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Effect of hypertonic media on the reversal of the hydrosmotic action of antidiuretic hormone in frog urinary bladder

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SUMMARY

The reversal of hydrosmotic response of isolated frog urinary bladder to oxytocin, theophylline and cyclic AMP is markedly slowed during incubation in hypertonic media. These results enforce the hypothesis that hypertonic media could exert their action by inhibiting the inactivation of some required factor more than by enhancing its activation.

Recent observations suggest that the hydrosmotic action of hypertonic media in frog urinary bladder is elicited *via* chemical steps also involved in the action of antidiuretic hormone. Responses to antidiuretic hormone and to hyperosmolarity are in many respects similar, and various agents known to modify the hormone response modify also the response to hypertonic media[1].

Hypertonic media, however, do not interact with the first steps of hormonal action, although their action is to some extent dependent upon the rate of activity of adenyl cyclase and the presence of cyclic AMP. Thus, the time course of the response to hyperosmolarity is considerably slowed by norepinephrine, a presumed inhibitor of adenyl-cyclase[2], but it can be shown that, once the response is developed, it is no longer reduced by this agent[1]. It thus appears that a normal rate of adenyl cyclase activity and the cyclic AMP level determine the time course of the response to hypertonic media, although they are not required for the biological effect to persist once it has developed.

If it is assumed that the time-limiting step of the response is the establishment of a new equilibrium between the inactive and active forms of some intermediary compound, the results observed with norepinephrine can be explained if it is admitted that hyperosmolarity blocks the destruction of the active form while cyclic AMP enhances its production.

The present note reports the existence of a slow-down of the reversal of the hydrosmotic action in the presence of hypertonic media, an observation that is in good agreement with such a mechanism of action.

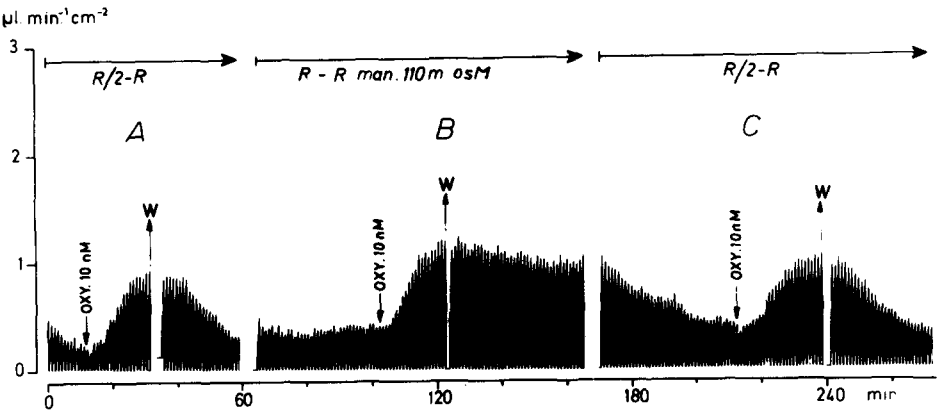

Fig. 1. Evolution of net water flux in response to oxytocin (OXY. 10 nM), and after the washout of the hormone (W). Comparison of the reversal of action in control media (A and C) and in hypertonic media (B). man. = mannitol.

Experiments were carried out on isolated bladders of *Rana esculenta*. Net water flux was recorded by a volumetric technique[3] and hormonal challenge realised with oxytocin (Syntocinon Sandoz).

Fig. 1 shows a typical recording. Reactions A and B were obtained in the presence of Ringer on the serosal side and half Ringer on the mucosal side. In Reaction B, the osmotic pressure was raised by the addition of mannitol (110 mOsm) to the serosal side, and the use of full Ringer as the mucosal medium.

Although no important rise in water net flux was observed with this low hyperosmolarity, it is clear that the reversal of hormonal action is slowed under these conditions.

In another series of experiments, we measured the half-time of reversal of hydrosmotic action (*i.e.* time at which the increase in water net flux is reduced to 50% of its maximal value, $t_{½\ R}$) after challenges with oxytocin, theophylline and cyclic AMP. In this series, full strength Ringer was used as the serosal medium and Ringer in which the NaCl concentration was reduced to 5.6 mM as mucosal medium. Serosal hyperosmolarity was obtained by the addition of mannitol (110 mOsm) to both the serosal and the mucosal media.

TABLE I

INFLUENCE OF HYPEROSMOLARITY ON REVERSAL TIME OF HYDROSMOTIC RESPONSE

Agent	*Half-time of action reversal (min)*★		*N*	*p*★★
	Control	*Experimental*		
Oxytocin (22 nM)	4.47 ± 0.26	9.06 ± 1.07	15	<0.001
Theophylline (5 mM)	5.44 ± 0.40	11.06 ± 0.53	16	<0.001
Cyclic AMP (5 mM)	4.70 ± 0.37	7.36 ± 0.43	10	<0.001

★Mean ± S.E.

★★Computed from differences between paired values.

Table I shows that hyperosmolarity increases the reversal half-time for the three types of stimuli, indicating, in agreement with earlier data[1,4], that the step controlled by hypertonic media is subsequent to the production of cyclic AMP. This slow-down of the reversal of hydrosmotic action is compatible with the hypothesis of a reduced rate of destruction of some active compound in hyperosmolar media.

The permeability of the membrane to water would thus be the result of a dynamic equilibrium controlled at multiple levels. One of these would be the equilibrium prevailing between production and destruction of cyclic AMP. The present results provide an approach to another level represented by an activation–inactivation equilibrium controlled directly or indirectly in one direction by cyclic AMP, and in the other by cellular tonicity. Whether this level is represented by the biological effector itself or by another intermediary compound is still a matter of conjecture.

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