Journal of Physiology (1992), **457**, *pp.* 131–142
With 5 figures
Printed in Great Britain

MECHANISM OF ACTION OF OXYTOCIN IN RAT VAGAL NEURONES: INDUCTION OF A SUSTAINED SODIUM-DEPENDENT CURRENT

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(*Received 10 December 1991*)

SUMMARY

1. The mechanism of action of oxytocin on vagal neurones of the rat was studied using single-electrode voltage-clamp recordings from brainstem slices. The ionic basis of the oxytocin-induced current was examined by changing the composition of the perfusion solution and by making use of channel blockers.

2. In neurones clamped at or near their resting potential, oxytocin generated a sustained, TTX-insensitive inward current whose peak amplitude was concentration related. This current was detectable at 10 nM, was half-maximal at about 100 nM and was maximal at micromolar concentrations of peptide.

3. The oxytocin current was inward over membrane potentials ranging from −110 to −20 mV and was voltage dependent, since it increased in magnitude as the membrane was depolarized from the resting potential toward less negative potentials.

4. Partial replacement of extracellular sodium by equimolar *N*-methyl-D-glucamine reversibly attenuated or suppressed the oxytocin current. By contrast, substituting part of extracellular chloride or blocking calcium currents did not modify it. Increasing the transmembrane potassium gradient was also without effect and none of the potassium channel blockers TEA, 4-amino pyridine (4-AP), apamin, caesium or barium affected the oxytocin current. This current is thus at least in part carried by sodium.

5. The activation of the oxytocin current as a function of the membrane potential could be quantitatively simulated using a Boltzmann equation, suggesting that oxytocin acts by inducing the opening of a voltage-dependent channel which can exist in either of two states, open or closed.

6. Lowering the extracellular calcium concentration from 2 to 0·1 mM, while keeping the magnesium concentration constant at 1 mM, enhanced the response to oxytocin. This low calcium-induced potentiation of the oxytocin current was 1·4–3-fold and was reversible.

7. We conclude that oxytocin increases the excitability of vagal neurones by generating a persistent, voltage-gated current which is sodium dependent, is insensitive to TTX and is modulated by divalent cations.

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INTRODUCTION

The nonapeptide oxytocin is a candidate neurotransmitter in the dorsal vagal complex of the medulla oblongata in rats. This region, which comprises the dorsal motor nucleus of the vagus nerve, the nucleus of the solitary tract and the area postrema, was shown to contain oxytocin-immunoreactive axons, arising from the paraventricular nucleus of the hypothalamus (Sawchenko & Swanson, 1982; Sofroniew, 1985). Axon terminals immunoreactive for oxytocin could be seen, at the electron microscopic level, both in the dorsal vagal nucleus and in the nucleus of the solitary tract (Voorn & Buijs, 1983; Siaud, Denoroy, Assenmacher & Alonso, 1989). In addition, high-affinity oxytocin binding sites were detected by autoradiography in the dorsal vagal nucleus (Dreifuss, Raggenbass, Charpak, Dubois-Dauphin & Tribollet, 1988; Elands, Beetsma, Barberis & De Kloet, 1988; Tribollet, Charpak, Schmidt, Dubois-Dauphin & Dreifuss, 1989). Some of these sites are located on vagal preganglionic motoneurones, since they became undetectable following ipsilateral vagotomy (Dubois-Dauphin, Raggenbass, Widmer, Tribollet & Dreifuss, 1992). These data suggest that the dorsal vagal nucleus is the target of a descending oxytocinergic pathway.

Other evidence supports the concept of a neurotransmitter role for oxytocin. Local microinjection of this peptide activated a majority of gastric-related vagal or solitary tract neurones *in situ* (McCann & Rogers, 1990). In brainstem slices, oxytocin could reversibly excite neurones in the dorsal vagal nucleus (Charpak, Armstrong, Mühlethaler & Dreifuss, 1984). The peptide acted by causing a direct, concentration-related membrane depolarization (Raggenbass, Dubois-Dauphin, Charpak & Dreifuss, 1987; Tribollet *et al.* 1989). This action was specific, since it could be mimicked by a selective oxytocin agonist (Charpak *et al.* 1984) and could be suppressed by a selective oxytocin antagonist (Dreifuss *et al.* 1988).

The morphological and electrophysiological studies cited above indicate that vagal motoneurones, or at least part of them, are endowed with functional oxytocin receptors whose activation causes an increase in neuronal excitability. In the present work we have studied the membrane mechanism of the oxytocin action. Using brainstem slices of young rats we have carried out single-electrode voltage-clamp recordings from vagal motoneurones. The ionic basis of the oxytocin-induced current was investigated by means of changes in the composition of the extracellular medium and by making use of a variety of channel blockers.

METHODS

Preparation

The animals used were 3- to 4-week-old male rats from the Sivz strain, a Sprague–Dawley-derived strain. Following stunning and decapitation, the brain was removed and the brainstem dissected out. Two to three coronal or parasagittal slices were cut on a vibrating microtome (Campden Instruments, London); they were 300–400 μm thick and contained the dorsal vagal complex. The slices were transferred to a thermoregulated (34–35 °C) recording chamber and incubated at the interface between an oxygenated humidified atmosphere and a perfusion solution, whose composition, unless otherwise stated, was the following (mM): 135 NaCl, 5 KCl, 15 $NaHCO_3$, 1 $MgSO_4$, 1·25 NaH_2PO_4, 2 $CaCl_2$ and 10 glucose. This solution was saturated with 95 % O_2–5 % CO_2 and had a pH of 7·35–7·45. In some experiments, sodium was partially replaced with *N*-methyl-D-glucamine (titrated to pH 7·4 with HCl) and chloride with sodium isethionate. Transmembrane

calcium currents were reduced by substituting the normal perfusion solution with a low calcium–high magnesium solution, which contained 0·1 mM calcium and 16 mM magnesium. The effect of lowering extracellular calcium on the oxytocin response was studied by perfusing the preparation with a solution containing 0·1 mM calcium and 1 mM magnesium. When barium was added to the perfusion solution, phosphates were omitted and $MgSO_4$ was replaced by $MgCl_2$.

Voltage-clamp recordings

Neurones were impaled with borosilicate micropipettes filled with 3 M potassium acetate (pH 7·4) and having tip DC resistances of 40–80 MΩ. The time constant of the micropipettes was 200–300 μs, whereas the membrane time constant of the impaled neurones was 15–35 ms. Single-electrode voltage-clamp recordings were carried out using an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA, USA). The switching frequency was 2–3 kHz and the headstage was continuously monitored. Membrane current and voltage were plotted on paper and stored on magnetic tape. In some cases, data were digitized on line using a home-made A/D converter and transferred to the hard disk of an IBM/AT-compatible personal computer.

Chemical compounds

Oxytocin was from Bachem (Bubendorf, Switzerland) or from Novabiochem (Läufelfingen, Switzerland). It was dissolved in the perfusion solution. Tetrodotoxin (TTX) and apamine were purchased from Sigma (St Louis, MO, USA) and tetraethylammonium bromide (TEA), 4-aminopyridine (4-AP), sodium isethionate and *N*-methyl-D-glucamine were from Fluka (Buchs, Switzerland).

RESULTS

Stable intracellular recordings were obtained from seventy-three oxytocin-sensitive vagal neurones, in brainstem slices from sixty animals. These neurones had resting membrane potentials in excess of −50 mV and input resistances of 40–300 MΩ (average, 151 ± 11 MΩ; mean ± S.E.M., $n = 28$). When voltage clamped at or near their resting membrane potential, they responded to oxytocin by generating an inward current whose peak amplitude was 11 ± 1 pA at 10 nM, 51 ± 6 pA at 100 nM and 103 ± 9 pA at 1 μM (mean ± S.E.M.; $n = 6$; Fig. 1). Raising the oxytocin concentration from 1 to 2 μM did not cause any further increase in the amplitude of the oxytocin current.

Steady-state current–voltage (*I–V*) curves were constructed, in the presence of tetrodotoxin (TTX) at 0·5–2 μM, by delivering to a voltage-clamped neurone 0·5–1·0 s-long voltage steps of varying amplitude and by measuring the current flowing through the membrane at the end of each step (Fig. 2*A*). The net current generated by oxytocin was computed by subtracting the control *I–V* curve from the *I–V* curve obtained in the presence of the peptide (Fig. 2*B*). In ten out of ten neurones, the oxytocin current was negative, i.e. inward, at all potentials tested (from −110 to −20 mV). No significant inactivation could be detected over the duration of the voltage steps. In six neurones, this current varied as a function of the membrane potential: its amplitude increased as the neuronal membrane was depolarized. In the neurone shown in Fig. 2*B*, for example, oxytocin generated an inward current of 40 pA at −106 mV and of 60 pA at −56 mV; when the membrane was depolarized from −56 to −26 mV, however, this current increased to 240 pA. The non-linearity of the oxytocin current suggests that, whatever its ionic composition, it is voltage dependent. A possible explanation of the fact that this voltage dependence could not be evidenced in all neurones is given in the Discussion. We found no correlation between the input resistance of the recorded cells and the voltage dependence of the oxytocin current.

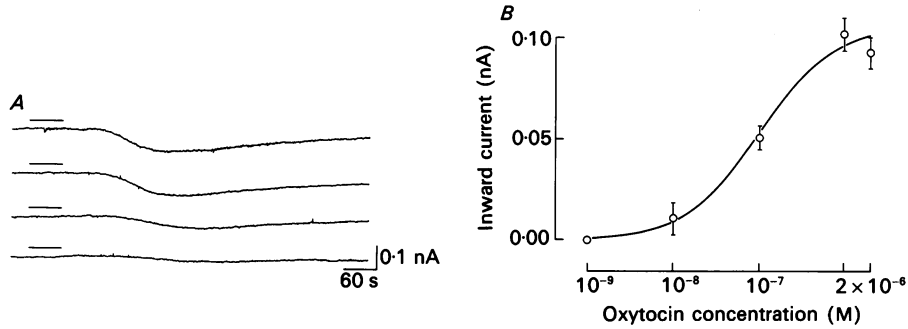

Fig. 1. Concentration dependence of the action of oxytocin on voltage-clamped vagal neurones. *A*, inward currents generated by oxytocin at 2000, 1000, 100 and 10 nM, from top to bottom. The peptide was added to the perfusion solution for the 1-min period represented by the horizontal bar above each trace. Note that at 10 nM, the evoked inward current was just detectable, whereas at 1 and 2 μM the currents were almost superimposable. Holding potential, −60 mV. *B*, concentration–response relationship obtained by pooling the data from eight neurones. Each point represents the average induced peak current; the vertical bars are S.E.M. ($n = 4$ for 2 μM; $n = 6$ for 1 μM, 100 nM and 10 nM oxytocin). The line is the best-fit curve, computed by assuming a Michaelis–Menten relationship between the peptide concentration and the induced peak current.

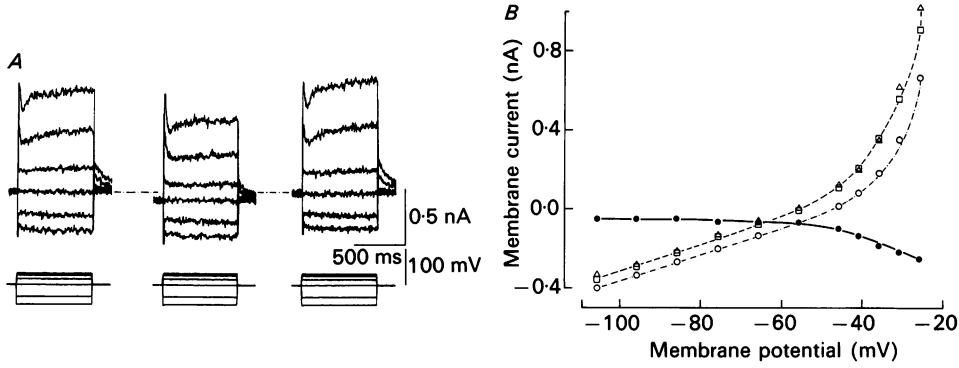

Fig. 2. Voltage dependence of the oxytocin current in a voltage-clamped vagal neurone. *A*, superimposed current traces, and the corresponding voltage steps, recorded in the control solution (left panel), in the presence of 1 μM oxytocin (middle panel) and in the control solution again (right panel). The holding potential was −56 mV; the holding current during the oxytocin test was −60 pA. TTX at 0·5 μM was present throughout the whole experiment. *B*, *I–V* curves measured in the control solution (□), in the presence of oxytocin (○) and in the control solution again (△). In each case, the total membrane current was measured at the end of each voltage step. The oxytocin current (●) was computed by subtracting the control *I–V* curve from the test *I–V* curve. Note that the oxytocin current was negative, i.e. inward, at each potential and that it increased in amplitude at depolarized potentials.

Ionic basis of the oxytocin-induced current

This was investigated by modifying the ionic composition of the perfusion solution. In six neurones, substitution of 72% of extracellular sodium by equimolar amounts of *N*-methyl-D-glucamine resulted in a 55–80% decrease in the oxytocin-

evoked current, this decrease being partially reversible (Fig. 3*A*). In two other neurones, replacement of 90% sodium by equimolar *N*-methyl-D-glucamine caused a decrease in the oxytocin current of 80 and 100%, respectively. This suggests that the oxytocin current is carried, at least in part, by sodium ions.

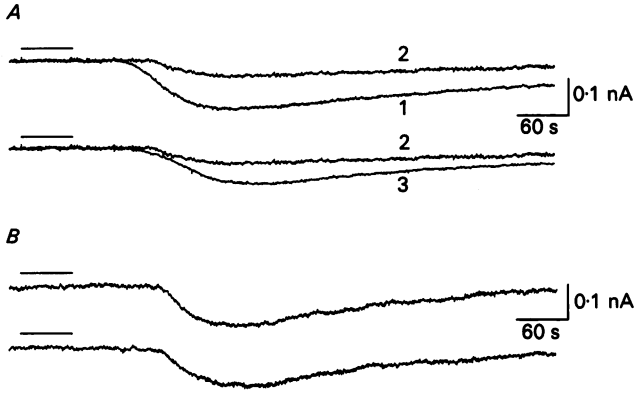

Fig. 3. Effects of partial sodium substitution and of a change in potassium concentration on the response to oxytocin in two voltage-clamped vagal neurones, *A* and *B*. *A*, current records showing the response to oxytocin at 1 μM in normal solution (trace 1), following substitution of 72% of sodium by equimolar *N*-methyl-D-glucamine (trace 2) and in the normal solution again (trace 3). Note that partial replacement of sodium caused a 65% decrease in the amplitude of the oxytocin current and that this effect was reversible. Holding potential, −60 mV. *B*, inward current evoked by oxytocin at 1 μM in normal solution, which contained 5 mM potassium (top trace), and in a solution containing 15 mM potassium (bottom trace). Note that in spite of a 3-fold decrease in the transmembrane potassium gradient, the oxytocin-induced current was unmodified. Holding potential, −52 mV.

A 3-fold increase in the extracellular potassium concentration – which, according to the Nernst equation, should displace the potassium equilibrium potential by 29 mV (at 35 °C) in the depolarizing direction – did not modify either the amplitude or the time course of the oxytocin-induced current in three neurones clamped at their resting membrane potential (Fig. 3*B*). In addition, the response to oxytocin persisted unchanged, or almost unchanged, in the presence of the following potassium channel blockers: TEA at 10 mM ($n = 4$); 4-AP at 1 mM ($n = 4$); barium at 1 mM ($n = 2$); caesium at 5 mM ($n = 2$) and apamin at 50 nM ($n = 2$). Thus, though it may contribute to the oxytocin current, potassium is probably not its major carrier.

Substitution of the normal solution, which contains 2 mM calcium and 1 mM magnesium, with a solution containing 0·1 mM calcium and 16 mM magnesium did not affect, to any significant extent, the oxytocin-induced response (tested on five neurones). Since in the low calcium–high magnesium solution synaptic transmission was blocked (Raggenbass *et al.* 1987), transmembrane calcium currents were greatly reduced or suppressed. We conclude that calcium ions do not play a major role in carrying the oxytocin current.

Chloride ions also probably did not contribute significantly to the oxytocin

current. Indeed, the response to the peptide persisted unchanged when 60 or 80% of sodium chloride in the perfusion solution was substituted with equimolar amounts of sodium isethionate ($n = 3$).

Simulation of the oxytocin current

As stated above, the non-linear behaviour of the oxytocin current as a function of the membrane potential suggests that this current is due to the activation of a

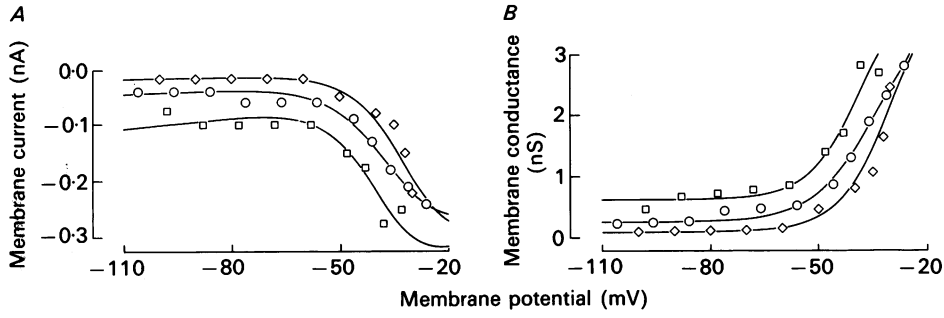

Fig. 4. Simulation of the oxytocin-induced current (*A*) and of the oxytocin-sensitive conductance (*B*) as functions of the membrane potential. Open symbols represent data obtained in three vagal neurones, among which is the neurone of Fig. 2 (○). The continuous lines are best-fit curves computed by assuming that the voltage-dependent activation of the oxytocin-sensitive conductance obeyed Boltzmann principles as explained in the text.

voltage-gated conductance. In order to determine whether this voltage-dependent activation was compatible with Boltzmann principles (Hodgkin & Huxley, 1952), we attempted to simulate quantitatively the voltage clamp data gathered from four neurones. The equations used were the following:

$$I_{OT} = g_{OT}(V - V_{OT}) \tag{1}$$

where I_{OT} is the oxytocin current, g_{OT} is the oxytocin-sensitive conductance, V is the membrane potential and V_{OT} is the reversal potential of the oxytocin current; and

$$g_{OT} = (1-a)\,g_{OT,max} + a g_{OT,max}\{1 + \exp[(V_0 - V)/V_s]\} \tag{2}$$

where $g_{OT,max}$ is the maximal oxytocin-sensitive conductance, V_0 is the membrane potential corresponding to a half-maximal activation and V_s is the slope factor. V_s is given by kT/ez, k being the Boltzmann constant, T the absolute temperature, e the elementary charge and z the gating charge. In writing eqn (2), we have defined g_{OT} as the sum of two components, one which is dependent on voltage and one which is not; a is the voltage-dependent fraction of g_{OT}.

The data for I_{OT}, as a function of voltage, are represented in Fig. 4*A* (open symbols; for the sake of clarity, only the data obtained from three neurones are shown). The corresponding values of g_{OT} were computed from eqn (1) and are shown in Fig. 4*B* (open symbols); it was assumed that $V_{OT} = 60$ mV, i.e. that the oxytocin current was exclusively, or almost exclusively, a sodium current. The results of the

simulation of g_{OT} are shown in Fig. 4*B*. The best-fit curves were computed using eqn (2). The average values for the parameters were the following: $g_{OT,max} = 4{\cdot}3 \pm 0{\cdot}3$ nS; $V_0 = -32 \pm 3$ mV; $V_s = 8 \pm 1$ mV; and $a = 0{\cdot}92 \pm 0{\cdot}03$ (mean ± S.E.M.; $n = 4$). The curves simulating I_{OT} were calculated from the best-fit curves for g_{OT} using eqn (1)

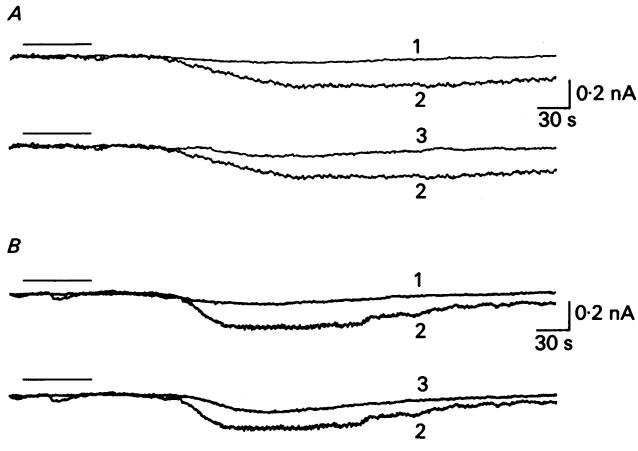

Fig. 5. Effect of lowering extracellular calcium concentration on the current generated by oxytocin in two vagal neurones, *A* and *B*. For each neurone, the superimposed traces 1 and 2 are voltage-clamp records of the oxytocin current obtained, respectively, in the normal solution (which contained 2 mM calcium and 1 mM magnesium) and in a low-calcium solution (which contained 0·1 mM calcium, the magnesium concentration being kept constant at 1 mM). The superimposed traces 2 and 3 represent, respectively, the oxytocin current in the low-calcium solution and following perfusion with the normal solution again. The oxytocin concentration was 1 μM in each experiment. Holding potentials were −59 mV for neurone *A* and −79 mV for neurone *B*. Note that, in both cases, in the low-calcium solution the amplitude of the oxytocin current increased markedly.

and are represented in Fig. 4*A*. The fact that the simulation procedure based on Boltzmann principles was successful suggests that oxytocin acts by inducing the opening of a voltage-dependent channel which has two states of conductance, open and closed, and whose gating charge $z = kT/eV_s$ is equivalent to three to four elementary charges.

The simulation was repeated under the assumption that the oxytocin-induced current was a mixed sodium–potassium current, having a reversal potential at 0 mV. The simulation was successful and gave the following values for the parameters: $g_{OT,max} = 19 \pm 3{\cdot}9$ nS; $V_0 = -28 \pm 4$ mV; $V_s = 7{\cdot}4 \pm 0{\cdot}9$ mV; and $a = 0{\cdot}94 \pm 0{\cdot}02$ (mean ± S.E.M.; $n = 4$). One can see that with respect to the case in which $V_{OT} = 60$ mV, the value of $g_{OT,max}$ increased by a factor of 4·5. This was expected, since the decrease in the driving force due to the reduction in V_{OT} had to be compensated by an increase in the oxytocin-sensitive conductance. By contrast, the parameters V_0, V_s and a were only slightly modified and the value of the estimated gating charge was not affected significantly.

It is worth noting that in order to fit the experimental data quantitatively, the

value of a had to be slightly less than 1. This is equivalent to assuming that, whereas a major fraction of g_{OT} was voltage sensitive, a minor fraction was not. The possible significance of this fact will be examined in the Discussion.

Effect of divalent cations on the oxytocin current

As stated above, the oxytocin-induced response persisted unmodified in the presence of a low calcium–high magnesium solution. However, when the extracellular calcium concentration was lowered (from 2 to 0·1 mM), while the magnesium concentration was kept constant (at 1 mM), the response to oxytocin was enhanced in nine out of ten neurones (Fig. 5). This low calcium-induced potentiation of the oxytocin current was 1·4- to 3-fold and could be reversed by perfusing the preparation with the normal solution again. This was probably not due to an indirect decrease in intracellular free calcium concentration because a similar decrease would have been brought about also following perfusion with a low calcium–high magnesium solution, i.e. containing 0·1 mM calcium and 16 mM magnesium. In this instance, however, no potentiation of the oxytocin effect was observed (see above). These results not only indicate that the oxytocin-induced current is modulated by extracellular calcium but also add further support to the notion that calcium ions do not themselves carry a significant part of the oxytocin current.

DISCUSSION

We have found that in vagal neurones of the rat, oxytocin generates a persistent, sodium-dependent current, which is voltage gated, TTX resistant and modulated by divalent cations. At least some of the neurones in which this current could be elicited were probably parasympathetic preganglionic motoneurones. In previous studies, indeed, some of the oxytocin-responsive vagal neurones could be identified as being preganglionic motoneurones either using morphological criteria (Raggenbass *et al.* 1987) or by antidromic invasion following electrical stimulation of vagal efferent axons (Dubois-Dauphin *et al.* 1992). In addition, vagal motoneurones can be recognized on the basis of their electrophysiological properties (Yarom, Sugimori & Llinas, 1985) and can be readily differentiated from adjacent hypoglossal motoneurones (Mosfeldt Laursen & Rekling, 1989). Solitary tract neurones, which are located dorsally to the vagal nucleus, bear V_1-type vasopressin receptors; they are sensitive to vasopressin but do not respond, or respond poorly, to oxytocin (Raggenbass, Tribollet, Dubois-Dauphin & Dreifuss, 1989).

The ion substitution experiments indicate that the current generated by oxytocin is, at least in part, carried by sodium and suggest that it reverses in polarity at positive values of the membrane potential. Other ions, in particular potassium ions, may also contribute to the oxytocin current. However, a quantitative estimation of their contribution can only be obtained by studying the effect of ion substitutions on the reversal potential of this current. In the present study, attempts to approach the reversal potential of the oxytocin current were hindered by the limited current-carrying properties of the intracellular microelectrodes.

To our knowledge, no data concerning the mechanism of action of oxytocin on mammalian neurones have been published to date. However, two recent studies have

dealt with the membrane effects of oxytocin in molluscan neurones. Oxytocin could either depolarize or hyperpolarize distinct classes of neurones in the snail *Helix pomatia* (Osipenko & Bogomoletz, 1989). The peptide-induced depolarization was associated with an increase in membrane conductance and was due to an inward current carried by chloride. The hyperpolarization was caused either by the blockade of a chloride current or by the activation of an outward potassium current. In identified neurones of the snail *Achatina fulica*, oxytocin induced a voltage-dependent, TTX-resistant sodium current (Funase, 1990). This effect was enhanced by a phosphodiesterase inhibitor, was attenuated by inhibitors of cAMP-dependent protein kinase and could be mimicked by intracellular injection of cAMP. This indicates that the oxytocin-induced changes in membrane properties were mediated by cAMP-dependent protein phosphorylation. Interestingly, the ionic composition of this cAMP-stimulated current bears some similarity to that of the current characterized in the present work.

We found that the amplitude of the oxytocin-induced current was increased when the extracellular calcium concentration was lowered. This indicates that in the normal physiological solution, which contains 2 mM calcium, this current is partially suppressed. The low calcium-induced potentiation of the oxytocin current took place provided the magnesium concentration was kept at 1 mM. Lowering external calcium while concomitantly increasing the magnesium concentration did not affect the amplitude of the oxytocin current (see Results). Therefore, this current is probably modulated by divalent cations rather than by calcium *per se*. Further studies are needed in order to determine the relative efficiency of different divalent cations in suppressing the oxytocin current and to establish whether the calcium-induced partial suppression of this current is dependent on voltage. Modulation by calcium of persistent sodium currents has been described in several systems. In molluscan neurones, slow inward currents could be generated either following application of a peptide, FMRFamide (Phe-Met-Arg-Phe-NH_2; Ichinose & McAdoo, 1988), or of dopamine (Matsumoto, Sasaki, Sato, Shozushima & Takashima, 1988) or by intracellular injection of cAMP (Gillette & Green, 1987; Kehoe, 1990). They were all carried by sodium, were TTX resistant and voltage dependent and were enhanced in low external calcium. In the rat, a sodium current could be evoked in non-myelinated fibres of the rat vagus nerve by activation of protein kinase C (Rang & Ritchie, 1988). This current, which was sustained and distinct from the current responsible for the rising phase of the action potential, was potentiated in a calcium-free perfusion solution.

The oxytocin current varied as a function of the membrane potential according to the Boltzmann relation. In order to fit quantitatively the behaviour of this current as a function of the membrane potential we had to assume that whereas a major fraction of the oxytocin-sensitive conductance was voltage dependent, a small fraction was not (see eqn (2) of the Results). For the four neurones used in the simulation procedure, the average amount of the oxytocin-sensitive conductance which was assumed to be independent of voltage was 5–10%. One possible interpretation of this fact is that the oxytocin-induced increase in sodium permeability is due to the opening of two distinct classes of channels: one which is dependent of voltage and one which is not. An alternative and more likely

explanation, however, is that our data are influenced by the imperfect space clamp conditions achieved in single-electrode voltage-clamp recordings (Johnston & Brown, 1983).

From the theory of finite linear cables, a steady-state voltage $V(0)$ applied at the soma of a neurone is attenuated along a dendrite according to the equation:

$$V(x) = V(0)\cosh[(l-x)/\lambda]/\cosh(l/\lambda) \quad (3)$$

where $V(x)$ is the voltage measured at a distance x from the soma, l is the dendritic length and λ is the length constant (Jack, Noble & Tsien, 1975). Let us define $L = l/\lambda$ as being the electrotonic length of the dendritic tree. Then, eqn (3) predicts that at the distal end of a dendrite for which $L = 1$ the voltage will be reduced to 65% of $V(0)$ and at the end of a dendrite with $L = 2$ the residual voltage will be 26%. For cat spinal motoneurones, L has been estimated as being between 1 and 2 (for a review, see Rall, 1977) and in a recent study, values of L ranging from 0·9 to 1·9 have been proposed for vagal neurones of the guinea-pig (Nitzan, Segev & Yarom, 1990). This would mean that in establishing I–V curves (using, for example, the procedure shown in Fig. 2), a voltage step of 40 mV applied at the soma would result in a voltage change of 25 mV at the end of the shortest dendrite and of 10 mV at the end of the longest dendrites. If we conjecture that part of the oxytocin-sensitive conductance is located distally along dendrites, such voltage changes may not be sufficient to alter appreciably the magnitude of the oxytocin current evoked at these sites. This distally located fraction of the oxytocin-sensitive conductance would thus appear as being independent, or nearly independent, of voltage.

As stated in the Results section, no significant voltage dependence of the oxytocin current could be detected in four out of ten vagal neurones. This suggests that in these neurones *most* of the sodium permeable channels opened by oxytocin were located on distal dendrites. Morphological studies, in which oxytocin-containing axon terminals were found to establish synaptic contacts with dendrites in the dorsal medulla of rats and guinea-pigs, support the notion that oxytocin may exert at least part of its effect by acting on receptors located on dendrites (Voorn & Buijs, 1983; Siaud *et al.* 1989). In order to confirm this notion, however, quantitative morphological data on the distribution of oxytocin receptors and/or of oxytocin-sensitive channels on the cell surface are needed.

The voltage dependence of the oxytocin current implies that the action of this peptide on vagal neurones is self-reinforcing. At or near the resting potential, the oxytocin current is only partially activated. This causes a depolarization, which in turn will activate more oxytocin current. As a consequence, the depolarization will increase. This oxytocin-induced depolarization would tend to displace the membrane potential towards the reversal potential of the oxytocin current, i.e. toward positive values. An excessive depolarization, however, will be prevented by the voltage-dependent activation of powerful outward currents, as evidenced by the outward-going rectifying properties of vagal neurone membranes (see Fig. 2*A*). These currents will counteract the effect of the oxytocin current and will tend to keep the neurone at a moderately depolarized, relatively stable membrane potential, at which it will fire at a regular rate (see current-clamp recordings of vagal neurones in the presence of oxytocin in Raggenbass *et al.* 1987 and Tribollet *et al.* 1989).

Recent work from our laboratory has shown that the facial nucleus of newborn rats is rich in high-affinity vasopressin binding sites, part of which are functional neuronal receptors of the V_1 type (Tribollet, Goumaz, Raggenbass, Dubois-Dauphin & Dreifuss, 1991). In antidromically identified facial motoneurones, vasopressin generated a concentration-dependent inward current which was persistent, voltage gated and TTX resistant (Raggenbass, Goumaz, Sermasi, Tribollet & Dreifuss, 1991). This current could be reduced or suppressed by partial substitution of extracellular sodium by *N*-methyl-D-glucamine or Tris. By contrast, it was not affected by reduction of calcium currents or by partial substitution of extracellular chloride. It was not modified by an increase in the transmembrane potassium gradient, nor was it suppressed by potassium channel blockers. The striking similarities between the oxytocin current characterized in the present work and the vasopressin-induced current in facial motoneurones suggest that although acting on distinct receptors, oxytocin and vasopressin can affect the bioelectrical properties of neuronal membranes by activating similar, or identical, sodium-permeable channels. What is not yet known is the second messenger which couples the receptors for these neuropeptides to the membrane channels and whether or not this coupling is the same for oxytocin and V_1-type vasopressin receptors.

This work was supported in part by the Swiss National Science Foundation (grant 31.28624.90). We thank Dr Hélène Widmer for help in the low-calcium experiments and Ms D. Machard and A. Cergneux for excellent technical and secretarial assistance.

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