Vasopressin-Induced Currents in Rat Neonatal Spinal Lateral Horn Neurons Are G-Protein Mediated and Involve Two Conductances

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INTRODUCTION

The nonapeptide arginine vasopressin (AVP) is commonly recognized as a hypothalamic neurohypophyseal peptide synthesized by magnocellular neurons of the supraoptic and paraventricular nuclei and released into the plasma from their posterior pituitary axon terminals to function as a circulating pressor agent and antidiuretic hormone. However, a differential expression of AVP receptors in brain (see Tribollet et al. 1991), detection of AVP in the cerebrospinal fluid (reviewed in Reppert et al. 1987), evidence of an influence of AVP and related peptides on behavior (reviewed in deWied et al. 1991), and demonstration of AVP-immunoreactive neurons and axons in CNS regions unrelated to posterior pituitary control (Buijs 1978) fostered the notion that vasopressin has a role in neural function, in particular neurotransmission. Supporting data come from electrophysiological evidence that AVP has an influence on neuronal excitability in a variety of CNS sites, including hippocampus (Mühlethaler et al. 1982), lateral septum (Raggenbass et al. 1988), brain stem (Mo et al. 1992; Palouzier-Paulignan et al. 1994; Raggenbass et al. 1991; Sun and Guyenet 1989), area postrema (Lowes et al. 1995), and spinal cord (Backman and Henry 1984; Ma and Dun 1985; Sermasi and Coote 1994). In confirmation of binding and molecular biological studies, these responses are mediated by AVP receptors of the V1 subtype (Lolait et al. 1995; Ostrowski et al. 1994; Tribollet et al. 1991, 1997).

In spinal cord, AVP is implicated in central regulation of cardiovascular and renal function (Crowley 1982; Noszczyk et al. 1993; Pittman et al. 1982; Riphagen and Pittman 1985a,b; 1989). Anatomical tracer and immunocytochemical studies indicate that a population of parvocellular AVP-synthesizing neurons in the hypothalamic paraventricular nucleus, a center for autonomic regulation, project to the brain stem and spinal cord where fibers in the thoracolumbar cord have a distribution around the central canal and intermedio-lateral column, the principal location of sympathetic preganglionic neurons (SPNs) (Hosoya et al. 1991; Saper et al. 1976; Sawchenko and Swanson 1982). Thus, because SPNs and other lateral horn neurons are physiologically relevant targets for an AVP innervation, there is interest in defining the properties of their AVP receptors. Although previous studies in adult cat in vivo (Backman and Henry 1984) and neonatal rat in vitro (Ma and Dun 1985; Sermasi and Coote 1994) reported excitatory or depolarizing actions of AVP on SPNs and lateral horn cells, ionic mechanisms were not defined. With the patch-clamp technique applied in neonatal spinal cord slices, we now report that AVP triggers a G-protein-mediated inward current in a majority (90%) of their posterior pituitary axon terminals to function as a circulating pressor agent and antidiuretic hormone.

METHODS

Preparation

Transverse slices of lower thoracic spinal cord from neonatal (11–22 days) Sprague-Dawley rats of either sex were prepared as...
follows. Under methoxyflurane-induced anesthesia, a dorsal laminectomy was performed to access and remove a 10- to 15-mm segment of the thoracolumbar spinal cord, which was then immersed in oxygenated (95% O₂, 5% CO₂) cooled (4°C) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 127 NaCl, 3.1 or 1.9 KCl, 0 or 1.2 KH₂PO₄, 1.3 MgCl₂ or MgSO₄, 2.4 CaCl₂, 26 NaHCO₃, 10 glucose, pH 7.3, 300 mosmol. Slices were cut at 400 μm on a vibratome and transferred to an incubation chamber filled with oxygenated ACSF at room temperature and stored for ≥1 h before transfer to a recording chamber where individual slices were continuously superfused with oxygenated ACSF (3–6 ml/min) at room temperature (23–26°C). In some experiments, slices were incubated for ≥18 h in either ACSF (control) or in ACSF containing pertussis toxin (PTX) at a concentration of 4–5 μg/ml, previously shown to inhibit G-protein-mediated responses in other slice preparations (Hsu 1996).

**Recording**

With the blind technique, recordings were obtained with borosilicate thin-walled micropipettes (BORO, BF150-110-10, Sutter, Novato, CA) pulled on a Flaming-Brown puller (P87) and filled with an internal solution containing (in mM) 130 K-gluconate, 10 KCl, 10 NaCl, 1 MgCl₂, 10 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, 1 ethyl glycol-bis-(β-aminoethyl ether)-N,N,N′,N″-tetraacetic acid, 2 Mg-ATP, 0.3 guanosine-5′-triphosphate (GTP), 2 Lucifer yellow, and tris(hydroxymethyl)aminomethane (Tris) base at pH 7.3; electrodes had resistances of 3–7 MΩ. In some experiments, GTP was omitted from the solution. In others, GTP was substituted with 0.2 mM GTP-γ-S, or 0.4 mM GMP-PNP, both nonhydrolyzable activators of G-proteins, or 0.8 mM GDP-β-S, which inhibits G-protein binding (Gilman 1987). Correction of liquid junction potential was applied to recorded membrane currents and voltages. An access resistance <15 MΩ was considered acceptable. Input resistance was determined from the linear slope (i.e., between −50 and −80 mV) of the current-voltage (I-V) relationships.

Whole cell current- and voltage-clamp recordings (holding potential −65 mV for all voltage-clamp recordings) obtained from lateral horn neurons were filtered at 2 kHz with an Axopatch-1D amplifier (Axon Instruments, Foster City, CA). Membrane current and membrane potential were continuously monitored on an oscilloscope, displayed on a pen recorder, and stored on videotape for later analysis. A TL-1 or Digidata 1200 interface and p-CLAMP (version 6) software (Axon Instruments) were used on-line to generate current and voltage commands.

**Application of agents**

Agents were dissolved in ACSF at known concentrations and applied to the slice by a computer-controlled superfusion system (DAD-12, ALA Scientific Instruments Inc., Westbury, NY). Agents included CsCl, BaCl₂, GTP, N-ethylmaleimide (NEM), Mg-ATP, Lucifer yellow, AVP, strophantidine, and TTX from Sigma (St. Louis, MO), [({Phe₇,Orn₈})-vasotocin], DDAVP, and [β-mercaptop-β,β-cyclopentamethylenepropionyl₁₁, O-Me-Tyr₇]-AVP, or Mannin compound (Manning et al. 1993) from American Peptide (Sunnyvale, CA), glibenclamide, 4AP, and TEA from RBI (Natick, MA), GTP-γ-S, GMP-PNP, and GDP-β-S from Calbiochem-Novabiochem (San Diego, CA), or RBI and PTX from List (Campbell, CA) or RBI. AVP was applied to the slice by a computer-controlled superfusion system by 10.220.33.2 on June 21, 2017 http://jn.physiology.org/ Downloaded from

**Data analysis**

Off-line analyses were performed with Clampfit version 6 (Axon Instruments). Statistical comparisons between control and experimental values were determined with both the paired or unpaired Student’s t-test and analysis of variance. Results are expressed as the means ± SE.

**Cell identification and morphology**

Slices containing cells injected with Lucifer yellow were transferred to a fixation medium (4% formaldehyde with 0.1 mM phosphate buffer) and stored overnight at 4°C. The following day slices were cleared for 45–60 min with dimethylsulfoxide and viewed under epifluorescence.

**RESULTS**

Observations were derived from a total of 273 lateral horn neurons. Within this population were 68 cells that we classified as SPNs on the basis of their antidromic activation (23/60 tested) after electrical stimulation of their axons in the ventral root exit and/or typical morphology with dendrites extending toward the central canal (45/100 tested) (cf. Shen and Dun 1990; Krupp and Feliz 1993; Pickering et al. 1991). SPNs had a resting membrane potential of −65.1 ± 0.8 mV and input conductance of 3.2 ± 0.2 nS, which differed little from that for all lateral horn neurons, where the mean resting membrane potential was −63.4 ± 0.5 mV (range −51 to −82 mV) and input conductance measured 3.6 ± 0.2 nS. In all cells, depolarizing current pulses elicited action potentials of 76 ± 1 mV (measured from threshold; width at half-amplitude of 1.6 ± 0.1 ms), and these were usually followed by fast and slow afterhyperpolarizations with amplitudes of 23.1 ± 0.7 mV (n = 258) and 13.8 ± 0.4 mV (n = 222), respectively. Whereas ~60% of lateral horn neurons displayed a hyperpolarization-activated time-dependent inward current (Iₖ) of varying degrees, this feature was seldom noted among the SPN population.

**AVP V₁ receptor-mediated depolarization and inward current**

A majority (90%) of 273 cells tested, including all 68 SPNs, responded to AVP. A typical response in current-clamp mode (n = 11) was a slowly rising (30–200 s) and prolonged (5–20 min) membrane depolarization (mean 14.5 ± 2.1 mV) that triggered a burst of action potentials on a plateau that lasted several minutes (Fig. 1A). Although an increase in baseline noise at the peak of the response suggested a possible presynaptic component, we focused on a postsynaptic site of action. This was confirmed in voltage clamp in ACSF containing 1 μM TTX where, at a holding potential of −65 mV, responsive cells featured a dose-dependent (0.01–1 μM) inward current, also with a prolonged time course (Fig. 1B). Applications of AVP at intervals <15 min could be seen to undergo a progressive reduction in amplitude, possibly reflecting receptor desensitization; therefore, when repetitive tests were utilized (e.g., Fig. 3C), intervals of 25–30 min were allowed between successive applications.

As anticipated for V₁-type AVP receptors, similar membrane depolarizations and/or inward currents (−25.4 ± 1.4
pA) were induced by application of a V₁ receptor agonist, [(Phe²,Orn⁸)-vasotocin], 1 μM, n = 12/14 cells, and reversibly blocked after pretreatment with a V₁ receptor antagonist (Manning compound; 0.1–1 μM; 14/14 cells). Only 2/9 cells responded to application of DDAVP, a V₂ receptor agonist, but with markedly reduced currents (–6.5 ± 1 pA) and at concentrations of 10–25 μM (not illustrated).

**Response to oxytocin**

Because oxytocin-immunoreactive fibers and oxytocin binding was observed in rat spinal cord (Rousselot et al. 1990; Tribollet et al. 1997), AVP-sensitive neurons were also tested for their response to oxytocin. Although cells were found to be responsive, oxytocin (1–2 μM for 60 s) induced significantly smaller membrane depolarizations when compared with 1 μM AVP (3 ± 0.8 mV vs. 15.4 ± 3.9 mV; n = 5; P < 0.05). Under voltage-clamp conditions, tests with a specific oxytocin agonist [Thr⁴,Gly⁷]-oxytocin (1–2 μM for 30–60 s) revealed small inward currents (–18 ± 3 pA) in three of seven tested cells. Thus neonatal lateral horn cells expressed more robust responses to AVP than to oxytocin (see also Sermasi and Coote 1994; but cf. Desaulles et al. 1995).

**Role of G-proteins**

Because AVP receptors are G-protein coupled, and to verify that these electrophysiological responses involved G-proteins, we first compared data where the recording pipette solutions lacked GTP. Although not achieving a level of significance, the mean AVP-induced inward current measured for 39 cells (–27.2 ± 2.3 pA) in the absence of GTP was lower than for 40 cells recorded in the presence of GTP (–33.8 ± 3.4 pA). We next loaded 17 cells (including 7 SPNs) with GTP-γ-S (200 μM), a nonhydrolyzable derivative of GTP that activates G-proteins in an irreversible manner (Gilman 1987). In 9/17 cells (including all 7 SPNs) a gradual inward current of –17.8 ± 3.9 pA was measurable at the 5-min interval after establishment of whole cell voltage clamp. Moreover, the response to the first application of AVP failed to demonstrate recovery (Fig. 2, A, bottom trace, and B, and Fig. 3A), still showing a significant residual current after 10 min (Fig. 2, B and D) and only a marginal response to a second AVP application after 25 min of wash (Fig. 3, A and C). Additionally, the initial AVP application resulted in a maximum current of –52.7 ± 6.1 pA, significantly greater than the –33.8 ± 3.4 pA measured in control cells (Fig. 2C). Similar results were obtained in five cells dialyzed with another G-protein activator, GMP-PNP (Fig. 2, C and D).

We also dialyzed nine cells with GDP-β-S (800 μM for 10 min), a stable analogue of GDP that competitively inhibits G-protein binding of and activation by GTP (Gilman 1987). In this group which included three SPNs, response (1±2 μM AVP (3 ± 0.8 mV vs. 15.4 ± 3.9 mV; n = 5; P < 0.05) reduction of 55% from the control horn cells expressed more robust responses to AVP than to oxytocin (see also Sermasi and Coote 1994; but cf. De-

![FIG. 1. Arginine vasopressin (AVP) induces a prolonged depolarization and dose-dependent inward current in lateral horn cells. A: whole cell current-clamp recording from a sympathetic preganglionic neuron (SPN, resting membrane potential -64 mV) illustrates that a 30-s AVP application (horizontal open bar) is followed by a slowly rising, prolonged, and reversible membrane depolarization, with spike discharges at the peak of the response. B: traces obtained in voltage-clamp mode (V₀ -65 mV) from another unidentified lateral horn neuron illustrate AVP-induced dose-dependent inward currents. Downward deflections are inward currents resulting from hyperpolarizing voltage pulses (10 mV, 400-ms duration). Right: histogram of data pooled from 134 neurons illustrate the dose-dependent increase of maximal inward current amplitude produced by AVP (0.01 μM, n = 5; 0.1 μM, n = 47; 1 μM, n = 82; for 20–60 s). Data are expressed as means ± SE in all figures.]
AVP activates different membrane conductances

Despite the increase in mean peak currents with higher AVP concentrations (Fig. 1B), we noted a marked variation among neurons in membrane conductances associated with the AVP responses, with some cells showing an obvious inward current but only a marginal conductance change. For 57 cells, we therefore looked at the profile of the net AVP current (obtained by subtraction of the I-V relationships obtained under control conditions and at the peak of a response) induced by 1 μM AVP (Fig. 5, A–D). The net AVP current for all cells revealed an overall reduction but no reversal in membrane conductance in the hyperpolarizing direction and amplitudes (Fig. 4, n = 10). Note the significant increase in cells pretreated with nonhydrolyzable analogues of GTP. In control cells the internal solution contained GTP.

*P < 0.05 and **P < 0.01, compared with values obtained without pretreatment.

(Fig. 4) revealed a significantly smaller mean AVP-induced inward current when compared with those from nine cells in control slices (−23.6 ± 7.6 pA vs. −53.4 ± 10.8 pA; P < 0.05). The duration of response in PTX-exposed cells was increased only marginally over controls. In view of the apparent PTX sensitivity of the response, we also tested the effects of NEM, a sulfhydryl-alkylating agent that has been shown to block G-protein-effector interactions by alkylating the α-subunits of PTX-sensitive GTP-binding proteins (Shapiro et al. 1994; Viana and Hille 1996). After a 5-min bath application of NEM (50 μM), AVP-induced currents were reduced to 34% of control amplitudes (Fig. 4, n = 5, P < 0.01, includes 1 SPN), an effect that was partly reversible (to 80% of control) in three of five neurons tested. The duration of the AVP-induced response was also affected, with residual current at 10 min being 18.1 ± 13.2% of the maximum for control versus 6.4 ± 6.4% for responses with NEM (P > 0.05).

These observations suggest that both PTX-sensitive and insensitive G-proteins participate in the AVP-induced current in neonatal spinal lateral horn neurons.
For one group of 21 cells (37%, 7 being identified as SPNs), $I-V$ plots converged near $-100 \text{ mV}$, with a net AVP-induced current that reversed at $-102.8 \pm 3.1 \text{ mV}$ (Fig. 6A, solid symbols); in 17 of these cells, membrane conductance decreased from $3.8 \pm 0.5$ to $3.2 \pm 0.4 \text{ nS}$ ($P < 0.01$) and was unchanged in the rest. Near resting membrane potentials, the shape of these plots suggested that the net AVP current was not particularly voltage sensitive in that its amplitude was linearly related to the driving force on $K^+$ ($V_m - E_K$). The linearity of this current assures that it would contribute substantially to the leak conductance of the membrane potential, even at rest.

Another group of 12 cells (21%, includes 4 identified as SPNs) displayed $I-V$ plots that also indicated a reversal of the net AVP-induced current, but at a more depolarized level of $-37.6 \pm 2.8 \text{ mV}$ (Fig. 6A, shaded symbols). For this group, measurements of membrane conductance revealed an increase in five cells (from $3.8 \pm 0.5$ to $4.4 \pm 0.7 \text{ nS}$; $P < 0.05$), a decrease in four cells (from $2.7 \pm 0.5$ to $2.3 \pm 0.5 \text{ nS}$; $P < 0.05$), and no change in the remainder. The $I-V$ relationships in these cells displayed outward rectification at potentials more positive than $-40 \text{ mV}$.

By contrast, the remaining group of 24 cells (42%, 3 identified as SPNs) had $I-V$ relationships and net AVP-induced currents that indicated no reversal (Fig. 6A, open symbols). In this group, 16 cells showed an AVP-induced decrease in membrane conductance (from $4 \pm 0.4$ to $3.4 \pm 0.4 \text{ nS}$; $P < 0.01$), and the remainder was without change. It is notable that the maximum inward current induced by AVP was significantly larger for cells with no reversal (Fig. 6B).

These data suggest that AVP can depolarize neonatal spinal lateral horn neurons, including SPNs, by at least two conductances. One is largely voltage independent, coupled with a decrease in membrane conductance and reverses close to the equilibrium potential for potassium ($-98 \text{ mV}$ in our conditions); the other has outward rectification, is coupled with a modest increase in membrane conductance, and reverses near $-40 \text{ mV}$.

**Ionic mechanisms**

In cells whose $I-V$ relationships demonstrated a reversal near $-100 \text{ mV}$, changing $[K^+]_{out}$ from 3.1 to 10 mM shifted
the AVP current reversal from $-106 \pm 3.1 \text{ mV}$ to $-69 \pm 2.6 \text{ mV}$, respectively (Fig. 7, A–C), as anticipated according to the Nernst equation. In ACSF containing 10 mM $[\text{K}^+]$, the net AVP-induced inward current (at a holding potential approximately $-65 \text{ mV}$) was substantially reduced from $-25.8 \pm 8.2 \text{ pA}$ (in normal ACSF) to $-10.8 \pm 4.2 \text{ pA}$ ($n = 4; P < 0.05$).

To characterize this potassium conductance(s) further, cells were tested for the effects of inhibitors of various $K^+$ currents. After switching to ACSF containing 2 mM barium, nine cells (including 3 identified SPNs) displayed a persistent mean inward current of $-18.3 \pm 2.6 \text{ pA}$, similar to that induced by AVP, accompanied by a reduction in membrane input conductance, from $2.7 \pm 0.2$ to $1.9 \pm 0.2 \text{ nS}$ ($P < 0.01$). The $I-V$ relationships indicated that this current decreased with hyperpolarization and reversed at a $V_h$ of approximately $-91 \text{ mV}$, close to the presumed $E_{K}$. (Fig. 8A), suggesting that the barium-activated inward current resulted from a decrease in one or more resting potassium conductances. Because of the similarity between the barium- and AVP-induced conductances (compare solid symbols in Fig. 6A with Fig. 8A), we hypothesized that AVP and barium might influence the same conductances. In fact, in five cells tested in ACSF that contained barium, we observed a significant reduction in the magnitude of the AVP-induced current (Fig. 8, B and C), whereas none of the cells tested with

**FIG. 5.** Net AVP-induced current. A: voltage-clamp trace (dotted line depicts holding current at $V_h = -65 \text{ mV}$) illustrates inward current in response to application of AVP. B and C: current responses to a series of voltage pulses (duration 600 ms, from $-130$ to $-20 \text{ mV}$ with 10-mV steps) applied before (control) and at the peak (AVP) of the AVP-induced response shown in A. D: $I-V$ plots constructed from values taken at the end of the pulse (open and closed symbols, representing control and drug-induced states, respectively). Net AVP-induced current (triangles) was determined by subtraction of the $I-V$ obtained at the peak of the AVP response (AVP) from that obtained before application of AVP (control). E: mean net AVP current for 57 cells indicates reduction in the hyperpolarizing direction but lacks an obvious reversal in this voltage range; note the rectification at potentials more positive than $-40 \text{ mV}$.

**FIG. 6.** Net AVP-induced inward current analysis indicates 3 patterns in $I-V$ relationships. A, open symbols: data from 24 cells with no obvious reversal potentials. Solid symbols: data from 21 cells where there was inward current that reversed close to $E_{K}$. Shaded symbols: data from 12 cells demonstrating an increase in membrane conductance with a reversal close to $-40 \text{ mV}$. B: amplitude histogram of the AVP-induced inward currents for the different conductance patterns. Note that the maximum amplitude for cells where no reversal was observed is significantly larger than that for cells with reversals around either $-100$ or $-40 \text{ mV}$. $*P < 0.05$, compared with values between cells with no reversal vs. cells with other $I-V$ patterns.
several other potassium channel blockers, including 4AP (5 mM), TEA (10 mM), and glibenclamide (20 μM), demonstrated a similar effect (Fig. 8C). The I-V relationship of the barium-sensitive component of inward current induced by AVP (obtained by subtracting the I-V plot obtained in normal media from that obtained in ACSF with barium, Fig. 8B) had characteristics similar to the net current produced by barium or AVP respectively, with a current that reversed at approximately −92 mV, close to the estimated $E_{K^+}$. On the other hand, the residual barium-insensitive component of the net AVP current had a quite different I-V relationship, with an opposite slope and a reduction to zero near −40 mV (Fig. 8B).

As indicated in Fig. 6A (shaded symbols), a population of neurons displayed another AVP-induced conductance that was outwardly rectifying and reversed near −40 mV (Fig. 6A, shaded symbols), a profile that is suggestive of a nonselective cationic conductance (c.f. Swandulla and Partridge 1990). One possibility for this conductance is $I_h$, a cesium-sensitive and nonselective cationic conductance that is permeable to both potassium and sodium ions (Pape 1996). As mentioned earlier, ~60% of cells (excludes SPNs) display a time-dependent, hyperpolarization-activated inward current of varying degrees (e.g., Fig. 9, A and C). To explore the contribution of this conductance to the AVP-induced current, we examined cells that showed no I-V reversals and/or reversals at approximately −40 mV. Anticipating a difference between their instantaneous and steady-state I-V relationships, we found that the magnitude of the net AVP-induced currents in 22 cells was independent of where they were measured and that the net AVP-induced inward current for cells with reversal around −40 mV was still active at potentials of −50 to −60 mV, where $I_h$ was already inactive (Fig. 6A, gray symbols, and Fig. 9, B and D). Additionally, the application of cesium (1 mM) blocked any $I_h$ conductance ($n = 8$) but was without effect on the AVP-induced current ($n = 4$). Furthermore, an $I_h$ conductance was also present in cells where the net AVP-induced current reversed close to $E_{K^+}$ ($n = 12$). Therefore these data suggest that the conductance underlying $I_h$ has little role in the proposed

![Figure 7](http://jn.physiology.org/)

**FIG. 7.** AVP-induced inward current is associated with a decrease in a potassium conductance. A: in normal artificial cerebrospinal fluid (ACSF), ramp analysis (voltage increases linearly from −110 to −40 mV in 7 s) reveals that AVP decreases the membrane conductance with a reversal potential close to the potassium equilibrium potential. B: in ACSF containing 10 mM potassium, reversal potential shifts toward the predicted $E_{K^+}$ (from −98 to −68 mV according to Nernst equation) C: histograms illustrate that the I-Vs during application of AVP (0.1–1 μM) in ACSF with [K+]o of 3.1 mM reverse at −106 ± 3.1 mV ($n = 15$) and the shift of this reversal to −69 ± 2.6 mV ($n = 4$) after increasing [K+]o to 10 mM.

![Figure 8](http://jn.physiology.org/)

**FIG. 8.** AVP-induced currents display both barium-sensitive and -insensitive components. A: data from 9 cells reveal a net barium-induced inward current (for calculation of net current see legend for Fig. 5). Note the resemblance between the barium and the AVP-induced current in Fig. 6A (solid symbols). B: data from 5 cells where AVP (1 μM for 20–50 s) were applied 1st in normal ACSF and (15–20 min later) after preincubation in ACSF containing barium (2 mM for 5–10 min). The I-V plots were obtained before and during each AVP application. Solid symbols represent the net AVP-induced current under control conditions. Net barium-insensitive AVP-induced current was calculated from I-Vs obtained during barium pretreatment and at the peak of the AVP response in ACSF containing barium (open squares); represents the residual AVP-induced current in the presence of barium. The net barium-sensitive AVP-induced current (open diamond) was obtained by subtracting the barium-insensitive from the control AVP-induced current. Note that the barium-insensitive plot displays an opposite slope when compared with the control or the barium-sensitive plot, suggesting that some other conductance likely contributes to this effect. C: summary histograms illustrate the influence of different potassium channel blockers on the AVP-induced inward current. In each cell, AVP was 1st applied (control), and then cells were exposed to ACSF containing blockers for 5–15 min before testing with a 2nd AVP application. The amplitude of AVP-induced inward current was measured at the maximum of response. *$P < 0.05$, compared with values obtained without pretreatment. Blockers included glibenclamide (GLB, 20 μM), 4-aminopyridine (4AP, 5 mM), tetraethylammonium chloride (TEA, 10 mM), cesium (Cs, 1 mM), and barium (Ba, 2 mM).
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mination effects, an additional inward current of
Ih, with a charge carrier that remains to be defined. When
closed symbols represent steady-state current (Iₛₛ). The net cur-
ents (square symbols), representing the difference be-
are virtually identical for measurements of instantaneous and steady-state currents and suggest minimal, if any, contribution of Ih to the AVP
response.
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DISCUSSION

These observations support a neuronal distribution for the AVP binding that was noted in neonatal spinal tissue (Tribollet et al. 1991). These data from the neonatal period indicate that a majority (90%) of lateral horn neurons express functional AVP receptors of the V₁ subtype, responding to a transient exogenous application of AVP with an inward current that results in membrane depolarization. As anticipated, our findings are consistent with a role for G-proteins in signaling at these neuronal AVP receptors. In addition, responses appear to involve both PTX-sensitive and -insensitive components, suggesting involvement of multiple G-proteins. Extending earlier reports of a depolarizing action of AVP on neonatal spinal neurons, our analyses of I-V relationships and net AVP-induced currents in individual cells led us to conclude that AVP receptors may induce membrane depolarization by altering one or both of two conductances. In one population of cells where the response to AVP resulted in a decrease in membrane conductance at resting levels, we concluded that the dominant component of the AVP-induced inward current is due to reduction in a barium-sensitive resting or leak potassium conductance that decreases at hyperpolarizing potentials and reverses at a potential close to the estimated EK. In another population of cells we noted that the AVP-induced inward current was associated with an increase in membrane conductance and involved an increase in a barium-insensitive conductance that reversed near −40 mV. We propose that the latter reflect a nonselective cationic conductance that is separate from Ih, with a charge carrier that remains to be defined. When individual cells expressed a predominance of one or other conductance, the profile of the net AVP currents permitted easy distinction. However, it appears that inward currents in some neurons can be mediated through both conductances, and their competition could explain both the parallel shifts observed in their I-V relationships and the uncertainty about net AVP-induced conductances. In fact, there are now several situations where postsynaptic actions of peptides or other transmitters increase a voltage-independent cation conductance and decrease an inward potassium conductance, for example, in hippocampus (Benson et al. 1988), brain stem (Bayliss et al. 1992; Dong et al. 1996; Shen and North 1992a, b), and spinal cord lateral horn (Kolaj et al. 1997).
It remains a matter of speculation as to why AVP receptors and oxytocin-immunoreactive fibers observed in the dorsal horn, central gray, and intermediolateral horn areas (e.g., Buijs 1978; Rousselot et al. 1990) are deemed to originate largely from parvocellular peptidergic neurons in the hypothalamic paraventricular nucleus (Cechetto and Saper 1988; Hosoya et al. 1991; Sawchenko and Swanson 1982; Swanson 1977; Swanson and McKeller 1979). Moreover, stimulation localized to PVN can increase the levels of AVP and oxytocin in spinal cord perfusates (Pittman et al. 1984) and produce a V1 receptor-mediated increase in renal sympathetic nerve activity in rat (Malpas and Coote 1994; Riphagen and Pittman 1989a,b) and cardioaccelerator and pressor responses in cat (Ciriello and Calaresu 1980). In spinal cord, most of the attention has focused on a role for AVP, in part because its intrathecal administration can be seen to alter cardiovascular and renal function (Riphagen and Pittman 1985a,b, 1989a,b), and its iontophoretic application in cat was shown to increase the excitability of SPNs (Backman and Henry 1984). These features are consistent with an AVP-induced membrane depolarization in neonatal SPNs and lateral horn neurons (see also Ma and Dun 1985; Sermasi and Coote 1994). Collectively, the data support the notion that AVP receptors may have an excitatory neurotransmitter/modulator role in autonomic outflow pathways. Although spinal lateral column neurons and SPNs are also responsive to oxytocin (Backman and Henry 1984; Desaulles et al. 1995), the present observations did not indicate a comparatively prominent role for oxytocin receptors in these neonatal neurons. However, the question remains as to whether these spinal AVP (and oxytocin) receptors have yet been reached by a descending peptidergic innervation at this stage of development.

In view of the preceding comment and other observations, alternative roles for AVP receptors that are unrelated to neurotransmission are worthy of consideration. With cerebrospinal fluid AVP demonstrating a circadian rhythmicity in several species (Reppert et al. 1987; Schwartz et al. 1983) at resting levels of 10–30 pg/ml in adult rat spinal subarachnoid space (e.g., Pittman et al. 1984) and ≤100 pg/ml in the preoptic recess in fetal lambs in utero (Stark and Daniel 1989), it is possible that AVP receptors may have a role in neuronal development. For example, in explanted spinal cord cultures, AVP, but not oxytocin, is reported to have a neurotrophic action (Iwasaki et al. 1991). Furthermore, the demonstration by Tribollet et al. (1994, 1997) that the expression of AVP binding in spinal cord can be regulated by sex steroids and by nerve injury (Tribollet et al. 1997) suggests that AVP receptors can be up- or down-regulated and apparently reexpressed under certain conditions, for example, after neuronal injury (Tribollet et al. 1994). It may be of interest in future studies to determine how these manipulations in AVP receptors correspond with the electrophysiological properties reported here.

Possible functions of AVP receptors in spinal cord

In the adult spinal cord, both AVP and oxytocin are proposed to have a neurotransmitter and/or neuromodulatory role in central regulation of autonomic function. Both AVP- and oxytocin-immunoreactive fibers observed in the dorsal horn, central gray, and intermediolateral horn areas (e.g., Buijs 1978; Rousselot et al. 1990) are deemed to originate largely from parvocellular peptidergic neurons in the hypothalamic paraventricular nucleus (Cechetto and Saper 1988; Hosoya et al. 1991; Sawchenko and Swanson 1982; Swanson 1977; Swanson and McKeller 1979). Moreover, stimulation localized to PVN can increase the levels of AVP and oxytocin in spinal cord perfusates (Pittman et al. 1984) and produce a V1 receptor-mediated increase in renal sympathetic nerve activity in rat (Malpas and Coote 1994; Riphagen and Pittman 1989a,b) and cardioaccelerator and pressor responses in cat (Ciriello and Calaresu 1980). In spinal cord, most of the attention has focused on a role for AVP, in part because its intrathecal administration can be seen to alter cardiovascular and renal function (Riphagen and Pittman 1985a,b, 1989a,b), and its iontophoretic application in cat was shown to increase the excitability of SPNs (Backman and Henry 1984). These features are consistent with an AVP-induced membrane depolarization in neonatal SPNs and lateral horn neurons (see also Ma and Dun 1985; Sermasi and Coote 1994). Collectively, the data support the notion that AVP receptors may have an excitatory neurotransmitter/modulator role in autonomic outflow pathways. Although spinal lateral column neurons and SPNs are also responsive to oxytocin (Backman and Henry 1984; Desaulles et al. 1995), the present observations did not indicate a comparatively prominent role for oxytocin receptors in these neonatal neurons. However, the question remains as to whether these spinal AVP (and oxytocin) receptors have yet been reached by a descending peptidergic innervation at this stage of development.

In view of the preceding comment and other observations, alternative roles for AVP receptors that are unrelated to neurotransmission are worthy of consideration. With cerebrospinal fluid AVP demonstrating a circadian rhythmicity in several species (Reppert et al. 1987; Schwartz et al. 1983) at resting levels of 10–30 pg/ml in adult rat spinal subarachnoid space (e.g., Pittman et al. 1984) and ≤100 pg/ml in the preoptic recess in fetal lambs in utero (Stark and Daniel 1989), it is possible that AVP receptors may have a role in neuronal development. For example, in explanted spinal cord cultures, AVP, but not oxytocin, is reported to have a neurotrophic action (Iwasaki et al. 1991). Furthermore, the demonstration by Tribollet et al. (1994, 1997) that the expression of AVP binding in spinal cord can be regulated by sex steroids and by nerve injury (Tribollet et al. 1997) suggests that AVP receptors can be up- or down-regulated and apparently reexpressed under certain conditions, for example, after neuronal injury (Tribollet et al. 1994). It may be of interest in future studies to determine how these manipulations in AVP receptors correspond with the electrophysiological properties reported here.

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