Biochimica et Biophysica Acta, 648 (1981) 267–274
Elsevier/North-Holland Biomedical Press

BBA 79434

CELLULAR pH AND WATER PERMEABILITY CONTROL IN FROG URINARY BLADDER

A POSSIBLE ACTION ON THE WATER PATHWAY

MARIO PARISI, RICARDO MONTOREANO, JACQUES CHEVALIER and JACQUES BOURGUET

Département de Biologie, C.E.N. de SACLAY, 91191 Gif-sur-Yvette Cedex (France)

(Received March 19th, 1981)

Key words: Antidiuretic hormone; Water permeability; pH; Temperature; 8-Bromo-cyclic AMP; (Frog bladder)

Mucosal acidification (from pH 8.1 to 6.0) reversibly inhibited the hydroosmotic responses to oxytocin, cyclic AMP and 8-bromo-cyclic AMP in frog urinary bladder. These inhibitory effects were only observed in the presence of a permeant buffer in the apical medium and could also be elicited by CO_2 bubbling, even when the mucosal pH was clamped at 8.1. Acid pH reduced the oxytocin-induced net water flux faster than norepinephrine or oxytocin removal and the difference was especially important at low temperature. The time course of recovery from acid pH inhibition was, at 20°C, similar to that of the hormonal action, but when the medium temperature was reduced to 6–7°C, the recovery from acid pH inhibition paradoxically became faster while the oxytocin action was markedly slowed down ($t_{1/2}$ of changes in net water fluxes (expressed in min): oxytocin addition at 20°C, 6.2 ± 0.9; at 6°C, 24 ± 3; oxytocin removal at 20°C, 4.7 ± 0.8; at 6°C, 22 ± 3; pH inhibition at 20°C, 2.6 ± 0.2; at 6°C 2.5 ± 0.2; recovery from pH 6 at 20°C, 6.5 ± 0.9; at 6°C, 2.7 ± 0.3). These results can be explained by accepting two main loci sensitive to medium acidification: (1) the cyclase system and (2) an intracellular, temperature-independent, post-cyclic AMP site. The fact that the intramembranous particle aggregates associated with the oxytocin-induced water permeability increase did not disappear after the flow inhibition by acid pH at low temperature suggests that the second effect could be located at the water channel itself.

Introduction

Recent studies have cast new light on the mechanism regulating water permeability in 'renal like' epithelial barriers. Progress came mainly from freeze-fracture studies showing that antidiuretic hormone (ADH) and related agents induce the appearance of intramembranous particle aggregates in the apical border of epithelial cells [1–4]. It has been proposed that these aggregates contain water channels which are at the origin of the observed increase in water permeability.

Different results suggest that the water permeability increase induced by ADH can be modulated at a post-cyclic AMP step: Cellular tonicity [5], cytosolic calcium concentration [6,7] and cellular pH [8,9] seem to play a role after cyclic AMP production but no clear evidence exists on the subcellular target of these agents. Mucosal acidification, at 20°C, inhibits the hydroosmotic response to oxytocin, serosal hypertonicity and cyclic AMP in frog urinary bladder and these inhibitory actions are accompanied by the disappearance of the intramembranous particle aggregates [9]. However, we have recently observed that the aggregates are still present when the ADH-induced water flux is inhibited by medium acidification at 6–7°C [10]. In an attempt to clarify the cellular loci of these actions, we present here a time-course study on the effects of medium pH at different temperatures. Results indicate that cellular pH controls one of the last steps in the chain of events started by the normal action, perhaps the water pathway itself.

0005-2736/81/0000–0000/$02.50

Methods

Frogs (*Rana esculenta*) originating from Central Europe were kept at 20°C in running tap water for at least 5 days before the experiments. The bladders were removed from pithed frogs and mounted horizontally between two lucite chambers, either with the mucosal or serosal border facing the upper solution (Fig. 1). The net water flux was measured with a modification of a previously described technique [11]: water was automatically withdrawn from or injected into the lower chamber to maintain a constant volume and the magnitude of this fluid movement, equivalent to the net flux, was recorded every minute. Two fragments of the same bladder were simultaneously tested in many cases. The serosal face of the tissue was bathed with a buffer containing (mM in each case) NaCl, 112; $CaCl_2$, 1.0; KCl, 5.0; and $NaHCO_3$, 2.5; pH 8.1, when saturated with air. The NaCl concentration was reduced to 5.6 mM on the mucosal side, making this solution largely hypotonic. The medium pH was continuously monitored in the upper chamber and, in some experiments, also in the lower one. Both solutions were vigorously stirred with a magnetic rod (lower chamber) or a Teflon helix (upper chamber). The medium pH was changed and clamped at a new value by adding the necessary amount of HCl or NaOH with a microsyringe device. In some experiments, $NaHCO_3$ was omitted from the mucosal solution or replaced by sodium phosphate (Na_2HPO_4, 1.5 mM) or Tris (1.5 mequiv./l). Experiments were performed at room temperature (20 ± 1°C) or at low temperature (6–7°C). In the last case the chamber was immersed in a thermostatically controlled bath.

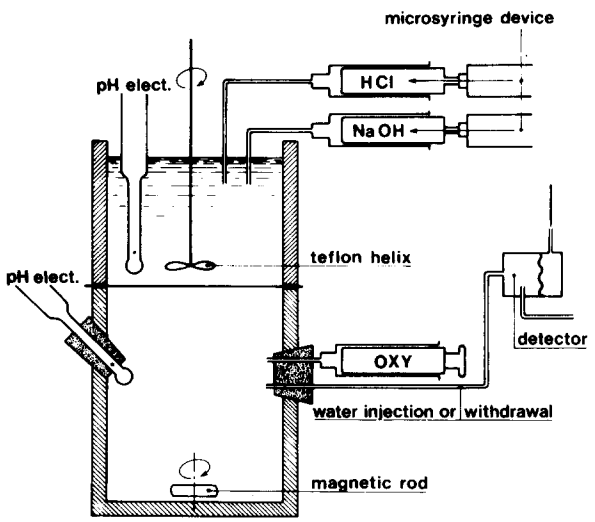

Fig. 1. Schematic representation of the experimental device employed. Frog urinary bladders were mounted between two lucite chambers with the mucosal or serosal border facing the upper solution. The net water flux was measured with a modification of a previously described technique [11]. See Methods for further details. elect., electrode.

Results

Time-course studies on oxytocin action at 20°C

Fig. 2 summarizes experiments in which the serosal border was facing the upper chamber. As previously reported [12], the onset of the response to the hormone had a sigmoidal shape (mean half-time ($t_{1/2}$) 6.5 min, see Table I). Once at the peak of the response to oxytocin, the water flux was reduced in three different ways: by removing the hormone, adding norepinephrine ($5 \cdot 10^{-5}$ M) or acidifying (from pH 8.1 to 6.0) the serosal bath. No differences were detected between the effect of hormonal withdrawal or norepinephrine addition, two situations in

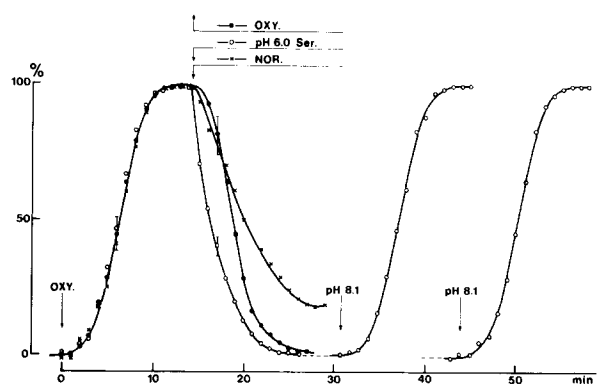

Fig. 2. Hydroosmotic response to oxytocin (OXY.) ($2.2 \cdot 10^{-8}$ M) at 20°C. At the peak of the hormonal action, the water flux increase was reversed in three different ways: by removing the hormone, adding norepinephrine (NOR.) ($5 \cdot 10^{-5}$ M) or acidifying the serosal bath. In the last situation, the serosal (Ser.) pH was returned to 8.1, at different times after acid pH inhibition. Mean of six experiments for each curve. Values are expressed as the percentage of maximal increase. Bicarbonate buffer was employed.

which the cyclase activity is reduced [13]. Decreases in water fluxed reflected rather the previously observed increases but the time course was slightly faster (Table I). Serosal acidification caused, on the contrary, an exponential and more rapid (Table I) decrease in the water flux. After returning to pH 8.1, in the presence of oxytocin, the water flux increased again, the time course being indistinguishable from the increase previously induced by oxytocin (Table I and Fig. 2).

As previously reported [9], mucosal acidification (from pH 8.1 to 6.0) strongly inhibited the oxytocin-induced increase in water flux in the presence of HCO_3^- (Fig. 3). Nevertheless, the flux returned to the initial values when the mucosal solution was vigorously aereated, even when the mucosal pH remained clamped at 6 by HCl microinjection. The inhibitory action of mucosal acidification was strongly depen-

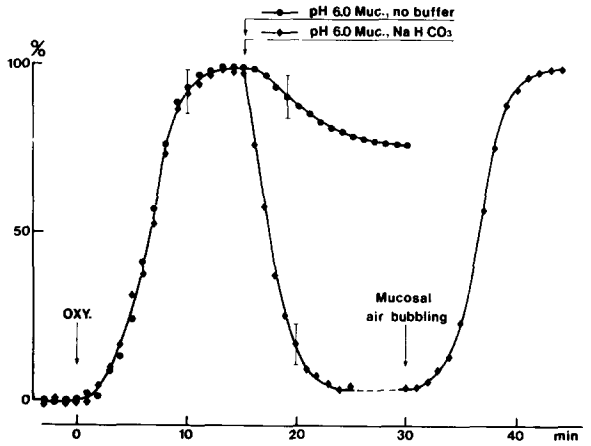

Fig. 3. Effect of mucosal (Muc.) acidification on the hydro-osmotic response to oxytocin (OXY.) in the presence or absence of bicarbonate in the apical bath. The mucosal solution was mechanically stirred. After complete inhibition in the presence of bicarbonate, vigorous air bubbling was established. Mean of six experiments for each condition.

TABLE I

HALF-TIMES OF VARIATIONS IN NET WATER FLUX INDUCED UNDER DIFFERENT CONDITIONS IN FROG URINARY BLADDER

Permeability increase				Permeability decrease			
Conditions	Temperature	n	$T_{1/2}$	Conditions	Temperature	n	$T_{1/2}$
oxy, serosal (2)	19–21	8	6.2 ± 0.9	Oxy washout (2)	19–21	6	4.7 ± 0.8
cAMP, serosal (7)	19–21	5	7.8 ± 0.8	cAMP washout	19–21	5	5.0 ± 0.3
8-b-cAMP, serosal (8)	19–21	4	7.9 ± 1.3	8-b-cAMP washout	19–21	4	5.2 ± 0.9
pH 8.1 serosal, after pH 6.0, under oxy (2)	19–21	6	6.5 ± 0.9	pH 6, serosal after oxy (2)	19–21	6	2.6 ± 0.2
				pH 6, mucosal after oxy (3)	19–21	7	2.4 ± 0.3
Air bubbling, mucosal pH clamped at 6.0 after oxy (3)	19–21	5	6.7 ± 1.0	pH 6, mucosal after cAMP (7)	19–21	7	2.8 ± 0.6
				CO_2 bubbling, pH clamped at 8.1 after 8 b-cAMP (8)	19–21	4	2.9 ± 0.3
Air bubbling, mucosal pH clamped at 6.0 after cAMP	19–21	5	2.4 ± 0.8	nor after oxy (2)	19–21	5	4.8 ± 0.9
				oxy washout (5)	6– 7	6	22.0 ± 3.0
Air bubbling, mucosal pH clamped at 6.0 under 8-b-cAMP (8)	19–21	4	2.8 ± 0.5	nor after oxy (5)	6– 7	6	24.0 ± 7.0
				pH 6, serosal, after oxy (5)	6– 7	8	2.5 ± 0.2
oxy, serosal (5)	6– 7	6	24.0 ± 3.0	pH 6, mucosal, after oxy	6– 7	4	1.8 ± 0.3
pH 8.1 serosal, after pH 6.0 under oxy (5)	6– 7	5	2.7 ± 0.3				

All experiments were carried out in the presence of bicarbonate buffer. Numbers in parentheses refer to corresponding figures. oxy, oxytocin ($2.2 \cdot 10^{-8}$ M), cAMP, cyclic AMP (10^{-2} M); 8-b-cAMP, 8-bromo cyclic AMP (10^{-3} M); nor, norepinephrine ($5 \cdot 10^{-5}$ M).

dent on the type of buffer present in the mucosal side. It was maximal in the presence of bicarbonate (mean inhibition 89 ± 9%, $n = 10$), also important when bicarbonate was replaced by phosphate (mean inhibition 50 ± 8%, $n = 5$), strongly reduced in the presence of Tris (mean inhibition 28 ± 5, $n = 5$) and almost absent when no buffer was added to the mucosal bath (mean inhibition 8 ± 4%, $n = 6$, see Fig. 3).

Mucosal acidification could induce a transepithelial variation in serosal pH, depending on the relative volumes of the serosal and mucosal baths and on the type of buffer employed. Fig. 4 shows three different situations: (1) In Fig. 4a, bicarbonate was present in the serosal side while the mucosal solution was not buffered. Mucosal acidification did not induce any significant inhibition of ADH-induced water flux and no detectable changes in serosal pH were observed. (2) When bicarbonate was present in the mucosal and serosal baths, a clear inhibition of the osmotic flow was registered, paralleled by a transepithelial acidification of the serosal solution (Fig. 4b). (3) Finally, in Fig. 4c, mucosal acidification blocked the water flux while no detectable variation in the serosal pH was observed (phosphate buffer was present on both sides).

Time-course studies on oxytocin action at low temperature

The time course of the hydroosmotic response to oxytocin is strongly dependent on medium temperature [14] and Fig. 5 shows the increase in the net water flux induced by hormonal stimulation at 6–7°C ($t_{1/2} = 24 \pm 3$ min). The reduction in water flux induced by oxytocin removal or norepinephrine addition was also strongly dependent on medium temperature, while the effect of serosal or mucosal acidification was, on the contrary, apparently not affected by cold (Table I and Fig. 5): flow inhibition was as rapid at 6 as at 20°C (HCO_3^- was present in both sides). When the pH was returned to 8.1 (maintaining oxytocin on the serosal side) a rather unexpected situation was observed: recovery was more rapid at 6–7°C than that observed at 20°C (Table I). Furthermore, while at 20°C recovery from acid pH inhibition was indistinguishable from that of hormonal stimulation, at low temperature the reversal time course showed a maximal slope immediately after raising of the medium pH (see Table I, cf. Figs. 1 and 6). Another difference was that at 20°C the magnitude of the recovery was rather independent of the duration of acid pH inhibition (Fig. 2), while at low temperature the recovery was smaller as the incubation time in acid pH increased (Fig. 6). Mucosal acidification at low temperature also inhibited the water flux, in the presence of HCO_3^-. As in the case of serosal acidification, this effect was independent of medium temperature (Table I), When mucosal pH was returned to 8.1 the increase in water permeability was similar to the recovery from serosal acid pH inhibition at 6–7°C.

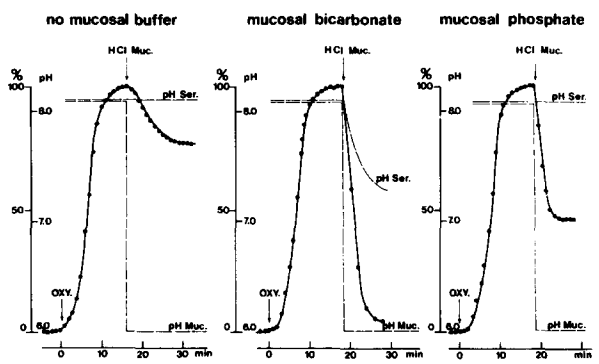

Fig. 4. Effect of mucosal (Muc.) acidification on the hydroosmotic response to oxytocin (OXY.) in the presence of different buffers. Medium pH was monitored in the serosal (pH Ser.) and mucosal (pH Muc.) sides. HCl was added to the mucosal bath (HCl Muc.) up to pH 6. Mean of six experiments for each condition.

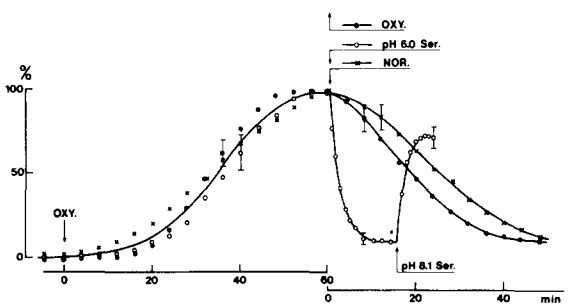

Fig. 5. Hydroosmotic response to oxytocin (OXY.) at 6°C. Once at the peak of the hormonal action the effects of hormonal removal, norepinephrine (NOR.) ($5 \cdot 10^{-5}$ M) and serosal (Ser.) acidification are compared. In the latter situation and after complete acid pH inhibition, pH 8.1 was restored. Bicarbonate buffer was employed. Mean of six experiments for each curve.

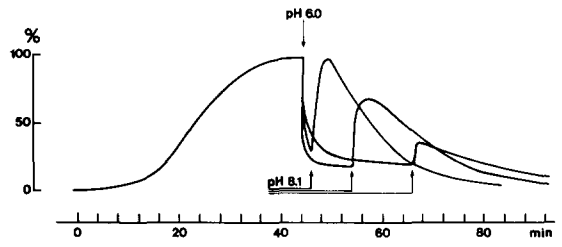

Fig. 6. Hydroosmotic response to oxytocin at 6°C. At the maximum of the hormonal action, HCl was added up to pH 6 in the serosal bath, while oxytocin was removed. pH 8.1 was restored at different times after acidification and hormonal removal.

Time-course studies on cyclic AMP and 8-bromo-cyclic AMP actions

Fig. 7 shows the time course of the response to cyclic AMP ($5 \cdot 10^{-3}$ M) at room temperature ($t_{1/2}$ = 7.2 ± 0.3 min, Table I). Mucosal acidification (pH 6.0, bicarbonate on both sides) reversibly inhibited, as in the case of oxytocin stimulation, water flux. Interestingly, recovery was similar to that observed after acid pH inhibition of the hormonal action at low temperature. Furthermore, it was more rapid that that observed after acid pH inhibition of the oxytocin action at room temperature and showed a maximal slope immediately after raising of the medium pH (see Figs. 2, 6 and 7 and Table I).

The hydroosmotic response to cyclic AMP is quite variable and large concentrations must be employed to elicit a significant increase in water flow. This is not the case with certain new cyclic derivatives. Experiments presented in Fig. 8 show the hydroosmotic response to 8-bromo-cyclic AMP (10^{-3} M). Tris-HCl buffer was employed in the mucosal bath during this experimental series. Acidification of the mucosal bath with HCl, up to pH 6.0, did not induce any detectable inhibition in the water flux. After returning to pH 8.1 (by washing the mucosal bath), 5% CO_2/95% O_2 bubbling was carried out and a clear inhibitory effect was recorded, even when mucosal acidification was prevented by NaOH injection. When CO_2 bubbling was again replaced by air bubbling, the water flux rapidly recovered to initial values, independently of pH on the mucosal side. Flux recovery started as soon as air replaced the gas mixture. The half-time of this new increase in water flux was similar to that observed during recovery from pH inhibition of cyclic AMP action at room temperature (see Table I).

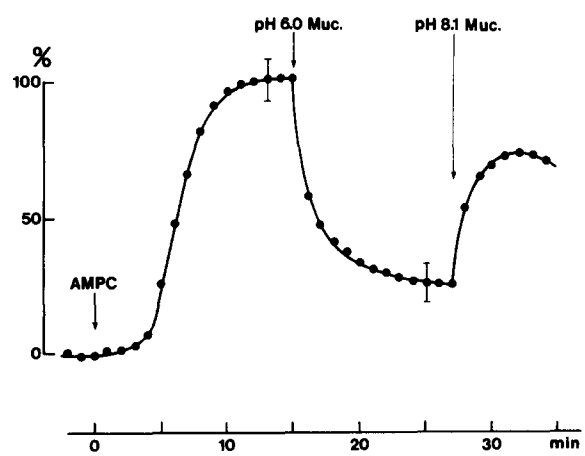

Fig. 7. Hydroosmotic response to cyclic AMP (AMPC) ($5 \cdot 10^{-3}$ M). At the peak of the nucleotide action, mucosal pH (pH Muc.) was lowered by adding HCl (bicarbonate was present in the mucosal side). As soon as the water flow stabilized at the new low value the mucosal pH was increased to 8.1. Mean of six experiments.

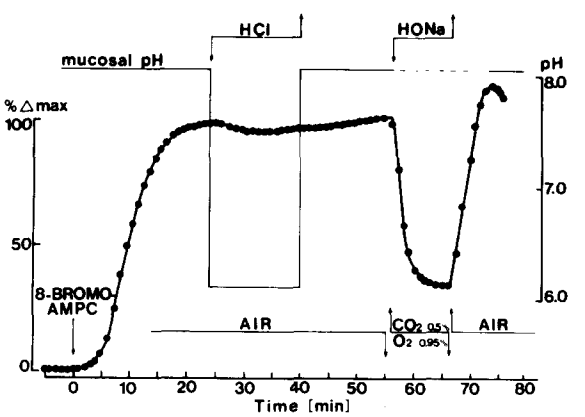

Fig. 8. Hydroosmotic response to 8-bromo-cyclic AMP (8-BROMO-AMPC) (10^{-3} M). Aereated Tris-HCl buffer was present in the mucosal bath. Once the response was fully developed, HCl was added to the mucosal solution up to pH 6.0 and maintained during 15 min. After returning to pH 8.1, air was replaced by a CO_2/O_2 mixture while mucosal pH variations were prevented by adding NaOH. Finally, air bubbling was reinstated.

Discussion

It has been previously suggested that medium acidification can inhibit the ADH-induced water permeability increase observed in the amphibian urinary bladder by two different mechanisms: (1) Blocking adenyl cyclase activity [15,16] and (2) interfering with a post-cyclic AMP step [9,17,18]. Nevertheless, no clear evidence has been presented until now on the site and mechanism of action of the latter effect.

Medium acidification affects an intracellular, apparently temperature-independent, post-cyclic AMP step

Studies on the turning on and off of the responses to oxytocin, cyclic AMP and serosal hyperotonicity (Refs. 5, 14 and 19; and the present study) indicate that the time course of the increase or decrease in the hydroosmotic flow depends on a rate-limiting step situated after cyclic AMP action. Results presented in Figs. 2 and 7 and Table I showing that the inhibition induced by medium acidification was more rapid than that elicited by norepinephrine action or agonist removal provide a first argument that medium acidification affects a post-cyclic AMP site, located after the rate-limiting step.

Responses to cyclic AMP and serosal hypertonicity are not more rapid than the response to oxytocin and are similarly affected by cold [19]. This shows that the rate-limiting step, located after cAMP action, is dependent on medium temperature. The inhibitory effect of medium acidification was temperature independent (Figs. 5 and Table I), within the limits of our experimental conditions. This also suggests that one of the effects of medium acidification must be located after the rate-limiting step.

Figs. 2–6 show that similar effects can be obtained under certain conditions by serosal or mucosal acidification. Nevertheless, it is evident that the presence of a permeant buffer seems a necessary condition for observing the inhibitory effects of mucosal acidification on the oxytocin-induced water flux. This is especially clear in the experiments presented in Fig. 3. Mucosal acidification can induce a reduction of serosal pH. Nevertheless, transepithelial pH modifications were not directly linked to the inhibition of the oxytocin-induced water flow: in the presence of phosphate buffer (2.5 mM), significant inhibition was observed in the absence of any transepithelial change in serosal pH (Fig. 4).

Finally, the experiments presented in Fig. 8 show that the acid pH inhibition of the bromo-cyclic AMP-induced hydroosmotic response seems clearly related to intracellular changes in H^+ activity. This result can explain previous contradictory reports [9,21] on the effect of mucosal acidification on the response to cyclic AMP and related agents. Inhibition will only be clearly observed when intracellular acidification is assured.

Cellular pH and intramembranous particle aggregates

There is experimental evidence that the intramembranous particle aggregates plugged into the apical border by hormonal action are closely related to a low-resistance water pathway. Freeze-fracture studies indicate that the influence of H^+ concentration on these aggregates depends on medium temperature. When water flux is inhibited by medium acidification at 20°C, the number of aggregates present in the membrane is proportionally reduced [9]. On the contrary, when pH inhibition is performed at 6–7°C, a clear dissociation between water fluxes and the number or surface of aggregates is observed: while the water permeability was strongly reduced, the number of aggregates remains almost unchanged [10].

The observation for the first time during incubation at low pH of a high aggregate number at a moment where permeability is already depressed would be naturally in line with the idea that water channels are not located in the aggregates. However, other explanations can be considered: for instance, cellular acidification can introduce a new resistance to water flux, in series with the water channel. Alternatively, it can be assumed that lowering the cellular pH modified the aggregates' fine structure and reduces their permeability.

A possible action of cellular pH on the water channel

Let us assume that medium acidification has, as previously mentioned, two main effects. First, adenyl cyclase activity is blocked, stopping the chain of events started by oxytocin and, as a consequence of this, the aggregates are removed from the apical border. The other effect would be on the water pathway itself, corresponding to the aggregates. This pathway would exist in two different states, either allow-

ing or not allowing significant water flux, and changes in cellular pH could induce the shift from one situation to the other.

After mucosal acidification at room temperature, the intramembranous particle aggregates disappear [9]. Furthermore, we have demonstrated (Fig. 2) that the recovery from acid pH inhibition of the oxytocin-induced water flow obeys, at room temperature, the same time course as does hormonal stimulation. We can explain these results, accepting that medium acidification would initially shift the water pathway permeability to the low-permeability state. Almost simultaneously and because of adenyl cyclase inactivation, these channels, represented by the aggregates, would disappear from the apical border. When the initial pH is restored, the aggregates must be again induced in the luminal membrane and this explains the observed time-course evolution, similar to that observed under hormonal stimulation.

Low-temperature experiments allowed us to dissociate effects of medium acidification. When the pH is reduced at low temperature, the aggregates' fine structure would change almost as rapidly as at room temperature but its removal from the apical border would be strongly slowed down by cold [10]. If the initial pH is restored after only a few minutes, the aggregates would be still present in the membrane and they would become operative very rapidly. Fig. 6 clearly shows that the fraction of the maximal flux recovered after returning to pH 8.1 was a function of the time spent in the presence of the acid medium.

Recovery of acid pH inhibition was more rapid in the presence of cyclic AMP derivatives than in the case of oxytocin, at room temperature (Table I). In this situation, the aggregates would not be removed by cyclase inhibition and the variation in water flux induced by changing medium pH would only be due to the opening and closing of the water channels. In this situation, as predicted, reversal from pH inhibition of the nucleotide-induced flow was, at room temperature, as rapid as the reversal from pH inhibition after the hormonal stimulus at 6°C.

In summary, freeze-fracture studies have shown that the aggregates were still present in the apical membrane when the water flux was inhibited by medium acidification at low temperature [10]. If we continue to accept, as suggested by strong experimental evidence, that the aggregates represent, in any way, the high-permeability water pathway induced by hormonal stimulation in the apical border, we must also accept that cellular pH can shift the water-permeation system * from a high-permeability to a low-permeability state. Cellular pH rather than H^+ concentration at the mucosal surface seems to control water permeability. If we accept a direct effect of medium pH on the aggregates, we must accept that this effect is on its internal surface. A similar effect of pH on gap junction ultrastructure and permeability has previously been reported [22].

We have proposed that the time course of the hormonal action results from the addition of permeability units that increase in number during the development of the permeability response [23]. If the mechanism now described is operative under physiological conditions the water permeability could be regulated in two different ways: modifying the number of water channels or regulating their permeability.

Acknowledgements

M.P. is a career investigator from the Centre National de la Recherche Scientifique (CNRS), France, and J.C. from the Institut National de la Santé et de la Recherche Médicale, INSERM, France. R.M. was an INSERM-invited investigator from the Universidad de Carabobo, Grupo Aragua, Facultad de Medicina, Maracay, Venezuela.

References

1 Chevalier, J., Bourguet, J. and Hugon, J.S. (1974) Cell Tissue Res. 152, 129–140
2 Kachadorian, W.A., Wade, J.B. and di Scala, V.A. (1975) Science 190, 67–69
3 Harmanci, M.C., Kachadorian, W.A., Valktin, H. and di Scala, V.A. (1978) Am. J. Physiol. 235, F440–F443
4 Brown, D., Grosso, A. and de Sousa, R.C. (1980) Biochim. Biophys. Acta 596, 158–164
5 Ripoche, P., Bourguet, J. and Parisi, M. (1973) J. Gen. Physiol. 61, 110–124
6 Taylor, A., Eich, E., Pearl, M. and Brem, A. (1979) INSERM Symp. Ser., vol. 85, pp. 167–174

* When we say 'water-permeation system' we refer to the association between the aggregates and the water channel that can be imagined, at the molecular level in different ways.

7 Hardy, M.A. (1978) J. Cell Biol. 76, 787–791
8 Hardy, M.A. (1979) Biochim. Biophys. Acta 552, 169–177
9 Parisi, M., Chevalier, J. and Bourguet, J. (1979) Am. J. Physiol., 237, F483–F489
10 Bourguet, J., Chevalier, J. and Parisi, M. (1981) in Water Transport Across Epithelia, Alfred Benzon Symposium 15 (Ussing, H.H., ed.), pp. 404–421, Munksgaard, Copenhagen
11 Bourguet, J. and Jard, S. (1964) Biochim. Biophys. Acta 88, 442–444
12 Bourguet, J. (1968) Biochim. Biophys. Acta 150, 104–112
13 Orloff, J. and Handler, J.S. (1967) Am. J. Med. 42, 757–768
14 Bourguet, J. (1966) J. Physiol. (Paris) 58, 476
15 Guliassy, P.F. and Edelman, I.S. (1965) Biochim. Biophys. Acta 102, 185–197
16 Bockaert, J., Roy, C. and Jard, S. (1972) J. Biol. Chem. 247, 7073–7081
17 Guliassy, P.F. and Edelman, I.S. (1967) Am. J. Physiol. 222, 740–746
18 Hardy, M. (1979) INSERM Symp. Ser. Vol. 85, 311–322
19 Ripoche, P. (1967) Mémoire pour le Diplome d'Études Superieures de Sciences Naturelles, Faculté de Sciences de l'Université de Paris
20 Malnic, G. and Steinmetz, P.R. (1976) Kidney Int. 9, 172–188
21 Carvounis, C.P., Levine, S.D. and Hays, R.M. (1979) Kidney Int. 15, 513–519
22 Perrachia, C. and Lillian, L.P. (1978) J. Cell Biol. 79, 217a
23 Parisi, M., Bourguet, J., Ripoche, P. and Chevalier, J. (1979) Biochim. Biophys. Acta 556, 509–523