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Reversible Inhibition by Lanthanum of the Hydrosmotic Response to Serosal Hypertonicity in Toad Urinary Bladder

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Summary. In the urinary bladder of amphibia, hypertonicity of the serosal bath (*SH*) evokes an increase in transepithelial water permeability, the characteristics of which resemble the response to antidiuretic hormone (ADH). The ionic dependency, in particular for Ca^{2+}, appears very similar for *SH*- and ADH-induced water fluxes. In the present experiments La^{3+} was used as a probe to study the Ca^{2+}-dependency of the hydrosmotic response to *SH* in isolated urinary bladder of the toad *Bufo marinus*.

Addition of La^{3+} (5 mM) on the serosal side of the membrane produced a significant and reversible increase in basal transepithelial water flux. The hydrosmotic response elicited by adding 250 mM mannitol to the serosal Ringer's solution was inhibited by 30% in the absence of serosal Ca^{2+}. Similarly, the hydrosmotic response to *SH* was inhibited by 37%, 30% and 40% when 5 mM La^{3+} was added to the serosal medium 30 min before, concommitantly with, or 60 min after induction of *SH*. The inhibition of transepithelial water flux observed in the absence of serosal Ca^{2+} or in the presence of serosal La^{3+} was reversible.

The results support a critical role for Ca^{2+} in the modulation of transepithelial water permeability in the urinary bladder of amphibia. Ca^{2+} presumably exerts its effects at a post-cyclic AMP step.

Key words: Toad urinary bladder, hypertonicity, water permeability, calcium, and lanthanum.

In the isolated urinary bladder of amphibia a hydrosmotic response can be elicited by increasing the osmolality of the serosal bath. This effect of serosal hypertonicity (*SH*) shares many common features with the hydrosmotic response induced in the same preparation by antidiuretic hormone (ADH). We have recently shown that the responses to ADH and to *SH* have similar ionic dependencies and that the presence of serosal Ca^{2+} is important in order to obtain a full response to either stimulus (Hardy, 1978; 1979). In many biological systems lanthanum

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(La^{3+}) has been shown to abolish the effects of Ca^{2+} and has proven to be a useful probe in elucidating the role of Ca^{2+} in certain physiological processes (Weiss, 1974). In the present experiments La^{3+} was used in order to further characterize the Ca^{2+}-dependency of the hydrosmotic response to *SH* across the isolated toad urinary bladder.

Materials and Methods

Water fluxes were measured by gravimetry in hemibladder sacs isolated from the toad *Bufo marinus*. The composition of the Ringer's solution and the methodology to measure the hydrosmotic response have been previously described (Hardy, 1979). Serosal hypertonicity was induced by adding 250 mM mannitol to the Ringer's solution bathing the serosal surface of the membrane. In all experiments the solution bathing the mucosal surface of the bladder was a 1/10 dilution of isotonic anuran Ringer. $LaCl_3$ was added to the serosal bath. When La^{3+} was used, the concentration of NaCl was iso-osmotically reduced in the Ringer. No precipitate or pH change of the Ringer's solution occurred after addition of La^{3+}. In some experiments quarterbladders, instead of hemibladders, were used. Water fluxes are expressed as means $\pm$ SEM of water lost per surface area of bladder per hour ($\mu l \cdot cm^{-2} \cdot h^{-1}$). Statistical significance was assessed by using Student's *t* test on paired hemibladders.

Results

The basal water flux across bladders exposed to isosmotic anuran Ringer on the serosal side and to 1/10 diluted Ringer on the mucosal surface was very low and ranged from 3 to 4 $\mu l \cdot cm^{-2} \cdot h^{-1}$. When mannitol was added to the serosal bath, an increase in transepithelial water flux was elicited which reached a peak 60 min after induction of *SH* and plateaued, thereafter, for 90 min at values between 50 and 70 $\mu l \cdot cm^{-2} \cdot h^{-1}$ (*see* Fig. 1).

When *SH* was induced in a Ca^{2+}-free serosal medium, the hydrosmotic response initially developed for the first 45 min at the same rate as in control hemibladders exposed to serosal Ringer containing 1 mM Ca^{2+}. In the absence of Ca^{2+}, however, the hydrosmotic response achieved at 60 min was significantly lower than in control bladders and the plateaus for experimental and control hemibladders averaged 36.5 ± 4.1 and 52.5 ± 4.8 $\mu l \cdot cm^{-2} \cdot h^{-1}$, respectively ($n=9$, $P<0.005$). This inhibition was completely reversible. Indeed, upon addition of Ca^{2+} to the serosal bath, the hydrosmotic response of preparations previously exposed for 45 min to Ca^{2+}-free Ringer increased immediately to the level achieved in control hemibladders.

Serosal concentrations of 1, 2.5, 5 and 12.5 mM La^{3+} were used to determine whether the effects obtained in the absence of serosal Ca^{2+} could be reproduced by La^{3+}. No inhibition of the hydrosmotic response to *SH* was observed with La^{3+} concentrations of 1 and 2.5 mM. Significant inhibition, however, occurred with 5 mM La^{3+}; 12.5 mM La^{3+} had no greater inhibitory effect than 5 mM.

Figure 1 presents the effects of 5 mM La^{3+} on the hydrosmotic response to *SH*. In the experiments depicted in Fig. 1*A*, at $t=15$ min, half of the hemibladders ($n=9$) were exposed to mannitol while the other half were exposed to mannitol and La^{3+} together. During the initial 30 min of observation, the water fluxes increased identically in both sets of preparation. At that point, however, the hydrosmotic response in the La^{3+}-treated hemibladders started to plateau, while in the control hemibladders it continued to increase until 60 min after the osmotic challenge. At $t=150$ min, water fluxes in control and experimental hemibladders averaged 57.5 ± 5.1 and 40.1 ± 2.1 $\mu l\cdot cm^{-2}\cdot h^{-1}$, respectively ($P<0.001$).

The effects of La^{3+} on the hydrosmotic response developed to *SH* are presented in Fig. 1*B*. In these experiments, mannitol was added to the serosal Ringer of all hemibladders ($n=18$). Sixty minutes after the osmotic challenge, the hydrosmotic response averaged 67.3 ± 3.0 $\mu l\cdot cm^{-2}\cdot h^{-1}$. Half of the preparations were then exposed to La^{3+}. This resulted in an immediate decrease in water flux. At $t=150$ min, water fluxes in control and La^{3+}-exposed hemibladders averaged 62.5 ± 2.5 and 37.5 ± 4.2 $\mu l\cdot cm^{-2}\cdot h^{-1}$, respectively ($P<0.001$).

The inhibition by La^{3+} of the hydrosmotic effects of *SH* is reversible. This is illustrated in Fig. 1*C*. In these experiments, at $t=15$ min, control hemibladders ($n=9$) were challenged with mannitol while the experimental preparations ($n=9$) were exposed to mannitol and La^{3+}. Forty-five minutes later (at $t=60$ min), water fluxes were significantly less in the experimental, 35.2 ± 6.1 $\mu l\cdot cm^{-2}\cdot h^{-1}$, than in the control hemibladders, 50.3 ± 7.3 $\mu l\cdot cm^{-2}\cdot h^{-1}$ ($P<0.05$). The experimental hemibladders were then transferred into La^{3+}-free hypertonic Ringer. This resulted in a progressive increase in water flux and, at $t=150$ min, the hydrosmotic responses were not different in control and in experimental hemibladders, 66.3 ± 6.5 and 62.5 ± 7.5 $\mu l\cdot cm^{-2}\cdot h^{-1}$, respectively ($P>0.2$).

Additional experiments were performed to determine whether La^{3+} had any effect on basal water flux and whether preincubation of bladder preparations with La^{3+} prior to mannitol exposure would modify the La^{3+} inhibition of the hydrosmotic response to *SH* or eventually result

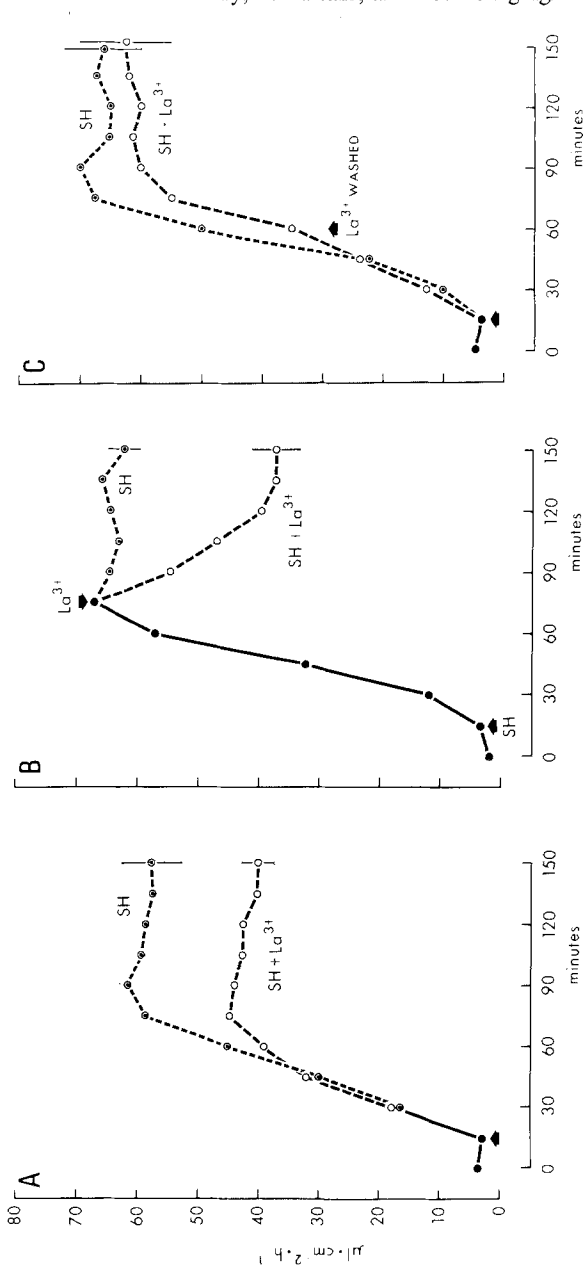

Fig. 1. Effects of Lanthanum on the hydrosmotic response to serosal hypertonicity. In all experiments control hemibladders (*SH* – closed and dotted circles) and experimental hemibladders ($SH + La^{3+}$ – open circles) were exposed to serosal hypertonicity (250 mM mannitol) from $t = 15$ to $t = 150$ min. La^{3+} (5 mM) was added on the serosal side of the experimental hemibladders at $t = 15$ min in experiments of *A* and at $t = 72$ min in experiments of *B*. In experiments of *C*, La^{3+} was added at $t = 15$ min and removed at $t = 60$ min. Nine bladders (18 hemibladders) were used in each set of experiments

in irreversible effects. These experiments were performed in quarterbladder sacs, each assigned to one of four groups to allow comparison between four different experimental conditions in preparations from the same bladder. Group 1 served as control and was challenged with mannitol for 60 min. Group 2 was exposed to La^{3+} for 30 min and washed in La^{3+}-free Ringer before exposure to *SH*. Group 3 was exposed to La^{3+}

Table 1. Effects of serosal La^{3+} on basal water flux and on the hydrosmotic response to serosal hypertonicity in quarterbladder sacs of the urinary bladder of the toad

Group	0	30 min	60 min	90 min	120 min
1	R 3.2±1.3	R 3.5±2.0	R 3.0±1.3	*SH* 34.5±4.3	*SH* 61.3±5.3
2	R 4.0±2.1	R-La^{3+} 12.2±3.1**	R 4.5±2.1	*SH* 32.1±5.2	*SH* 58.9±6.1
3	R 3.7±1.0	R 2.2±1.0	R-La^{3+} 13.7±3.1*	*SH*-La^{3+} 29.9±6.1	*SH*-La^{3+} 38.5±4.3*
4	R 4.2±2.3	R 4.2±2.5	R-La^{3+} 14.1±2.2*	*SH*-La^{3+} 31.9±5.4	*SH* 60.2±6.1

Results indicate mean water fluxes ±SEM in $\mu l \cdot cm^{-2} \cdot h^{-1}$. The composition of serosal Ringer's solution is indicated for each time period. R = isotonic anuran Ringer; R-La^{3+} = R plus 5 mM La^{3+}; *SH* = serosal hypertonicity (R + 250 mM mannitol); *SH*-La^{3+} = *SH* plus 5 mM La^{3+}. Mucosal solution: R/10. Statistical significance was determined by Student's *t* test for paired data, comparing results in groups 2, 3 and 4 with group 1 for each time period (*: $P<0.001$; **: $P<0.01$). There were 8 quarterbladders in each group.

for 30 min before and 60 min during *SH*. Group 4 was exposed to La^{3+} 30 min before and 30 min during *SH* before being washed with La^{3+}-free hypertonic solution.

Results of these experiments are presented in Table 1. Serosal addition of 5 mM La^{3+} resulted in a significant increase in basal water flux across the bladder preparations (Groups 2, 3, and 4). This increase was entirely reversible (Group 2). Preincubation with La^{3+} had no effects on the inhibition by La^{3+} of the hydrosmotic response to *SH*, and preincubation with La^{3+}, before the osmotic challenge, did not accelerate the inhibition of water transport. Indeed, both without (Fig. 1 *A* and *C*) and with preincubation (Groups 3 and 4), La^{3+} had no significant effect on the hydrosmotic response to *SH* during the first 30 min of the osmotic challenge. On the other hand, inhibition of the hydrosmotic response in La^{3+}-preincubated preparations averaged 37% at 60 min and was highly significant (Group 3 *vs.* Group 1). This inhibition, however, compares closely with that observed after 60 min in the absence of preincubation (Fig. 1 A). With and without preincubation, the inhibition by La^{3+} of the response to *SH* was entirely reversible (*see* Fig. 1 *A* and Groups 3 and 4).

Discussion

Our experiments clearly demonstrate that, in the absence of serosal Ca^{2+} or in the presence of serosal La^{3+}, the hydrosmotic response to

SH is inhibited. Under both conditions the inhibition was of similar magnitude and was completely reversible. Since La^{3+} also inhibited the developed hydrosmotic response, its effects are unlikely the result of an inhibition by La^{3+} of the adenyl cyclase system (*c.f.* Bourguet, Ripoche & Parisi, 1974; Kregenow, Robbie & Orloff, 1976; Hardy, 1979).

In contrast, with the inhibition of *SH*-induced water fluxes, a small albeit highly reproducible and significant stimulation by La^{3+} of the basal water flux was observed. The results of the present experiments in the isolated toad bladder on water transport are at variance with observations in the isolated frog bladder. In the latter preparation, serosal La^{3+} had no effect on either basal water flux or on the hydrosmotic response to *SH* (Wietzerbin, Lange & Gary-Bobo, 1974). On the other hand, others have reported a stimulating effect of La^{3+} on basal water flux in toad skin and bladder which occurred without any increase in cellular cyclic AMP, while La^{3+} has also been shown to blunt the hydrosmotic response to ADH and to decrease the ADH-induced increase in cyclic AMP (Wong, Bedwani & Cuthbert, 1972; Marguerat & de Sousa, 1975).

A dual effect of La^{3+} in the same biological preparation (stimulation of basal activity and inhibition of stimulated activity) is not without precedent and has been reported in at least two other biological systems with Ca-mediated processes: (i) in frog neuromuscular junctions, La^{3+} increases the frequency of miniature end-plate potentials but blocks the evoked release (Heuser & Miledi, 1971); (ii) in nerve terminals of guinea pig vas deferens, La^{3+} increases basal noradrenaline output but blunts the Ca-stimulated release (Nakazato, Onoda & Ohga, 1977).

It has been shown that La^{3+} does not permeate cell membranes (Erlij & Martinez-Palomo, 1972; Henrikson, 1974; Szász *et al.*, 1978) but that its effects are mainly due to the displacement of extracellular Ca^{2+} and a decreased cell membrane permeability to Ca^{2+} (Weiss, 1974; van Breemen, Hwang & Siegel, 1977). Thus, the present results emphasize the critical role of Ca^{2+} in the modulation of transepithelial water permeability in urinary bladder of amphibia (Hardy, 1978). Mode(s) and site(s) of action still need clarification. However, the characteristics of the La^{3+} effects on the hydrosmotic response to serosal hyerptonicity suggest that Ca^{2+} exerts a critical influence on some mechanism located at a post-cyclic AMP step in the intracellular chain of biochemical events involved in the transepithelial transport of water.

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