J. Membrane Biol. 116, 57–64 (1990)

The Journal of
Membrane Biology

Effects of PCMBS on the Water and Small Solute Permeabilities in Frog Urinary Bladder

Cristina Ibarra, Pierre Ripoche, Mario Parisi,† and Jacques Bourguet

Biomembranes, Service de Biologie Cellulaire, Départment de Biologie, Centre d'Etudes Nucléaires de Saclay-91191 Gif-Sur-Yvette, France, †Seccion de Biomembranas, Departamento de Fisiologia, Facultad de Medicina, Universidad de Buenos Aires, Argentina

Summary. It has been reported that PCMBS (*p*-chloromercuribenzene sulfonate) blocks the water permeability of red cells and of the tubular kidney membranes. In this study we compare the effects of this mercurial compound on the permeability of water and other small solutes in the frog urinary bladder.

We observed that: (*i*) 5 mM PCMBS applied at pH 5.0 to the mucosal side inhibited the net and unidirectional water fluxes induced by oxytocin without changing the $\Delta P_f/\Delta P_d$ ratio. (*ii*) The oxytocin-induced urea and Na^+ influxes were also inhibited by PCMBS. (*iii*) The unidirectional Cl^- movement was first reduced and then increased during the course of PCMBS treatment. (*iv*) The short-circuit measured at low mucosal Na^+ concentration (10 mM), diminished continuously, whereas the transepithelial resistance first increased and then diminished. (*v*) Mannitol, raffinose, α-methyl-glucose, antipyrine, caffeine and Rb^+ movements were not changed significantly during the first 26 min of the water permeability inhibition. In conclusion: (*i*) The ADH-sensitive water, urea and Na^+ transport systems were inhibited by PCMBS, (*ii*) PCMBS did not induce a nonspecific and general effect on the permeability of the membrane during the development of the water permeability inhibition, and (*iii*) in terms of water channels, the inhibition of water transport with the maintenance of a high P_f/P_d ratio suggests that PCMBS closes the water channels in an all or none manner, reducing their operative number in the apical border of frog bladder.

Key Words water transport · PCMBS · amphibian urinary bladder · oxytocin

Introduction

Antidiuretic hormone (ADH) increases the permeability of the apical membrane of amphibian urinary bladder epithelial cells to water (*see* Handler, 1988; Bourguet et al., 1989). The hormone also stimulates the transepithelial active sodium transport and increases the permeability of the bladder to urea and other solutes (Leaf & Hays, 1962). The increase in water permeability is thought to involve the insertion of water channels in the apical membrane. Morphological studies have indicated that water channels are contained within particle aggregates stored in vesicular structures underlying the apical membrane (Chevalier, Bourguet & Hugon, 1974; Kachadorian, Wade & DiScala, 1975). According to this hypothesis, the hormonal action, mediated by cAMP, induces the fusion of vesicles with the membrane, allowing the transfer of the aqueous channels to the luminal membrane (Harris & Handler, 1988).

Although water permeability seems to be insensitive to most transport reagents, it is reduced in certain conditions by mercurial sulfhydryl reagents such PCMBS (*p*-chloromercuribenzene sulfonate). Macey, Karan and Farmer (1972) found that in red cells micromolar concentrations of PCMBS decrease water permeability while increasing the activation energy (E_a) to water transfer. In this situation the ratio between the osmotic permeability (P_f) and the diffusional permeability (P_d) for water across the membrane decreased and became no different from one. Whittembury et al. (1984); Pratz, Ripoche and Corman (1986); Verkman and Wong (1987) and Whittembury, Carpi-Medina and Gonzalez (1987) have also reported inhibitory effects of PCMBS on water transport concomitant with an increase of E_a and decrease of P_f/P_d ratio in kidney proximal tubules.

We have already reported (Ibarra, Ripoche & Bourguet, 1989) a PCMBS inhibition of water movement through frog bladder stimulated by oxytocin (OXY). This inhibition was accompanied by an augmentation of the aggregate density in the apical membrane. Our findings closely parallel those reported by Hoch et al. (1989) in toad bladder and confirm the hypothesis that PCMBS directly blocks the ADH-induced water channels in frog bladder.

The present study was focused on the effect of PCMBS on osmotic and diffusional water permeabilities (ratio P_f/P_d) and on the transepithelial urea, Na^+, Cl^-, K^+ and other small solute movements.

We found that PCMBS present in the mucosal bath of the oxytocin-stimulated bladder inhibits water permeability while the $\Delta P_f/\Delta P_d$ ratio remains unchanged. The inhibitory action of PCMBS extends to urea and Na^+ permeabilities to different time courses with respect to water. Our studies suggest that PCMBS reacts with the apical sulfhydryl groups associated with the ADH-sensitive water, urea and Na^+ transport systems and, in different ways, modifies their function. For the water channels, this mercurial compound would appear to reduce the number of operative units in the apical membrane.

Materials and Methods

Frogs (*Rana esculenta*) were kept at 20°C in running tap water for at least five days before the experiments. Urinary bladders were removed and mounted horizontally on a nylon mesh between two Lucite chambers, the mucosal border facing downwards. The exposed bladder area was 3.1 cm^2. The serosal bath was a saline solution (2 ml) containing (in mM): NaCl 112; KCl 5; $CaCl_2$ 1; $NaHCO_3$ 2.5; at pH 8.1 when aerated. The mucosal bath (12 ml) was a MES(2(N-morpholino)ethanesulfonic acid) (20 mM)-Tris (Tris hydroxymethyl aminomethane) buffer at pH 5.0.

Diffusional permeability coefficients of electrolytes and nonelectrolytes were determined as previously described (Leaf & Hays, 1962). Briefly, radiotracers were added to the mucosal medium up to a final concentration of 1 μCi/ml. The serosal bath solution was then completely removed at 2-min intervals and replaced with fresh unlabeled solutions. Unidirectional fluxes (J_{ms}) were estimated from the rate of isotope appearance in the serosal medium. The permeability coefficients (P, cm/sec) were calculated from the J_{ms} and the applied concentration in the mucosal medium. Diffusional water permeability coefficient (P_d), obtained from unidirectional water fluxes, was corrected for unstirred layer effect as in previous studies (Parisi & Bourguet, 1983). The net water flux (J_w) was recorded minute by minute as described by Bourguet and Jard (1964). The osmotic permeability coefficient (P_f) was calculated from J_w and the applied osmotic gradient.

In short-circuit current (I_{sc}) experiments, bladders were mounted vertically between two Lucite chambers. The serosal solution contained, in addition to the previously mentioned components, 10 mM glucose and the mucosal bath contained MES-Tris buffer at pH 5.0 with 10 mM NaCl. The bladders were aerated continuously. Transepithelial electrical potentials (V_t) and short-circuit current were measured by the usual techniques (Spooner & Edelman, 1976). To evaluate the time course evolution of I_{sc}, the ratio between each recorded I_{sc} and the I_{sc} value recorded at the beginning of the experiment ($I_{sc}(t)/I_{sc}(0)$) was calculated for each bladder. Transepithelial total resistance (R_t) was calculated as the ratio of open circuit V_t to I_{sc}. To analyze R_t changes, $R_t(t)/R_t(0)$ were also calculated.

Radioactive chemicals used in this study were obtained as follows: ^3HOH, ^{14}C-urea and ^{36}Cl from CEN-Saclay, France; ^{14}C-mannitol, ^{14}C-α-methyl glucose, ^{14}C-caffeine, ^{14}C-antipyrine and ^{22}Na from Amersham, UK; ^{14}C-raffinose from NEN, FRG.

PCMBS (*p*-chloromercuribenzene sulfonate) was obtained from Sigma, St. Louis, MO. Oxytocin (Sandoz, Switzerland) was employed as an antidiuretic hormone analog.

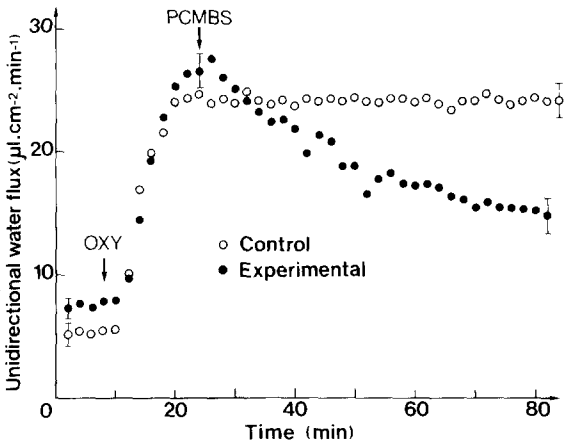

Fig. 1. Effect of PCMBS on the unidirectional water flux stimulated by oxytocin (2.2×10^{-8} M). PCMBS (5 mM) was added to the mucosal bath of one hemibladder at the peak of the oxytocin response (●), the other hemibladder was used as control (only MES-Tris buffer at pH 5.0 in the mucosal bath) (○). Each curve is the mean of seven experiments and the vertical bars correspond to the standard error of means (SEM)

Results

Inhibition of Unidirectional Water Fluxes by PCMBS

Unidirectional water fluxes (J_{ms}) were measured in oxytocin (2.2×10^{-8} M) stimulated bladders bathed with MES-Tris buffer pH 5.0 on the mucosal side. In a previous paper (Ibarra et al., 1989) we reported that MES-Tris buffer pH 5.0 in the mucosal bath did not modify either water permeability or the hydrosmotic response to ADH. MES-Tris buffer at pH 5.0 was employed for two reasons: (*i*) PCMBS inhibitory effects are only observed at low pH, and (*ii*) the use of a nonpermeant buffer in the mucosal bath prevents cell acidification from having an inhibitory action on the response to ADH as previously described (Parisi, Wietzerbin & Bouguet, 1983). The effects of 5 mM mucosal PCMBS are shown in Fig. 1 (mean curve of seven experiments). Before PCMBS treatment, the J_{ms} values measured in the presence of oxytocin, were similar to those previously reported when Ringer solutions (pH 8.1) bathed both sides of the hormone-stimulated bladders (Parisi et al., 1979). In the presence of PCMBS, a progressive inhibition of J_{ms} was observed. Since the same degree of inhibition was found when the effects of PCMBS on the net water flux were measured in the same experimental conditions (Fig. 3 in Ibarra et al., 1989), it was interesting to determine both unidirectional and net water fluxes in the same bladder at different times before and after PCMBS

Table 1. $\Delta P_f/\Delta P_d$ values at different times before and after PCMBS addition[a]

	$\Delta P_f/\Delta P_d$		
t (min)	Before PCMBS	t (min)	After PCMBS
0		18	10.9
2	14.0	22	19.7
4	12.3	26	18.0
6	21.7	30	13.2
8	18.9	34	18.5
10	18.8	38	12.4
12	13.5	42	11.5
14	9.8	46	15.6
16	9.7	50	11.2
		54	12.1
		58	11.0
$\bar{X} \pm$ SEM:	14.8 ± 1.5		14.0 ± 0.7

[a] Mean of three experiments.
At $t = 0$, oxytocin (2.2×10^{-8} M) was added in the serosal bath. After 16 min of hormonal stimulation, 5 mM PCMBS was added in the mucosal bath.

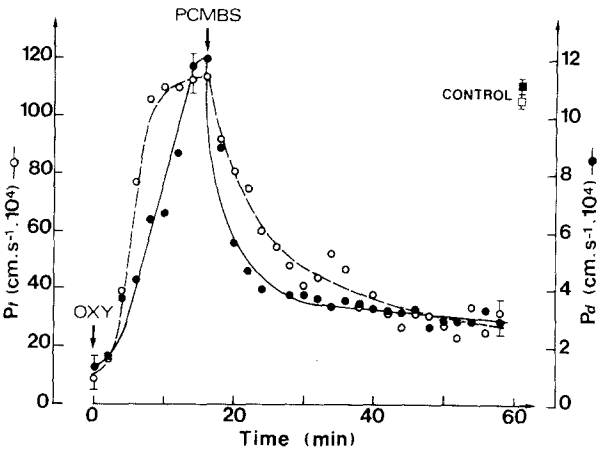

Fig. 2. Inhibition of the osmotic (P_f) and diffusional (P_d) water permeability by PCMBS. Two fragments of the same bladder were bathed with Ringer solution, pH 8.1 (serosal side) and MES-Tris solution, pH 5.0 (mucosal side). Unidirectional and net water fluxes were simultaneously measured in each fragment and the diffusional (●) and osmotic (○) permeabilities were calculated. At the maximal hydrosmotic response to oxytocin, 5 mM PCMBS was mucosally added to one fragment (circles) whereas the other was maintained without PCMBS (control, squares). Each curve is the mean of three experiments

treatment, to compare the evolutions of the corresponding diffusional and osmotic permeabilities during PCMBS action.

Effect of PCMBS on the $\Delta P_f/\Delta P_d$ Ratio

Unidirectional and net water fluxes were simultaneously measured in three bladders stimulated by oxytocin, before and after addition of PCMBS to the mucosal bath. Diffusional permeability (P_d) and osmotic permeability (P_f) coefficients were calculated from the corresponding unidirectional and net water fluxes. The results obtained are shown in Fig. 2. PCMBS (5 mM) significantly decreased both permeabilities with similar time evolutions. There was a 50% inhibition of diffusional permeability after 6 min of PCMBS incubation and of osmotic permeability after 10 min incubation. It has been suggested that the $\Delta P_f/\Delta P_d$ ratio indicates the physical state of the water permeation pathway; it is therefore important to ascertain whether this ratio is modified by the presence of the inhibitor. At various intervals before and after PCMBS treatment therefore, ΔP_f and ΔP_d (ΔP_f and ΔP_d being the differences between the permeability values at a given time after oxytocin stimulation and at zero time) were calculated and compared. The results are shown in Table 1. In the presence of PCMBS, the $\Delta P_f/\Delta P_d$ ratio maintained approximately constant high values (range: 10–20), similar to those obtained before PCMBS addition.

Effect of PCMBS on the Unidirectional Water Movement in Fixed Urinary Bladders

Bladders exposed on the serosal and mucosal surfaces, to 2.5% glutaraldehyde for 20 min after oxytocin challenge remain highly permeable (Jard et al., 1966; Eggena, 1972). This phenomenon is illustrated in Fig. 3: bladders fixed in the presence of oxytocin, remained permeable to water even in the absence of hormone. After fixation, the bladders were carefully rinsed and the serosal and mucosal sides bathed with Ringer solution at pH 8.1 and MES-Tris solution at pH 5.0, respectively.

Net and unidirectional water fluxes were simultaneously measured before and after adding 5 mM PCMBS to the mucosal bath. It was observed that in these conditions PCMBS failed to depress either net or unidirectional water fluxes (Fig. 3).

Effect of PCMBS on Unidirectional Mucosal to Serosal Urea Movement

It is well known that the presence of antidiuretic hormone stimulates urea movement across the urinary bladder (Leaf & Hays, 1962; Parisi & Candia, 1977). When 5 mM PCMBS was added to the mucosal bath 40 min after oxytocin stimulation, the urea

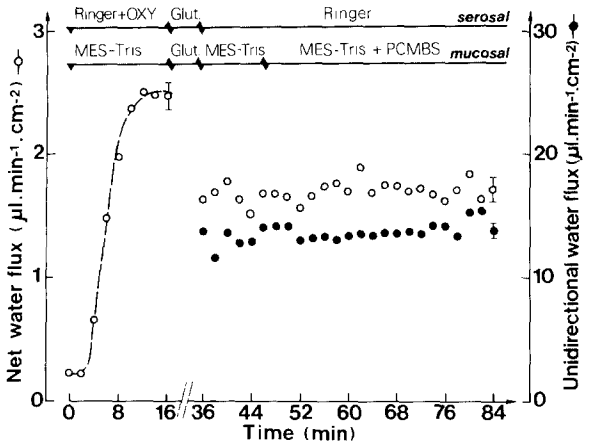

Fig. 3. Influence of fixation on PCMBS inhibition. Three bladders were fixed by 2.5% glutaraldehyde (20 min, mucosal and serosal sides) following exposure to oxytocin (2.2×10^{-8} M). After stabilization of the fluxes, 5 mM PCMBS was added on the mucosal side. Net (○) and unidirectional (●) water fluxes were measured

permeability (P_{urea}) dropped significantly to approximately 50% of the control value in about 7.5 min (Fig. 4*b*). This inhibition was greater and faster than that observed in the case of water (Table 2). Inhibitory effects on the P_{urea} were also observed at PCMBS concentrations 10 times more dilute (Fig. 4*a*) but in these conditions there was no significant effect of PCMBS on the oxytocin-induced water permeability in these bladders (Table 2). An interesting observation was the difference in the time courses of the hormonal responses of P_d and P_{urea}. In the case of water permeability, we confirmed the sigmoidal curve previously reported (Parisi et al., 1979). The half time of the response to oxytocin was 5.8 ± 0.3 min (Fig. 4). For P_{urea} however, measured in the same bladders, we recorded the same sigmoidal curve but starting significantly later (half time of the oxytocin response 18.1 ± 0.4 min, $n = 7$) (Fig. 4).

Effects of PCMBS on Ionic Movements

At the same time as inhibiting the water and urea permeabilities PCMBS might also alter the ionic transfer across urinary bladders. To test this possibility, we studied the short circuit current (I_{sc}), transepithelial resistance (R_t) and Na^+, Cl^-, and Rb^+ permeabilities in the presence and absence of PCMBS.

Effect on the I_{sc} and R_t

Figure 5 shows the mean values for I_{sc} and R_t in three experiments in which 5 mM PCMBS was added to the mucosal bath (pH 5.0) of oxytocin-stimulated bladders. The addition of the mercurial compound followed an oxytocin stimulation of 12 min. The effects of PCMBS on I_{sc} and R_t are expressed in terms of the time-normalized ratios $I_{sc}(t)/I_{sc}(0)$ and $R_t(t)/R_t(0)$. A gradual decline of $I_{sc}(t)/I_{sc}(0)$ ratio was observed, it reached negative values about 30 min after the beginning of PCMBS treatment. The diminution of I_{sc} was accompanied for approximately the first 10 min by an increase of the $R_t(t)/R_t(0)$ ratio, after which R_t decreased progressively to 40% of the control values 30 min later.

Table 2. Comparison of the percentage and half time PCMBS inhibition between water and urea permeabilities

		PCMBS 0.5 mM		PCMBS 5 mM	
	n	% inhibition	$t_{1/2}$ (min)	% inhibition	$t_{1/2}$ (min)
Water	3	7 ± 2	ND	41 ± 2	13.5 ± 0.4
Urea	3	47 ± 5	8.5 ± 0.7	73 ± 2	7.5 ± 0.5

% of inhibition of water and urea permeability after 30 min of treatment by PCMBS.

Effect on the Unidirectional Na^+ Movement

To test whether the PCMBS effects on I_{sc} and R_t are related to Na^+ transport, the Na^+ unidirectional fluxes were determined with 10 mM Na^+ in the mucosal compartment. After a 10-min equilibration period, the bladders were exposed to oxytocin for 10 min. Thereafter 5 mM PCMBS was added to the mucosal bath for 60 min. Fig. 6 shows the mean curve of four experiments. As expected, there was a significant increase of Na^+ transfer after oxytocin stimulation. This increase was totally abolished 4 min after the PCMBS treatment, after which the P_{Na} values remained approximately constant to the end of the experiments.

Effect on the Unidirectional Cl^- Movement

To determine whether PCMBS altered the transepithelial Cl^- movement across the bladder, unidirectional Cl^- fluxes from mucosal to serosal baths were measured before and after PCMBS treatment. The results are expressed in terms of Cl^- permeability (P_{Cl}) and the mean curve for 10 experiments is shown in Fig. 6. After oxytocin addition, Cl^- unidirectional fluxes had a tendency to increase but this was not significant. When PCMBS was added to the mucosal bath, two significant effects were observed: an initial diminution of the P_{Cl} values (by 50% in about 6 min) and then a gradual increase

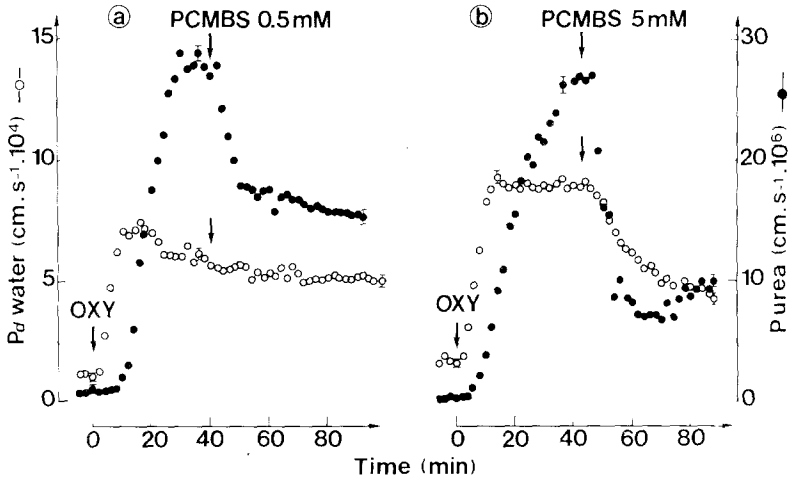

Fig. 4. Comparison of the effects of PCMBS on the water and urea permeabilities. Water (open symbols) and urea (filled symbols) permeabilities were simultaneously determined in paired oxytocin-stimulated hemibladders with PCMBS. Means of three experiments ± SEM for each curve

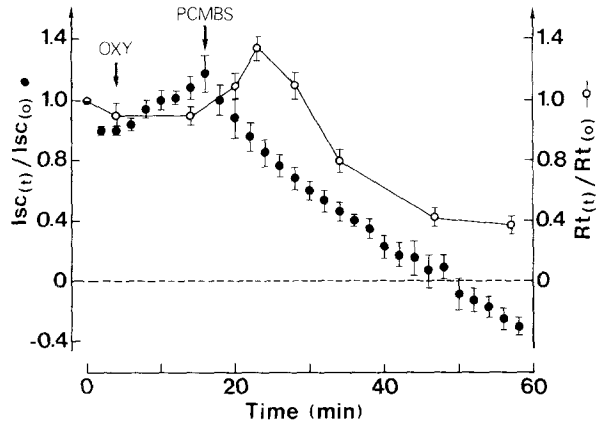

Fig. 5. Time course of the effect of PCMBS on transepithelial resistance (○) and short-circuit current (filled circles). PCMBS (5 mM) was added to the mucosal bath after 12 min of oxytocin stimulation. The resistance was calculated as the ratio of the spontaneous V_t/I_{sc}. Mean curve of three experiments

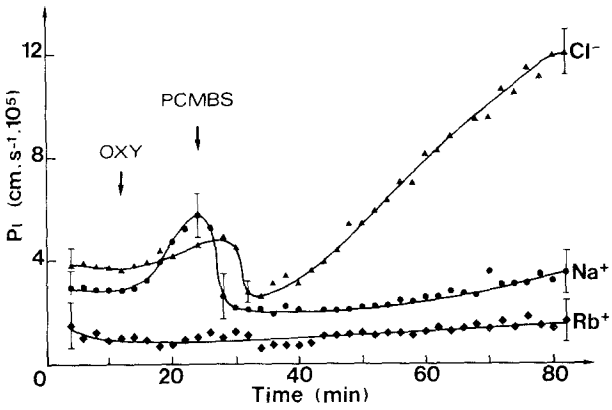

Fig. 6. Effects of PCMBS on the ionic permeabilities (P_l) in the presence of oxytocin. Unidirectional ionic fluxes were measured in two fragments of the same bladder. After 12 min of incubation by oxytocin (2.2×10^{-8} M), 5 mM PCMBS was added in the mucosal bath of the experimental fragment. Mean curve represents 10, 4 and 3 experiments, respectively, for Cl^-, Na^+, and Rb^+. Vertical bars are SEM

reaching three times the resting values 60 min after the beginning of PCMBS treatment.

Effect on the Unidirectional Rb^+ Movement

Unidirectional K^+ movement across the frog bladder estimated from the transfer of Rb^+ as a radioactive label, was also studied in the presence and absence of 5 mM PCMBS. The results are plotted as a function of time in Fig. 6. No differences in the Rb^+ permeability (P_{Rb}) were observed before and after 60 min of PCMBS treatment.

Concomitant measurements of unidirectional water fluxes in the chamber in which the permeability of Na^+, Rb^+, or Cl^- were studied, showed the usual inhibition in the presence of PCMBS.

Effect of PCMBS on the Diffusion of Hydrophilic and Lipophilic Solutes

Hydrophilic Solutes

The effects of 5 mM PCMBS on the movement of hydrophilic solutes were determined by the same protocol as that used in the study of water movements. With none of the solutes studied was the permeability increased by oxytocin (Fig. 7). Moreover, there was no inhibition of the raffinose, mannitol and α-methyl-glucose permeabilities (P_s) after PCMBS addition. Indeed, a small but consistent increase in the transfer of these solutes in untreated bladders was found in all cases. These increases became significant after 26 min of PCMBS incuba-

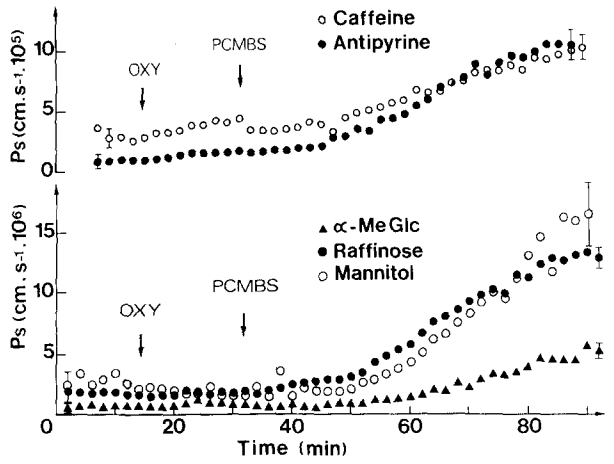

Fig. 7. Effect of PCMBS on the diffusional permeability coefficient of hydrophilic and hydrophobic solutes. Unidirectional mucosal to serosal fluxes were determined in bladders stimulated by oxytocin (2.2×10^{-8} M) before and after 5 mM PCMBS added in the mucosal bath. Each curve is the mean of the following bladders: three for caffeine, three for antipyrine, three for α-methyl-glucose, 13 for mannitol and nine for raffinose. Vertical bars correspond to SEM

tion for the raffinose (nine experiments) and mannitol (13 experiments) and after 44 min incubation for α-methyl-glucose (three experiments) (Fig. 7).

Lipophilic Solutes

The diffusion of lipophilic solutes across the bladder was determined by the same method as hydrophilic solutes. Caffeine and antipyrine were chosen as lipophilic solutes since their oil/water partition coefficients are greater than 1×10^{-3}. For these solutes, oxytocin produced a small increase of their permeability values (Pietras & Wright, 1974). After addition of PCMBS, a gradual increase of both permeabilities, only significant after 60 min of treatment was observed (Fig. 7, mean curve of three experiments for each solute).

In both series of experiments, unidirectional water fluxes determined simultaneously in the same chambers showed the previously described inhibition by PCMBS.

Discussion

Previous studies on the amphibian urinary bladder have shown that PCMBS decreases the water osmotic flux induced by oxytocin while maintaining the intramembrane particle aggregates (IMA) on the surface of the apical membrane (Hoch et al., 1989; Ibarra et al., 1989). In the present study we have characterized the effects of this mercurial agent on the water pathway.

Strong evidences indicate that in ADH-sensitive epithelia, the hormone induces the appearance of a new water pathway in the apical membrane. After ADH action, both, osmotic (P_f) and diffusional (P_d) permeabilities increased and P_f/P_d ratio became significantly higher ($11 < P_f/P_d < 18$) than in absence of the hormone ($P_f/P_d = 1$) (Parisi & Bourguet, 1983; Levine, Jacoby & Finkelstein, 1984). It has been suggested that this ratio would indicate the functional state of the water channels present in the apical membrane of ADH-stimulated cells. A P_f/P_d ratio much more than one has also been found in red cells (Moura et al., 1984) and kidney proximal tubules (Verkman & Wong, 1987), even though the experimental approach was completely different, supporting the hypothesis of the existence of water channels in these preparations.

When PCMBS was applied to the mucosal side of oxytocin-stimulated bladders, this mercurial compound inhibited the increase of the diffusional (P_d) and osmotic (P_f) water permeabilities induced by the hormone while the $\Delta P_f/\Delta P_d$ ratio remained constant between 10 to 20. Similar inhibition has been reported in the red cells (Macey, 1984; Moura et al., 1984). In these cells, P_f and P_d were proportionally reduced whereas the activation energy for water transfer increased up to the values observed in nonporous lipid membranes (*see* Finkelstein, 1987). These authors advanced the hypothesis that PCMBS closes down the water pores present in the cell membrane in an all or none manner. Accepting that PCMBS does not alter the lipid bilayers (*see below*), our results in frog bladder confirm this hypothesis.

In order to analyze the specificity of the PCMBS inhibition on the water permeability, we have examined the PCMBS influence on the other transport systems.

PCMBS also inhibits the urea transfer but with a time and concentration dependence different from those observed in the case of water. Although water and urea transports are strongly stimulated by oxytocin in amphibian bladder, certain evidence indicates that urea crosses the membrane by special facilitated diffusion pathways and that it is not transported by water channels (Levine, Franki & Hays, 1973; Parisi & Candia, 1977; Eggena, 1983). In our studies, PCMBS inhibits urea movement much more rapidly and at a much lower concentration than it does water movement, suggesting that the two pathways are distinct. Thus, the fact that PCMBS inhibits both transport systems would appear to be related to the high reactivity of organic mercurials with sulfhydryl groups and their pres-

ence in various transport systems. In red cells, similar effects of PCMBS on both water and urea permeabilities have been reported (Macey & Farmer, 1970; Solomon et al., 1983).

For Na^+ transfer, however, our results are different from those found with erythrocytes (Rega, Rothstein & Weed, 1967; Grinstein & Rothstein, 1978). We found that the increase in the Na^+ influx induced by oxytocin (Frazier, Dempsey & Leaf, 1962) was inhibited 4 min after the addition of PCMBS to the mucosal bath. During this period, a decrease in the I_{sc} and an increase in the R_t was recorded. Earlier studies in amphibian tight epithelia showed a stimulation of apical Na^+ permeability with 10 to 50 times lesser PCMBS concentration. These effects were attributed either to specific modifications of luminal sulfhydryl groups associated with Na^+ transport, in the toad bladder (Spooner & Edelman, 1976) or to increases of conducting Na^+ channels by Na^+ self-inhibition removal, in the frog skin (Li & Lindemann, 1983). In our preparations, 5 mM PCMBS at pH 5.0 produces an inhibition more than a stimulation of the Na^+ permeability. A possible explanation is that PCMBS at this concentration may directly and deeply alterate the Na^+ channels structure. Nevertheless, it is not clear whether these alterations also affect the Na^+ pumps located in the basolateral cell membrane. The fact that PCMBS penetrates very poorly and slowly into the cell (Frenkel, Ekblad & Edelman, 1975) and that it totally inhibits the hormone-stimulated Na^+ transfer in 4 min, point to an action of the drug on the luminal Na^+ channel.

An unexpected finding in these studies was the significant effects of PCMBS on the Cl^- permeability. There was a significant diminution in Cl^- transfer during the first 6 min after PCMBS addition, followed by a gradual increase in this parameter. Cl^- influxes were not measured under short-circuit conditions. Thus, the initial reduction in the Cl^- transfer induced by PCMBS would probably well reflect the inhibitory action of PCMBS on Na^+ transport. In addition to this indirect effect, our results suggest that PCMBS increases the paracellular Cl^- pathway described by Civan and DiBona (1977) as the principal route for chloride in toad bladder. Moreover, the PCMBS effect appears to be specific for anions, since Rb^+ and Na^+ permeabilities remained constant during the significant increase in the Cl^- movement. Since a Cl^- conductance has been found in the apical membrane of amphibian epithelial cells (Navarte & Finn, 1980; Nelson, Tang & Palmer, 1984) an effect of PCMBS on the transcellular Cl^- pathway, possibly across mitochondria-rich cells, cannot be excluded.

The movements of a number of nonelectrolytes in addition to urea were also affected by PCMBS, but in all cases there was an increase rather than a reduction in the permeability coefficients. Furthermore these effects became significant within 25–45 min after the beginning of PCMBS treatment. The tested molecules were hydrophilic (raffinose, mannitol and α-methyl-glucose) or lipophilic (caffeine and antipyrine). These nonelectrolytes are recognized as probes of membrane structure (Pietras & Wright, 1974). The fact that PCMBS did not modify their permeabilities during the first 26 min of treatment indicates that the hydrophilic and lipophilic selectivity of the membrane remained virtually unchanged during this period of time.

In conclusion, the present results show that PCMBS dramatically inhibits the osmotic and diffusional water permeabilities but has no effect on the $\Delta P_f/\Delta P_d$ ratio. These findings, together with the persistence of the IMA in the apical membrane (Ibarra et al., 1989), suggest that this mercurial compound closes the water channels in an all or nothing manner. PCMBS also inhibits other specific transport systems such as urea and Na^+. Although the time course of inhibition was different from that of water permeability, all these actions were observed during the first 26 min after PCMBS addition. After this time, the general permeability properties were altered, as it is shown by the hydrophilic and lipophilic permeability studies.

We wish to thank Mrs. Michelle Lucarain for drawing and preparing the manuscript and Mrs. Bethy Maetz for improvements to the text.

References

Bourguet, J., Chevalier, J., Parisi, M., Ripoche, P. 1989. Water permeability of amphibian urinary bladder. *In:* Water Transport in Biological Membranes. From Cells to Multicellular Barrier Systems. G. Benga, editor. Vol. 2, pp. 169–196. CRC Press, Boca Raton (FL)

Bourguet, J., Jard, S. 1964. Un dispositif automatique de mesure et d'enregistrement du flux net d'eau à travers la peau et la vessie des amphibiens. *Biochim. Biophys. Acta* **88:**442–444

Chevalier, J., Bourguet, J., Hugon, J. 1974. Membrane associate particles: Distribution in frog urinary bladder epithelium at rest and after oxytocin treatment. *Cell Tissue Res.* **152:**129–140

Civan, M.M., DiBona, D.R. 1977. Pathways for movement of ions and water across toad urinary bladder. *J. Membrane Biol.* **38:**359–386

Eggena, P. 1972. Osmotic regulation of toad bladder responsiveness to neurohypophyseal hormones. *J. Gen. Physiol.* **60:**665–678

Eggena, P. 1983. Selective fixation with glutaraldehyde of ADH-induced urea permeability sites in toad bladder. *Proc. Soc. Exp. Biol. Med.* **173:**244–251

Finkelstein, A. 1987. The red cell membrane. *In:* Water Movement Through Lipid Bilayers, Pores, and Plasma Mem-

branes. Theory and Reality. pp. 166–183. John Wiley & Sons, New York

Frazier, H.S., Dempsey, E.F., Leaf, A. 1962. Movement of sodium across the mucosal surface of the isolated toad bladder and its modification by vasopressin. *J. Gen. Physiol.* **45:**529–543

Frenkel, A., Ekblad, E.B.M., Edelman, I.S. 1975. Effects of sulfhydryl reagents on basal and vasopressin-stimulated Na^+ transport in the toad bladder. *In:* Biomembranes. H. Eisenberg, E. Katchalski-Katzir, and L.A. Manson, editors. Vol. 7, pp. 61–80. Plenum, New York

Handler, J. S. 1988. Antidiuretic hormone moves membranes. *Am. J. Physiol.* **255:**F375–F382

Grinstein, S., Rothstein, A. 1978. Chemically-induced cation permeability in red cell membrane vesicles. *Biochim. Biophys. Acta* **508:**236–245

Harris, H.W., Jr., Handler, J.S. 1988. The role of membrane turnover in the water permeability response to antidiuretic hormone. *J. Membrane Biol.* **103:**207–216

Hoch, B.S., Gorfien, P.C., Linzer, D., Fusco, M.J., Levine, S.D. 1989. Mercurial reagents inhibit flow through ADH-induced water channels in toad bladder. *Am. J. Physiol.* **256:**F948–F953

Ibarra, C., Ripoche, P., Bourguet, J. 1989. Effect of mercurial compounds on net water transport and intramembrane particle aggregates in ADH-treated frog urinary bladder. *J. Membrane Biol.* **110:**115–126

Jard, S., Bourguet, J., Carasso, N., Favard, P. 1966. Actions de divers fixateurs sur la perméabilité et l'ultrastructure de la vessie de grenouille. *J. Microsc.* **5:**31–36

Kachadorian, W., Wade, J., DiScala, V. 1975. Vasopressin-induced structural change in toad bladder luminal membrane. *Science* **190:**67–69

Leaf, A., Hays, R.M. 1962. Permeability of the isolated toad bladder to solutes and its modification by vasopressin. *J. Gen. Physiol.* **45:**921–932

Levine, S., Franki, N., Hays, R.M. 1973. Effect of phloretin on water and solute movement in the toad bladder. *J. Clin. Invest.* **52:**1435–1442

Levine, S., Jacoby, M., Finkelstein, A. 1984. The water permeability of toad urinary bladder. *J. Gen. Physiol.* **83:**543–561

Li, J., Lindemann, B. 1983. Chemical stimulation of Na^+ transport through amiloride-blockable channels of frog skin epithelium. *J. Membrane Biol.* **75:**179–192

Macey, R.I. 1984. Transport of water and urea in red blood cells. *Am. J. Physiol.* **246:**C195–C203

Macey, R.I., Farmer, R.E.L. 1970. Inhibition of water and solute permeability in human red cells. *Biochim. Biophys. Acta* **211:**104–106

Macey, R.I., Karan, D.M., Farmer, R.E.L. 1972. Properties of water channels in human red cells. *In:* Biomembranes. Vol. 3: Passive Permeability of cell membranes. F. Krenzer and J.F.G. Slegers, editors. pp. 331–340. Plenum, New York

Moura, T.F., Macey, R.I., Chien, D.Y., Karan, D., Santos, H. 1984. Thermodynamics of all-or-none water channel closure in red cells. *J. Membrane Biol.* **81:**105–111

Navarte, J., Finn, A.L. 1980. Anion-sensitive sodium conductance in the apical membrane of toad urinary bladder. *J. Gen. Physiol.* **76:**69–81

Nelson, D.J., Tang, J.M., Palmer, L.G. 1984. Single-channel recordings of the apical membrane chloride conductance in A6 epithelial cells. *J. Membrane Biol.* **80:**81–89

Parisi, M., Bourguet, J. 1983. The single-file hypothesis and water channels induced by antidiuretic hormone. *J. Membrane Biol.* **71:**189–193

Parisi, M., Bourguet, J., Ripoche, P., Chevalier, J. 1979. Simultaneous minute by minute determination of unidirectional and net water fluxes in frog urinary bladder. *Biochim. Biophys. Acta* **556:**509–523

Parisi, M., Candia, O. 1977. Urea uptake and translocation in toad urinary bladder. The effect of antidiuretic hormone. *J. Membrane Biol.* **36:**373–387

Parisi, M., Wietzerbin, J., Bourguet, J. 1983. Intracellular pH, transepithelial pH gradients, and ADH-induced water channels. *Am. J. Physiol.* **244:**F712–F718

Pietras, R., Wright, E. 1974. Non-electrolyte probes of membrane structure in ADH-treated toad urinary bladder. *Nature (London)* **247:**222–224

Pratz, J., Ripoche, P., Corman, B. 1986. Evidence for proteic water pathways in the luminal membrane of kidney proximal tubule. *Biochim. Biophys. Acta* **856:**259–266

Rega, A.F., Rothstein, A., Weed, R.I. 1967. Erythrocyte membrane sulfhydryl groups and the active transport of cations. *J. Cell. Physiol.* **70:**45–52

Solomon, A.K., Chasan, B., Dix, J.A., Lukacovic, M.F., Toon, M.R., Verkman, A.S. 1983. The aqueous pore in the red cell membrane: Band 3 as a channel for anions, cations, nonelectrolytes, and water. *Ann NY Acad. Sci.* **414:**97–124

Spooner, P.M., Edelman, I.S. 1976. Stimulation of Na^+ transport across the toad urinary bladder by *p*-chloromercuribenzene sulfonate. *Biochim. Biophys. Acta* **455:**272–276

Verkman, A.S., Wong, K.R. 1987. Proton nuclear magnetic resonance measurement of diffusional water permeability in suspended renal proximal tubules. *Biophys. J.* **51:**717–723

Whittembury, G., Carpi-Medina, P., Gonzalez, E., Linares, E. 1984. Effect of *para*-chloromercuribenzene sulfonic acid and temperature on cell water osmotic permeability of proximal straight tubules. *Biochim. Biophys. Acta* **775:**365–373

Whittembury, G., Carpi-Medina, P., Gonzalez, E. 1987. Channels for water flow in epithelia: Characteristics and regulation. *Acta Physiol. Pharmacol. Latinoam.* **37:**555–563

Received 6 September 1989; revised 28 November 1989