Vasopressin Generates a Persistent Voltage-dependent Sodium Current in a Mammalian Motoneuron

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During the period of life that precedes weaning, the facial nucleus of the newborn rat is rich in ^3H -vasopressin binding sites, and exogenous arginine vasopressin (AVP) can excite facial motoneurons by interacting with V_1 (vasopressor-type) receptors. We have investigated the mode of action of this peptide by carrying out single-electrode voltage-clamp recordings in coronal brainstem slices from the neonate. Facial motoneurons were identified by antidromic invasion following electrical stimulation of the genu of the facial nerve. When the membrane potential was held at or near its resting level, vasopressin generated an inward current whose magnitude was concentration related; the lowest peptide concentration still effective in eliciting this effect was 10 nM. The vasopressin-induced current, I_{AVP} , was resistant to tetrodotoxin (TTX) and was insensitive to a reduction in extracellular calcium concentration. It was sustained, was inward at all potentials tested (-120 to -25 mV), and increased in magnitude during depolarization. I_{AVP} was not generated by the blockade of a potassium current, because it did not reverse at hyperpolarized potentials, was not affected by a two-fold increase in the transmembrane potassium gradient, and was not modified by the potassium channel blockers tetraethylammonium bromide (TEA), 4-aminopyridin (4-AP), barium, cesium, quinine, glibenclamide, and apamin. Also, I_{AVP} was not affected by changes in the transmembrane chloride gradient. In contrast, it could be reduced by partially substituting extracellular sodium with equimolar *N*-methyl-D-glucamine or Tris. Our results suggest that vasopressin increases the excitability of facial motoneurons by generating a persistent sodium-dependent membrane current that is voltage gated and TTX resistant.

The nonapeptide arginine vasopressin, which is synthesized in the hypothalamus and secreted into the circulation at the level of the neurohypophysis, exerts a variety of peripheral effects. Thus, by acting on V_1 -type, phospholipase C-coupled receptors, it causes vasoconstriction and stimulates glycogenolysis in hepatocytes; by binding to V_2 -type, adenylate cyclase-coupled receptors, it enhances water reabsorption in the kidney (Jard, 1983; Manning et al., 1987). Immunocytochemical, autora-

diographical, and biochemical studies have suggested that vasopressin probably also plays a role as a neurotransmitter/neuromodulator (Audigier and Barberis, 1985; Buijs, 1987; Dubois-Dauphin and Zakarian, 1987; Van Leeuwen, 1987; Freund-Mercier et al., 1988; Poulin et al., 1988; Tribollet et al., 1988), and on the basis of behavioral studies, it has been claimed that this peptide may influence memory storage and retrieval (De Wied, 1980). Electrophysiological studies have indicated that vasopressin can directly excite selected populations of central neurons (Suzue et al., 1981; Mühlethaler et al., 1982; Ma and Dun, 1985; Peters and Kreulen, 1985; Raggenbass et al., 1987, 1988, 1989; Carette and Poulain, 1989; Liou and Albers, 1989; Sun and Guyenet, 1989). Although some information is available concerning the cellular mode of action of vasopressin as a hormone (Mollard et al., 1988; Van Renterghem et al., 1988; Korbmacher et al., 1989; Martin et al., 1989), the membrane mechanism by which vasopressin affects neuronal bioelectrical activity is not yet known. We have approached this problem by recording from vasopressin-sensitive facial motoneurons from the newborn rat (Tribollet et al., 1991), using the *in vitro* brain slice technique and the single-electrode voltage-clamp method.

Materials and Methods

Intracellular recordings. The animals used were 2–10-d-old male rats from a Sprague-Dawley-derived strain. Coronal brainstem slices, 300–400 μm thick, were cut using a vibratome, transferred to a recording chamber, and perfused, at 34–35°C, with a solution containing (in mM) 135 NaCl, 15 NaHCO₃, 1 MgSO₄, 1.25 KH₂PO₄, 2 CaCl₂, 10 glucose, and, unless otherwise stated, 5 KCl. This solution was saturated with 95% O₂/5% CO₂ (pH, 7.35–7.45). A low-calcium, high-magnesium solution, containing 0.1 mM CaCl₂ and 16 mM MgSO₄, was used in some experiments to block synaptic transmission and to reduce membrane calcium currents. When barium was added to the solution, phosphates were omitted, and MgSO₄ was replaced by MgCl₂. *N*-methyl-D-glucamine and Tris, which were used in sodium ion substitution experiments, were titrated to pH 7.4 with concentrated HCl. Microelectrodes contained either 3 M KCl or 3 M K-acetate (pH, 7.4; 30–80 M Ω); in some experiments, facial neurons were cesium-loaded by impalement with microelectrodes filled with 2 M CsCl or Cs-acetate (pH, 7.4). Recordings were done using an Axoclamp-2A amplifier. In the single-electrode voltage-clamp mode, the switching frequency was 2–3 kHz, and the headstage was continuously monitored. Membrane potential and current were plotted on paper on line. Some data were digitized using a homemade A/D converter and stored on the hard disk of an IBM-AT-compatible computer for further analysis. Antidromic activation of facial neurons was achieved by delivering constant current pulses (10–100 μA , 0.1 msec) to the genu of the facial nerve. The elicited action potentials were considered to be due to antidromic invasion if (1) they arose at fixed latency following stimulation, (2) they followed high-frequency stimulation, and (3) they could dissociate, in double-shock studies, into initial segment and somatodendritic components.

Light microscopic autoradiography. Following dissection, brain tissue was frozen in 2-methylbutane at -25°C . Series of 15- μm -thick sections were cut in a cryostat, mounted on gelatin-coated slides, and, after

Received Oct. 1, 1990; revised Jan. 2, 1991; accepted Jan. 4, 1991.

This work was supported in part by Grant 31.28624.90 from the Swiss National Science Foundation. We thank Dr. M. M. Manning for the gift of the selective oxytocin agonist, Dr. D. Bertrand for helpful discussions, and Mrs. D. Machard for excellent technical assistance.

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Figure 1. Vasopressin binding sites in the facial nucleus of a newborn rat. Autoradiograms in *A* and *B* were obtained by incubating coronal brainstem sections with ^3H -vasopressin (*A*) or with ^3H -vasopressin supplemented with an excess of nonradioactive vasopressin (*B*). *C* and *D* show the ventrolateral region on the right side of *A* at a higher magnification. *D* is the cresyl violet-stained section used to generate autoradiograms *A* and *C*. Note that labeling is most intense in the area containing large facial-motoneuron perikarya. Scale bars: *A* and *B*, 1 mm; *C* and *D*, 0.5 mm.

drying, stored at -80°C . Prior to the binding procedure, the sections were slightly fixed for 5 min in 0.2% paraformaldehyde in phosphate-buffered saline (PBS; pH, 7.4). Each slide was incubated for 1 hr at room temperature in 400 μl of a solution containing 50 mM Tris-HCl (pH, 7.4), 0.025% bacitracin, 5 mM MgCl_2 , and 0.1% BSA and supplemented with 3 nM ^3H -vasopressin and 5 nM $\text{HO}[\text{Thr}^4, \text{Gly}^7]\text{oxytocin}$. The presence of this last compound, a selective oxytocin agonist, prevented the labeling by ^3H -vasopressin of oxytocin binding sites. In order to determine non-specific binding, adjacent sections were incubated in the same solution as above but supplemented with 1 μM nonradioactive vasopressin. The slides were washed in ice-cold medium, rinsed in distilled water, dried, and placed in contact with tritium-sensitive films in an x-ray cassette for 3–4 months. Films were developed for 5 min in Kodak D19, and sections were stained with cresyl violet.

Chemical compounds. Vasopressin (8-L-arginine vasopressin) was purchased from Bachem (Bubendorf, Switzerland). ^3H -vasopressin (specific activity, 67 Ci/mmol) was from Du Pont-New England Nuclear (Boston, MA) and was purified by HPLC and affinity chromatography (Pradelles et al., 1972). The oxytocin-selective agonist $\text{HO}[\text{Thr}^4, \text{Gly}^7]\text{oxytocin}$ ([1-(L-2-hydroxy-3-mercaptopropionic acid), 4-threonine, 7-glycine]oxytocin; Lowbridge et al., 1977) was kindly provided by Dr. M. M. Manning (Department of Biochemistry, Medical College of Ohio, Toledo, OH). Tetrodotoxin (TTX), glibenclamide, apamine, and quinine hydrochloride were from Sigma (St. Louis, MO). Tetraethylammonium bromide (TEA), 4-aminopyridin (4-AP), Na-isethionate, and *N*-methyl-D-glucamine were from Fluka (Buchs, Switzerland).

Results

The facial nucleus of the newborn rat, and particularly its intermediate part, contains specific, high-affinity binding sites for ^3H -vasopressin (Fig. 1). The density of these sites is maximal shortly after birth and decreases during the third week of postnatal life; no binding sites can be detected in the adult animal. At least part of these sites represent functional neuronal receptors, whose pharmacological profile is indistinguishable from that of V_1 (vasopressor-type) peripheral receptors (Tribollet et al., 1991). Because facial motoneurons can be stably impaled due to their large dimensions, we chose to study the membrane mechanism underlying the neuronal action of vasopressin by recording from these cells in brainstem slices from newborn rats.

Intracellular recordings were obtained from 193 neurons located in the region of the facial nucleus in slices from 66 animals.

These cells had resting membrane potentials negative to -50 mV and fired no action potentials at rest. Their average input resistance was 25 ± 12 M Ω (mean $\pm$ SD; $n = 180$). Eighty-seven neurons could be activated antidromically following electrical stimulation of the genu of the facial nerve and were thus identified as facial motoneurons (Fig. 2*B*). The average input resistance of these identified motoneurons was 25 ± 11 M Ω (mean $\pm$ SD; $n = 84$). Vasopressin, dissolved in the perfusion solution, affected 82% of the recorded neurons. Of the identified motoneurons, 84% responded to the peptide. When the membrane potential was voltage clamped at or near its resting level, vasopressin generated a sustained inward current (Fig. 2*A*); under current-clamp conditions, the peptide caused a membrane depolarization (see Fig. 5*B*). These effects were fully reversible. The peak current evoked by vasopressin, added to the perfusion solution at 1 μM for 30 sec, ranged from 0.1 to 1.4 nA, and the peak depolarization ranged from 3 to 20 mV. The action of vasopressin was concentration dependent, the lowest peptide concentration still effective in eliciting an effect being 10 nM (except in one neuron, where a measurable effect was evoked by vasopressin at 1 nM; see Fig. 2*A*). Thus, at 10 nM, 100 nM, and 1 μM , the vasopressin-induced peak current was 0.08 ± 0.02 , 0.31 ± 0.07 , and 0.63 ± 0.12 nA (mean $\pm$ SEM; $n = 4$), respectively, and the evoked peak depolarization was 2.2 ± 0.4 , 7.6 ± 1.2 , and 12 ± 2.4 mV (mean $\pm$ SEM; $n = 5$), respectively. No tachyphylaxis was observed upon repeated application of vasopressin.

The effect of vasopressin was insensitive to TTX present in the perfusion solution at 0.5–2 μM (see Fig. 3; tested on 30 neurons); at these toxin concentrations, sodium-dependent action potentials were abolished. The peptide effect persisted when the normal solution was replaced with a low-calcium, high-magnesium solution (Fig. 2*B*; tested on eight neurons). During perfusion with the latter solution, transmembrane calcium currents were greatly reduced, as indicated by the disappearance of TTX-resistant action potentials and of evoked synaptic potentials. Thus, the action of the peptide is postsynaptic, and the vasopressin-induced response is probably not due to the activation either of a TTX-sensitive sodium current or of a calcium current.

Facial neurons were voltage clamped, and current–voltage (I/V) relations were determined by measuring the total membrane current at the end of voltage command steps lasting 0.5–1 sec delivered before, during, and after the peptide-induced response (see Fig. 3A₁). The vasopressin-induced current, I_{AVP} , was computed by subtracting the control I/V curve from the curve obtained in the presence of the peptide (see Fig. 3A₂). In 16 out of 25 sensitive neurons, vasopressin affected the I/V relation by causing an *apparent* increase in membrane resistance, which was most pronounced at depolarizing voltage steps (Fig. 3A₁). No inactivation of I_{AVP} could be detected over the duration of the voltage steps. I_{AVP} was inward at all potentials tested, which ranged from -120 to -25 mV, and the magnitude of this current increased progressively as the potential was displaced in the depolarizing direction (Fig. 3A₂,B).

Command steps to potentials positive to -20 mV evoked powerful outward currents, which were often unstable and which could be reduced, but not suppressed, by perfusing a cocktail of potassium channel blockers and a low-calcium, high-magnesium solution. At these potentials, I_{AVP} could not be reliably measured by the subtraction method illustrated in Figure 3.

The voltage dependence of I_{AVP} might suggest that vasopressin acted by blocking an outward current whose reversal potential is more negative than the resting membrane potential (i.e., a potassium or a chloride current). Three lines of evidence indicate, however, that a potassium current is probably not implicated in the vasopressin effect: (1) As stated above, I_{AVP} did not reverse in polarity at hyperpolarized potentials (see Fig. 3A₂,B). (2) In seven out of seven neurons, which were current or voltage clamped at their resting membrane potential, the responses to vasopressin recorded in 3.25 mM and 7.25 mM extracellular potassium were superposable. (3) None of several potassium channel blockers (Castle et al., 1989; Dreyer, 1990) could suppress the response to vasopressin. Thus, TEA, at 10 mM, blocked the delayed rectifier, as evidenced by the induced increase in the duration of TTX-resistant action potentials, but failed to affect the action of the peptide (see Fig. 3A; $n = 14$). 4-AP, at 1 mM, enhanced the frequency and the amplitude of spontaneous synaptic potentials but did not affect the response to vasopressin (see Fig. 3A; $n = 11$). Barium, at 1 mM, also had a stimulatory action on synaptic input, and cesium, at 5 mM, suppressed a time-dependent inward rectifier, but neither cation had any effect on the peptide-induced current ($n = 10$). Moreover, vasopressin responsiveness was still present in 8 out of 11 cesium-loaded facial motoneurons (Fig. 4). Quinine, at 200 μ M, did not interfere with the vasopressin-induced response ($n = 4$). Glibenclamide, a blocker of ATP-sensitive potassium channels, at 1 μ M ($n = 5$), and apamin, a blocker of small-conductance calcium-dependent potassium channels, at 50 nM ($n = 4$), were also without effect on the peptide-evoked current.

Twelve vasopressin-sensitive facial neurons were recorded using micropipettes filled with KCl instead of K-acetate. Following impalement, spontaneous IPSPs were converted to depolarizing potentials, indicating that the transmembrane chloride gradient had been reversed. However, the polarity of the response to vasopressin, determined during either current- or voltage-clamp recordings, was similar to that observed when using K-acetate-filled micropipettes. Moreover, the effect of the peptide was not modified when 60% of external NaCl was replaced by an equimolar amount of Na-isethionate (tested on three neurons). This indicates that a chloride current does not contribute significantly to the vasopressin-induced response.

Figure 2. Concentration-dependent effect of vasopressin on a voltage-clamped facial neuron (*A*) and lack of effect of a change in extracellular calcium concentration on the vasopressin-induced response in an antidromically identified facial motoneuron (*B*). *A*, Voltage-clamp records of the membrane current activated by vasopressin at 1 μ M and 100, 10, and 1 nM, from top to bottom. The horizontal bar above each record indicates the time during which the peptide was added to the perfusion solution. The holding potential was -62 mV. *B*: Upper traces, Three superimposed current-clamp records, showing the neuronal response to pairs of shocks delivered to the genu of the facial nerve at 14-msec intervals. The stimulus artifacts are marked by asterisks. Note that, following the second stimulus in each pair, the evoked action potential dissociated into initial segment and somatodendritic components; due to refractoriness of the soma, the latter component was absent from some of the records. This indicates that action potentials were evoked by antidromic invasion of the neuronal soma. During these recordings, the preparation was perfused with the normal solution. Middle and lower traces, Voltage-clamp records from the same neuron, showing the current generated by 1 μ M vasopressin during perfusion with the normal solution and with a low-calcium, high-magnesium solution, respectively. Holding potential, -61 mV.

I_{AVP} could be an inward current whose reversal potential is positive to the resting membrane potential. In this case, however, in order to account for its voltage dependence (see Fig. 3A₂,B), I_{AVP} has to be voltage gated: Its activation would be weak, or negligible, at hyperpolarized potentials and would increase following depolarization. Because calcium ions do not contribute significantly to the response to vasopressin (see above),

Figure 3. Voltage dependence of the vasopressin-specific current. *A₁*, Superimposed current responses, evoked by 750-msec voltage command steps (bottom panel), and recorded in the absence (top panel), in the presence (second panel), and following washout of 1 μ M vasopressin (third panel). Note that the action of the peptide was accompanied by an apparent increase in membrane resistance and that this effect was more pronounced at depolarized voltage levels. The recordings were carried out while the preparation was perfused with a solution containing 1 μ M TTX, 10 mM TEA, and 1 mM 4-AP. The neuron was clamped at its resting membrane potential, -75 mV; the holding current in the presence of vasopressin attained -0.2 nA. *A₂*, *I/V* relations determined before (open squares), during (open circles), and after (open triangles) the peptide-induced effect. Each point represents the total current flowing through the membrane at the end of each of the 750-msec voltage steps shown in *A₁*. The vasopressin-specific current, I_{AVP} (solid circles), was obtained by subtracting the control *I/V* curve from the curve determined in the presence of the peptide. *B*, I_{AVP} from a sample of four further neurons, represented by solid squares, diamonds, circles, and triangles, respectively. The perfusion solutions contained 1 μ M TTX; in one case, 10 mM TEA was also added (solid triangles). Note, in *A₂* and *B*, that I_{AVP} was inward over the entire range of potentials tested and increased in magnitude as the membrane was depolarized; in one case, it apparently began to decrease in magnitude at potentials positive to -40 mV (*B*, solid diamonds).

I_{AVP} could be a sodium current. To test this hypothesis, we compared the peptide-induced response recorded in the physiological solution with that recorded in the presence of reduced extracellular sodium concentration. When 54% sodium was replaced by equimolar *N*-methyl-D-glucamine, or when 45% sodium was replaced by equimolar Tris, the vasopressin-induced peak current decreased reversibly by 40–50% (Fig. 5*A*; $n = 7$). Substitution of 72% sodium with equimolar amounts of either *N*-methyl-D-glucamine or Tris resulted in a 60–85% reduction of the response to vasopressin, recorded under either current- or voltage-clamp conditions (Fig. 5*B*; $n = 8$). The effectiveness of these ionic substitutions was certified by the fact that, during low-sodium perfusion, action potentials, evoked by antidromic invasion or by intracellular current injection, had a reduced amplitude or even disappeared. These data show that I_{AVP} is

sodium dependent. Because the reversal potential of this current is not known, they do not indicate whether it is a pure sodium current or a mixed sodium–potassium current. However, while a two- to three-fold reduction in the transmembrane sodium gradient greatly reduced the response to vasopressin, a more than two-fold reduction in the transmembrane potassium gradient did not affect it (see above). This suggests, though does not prove, that the current generated by this peptide is mainly carried by sodium ions.

Discussion

We have found that in facial motoneurons from the newborn rat the neuropeptide vasopressin can generate a sodium current that is voltage gated, noninactivating (or very slowly inactivating), and TTX resistant. To our knowledge, it is the first time

Figure 4. Persistence of the response to vasopressin in a cesium-loaded, antidromically identified facial motoneuron. *A*, Superimposed current-clamp records showing the response of the motoneuron to pairs of stimuli given to the genu of the facial nerve at 5-msec intervals (left) and at intervals of 4, 6, 8, and 10 msec (right). Asterisks mark the first stimulus artifact in each pair. Note the increased duration of the evoked action potentials, particularly visible on the second one in each pair, due to the cesium-induced blockade of the delayed rectifier. *B*, Voltage-clamp record of the membrane current generated in the same neuron by vasopressin at 1 μ M, 100 nM, and 10 nM, from top to bottom. The horizontal bar above each record indicates the time during which the peptide was added to the perfusion solution. Holding potential, -62 mV.

that such a current, which we have called I_{AVP} , is detected in a mammalian central neuron. Although we cannot exclude the possibility that vasopressin exerts other effects on facial motoneurons, the generation of I_{AVP} probably constitutes the main mechanism by which vasopressin, acting on V_1 -type receptors, increases the excitability of these neurons.

Besides being regulated by vasopressin, I_{AVP} is controlled by voltage. The peptide-induced enhancement in the membrane permeability to sodium is more pronounced at depolarized potentials than at the resting potential. Thus, though vasopressin causes an increase in the chord conductance, it induces a decrease in the slope conductance at or near the resting potential. This explains the apparent rise in membrane resistance brought about by the peptide (see Fig. 3), this resistance being defined, operationally, as the inverse of the slope conductance. The steady-state relation between I_{AVP} and voltage can be quantitatively simulated by assuming that the voltage dependence of its gating obeys the Boltzmann equation (Hodgkin and Huxley, 1952):

$$g = g_{\max} / \{1 + \exp [(V_0 - V)/V_s]\}, \quad (1)$$

where $g_{\max}$ is the maximal conductance; V_0 , the membrane potential at which the conductance is half-maximal; and V_s , the Boltzmann slope constant. Let us apply the simulation procedure to the data obtained for the neuron of Figure 3*A*. As a first step, the data of I_{AVP} for this cell (see Fig. 3*A*, solid circles) are used to calculate the corresponding chord-conductance values by the relation

$$g = I / (V - V_r), \quad (2)$$

where I is the membrane current, and V_r , its reversal potential, which is assumed to be 60 mV for I_{AVP} . The calculated chord-

Figure 5. Effects of changes in the extracellular sodium concentration on two facial neurons (*A* and *B*). *A*, Voltage-clamp records of the membrane current induced by 1 μ M vasopressin in the normal solution (trace 1), in a solution in which 54% of extracellular sodium was replaced by equimolar *N*-methyl-D-glucamine (trace 2), and 6 min after perfusing the preparation with the normal solution again (trace 3). The horizontal bar above each record indicates the time during which the peptide was added to the perfusion solution. Holding potential, -55 mV. *B*, Current-clamp records showing the membrane depolarization induced by 1 μ M vasopressin in the normal solution (top trace), following substitution of 72% extracellular sodium with equimolar *N*-methyl-D-glucamine (second trace), and 7 min (third trace) or 28 min (bottom trace) after reintroducing the normal perfusion solution. Resting membrane potential, -61 mV. Action potentials in the third and bottom traces are truncated due to the low-frequency response of the oscillograph. Note that, in both *A* and *B*, lowering the extracellular sodium concentration caused a reversible reduction in the amplitude of the vasopressin-induced response. Note also, in *B*, the reversible disappearance of spontaneous synaptic potentials during perfusion with the low-sodium solution.

conductance values are represented by the solid circles in Figure 6, top. (In assuming $V_r = 60$ mV, we have postulated, quite arbitrarily, that I_{AVP} is a pure or nearly pure sodium current. Provided it is much more positive than the resting membrane potential, the exact value of V_r does not affect the qualitative outcome of the simulation procedure. However, it influences the value of the parameters V_0 and V_s .) As a second step, the best-fit curve to the conductance values is generated using Equation 1, with $g_{\max} = 15$ nS, $V_0 = -51$ mV, and $V_s = 11$ mV. This curve is represented by the solid line in Figure 6, top. As a last step, the theoretical peptide-induced current is computed by solving Equation 2 with respect to I . This computed current is plotted in Figure 6, bottom (solid line), together with the experimental values of I_{AVP} (solid circles).

The result of the curve fitting suggests that the amplitude of I_{AVP} is regulated by the voltage-dependent gating of an ionic channel that can be in one of two states, open or closed. Because the slope factor V_s is given by kT/ez , where k is the Boltzmann constant; T , the absolute temperature; e , the elementary charge; and z , the gating charge for opening the channel, and because

Figure 6. Mathematical simulation of the vasopressin-induced conductance change (solid line, top) and membrane current (solid line, bottom) as a function of the membrane potential. The solid circles are data points obtained for the neuron of Figure 3A and represent the vasopressin-induced change in chord conductance (top) and the vasopressin-induced membrane current, I_{AVP} (bottom). The best-fit curves were computed by assuming that the voltage dependence of the vasopressin-sensitive conductance obeys the Boltzmann equation. For a detailed description of the calculations, see Discussion.

at 35°C, $kT/e = 26.5$ mV, by taking $V_s = 11$ mV (see above) one can estimate that the gating charge is equivalent to about 2.5 elementary charges. By virtue of its voltage dependence, I_{AVP} could play a role in amplifying, in a self-reinforcing manner, depolarizing signals induced by synaptic currents. Because of its persistence, I_{AVP} could also contribute to the generation of sustained depolarizing potentials and/or modulate the repetitive firing of facial motoneurons. More detailed biophysical and functional studies of the membrane properties of facial motoneurons are needed in order to test these conjectures.

An early attempt to characterize the mode of action of vasopressin was carried out in supraoptic neurons from the guinea pig (Abe et al., 1983). Vasopressin could depolarize these neurons, but contrary to what was found in the present work, this effect was independent of voltage and was not accompanied by any change in membrane resistance. It was not affected by changes in extracellular calcium, potassium, or chloride and was only slightly reduced following sodium depletion.

Noninactivating or slowly inactivating sodium-dependent

currents have been detected in some mammalian neurons, including cerebellar Purkinje cells, inferior olive neurons, neocortical neurons, and hippocampal pyramidal neurons (French et al., 1990; for a recent review, see Llinas, 1988). These currents, which are enhanced by membrane depolarization, are blocked by micromolar concentrations of TTX and do not require the presence of an agonist in order to be activated. They are thus different from the vasopressin-induced current characterized in the present work. TTX-resistant sodium currents have been recorded in denervated skeletal muscle cells (Harris and Thesleff, 1971; Paponne, 1980) as well as in muscle cells developing *in vitro* (Frelin et al., 1984; Gonoï et al., 1985; Weiss and Horn, 1986). Contrary to I_{AVP} , however, these currents inactivate rapidly, with time constants comparable to those of TTX-sensitive sodium currents in innervated adult muscle.

Sodium-dependent, TTX-resistant currents have been characterized in invertebrate neurons and, more recently, also in some mammalian central neurons. Thus, the egg-laying hormone (ELH) of *Aplysia* excites an identified motoneuron in this species by inducing a persistent inward current that is not affected by changes in extracellular calcium, potassium, or chloride concentrations, is resistant to potassium channel blockers, and is reduced, or suppressed, when extracellular sodium is lowered (Kirk and Scheller, 1986). This current has a negative slope between -40 and -25 mV and reverses at potentials ranging from -28 to $+46$ mV. It bears some striking similarities to I_{AVP} . The tetrapeptide FMRFamide and the vasopressin-related peptide oxytocin can elicit, in *Aplysia* and in the snail *Achatina fulica*, respectively, a slow inward, voltage-dependent current largely carried by sodium (Ichinose and McAdoo, 1988; Funase, 1990). At variance with both I_{AVP} and the ELH-induced current, these currents are modulated by extracellular calcium ions. Persistent, voltage-dependent sodium currents can also be activated by intracellular injection of cAMP or cGMP in neurons from several gastropod species (Green and Gillette, 1983; Connor and Hockberger, 1984). Interestingly, lysine vasopressin can induce a bursting activity in a neuron of the land snail *Otala* by eliciting a voltage-dependent, slowly inactivating inward current (Barker and Smith, 1976); however, the ionic nature of this current has not been determined. Recording from locus coeruleus neurons in rat brain slices, Wang and Aghajanian (1987) found that 8-Br-cAMP, a membrane-permeable analog of cAMP, and forskolin, an activator of adenylate cyclase, elicited a current that was inward over the potential range of -120 to -50 mV, was accompanied by a slight increase in conductance, was resistant to TTX and to calcium channel blockers, and was reduced by decreasing extracellular sodium concentration. In these same neurons, vasoactive intestinal peptide (VIP) generated a similar inward current (Wang and Aghajanian, 1990). The effects of cAMP-active agents and of VIP were nonadditive and were both attenuated by a specific protein inhibitor of cAMP-dependent protein kinase, suggesting that the VIP-induced inward current was mediated by cAMP.

Though the intracellular events associated with the electrophysiological effect of vasopressin on facial motoneurons have yet to be elucidated, the existence of points of similarity between I_{AVP} and cyclic-nucleotide-dependent slow sodium current raises the possibility that vasopressin may exert its neuronal action by activating a receptor-coupled adenylate cyclase. In accordance with this conjecture, Abe et al. (1983) reported that dibutyryl cAMP could mimic the depolarizing effect of vasopressin on guinea pig supraoptic neurons and that the content

of cAMP in hypothalamic slices increased following incubation with the peptide. These findings, however, have not been confirmed by other workers. By binding to V_1 -type receptors, vasopressin strongly activated the metabolism of inositol phospholipids without affecting cAMP concentration in superior cervical ganglia of the rat (Bone et al., 1984; Kiraly et al., 1986) and caused a moderate increase in phosphoinositide hydrolysis in rat hippocampus (Stephens and Logan, 1986), medulla oblongata (Moratalla et al., 1988), and septum (Shewey and Dorsa, 1988; Lebrun et al., 1990). Thus, most of the existing evidence supports the notion that the interaction of agonist with V_1 vasopressin receptors present in the nervous system leads to the activation of a phospholipase C, as is the case in the periphery (Jard, 1983). One should keep in mind, however, that vasopressin could indirectly influence cAMP metabolism. Thus, vasopressin facilitated the stimulation of adenylate cyclase activity by dopamine in the rat caudate nucleus but had no effect per se on the activity of this enzyme (Courtney and Raskind, 1983). In pituitary corticotrophs, vasopressin triggered an increase in cytosolic calcium and stimulated phosphoinositide turnover, yet it potentiated the cAMP production induced by corticotropin-releasing factor (CRF). This facilitation has been attributed to the fact that the vasopressin-activated protein kinase C probably suppresses the activity of a guanine-nucleotide-binding protein, which in turn exerts an inhibitory regulatory action upon the CRF-dependent adenylate cyclase complex (Leong, 1988; Carvallo and Aguilera, 1989).

Various effects of vasopressin on membrane currents of non-neuronal cells have been recently described. The vasopressin-induced stimulation of adrenocorticotropin (ACTH) from ACTH-secreting pituitary adenoma cells was accompanied by an increase in amplitude and duration of the calcium-dependent action potential fired by these cells (Mollard et al., 1988). This effect was probably due to a selective enhancement of a nifedipine-sensitive, slowly inactivating (L-type) calcium current component. Vasopressin could stimulate insulin-secreting cells by inducing a membrane depolarization and by generating calcium spikes and a rise in intracellular calcium concentration (Martin et al., 1989). These actions took place via a peptide-evoked closure of ATP-sensitive potassium channels that, in these cells, are largely responsible for the maintenance of the resting membrane potential. The effects of vasopressin on both ACTH- and insulin-secreting cells are at variance with those described in the present work, because no significant contribution either of calcium currents or of potassium currents—and in particular, of ATP-sensitive potassium currents—to I_{AVP} could be evidenced. Cultured aortic smooth-muscle cells have been shown to respond to vasopressin in a biphasic way, in which a transient hyperpolarization was followed by a sustained depolarization. The initial hyperpolarization was caused by the activation of a calcium-sensitive potassium conductance and by the inhibition of an L-type calcium conductance (Van Renterghem et al., 1988; Korbmacher et al., 1989). The late depolarization was associated with the opening of a channel permeable to sodium, calcium, and potassium. The inward current flowing through this nonselective cationic channel reversed at 5 mV, was not affected by changes in intracellular concentration of calcium or inositol 1,4,5-triphosphate, was not suppressed by the blockade of potassium channels, and was independent of the transmembrane chloride gradient (Van Renterghem et al., 1988). Although its voltage dependence is not known, and apart from the fact that it can in part be carried by calcium ions, this

current bears some resemblance with the vasopressin-induced current characterized in the present work.

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