based on observations that $\sim 70\%$ of cells from slices at ages P8–P10 demonstrate evidence of opening of nonselective cationic channels, whereas $\sim 90\%$ of cells from slices at ages P18–P20 reveal AVP-induced inward currents associated with reduction in a potassium conductance. Among ventral horn interneurons, the smaller sample from the older age group currently precludes our definition of a similar trend.

AVP-induced conductances seem to vary among cell types. In hypoglossal motoneurons, AVP induces a noninactivating inward current that reverses around $\sim 215 \text{ mV}$ (Palouzier-Paulignan et al. 1994). In subfornical organ, AVP inhibits both a delayed rectifier $I_{K}$ and a transient outward current $I_{A}$ (Washburn et al. 1999). In facial motoneurons AVP induces depolarization and inward current via a persistent voltage-dependent sodium current (Ragenbass et al. 1991). In horizontal limb of diagonal band of Broca neurons, AVP modulates conductance through $I_{C}$ (Easaw et al. 1997). In cultured aortic A7r5 cells, AVP modulates activity by acting on different types of ionic conductances, including a late nonselective cationic channel that reverses at 5 mV (Byron and Taylor 1995; Thibonnier et al. 1991; Van Renterghem et al. 1991). Activation of AVP receptors can increase calcium influx through high-voltage-activated calcium channels, as observed in area postrema neurons (Hay et al. 1996), cerebral cortex, and hippocampus (Chen et al. 2000; Mihara et al. 1999). Thus AVP receptors may be coupled with different signaling mechanisms and ion channels in different types of neurons. The present study did not undertake ion substitution or pharmacological blockade to characterize in more detail the AVP-induced conductances.

AVP responses that we refer to as presynaptic comprise two...
FIG. 8. Schema to illustrate possible distribution of AVP receptors in motoneurons and glutamatergic (Glu) and glycinergic/?GABAergic (Gly) interneurons in neonatal rat spinal cord. The response to activation of postsynaptic vasopressin V1 type receptors is illustrated as a reduction in a potassium conductance (in motoneurons) and/or increase in a nonselective cationic channel conductance (interneurons). Mechanisms mediating AVP’s actions in presynaptic terminals remain to be defined.

categories. In one situation, the term indicates synaptic inputs to the recorded neuron from activated gluminergic/?GABAergic and glutamatergic interneurons; these are responsible for the increase in baseline “noise” and can be blocked with tetrodotoxin. A recent report (Omura et al. 1999) noted that AVP can cause a reduction in the amplitude of gluminergic IPSPs in hippocampus, an effect not observed in our spinal cord study. In the other situation, receptors responsible for the AVP-induced increase in mIPSCs and mEPSCs are considered as truly presynaptic and presumed to be located on the axon terminals of inhibitory interneurons that synapse on motoneurons and on axon terminals of glutamatergic and gluminergic/?GABAergic neurons that synapse on interneurons. These distributions are schematically summarized in Fig. 8.

Presynaptic conductances are deemed important in the regulation of transmitter release (Meir et al. 1999). Our data indicate the presence of presynaptic AVP receptors that can change the frequency but not the amplitude of mIPSCs and mEPSCs, i.e., modulate release of inhibitory and excitatory transmitters. Mechanisms remain to be identified but may include enhancement of calcium currents and/or closing of potassium channels in presynaptic terminals. Verification will pose a challenge.

An obvious question relates to the endogenous origin of the ligand (AVP) for these V1 receptors. In the adult CNS, one source could be through excytosis from terminals of hypothalamic AVP-synthesizing paraventricular neurons whose axons can be shown to project to spinal lateral and ventral horn areas (Hallbeck and Blomqvist 1999; Hallbeck et al. 1999; Wagner and Clemens 1993). These pathways may mediate release of neurohypophysial peptides in spinal cord perifusates following supraspinal stimulation (Pittman et al. 1984). It remains uncertain whether such neuronal connections are established or are sufficiently abundant in neonatal spinal cord. AVP has not been detected in dorsal root ganglia neurons (Hallbeck et al. 1996, 1999), an argument against local synthesis and release. Not to be overlooked, however, is AVP that circulates in cerebrospinal fluid with a circadian rhythmity that depends on the integrity of the hypothalamic suprachiasmatic nucleus (Reppert et al. 1987; Schwartz et al. 1983). Reported levels vary from 10 to 30 pg/ml in adult rat spinal subarachnoid space (e.g., Pittman et al. 1984) ≤100 pg/ml in the preoptic recess in fetal lambs in utero (Stark and Daniel 1989), sufficient to activate these receptors.

What role may such widely distributed AVP receptors have in spinal cord function? Observations to date leave little doubt that their activation can enhance neuronal excitability, thereby rendering neurons more responsive to excitatory inputs. In neonatal tissue, this may have important consequences for neuronal survival and development. Of note are reports of impaired neural functions in the vasopressin-deficient Brattleboro rat (reviewed in Bohus and de Weid 1998). AVP does influence neuronal growth and development in various preparations (reviewed in Carter et al. 1993), including hippocampal and cortical neuronal cultures (Brinton et al. 1994; Chen et al. 2000; Tarumi et al. 2000), and accelerates neurite outgrowth from cultured embryonic neurons (Brinton and Gruener 1987). Interestingly, AVP, but not oxytocin, is reported to have a neurotrophic action in cultured explants of spinal cord (Iwasaki et al. 1991). AVP belongs to a family of vasoactive and mitogenic peptides that participate in physiological and pathological cell growth and differentiation (Van Biesen et al. 1996) and can activate a set of kinases that are known for their importance in cell survival (Thibonnier et al. 2000). The observation that the expression of AVP binding and V1 receptors can be regulated by sex steroids and by nerve injury (Chritin et al. 1999; Tribollet et al. 1994, 1997) is perhaps an additional reflection of the multiple roles that AVP receptors impart in target neurons. Studies of the distribution pattern and responses to activation of AVP receptors in the mature spinal cord and in response to injury (cf. Tribollet et al. 1994) may provide some insight on these issues.

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REFERENCES


