Biochimica et Biophysica Acta 905 (1987) 399–408
Elsevier

BBA 73800

Intracellular Ca^{2+} concentration and the antidiuretic hormone-induced increase in water permeability: effects of ionophore A23187 and quinidine

M. Parisi, C. Ibarra and M. Porta

Sección de Biomembranas, Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires (Argentina)

(Received 25 August 1987)

Key words: cyclic AMP; Prostaglandin; Water exchange; Ionophore A23187; Calcium ion, intracellular; (Frog bladder)

The hydroosmotic responses induced by oxytocin and 8-bromo-cyclic AMP, in frog and toad urinary bladders, were recorded minute by minute. ^{3}HHO and ^{45}Ca unidirectional fluxes as well as prostaglandin B_2 liberation were also measured. It was observed that: (1) Addition of the calcium ionophore A23187 or quinidine to the serosal bath inhibited the response to oxytocin, but not to 8-bromo-cyclic AMP, while increasing propstaglandin E_1 liberation into the serosal but not into the mucosal bath. (2) Addition of A23187 to the mucosal bath induced a transient and temperature-dependent inhibition of the response elicited by 8-bromo-cyclic AMP. The time-course of this reduction in water permeability and its sensitivity to medium temperature were similar to those observed after the withdrawal of agonist, but clearly different of those observed after intracellular acidification. (3) The hydroosmotic response was also transitorily inhibited when the Ca^{2+} concentration was step-changed in the mucosal bath. (4) When added to the mucosal or to the serosal baths, the ionophore increased either the apical or the laterobasal Ca^{2+} permeabilities. It is concluded that manipulation of intracellular Ca^{2+} interferes with the hydroosmotic response at two different levels. (1) A first target point located 'pre-cyclic-AMP production'. This effect would be mediated by prostaglandin liberation. (2) A second target point located after cyclic AMP production and before the 'temperature-dependent rate-limiting step'. This effect is probably related to the mechanism controlling the insertion and removal of water channels.

Introduction

Antidiuretic hormone (ADH) and its intracellular second messenger, cyclic adenosine monophosphate (cyclic AMP) [1], increase the water permeability of their target membranes. Experimental evidence, coming from both ultrastructural and biophysical studies [2–5], indicates that this action is associated with the transfer of water channels from cytoplasmic vesicles into the apical plasma membrane of granular cells [6,7]. Microtubules and microfilaments seem to be involved in this 'membrane traffic' process [8,9] and Ca^{2+} has been implicated in cytoskeletal functions [10]. Furthermore, membrane fusion events also depend on Ca^{2+} concentration.

Previous information from experiments in which intracellular Ca^{2+} was manipulated employing the Ca^{2+} ionophore A23187 or quinidine indicates that cytosolic Ca^{2+} concentration plays a role in the ADH-induced response [11,12]. Nevertheless, several points remain obscure or controversial: (1) Are the responses to ADH and cyclic AMP in-

Abbreviation: ADH, antidiuretic hormone.

Correspondence: M. Parisi, J.E. Uriburu 166, 1027 Buenos Aires, Argentina.

hibited [12] or potentiated [11] by A23187 or quinidine? (2) Are the ionophore actions restricted to 'pre-cyclic-AMP' steps [13] and mainly related to liberation of prostaglandins [14,15]? (3) If a 'post-cyclic-AMP' action can be demonstrated, which would be the 'target point' for this Ca^{2+}-mediated effect?

We have now re-examined the effects of ionophore A23187 and quinidine on the response to oxytocin and 8-bromo-cyclic AMP in the amphibian urinary bladders. Net water fluxes were recorded minute by minute, allowing a time-course study of changes in water permeability. ^{3}HHO and ^{45}Ca unidirectional fluxes were also mesured, while intracellular Ca^{2+} manipulation was attempted in different ways. Finally, liberation of prostaglandins was tested under the different experimental conditions employed.

Materials and Methods

European frogs (*Rana esculenta*) or South American toads (*Bufo arenarum*) were employed in different experiments. Most experimental conditions were tested in both species, and no significant differences were observed.

Net water fluxes

Urinary bladders were removed from pithed frogs or toads and horizontally mounted (serosal or mucosal side upwards) as flat sheets between two twin lucite chambers. The saline solution bathing the serosal side of the bladder contained (mM): NaCl, 112; KCl, 5; $CaCl_2$, 1; $NaHCO_3$, 2.5 (pH 8.1 when bubbled with air). An osmotic gradient was imposed by reducing the NaCl concentration in the mucosal solution to 5.2 mM. The net water fluxes were measured with a modification of a previously described technique [16,17]. Water was automatically injected into or sucked from the lower chamber to maintain a constant volume and the magnitude of this fluid movement, equivalent to the net flux, was recorded every minute. Free access to the mucosal or to the serosal bath (upper chamber) was alternatively assured.

Unidirectional water fluxes

Unidirectional water fluxes were measured with a technique previously employed and discussed [18]. Everted bladder sacs were attached to a polyethylene cannula and filled with about 6 ml of saline. The sacs (mucosal side outwards) were inmersed for 40 s in a buffer solution containing 10 μCi/ml of ^{3}HHO and 1 μCi/ml of [^{14}C]mannitol, washed for 0.5 s in the chilled non-radioactive solution and transferred to a preweighed empty beaker in which the sac was cut open and the serosal fluid collected. 500-μl samples of this fluid were analysed in a liquid scintillation counter. The volume of the sac was calculated from its weight and an estimation of its surface was made assuming a spherical shape. After correction for the remaining adherent layer by subtracting the mannitol space, the unidirectional water flux was calculated and expressed in $\mu l \cdot min^{-1} \cdot cm^{-2}$. In most experiments, paired sacs were prepared from each bladder and fluxes in the tested situation were expressed as a percentage of those observed in the control.

Unidirectional Ca^{2+} fluxes

Frog or toad urinary bladders were horizontally mounted as for net water measurements. Either the mucosal or the serosal surface was facing the upper bath in different experiments. The lower chamber volume (stirred with a magnetic bar) was 12 ml and the upper one 2 ml. ^{45}Ca (1 μCi/ml as $CaCl_2$) was added to the lower chamber at the beginning of the experiments and the upper volume was removed every 10 min. ^{45}Ca activity was measured by scintillation counting. The transepithelial Ca^{2+} permeability coefficient was then calculated (exposed area 3.14 cm^2) and expressed in cm/s. In some experiments [^{14}C]mannitol permeability was simultaneously tested.

Determination of prostaglandin release

Bladders were vertically mounted between two symmetric twin lucite chambers. The exposed area was 3 cm^2 and the chamber volumes 5 ml. Samples (150 μl) were taked by triplicate at 30 min intervals and prostaglandin E_2 concentration was measured with a New England Nuclear (^{125}I) RIA kit (NEK-020A).

The term ADH is used throughout the text as a generic expression covering the hydroosmotic action of oxytocin and other neurohypophyseal peptides.

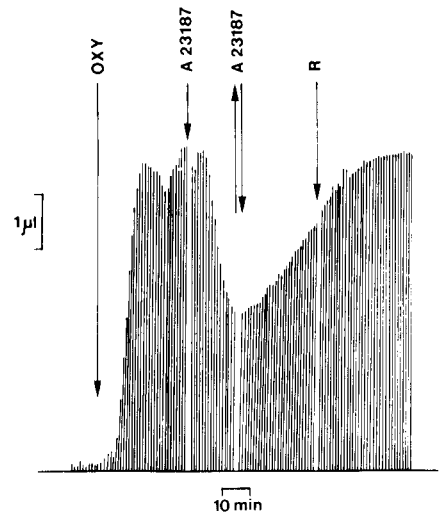

Fig. 1. The hydroosmotic response to oxytocin (OXY, 10^{-8} M) in a frog urinary bladder. Effect of mucosal A23187 ($1.5 \cdot 10^{-5}$ M). At the second arrow the A23187 solution ws replaced by a fresh one. R: A23187 withdrawal.

Results

The effects of the Ca^{2+} ionophore A23187 on the hydroosmotic response to oxytocin

In a first type of experiment, A23187 (Calbiochem) was added, either to the mucosal or to the serosal bath, once the hydroosmotic response to oxytocin (Sandoz) was fully developed.

Addition of A23187 to the mucosal bath. Fig. 1 records a typical experiment. The height of each vertical stroke indicates the every minute value of the net water flux. At the top of the response to oxytocin (OXY, $1 \cdot 10^{-8}$ M) 5 µl of a A23187 solution ($1.5 \cdot 10^{-5}$ M) were added to the mucosal chamber (the open one in these experiments). This induced a transient reduction in water permeability, while 5 µl of the carrier solution (DMSO/ethanol, 1 : 1) had no significant effect (not shown). Replacement of the A23187-containing solution by a fresh one, with the same or higher ionophore concentration (2- or 3-fold increase), did not change the observed time-course (regardless of the time elapsed after A23187 addition, see Fig. 4.

Fig. 2 represents the mean curve for eight experiments. The development of the response to oxytocin was not represented and the graph starts at the top of the hydroosmotic response. The inhibitory phase shows a time-course reminiscent of that observed during oxytocin washout [19]. Maximal inhibition represented 47% of the hydroosmotic response (Table I) with a half-time of 5 ± 0.4 min.

It has been previously reported that at least

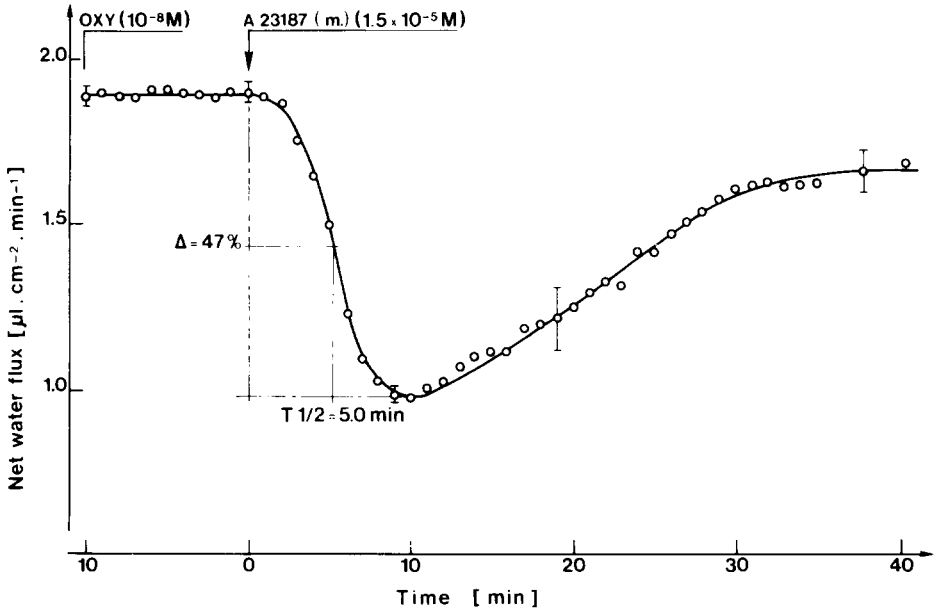

Fig. 2. The graph starts at the top of the response to oxytocin (OXY). Net water fluxes, mean curve for eight experiments. A23187 was added to the mucosal (m.) bath.

part of the effects observed after incubation with A23187 can be due to liberation of prostaglandins [15]. In a series of experiments we challenged the bladders with oxytocin (10^{-8} M) plus an inhibitor of prostaglandin liberation (indomethacin, $5 \cdot 10^{-5}$ M). As reported in Table I, no significant changes in the inhibitory effect of mucosal A23187 were observed.

Addition of A23187 to the serosal bath. When the ionophore ($1.5 \cdot 10^{-5}$ or $2.2 \cdot 10^{-5}$ M) was added to the serosal bath, at the top of the response to oxytocin, a reduction in the hydroosmotic response was also observed (Fig. 5). The time-course of the decay was again similar to that observed during oxytocin washout (half-time 5.0 $\pm$ 0.4 min). Nevertheless, the inhibitory effect did not show the spontaneous recovery observed when A23187 was added to the mucosal bath. Another difference was registered when indomethacin was added together with oxytocin. In this situation the inhibitory effect of A23187 was prevented (Table I).

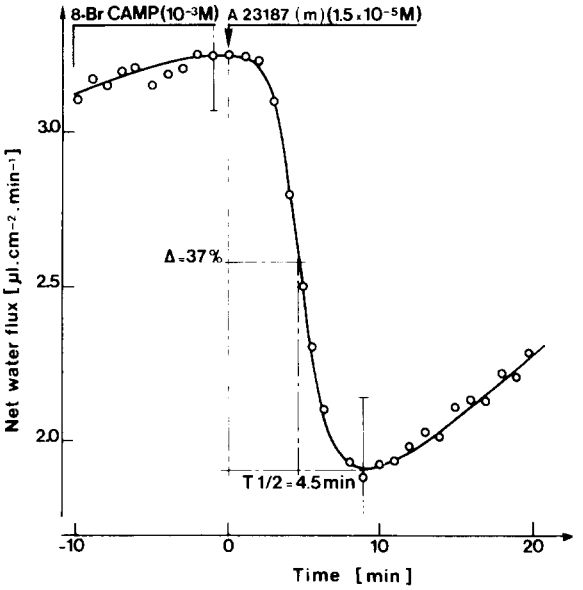

Fig. 3. Net water fluxes, mean curve for 12 experiments. The graph starts at the top of the response to 8-bromocyclic AMP (8-Br CAMP). A23187 was added to the mucosal (m.) bath.

The effects of the Ca^{2+} ionophore A23187 on the hydrostatic response to 8-bromo-cyclic AMP

Cyclic AMP is the accepted second messenger for ADH. Fig. 3 shows the effects of A23187 ($1.5 \cdot 10^{-5}$ M), when added to the mucosal bath, on the response elicited by 8-bromo-cyclic AMP (10^{-3}, Sigma), a potent analog of the nucleotide. The development of the response was not represented (mean curve for 12 experiments). The inhibitory phase (37% at the maximum, Table I) had characteristics (half-time 4.5 $\pm$ 0.6 min) similar to those observed during oxytocin withdrawal.

Fig. 4 shows an experiment (representative of six) in which two fragments of the same urinary bladder were tested, one at 20 °C and the other at 12 °C. It was observed that: (1) As previously reported for cyclic AMP [20] the response to 8-bromo-cyclic AMP was slower at 12 °C (lower curve, half-time 18 $\pm$ 3 min, $n = 6$) than at 20 °C. (upper curve, half-time 7.5 $\pm$ 0.6 min, $n = 6$). (2) The time-course of the inhibitory action of A23187 was also slowed down by reduction in medium temperature (half-time for maximal inhibition 4.0 $\pm$ 1.0 min at 20 °C and 22 $\pm$ 3 min at 12 °C). (3)

TABLE I

EFFECTS OF A23187, QUINIDINE AND INDOMETHACIN ON THE HYDROSMOTIC RESPONSE TO OXYTOCIN AND 8-BROMO-CYCLIC AMP

Results represent, for agents tested after the agonist, the per cent variation ($\pm$S.E.) induced on the previously developped hydroosmotic response (measured as shown in Fig. 2, for example). For experiments in which A23187 was added before the agonist, the response is expressed as the percentage of a control response to the agonist observed in a simultaneously stimulated hemibladder.

Condition	Serosal A23187 (10^{-5} M)		Mucosal A23187 (10^{-5} M) after agonist	Serosal quinidine ($2 \cdot 10^{-4}$ M) after agonist
	before agonist	after agonist		
Oxytocin (10^{-8} M)	-40 ± 6 (6)	-37 ± 6 (12)	-47 ± 5 (12)	-42 ± 4 (8)
8-Bromo-cyclic AMP (10^{-3} M)	-28 ± 5 (6)	-3 ± 6 (10)	-37 ± 7 (12)	$+33 \pm 6$ (8)
Indomethacin ($5 \cdot 10^{-6}$ M) + ocytocin ($2.2 \cdot 10^{-8}$ M)	–	-8 ± 7 (6)	-35 ± 4 (6)	-10 ± 6 (6)

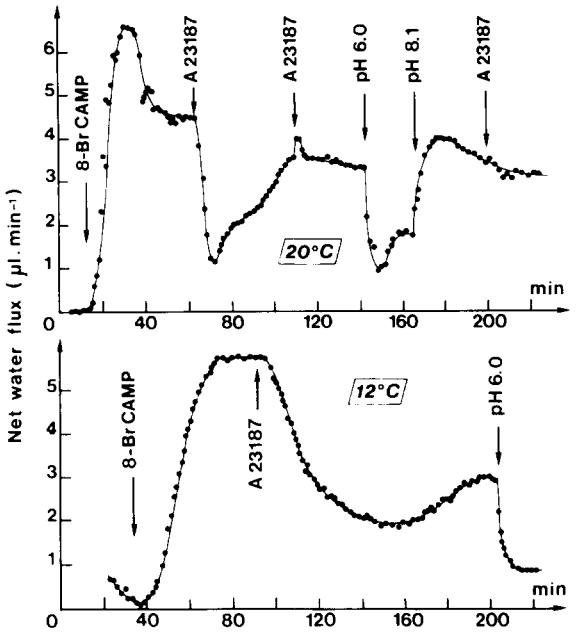

Fig. 4. Net water fluxes across two fragments of the same frog urinary bladder, measured at 20 and 12° C. After 8-bromo-cAMP stimulation (10^{-3} M), A23187 ($1.5 \cdot 10^{-5}$ M) was added to the mucosal bath. Second and third additions of A23187 (at 20° C) increased the ionophore concentration to $3 \cdot 10^{-5}$ and $4.5 \cdot 10^{-5}$ M. Medium acidification was obtained by bubbling 5% CO_2 in the mucosal side.

A23187 additions subsequent to the initial one (concentration went up to $3 \cdot 10^{-5}$ and $4.5 \cdot 10^{-5}$ M) were ineffective. (4) Mucosal acidification to pH 6 (CO_2 bubbling) reversibly inhibits the response to cyclic AMP [21,22]. We show now that pretreatment with A23187 did not interfere with the mucosal acidification effect. It can be also observed that, as previously reported [17], the inhibitory effect of low pH was not significantly dependent on medium temperature.

Serosal A23187 ($1.5 \cdot 10^{-5}$ or $2.2 \cdot 10^{-5}$ M) did not inhibit the response to 8-bromo-cyclic AMP (Fig. 6, Table I). This indicates a striking asymmetry between the effects of the ionophore when added to the mucosal or to the serosal bath. However, this asymmetry disappeared when the ionophore was added before the nucleotide (Table I).

The effects of quinidine on the hydroosmotic response

Addition of quinidine to the serosal side ($2 \cdot 10^{-4}$ M), on the previously developed response to oxytocin, had an inhibitory effect (Fig. 7, half-time 5.3 ± 0.3 min, $n = 9$). This inhibition was prevented, as in the case of serosal A23187, when indomethacin was added together with oxytocin (Table I).

The effect of serosal quinidine on the resposne to 8-bromo-cyclic AMP is shown in Fig. 8 ($n = 6$). A slow but clear increase in the hydroosmotic response was observed. Nevertheless, in a second series, in which the water flux was followed during a longer period, a biphasic response was detected. After an initial increase up to 120% of the initial value (20 min after quinidine addition) the flux slowly went down to 70 + 5% of the control (40 min after addition of drug, $n = 6$).

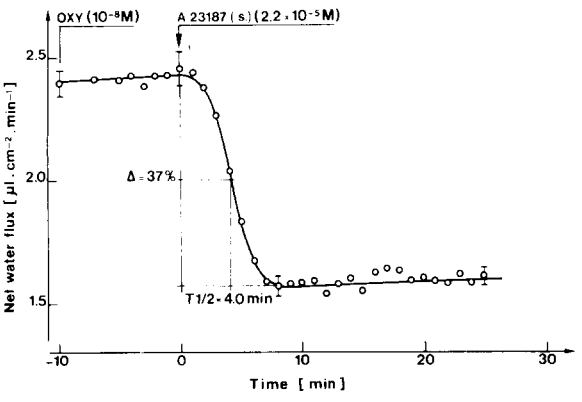

Fig. 5. Net water fluxes, mean curve for 8 experiments. The graph starts at the top of the response to oxytocin (OXY, 10^{-8} M). A23187 has been added to the serosal (s.) bath.

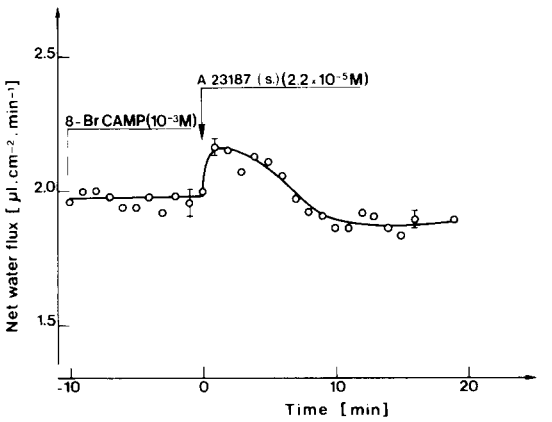

Fig. 6. Net water fluxes, mean curve for 8 experiments. The graph starts at the top of the response to 8-bromo-cAMP. A23187 was added on the serosal (s.) side.

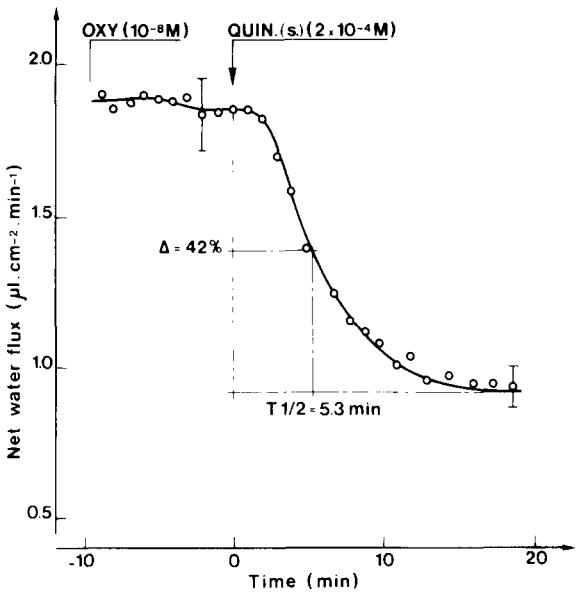

Fig. 7. Net water fluxes, mean curve for nine experiments. The graph starts at the top of the response to oxytocin (OXY). Quinidine (QUIN) was added to the serosal (s.) bath.

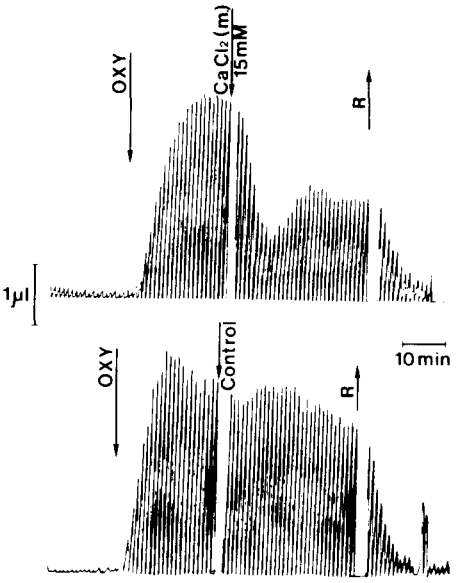

Fig. 9. Net water fluxes across two fragments of the same bladder. At the top of the response to oxytocin (OXY, 10^{-8} M), $CaCl_2$ concentration was increased to 15 mM in the mucosal bath (upper graph). To minimize changes in the transepithelial gradient 45 mM mannitol, initially added to the employed hypotonic buffer, were simultaneously removed. In the control fragment (lower graph) the mucosal bath was replaced by a fresh solution R: oxytocin withdrawal.

Effects of changing mucosal Ca^{2+} concentration on the hydroosmotic response to oxytocin

The effect of changing mucosal Ca^{2+} concentration was tested in both net and unidirectional water fluxes.

Net water fluxes. Fig. 9 shows a typical experiment ($n = 5$). At the top of the response to oxytocin, the $CaCl_2$ concentration in the apical medium was increased from 1 to 15 mM. A reduction in the net water flux, having a time-course similar to that when the Ca^{2+} ionophore was added to the mucosal bath was observed. In this experiment, the mucosal bath initial contained the previously described hypotonic buffer, to which 45 mM mannitol was added. When the $CaCl_2$ concentration was increased, mannitol was eliminated, to minimize changes in the osmotic gradient (even if 45 mM mannitol is not perfectly isosmotic with 15 mM $CaCl_2$). In the control fragment the mucosal bath was just changed for a fresh solution.

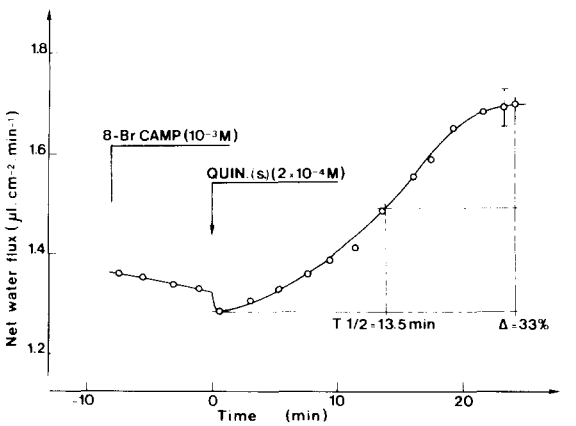

Fig. 8. Net water fluxes, mean curve for six experiments. The graph starts at the top of the response to 8-bromo-cAMP. Quinidine (QUIN) was added to the serosal (s.) bath.

Unidirectional ^3HHO fluxes. To avoid any influence of regulatory processes associated with changes in the net water flux [23], unidirectional ^3HHO experiments were performed in the absence of an osmotic gradient. Paired everted bladder sacs (mucosal side outside) were incubated during 20 min in the presence of oxytocin (serosal side). Then one of the sacs (experimental) was transferred to a bath in which NaCl was isosmotically replaced by different concentrations of $CaCl_2$,

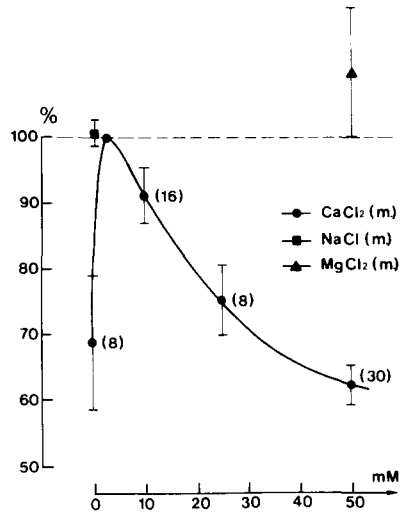

Fig. 10. Data obtained from ^{3}HHO uptake by everted bladder sacs stimulated by oxytocin (10^{-8} M). Paired sacs were prepared from each bladder. One was tested in the presence of the standard hypotonic solution in the mucosal side (1 mM $CaCl_2$, 112 mM NaCl) and taken as a control. The other (experimental) was tested in different mucosal $CaCl_2$, $MgCl_2$ or NaCl concentrations. The points in the graph are mean values (± S.E.) for the experimental sacs, expressed as a percentage of the control ones.

$MgCl_2$ or mannitol. The other sac (control) was changed to a fresh buffer solution. 10 min later the unidirectional ^{3}HHO fluxes were measured for 40 s, as described in Materials and Methods (net water flux experiments have shown that the inhibitory effect was maximal 10 min after the change in $CaCl_2$ concentration).

The results obtained with the experimental sac are presented as a percentage of the control (Fig. 10). A clear dependence of water permeability on mucosal $CaCl_2$ concentration was observed. Both an increase and a decrease (nominally no $CaCl_2$ in the buffer) reduced the unidirectional water flux in bladders previously permeabilized with oxytocin. Replacement of NaCl by mannitol (square) or 45 mM $MgCl_2$ (triangle) did not modify the water permeability (values were expressed as a percentage of the control sac).

When similar experiments were performed in non-stimulated bladders, no changes in water permeability were observed under any of the conditions tested.

Liberation of prostaglandins into the incubation medium

The results obtained are summarized in Table II. It can be observed that spontaneous liberation of prostaglandins into the serosal bath did not change during two consecutive 20 min periods. Mean values under oxytocin were lower, but the differences were, under our experimental conditions, not statistically significant. Serosal A23187 induced a significant increse of prostaglandin E_1 liberation into the serosal bath. On the contrary, mucosal A23187 did not increase prostaglandin E_2 liberation significantly, either into the mucosal or into the serosal bath. Finally, it can be noted that quinidine also induced liberation of prostaglandins into the serosal bath.

TABLE II

LIBERATION OF PROSTAGLANDINS INTO THE INCUBATION MEDIUM (pg prostaglandin E_2/100 µl per 40 min)

n	Side tested	1st period	2nd period	Difference
		control	control	
5	S	448 ± 62	527 ± 42	+89 ± 90 n.s.
		oxytocin	oxytocin	
8	S	331 ± 66	391 ± 73	+60 ± 33 n.s.
		control	oxytocin	
6	S	427 ± 71	370 ± 65	−57 ± 48 n.s.
		oxytocin	A23187(serosal) 10^{-5} M	
9	S	349 ± 35	1897 ± 430	+1547 ± 407 $P < 0{,}01$
		oxytocin	A23187(mucosal) 10^{-5} M	
5	M	336 ± 17	316 ± 23	−19.8 ± 18 n.s.
5	S	342 ± 21	517 ± 111	+174 ± 69 n.s.
		oxytocin	quinidine	
6	S	354 ± 21	670 ± 37	+336 ± 87 $P < 0.05$

Effects of A23187 in the transepithelial calcium permeability

Fig. 11 shows the effects of addition of A23187 ($1.5 \cdot 10^{-5}$ M) on transepithelial calcium permeability, calculated from ^{45}Ca unidirectional fluxes. The upper part of the figure represents mucosal to serosal calcium fluxes measured during 10-min periods (see Materials and Methods). The addition of ionophore to the mucosal side significantly increased the transepithelial calcium movement, which stabilized at a new higher value. Subsequent A23187 addition to the serosal bath induced a second and significant increase in calcium permeability.

The lower part of the figure shows calcium permeabilities calculated from serosal to mucosal unidirectional ^{45}Ca fluxes. In this situation, addition of the ionophore to the serosal bath did not significantly increase the transepithelial calcium movement. Nevertheless, when A23187 was subsequently added to the mucosal bath, the observed increase was similar to that registered when the ionophore was added to both compartments in the experiments previously described (upper part of the figure). No significant effect of A23187 on mannitol permeability was observed (not shown).

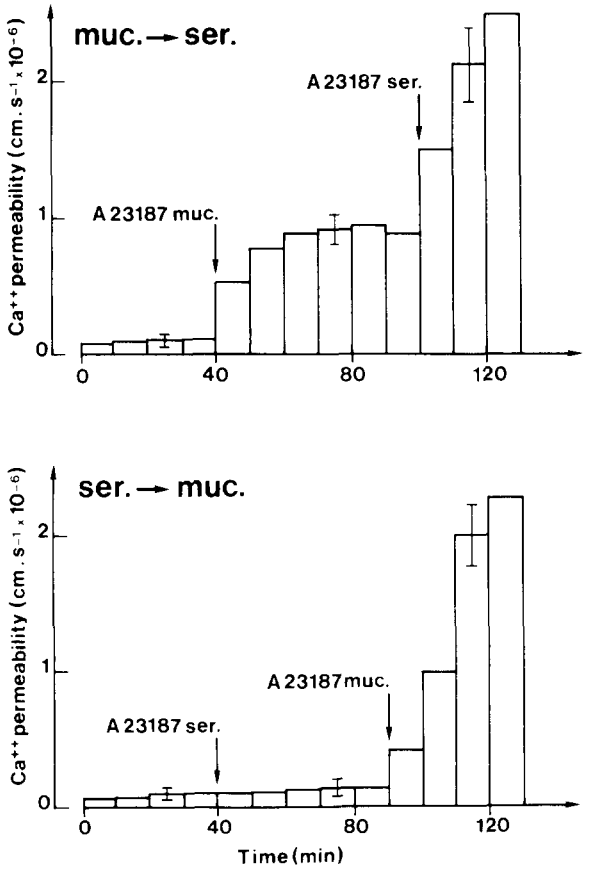

Fig. 11. Transepithelial Ca^{2+} permeability calculated from ^{45}Ca unidirectional fluxes. Upper graph: mucosal to serosal flux; lower graph: serosal to mucosal flux. A23187 ($1.5 \cdot 10^{-5}$ M) was added either to the mucosal (muc) or to the serosal (ser) bath.

Discussion

The results presented here provide evidence indicating that cytosolic Ca^{2+} interacts with the hydroosmotic response to ADH at least at two different levels. The evidence supporting this view comes from the effects of the calcium ionophore A23187 and quinidine. In the case of the ionophore, addition to the serosal bath affected only those steps before cyclic AMP production. In contrast, its addition to the mucosal bath clearly interfered with 'post-cyclic-AMP' steps.

A23187 and Ca^{2+} permeability in amphibian urinary bladder

The average level of cytosolic free Ca^{2+} (as measured by employing quin2 and fura2 in isolated toad bladder cell suspensions) dramatically rises after A23187 [32]. The experiments presented here clearly show that A23187 increased transepithelial calcium permeability (Fig. 11) with no change in mannitol transfer. While the addition of ionophore to the mucosal bath increased the mucosal to to serosal calcium transfer, incubation with serosal A23187 did not change the serosal to mucosal movement. It seems, however, plausible that, even in this last situation, A23187 was modifying the permeability of serosal membrane to Ca^{2+}. Subsequent addition of the ionophore to the mucosal bath (Fig. 12, lower part) induced a huge increase in the transepithelial calcium flux. The total change in Ca^{2+} permeability was similar to the one observed when A23187 was first added to the mucosal and then to the serosal baths. Pretreatment of the serosal surface must modify Ca^{2+} permeability, but this was probably not reflected in the transepithelial movement, because the

mechanism responsible for Ca^{2+} extrusion is located in the laterobasal membrane.

It can be concluded that the addition of A23187 to the mucosal or to the serosal bath induces an increase in cytosolic Ca^{2+} concentration.

Intracellular Ca^{2+} and 'pre-cyclic-AMP' steps

Prostaglandins inhibit the hydroosmotic response to ADH but not to its second messenger, cyclic AMP [25]. It has been also reported that inhibition of prostaglandin liberation potentiates the response to ADH [26,27]. Table I shows that the inhibitory effect of serosal A23187 or quinidine on the oxytocin-induced hydroosmotic response can be prevented by indomethacin. Furthermore, incubation with serosal A23187 or quinidine dramatically increased liberation of prostaglandin E_2 into the serosal bath (Table II), confirming and extending previous observations (Forrest et al. [14]; Erlij et al. [15]). The physiological role of prostaglandin biosynthesis and its regulation by ADH is a complex problem [28,29]. Nevertheless, we have observed an inhibitory effect of serosal A23187 or quinidine on the response to oxytocin but not on the response to 8-bromo-cyclic AMP. We can then reasonably conclude that the actions of serosal A23187 and quinidine on the hydroosmotic response to oxytocin are due to prostaglandin E_2 liberation and are probably mediated by an increase in intracelular Ca^{2+} [13]. A similar mechanism has been proposed by Erlij et al. [15] to explain the action of A23187 on sodium transport in epithelial barriers.

Intracellular Ca^{2+} concentration and 'after cyclic AMP' steps

Two experimental results suggest that the inhibitory action of mucosal A23187 on the response to 8-bromo-cyclic AMP is mediated by an increase in intracellular Ca^{2+} concentration. (1) Mucosal A23187 increased the transepithelial Ca^{2+} permeability. (2) An increase in the apical Ca^{2+} concentration mimicked the effect of A23187 on the 8-bromo-cyclic-AMP-induced hydroosmotic response. Furthermore, it was observed that either an increase or a decrease in mucosal Ca^{2+} specifically inhibited the oxytocin-induced water flux, suggesting the existence of a 'critical' Ca^{2+} concentration to obtain a maximal response.

As in the case of A23187, quinidine has been previously employed to test the role of Ca^{2+} in the response to ADH [12,31]. Besides its inhibitory action on oxytocin, due to liberation of prostaglandins, quinidine had a stimulatory effect on 8-bromo-cyclic AMP that could be followed by an inhibitory one. These results suggest that an increase in Ca^{2+} concentration will first potentiate and then will inhibit the hydroosmotic response. This points again to the existence of a 'critical' Ca^{2+} concentration for a maximal ADH action. In the case of A23187, at least under the conditions here described, the change in intracellular Ca^{2+} would be important and we would drop directly in the inhibitory levels. This could also explain the previously reported potentiation of the hydroosmotic response by A23187 under certain experimental conditions [11].

The inhibitory action of mucosal A23187 on the response to 8-bromo-cyclic AMP was temperature-dependent and reminiscent of the offset of the reaction that follows agonist withdrawal. The effect can then be located before the 'temperature-dependent rate-limiting step' [33,34] that has been identified as the mechanism controlling the plugging-in and removal of water channels [24].

Intracellular Ca^{2+} and intracellular pH

It has been proposed that the inhibitorty actions of cellular acidification on the hydroosmotic response could be mediated by a change in intracellular Ca^{2+} concentration [11,30]. The experiment presented in Fig. 4 clearly shows that the effects of pH and mucosal A23187 can be separated. Previous results have shown that the inhibitory effects of cellular acidification are related in part to an effect on adenylate cyclase, in part to interference with the mechanism of channel insertion and retrieval and in part to changes in the permeability status of the water channel [4,17,19,35]. Results with CO_2 bubbling probably reflect the effects of pH decrease on the water channels that, once inserted in the apical membrane, could switch from an open to a closed configuration [17,19]. The present results suggest that this effect is not mediated by a change in intracellular Ca^{2+} concentration. On the other hand, the slower effects of cellular acidification [17,19] as well as the action of mucosal A23187,

can be reasonable ascribed to a change in intracellular Ca^{2+} and related to the mechanism controlling the channels' insertion and removal.

In summary, we can conclude that A23187 and quinidine affect either 'pre-' or 'post-cyclic-AMP' steps. The action located before cyclic AMP production is an inhibitory one, mediated by liberation of prostaglandins. The action located after cyclic AMP production inhibits or potentiates the hydroosmotic response. This probably depends on the magnitude of the change in the intracellular Ca^{2+} concentration.

References

1 Handler, J.S., Butcher, R.W., Sutherland, E.W. and Orloff, J. (1965) J. Biol. Chem. 240, 4524–4526
2 Chevalier, J., Bourguet, J. and Hugon, J.S. (1974) Cell Tiss. Res. 152, 129–140
3 Kachadorian, W.A., Wade, J.B. and DiScala, V.A. (1975) Science 190, 67–69
4 Parisi, M., Bourguet, J., Ripoche, P. and Chevalier, J. (1979) Biochim. Biophys. Acta 556, 509–523
5 Parisi, M. and Bourguet, J. (1983) J. Membrane Biol. 71, 189–193
6 Muller, J., Kachadorian, W.A. and DiSacala, V.A. (1980) J. Cell. Biol. 85, 85–95
7 Wade, J.B., Stetson, D.L. and Lewis, S.A. (1981) Ann. N.Y. Acad. Sci. 372, 106–117
8 Taylor, A., Mamelak, M., Reaven, N. and Maffly, R. (1973) Science 181, 347–350
9 Parisi, M., Pisam, M., Merot, J., Chevalier, J. and Bourguet, J. (1985) Biochim. Biophys. Acta 817, 333–342
10 Pearl, M. and Taylor, A. (1985) Biol. Cell 55, 163–172
11 Hardy, M.A. (1978) J. Biol. Chem. 240, 4524–4526
12 Taylor, A., Eich, E., Pearl, M. and Boem, A. (1979) INSERM Symposium Series 85, 167–174
13 Yorio, T., Sheryl, L.H., Hodges, D.H. and Caffrey, J.L. (1983) Biochem. Physiol. 32, 1113–1118
14 Forrest, J.N., Scheider, J.C. and Goodman, D.B.P. (1982) J. Clin. Invest. 69, 499–506
15 Erlij, D., Gersten, L., Stebba, G. and Schoen, H.F. (1986) Am. J. Physiol. 250, C629–C636
16 Bourguet, J. and Jard, S. (1964) Biochim. Biophys. Acta 88, 442–444
17 Parisi, M., Montoreano, R., Chevalier, J. and Bourguet, J. (1981) Biochim. Biophys. Acta 648, 267–274
18 Parisi, M. and Piccinni, Z. (1973) J. Membrane Biol. 12, 227–246
19 Parisi, M., Lacaz-Vieira, F., Wietzerbin, J. and Bourguet, J. (1984) Braz. J. Med. Biol. Res. 17, 341–347
20 Ripoche, P. (1967) Mémoire pour le Diplome d'Etudes Superieurs de Sciences Naturelles. Université de Paris.
21 Parisi, M., Chevalier, J. and Bourguet, J. (1979) Am. J. Physiol. 237, F483–F489
22 Parisi, M. and Bourguet, J. (1984) Am. J. Physiol. 246, C157–C159
23 Parisi, M., Ripoche, P., Prevost, G. and Bourguet, J. (1981) Proc. N.Y. Acad. Sci. 372, 144–158
24 Chevalier, J., Parisi, M. and Bourguet, J. (1983) Cell Tiss. Res. 228, 345–555
25 Orloff, J., Handler, J.S. and Bergstrom, S. (1965) Nature (LOndon) 205, 397
26 Flores, A. and Sharp, G.W.G. (1972) Am. J. Physiol. 233, 1392–1398
27 Parisi, M. and Piccinni, Z. (1972) Biochim. Biophys. Acta 279, 209–212
28 Orloff, J. and Zusman, R. (1978) J. Membrane Biol. 40, 297–304
29 Schlondorff, D. and Satriano, J.D. (1985) Am. J. Physiol. 248, F454–F458
30 Taylor, A. and Windhager, E.E. (1979) Am. J. Physiol. 236, F507–F512
31 Arruda, J.A.L. and Sabatini, S. (1980) J. Membrane Biol. 55, 141–147
32 Taylor, A., Pearl, M. and Crutch, D. (1985) Mol. Physiol. 8, 43–58
33 Bourguet, J. (1966) J. Physiol. (Paris) 58, 476–476
34 Bourguet, J. (1968) Biochim. Biophys. Acta 150, 104–112
35 Parisi, M., Wietzerbin, J. and Bourguet, J. (1983) Am. J. Physiol. 244, F712–F718