VASOPRESSIN-LIKE EFFECTS OF ADENOSINE 3',5',-PHOSPHATE (CYCLIC 3',5'-AMP) AND THEOPHYLLINE IN THE TOAD BLADDER

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Vasopressin exerts a unique effect on frog skin (Ussing and Zerahn, 1951) and toad bladder (Leaf, 1960; Bentley, 1958). Addition of the hormone to the serosal surface of the bladder results in an increase in the net flow of water along an osmotic gradient and a rise in both short-circuit current and potential difference (P.D.) across the membrane. These effects are specific for neurohypophyseal extracts, are reversible, and can be prevented by acidification of the bathing medium or the addition of certain sulfhydryl blocking agents (Rasmussen et al, 1960). Vasopressin also stimulates the release of hydrocortisone from the adrenal (Hilton et al, 1960) as does ACTH, and glucose from liver (Bergen et al, 1960) as does epinephrine. Since the effects of the latter hormones involve the intermediacy of cyclic 3',5'-AMP (C-AMP) and phosphorylase activation (Sutherland and Rall, 1960; Haynes, 1958) the present studies were designed to test the hypothesis that vasopressin alters permeability by stimulating the production of cyclic AMP from ATP in toad bladder. Since conversion of C-AMP to inactive 5' AMP is prevented by theophylline (Butcher and Sutherland, 1959) the effect of theophylline on the toad bladder was also examined.

Methods: The bilobed bladder of the toad Bufo marinus, was divided into two separate sacs. Sides B and D served as controls for sides A and C respectively (Tables 1 & 2). Each sac was filled with Ringer's solution diluted 5 fold with distilled H_2O and then suspended in aerated undiluted Ringer's (Bentley, 1958). Net water movement from inside to out was estimated by weighing the sacs in air and observing the weight loss (equivalent to outward movement of water into the surrounding bath) in

successive 30 minute periods. Short-circuit current and P.D. were measured according to the technique of Ussing and Zerahn, 1951.

Results and Conclusions: Tables 1 and 2 summarize the results from individual periods in separate experiments.

Table 1 NET WATER MOVEMENT ALONG AN OSMOTIC GRADIENT

A		B		C		D	
Solution	wt. loss mg/min	Solution	wt. loss mg/min	Solution	wt. loss mg/min	Solution	wt. loss mg/min
Ringer's	1.0	Ringer's	1.0	10^{-2}M C-AMP	34.5	200 mU/ml vasopressin	32.8
10^{-3}M C-AMP	14.0	10^{-3}M 5' AMP	0.6	10^{-2}M theophylline	18.2		
10^{-3}M C-AMP	13.3	10^{-3}M ATP	0.9			Ringer's	1.2
500 μU/ml vasopressin	12.0	Ringer's	1.0				

Table 2 EFFECT OF pH 6.5 AND N-ETHYL-MALEIMIDE ON WATER MOVEMENT

A pH 6.5		B pH 7.4		C NEM 10^{-3}M		D NO NEM	
Solution	wt. loss mg/min	Solution	wt. loss mg/min	Solution	wt. loss mg/min	Solution	wt. loss mg/min
Ringer's	0.8	Ringer's	0.4	10^{-3}M C-AMP	2.5	10^{-3}M C-AMP	7.8
1 mU/ml vasopressin	1.0	1 mU/ml vasopressin	16.4	10^{-2}M theophylline	0.5	10^{-2}M theophylline	27.0
10^{-2}M theophylline	1.7	10^{-2}M theophylline	11.1	5 mU/ml vasopressin	1.2	5 mU/ml vasopressin	22.8
10^{-3}M C-AMP	8.2	10^{-3}M C-AMP	10.2				

Table 1: Addition of 10^{-3}M C-AMP to the serosal surface of the bladder sac produced a significant increase in water movement along the osmotic gradient. The effect may be reversed by replacement of the sac in normal Ringer's. No effects of 10^{-3}M ATP or 5' AMP were observed. 500 μU/ml of vasopressin produced equivalent changes in water movement. 10^{-2}M C-AMP on the other hand elicited a maximal increase in water movement similar to that caused by 200 mU/ml of vasopressin. 10^{-3}M C-AMP also increased both the P.D. and

short-circuit current in a fashion which was qualitatively similar to that provided by vasopressin although less sustained. 10^{-2}M theophylline which blocks the conversion of C-AMP to inactive 5' AMP in homogenates, also mimicked the response to vasopressin with respect to water flow, current and P.D. Vasopressin, C-AMP, and theophylline were ineffective if added to the mucosal surface of the bladder. The theophylline effect was additive to that of either 10^{-3}M C-AMP or submaximal doses of vasopressin. Furthermore C-AMP increased the effectiveness of submaximal doses of vasopressin.

Table 2: Acidification of the bathing medium blocked the effect of vasopressin and theophylline on water movement but not that of C-AMP. This is of interest since conversion of ATP to C-AMP is suppressed below pH 7.0 (Sutherland, 1961). N-ethyl-maleimide prevented the effect of all three agents on water movement.

The similarity of effects of C-AMP, theophylline, and vasopressin on both water movement and the electrical changes in toad bladder strongly suggest that the latter hormone exerts its effect in this tissue and perhaps in the kidney by stimulating the production and accumulation of C-AMP. Studies are in progress in collaboration with Dr. E. W. Sutherland to determine directly the effects of vasopressin on C-AMP production in bladder and renal tissue as well as the effects of the hormone on phosphorylase activation in these tissues.

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