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Mechanism of the 'antidiuretic hormone-like' action of hypertonic media on the frog urinary bladder

Incubation in hypertonic media has been reported to alter considerably the permeability of various epithelia: the d.c. resistance of frog skin is lowered to 20% of its normal value in twice isoosmotic urea solutions[1,2]; the resistance of the cell membrane to water increases linearly and reversibly with the osmolarity of bathing solution in the rabbit gall bladder[3] and active Na^+ transport is inhibited and water permeability increased in the amphibian urinary bladder when incubating media are made hypertonic with nonpermeant solutes[4]. The results reported in this paper suggest that in frog urinary bladder the action of hyperosmotic media is elicited *via* a chemical reaction, also involved in the mechanism of action of antidiuretic hormone.

Urinary bladders of *Rana esculenta*, mounted between two lucite chambers, were bathed on the serosal side with a Ringer solution and on the mucosal side with the same solution in which the NaCl concentration was reduced to 5.6 mM. The net flux of water was measured by a technique previously described[8]. In each case, hemibladder from the same frog was taken as a control.

The increase in osmolarity was obtained by addition of 220 mosM mannitol on the serosal side. In other experiments, both mucosal and serosal osmotic pressures were increased simultaneously, in order to avoid any variation in the transepithelial osmotic pressure difference.

The hydrosmotic responses to oxytocin (Syntocinon, Sandoz), adenosine 3',5'-monophosphate (cyclic AMP; Schwarz Bioresearch Lab.) and theophylline (Serlabo) were also tested.

The record in Fig. 1 illustrates responses to three kinds of stimuli obtained in sequence on the same preparation. Response A was elicited by the addition of mannitol to both serosal and mucosal media. In B, on the contrary, the serosal solution alone was made hypertonic. In both cases the hydrosmotic effect was comparable, as previously reported by BENTLEY[4]; the higher magnitude of the response in B was due to

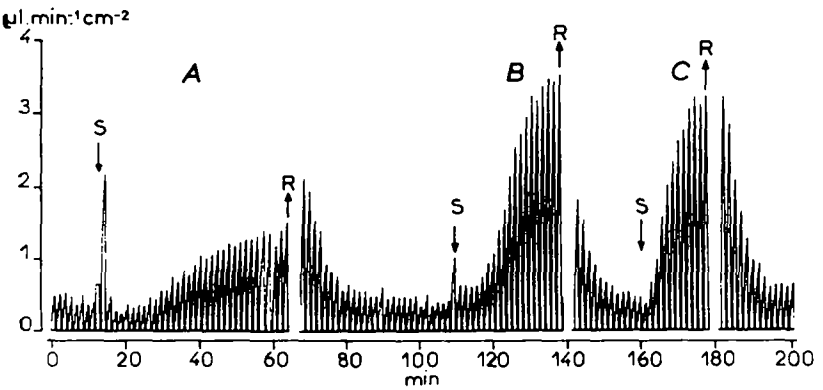

Fig. 1. Evolution of water net flux in response to (A) an increase of osmotic pressure (220 mosM) in both mucosal and serosal bathing solutions (S = stimulation; R = washout); (B) the same osmotic pressure increase of serosal solution alone; (C) addition of 10 nM oxytocin to serosal medium.

the increase of the transepithelial osmotic pressure. In this case, when mannitol was washed out at the peak of the response, the driving force decreased to 50% and accordingly, the net flux of water dropped immediately to half of its value. After that, the decrease in the permeability was slow and similar to that observed in A. As shown in C, the reaction to oxytocin was not impaired by previous stimulation by hypertonicity and the response to hormone (C) and to hyperosmolarity (A and B) were strikingly similar.

Usually, however, the response to hyperosmolarity was slower than that to oxytocin, the mean half times being 18.9 ± 1.7 min ($n = 24$) and 7.3 ± 0.35 ($n = 24$), respectively. As reported for oxytocin[5], the time course of the response was dependent on the temperature of the incubation medium (mean half time: 5.9 ± 0.2 min at 30°; 13.0 ± 1.6 min at 20°; mean $Q_{10} = 2.38 \pm 0.38$; $n = 7$); the response was fully reversible. Moreover reactivity of the bladder to hyperosmolarity depended upon the condition in which the animals were kept; responses of bladders from frogs maintained at 22° were 5 times higher (mean increase of net flux 2.24 ± 0.23 $\mu l \cdot min^{-1}$; $n = 10$) than that of bladders from animals kept at 2° (0.409 ± 0.08; $n = 26$).

In view of the similarity between the responses to hyperosmolarity and oxytocin, the effect of agents known to modify the action of the hormone was investigated[6]. After incubation with 10 μM norepinephrine, the response to oxytocin was reduced in magnitude to 62.9 ± 5.4% of control ($n = 5$), without any change in its time course (Fig. 2b). A comparable inhibition to 66.4 ± 9.4% of control ($n = 10$) was observed with hyperosmolarity (Fig. 2a) and in this case the response was also slowed (mean half time: 66.4 ± 0.40 min against 12.4 ± 0.75 min in this series control; $n = 10$). Norepinephrine was also tested at the same concentration once the responses were developed. Contrary to the oxytocin response (Fig. 3b) that was reduced to 37.2 ± 7.4% ($n = 7$) the mannitol response (Fig. 3a), once developed, was no longer lowered by norepinephrine. The effect of norepinephrine on the mannitol response was completely prevented by 0.1 mM phentolamine, an agent known to block α adrenergic receptors but not by 0.1 mM dichloroisoproterenol which blocks β receptors.

Conversely the stimulation by hyperosmolarity was potentiated by various treatments increasing the cellular concentration of cyclic AMP, which is the presumed mediator of the hormonal action: activation of adenylcyclase by neuropeptides, inhibition of phosphodiesterase by theophylline or, more directly, incubation in the presence of cyclic AMP in the serosal medium. These three compounds given at threshold doses were found to increase the magnitude of the response to a subsequent challenge by hyperosmolarity, especially on bladders from animals kept at 2° (Table I).

TABLE I

EFFECT OF PRETREATMENT BY DIFFERENT AGENTS ON THE RESPONSE TO HYPERTONIC MEDIA

Pretreatment by liminar concentrations of	*Increase of the net flux of water due to hypertonicity* ($\mu l \cdot min^{-1} \cdot cm^{-2}$)	
	Without pretreatment	*With pretreatment*
Oxytocin, 10 nM ($n = 9$)	0.39 ± 0.07	1.32 ± 0.15
Cyclic AMP, 10 mM ($n = 9$)	0.41 ± 0.07	1.12 ± 0.14
Theophylline, 1 mM ($n = 13$)	0.71 ± 0.08	1.81 ± 0.40

These results suggest that response to hyperosmolarity is not a purely physical process. The interactions observed with agents known to modify either production or destruction of cyclic AMP indicate that one of the chemical steps implicated in the mechanism of action of oxytocin is also involved in the response to hyperosmolarity.

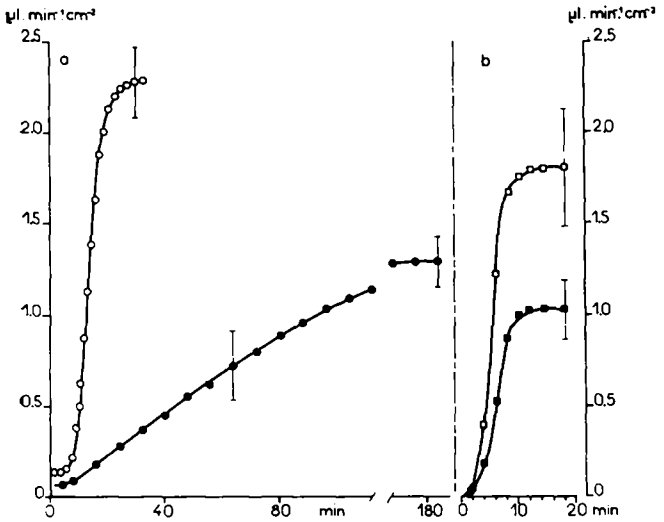

Fig. 2. Influence of 10 μM norepinephrine on mean responses to hyperosmolarity (220 mosM mannitol) (a) and to 10 nM oxytocin (b). ○ and □, responses of control hemibladders; ● and ■, responses in presence of norepinephrine added to the serosal medium 15 min before stimulation.

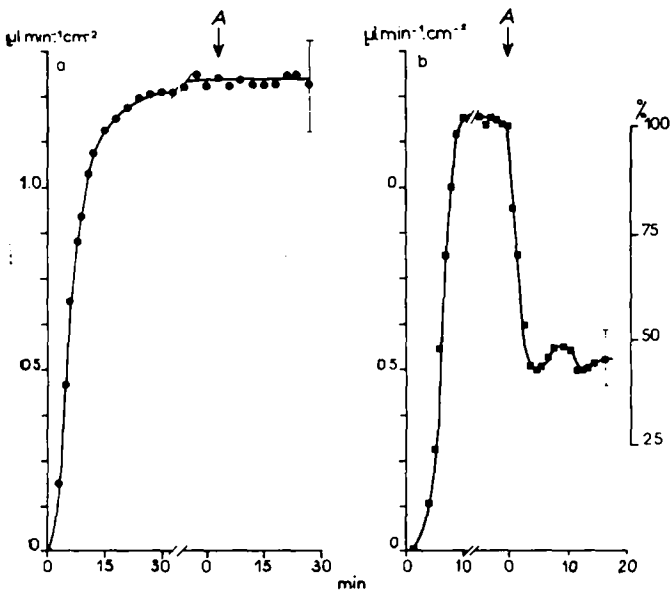

Fig. 3. Influence of 10 μM norepinephrine (A) added to developed responses to 220 mosM mannitol (a) and to 10 nM oxytocin (b).

However, lowering cyclic AMP levels by norepinephrine slows the response without reversing it once developed; it is therefore suggested that a high level of cyclic AMP is a facilitating but not a necessary condition to the response to hyperosmolarity.

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