THE ROLE OF ADENOSINE 3',5'-PHOSPHATE IN THE ACTION OF VASOPRESSIN ON WATER PERMEABILITY OF TOAD BLADDERS

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Although the antidiuretic action of vasopressin in intact animals has been recognized for many years, the chemical mechanism by which this hormone exerts its characteristic effects on water balance remained obscure. Recently, however, Schwartz, Rasmussen and coworkers (Schwartz, 1960; Rasmussen, 1963) have reported that the addition of vasopressin to isolated toad bladders in vitro results in dramatic increases in the rate of passive diffusion of water through the walls of these organs. Moreover, these authors have suggested that this action of vasopressin is mediated by an initial thiol-disulfide interchange reaction between the disulfide ring of the hormone and a tissue sulfhydryl group; it has been proposed that this primary reaction initiates a chain of thiol-disulfide interchange reactions which per se alter the integrity or permeability of the tissue diffusion barrier. This stimulating work has aroused interest widely not only because of its intrinsic merit, but because of its possible extension to the action of the structurally similar hormone insulin. (Cadenas, 1961).

One puzzling facet of this problem has been the observation that adenosine 3',5'-phosphate (cyclic AMP), caffeine, and theophylline exert effects similar to that of vasopressin on toad bladder permeability. (Orloff, 1962). Moreover, it has been reported that vasopressin stimulates the net formation of cyclic AMP by kidney homogenates. (Brown, 1963). These observations suggested a mode of action of vasopressin alternative to that

proposed by Schwartz, Rasmussen and coworkers. It seemed possible that the action of vasopressin might be mediated via stimulation of the net formation of cyclic AMP; if so, then the similar effects on water flux of such chemically diverse agents as vasopressin, caffeine and cyclic AMP would be explicable on a common biochemical basis.

The elegant studies of Sutherland and coworkers (Davoren and Sutherland, 1963) revealed that the enzyme adenyl cyclase, which catalyzes the conversion of ATP to cyclic AMP, is stimulated by certain catecholamines such as epinephrine and is powerfully inhibited by the analogue dichloro-isoproterenol (DCI). Furthermore, the hydrolysis of cyclic AMP is inhibited by theophylline or caffeine. If vasopressin acted by causing an increased net production of cyclic AMP, then it would be predicted that DCI would inhibit the stimulation of water flux caused by vasopressin or theophylline but would influence to a lesser extent the action of added cyclic AMP. The results reported here support this hypothesis.

Materials and Methods. Bladders of Bufo marinus toads were excised, bisected, and tied onto glass tubes to form sacs with their serosal surfaces on the exterior; by this means one-half of each bladder could be used as a control for the other. (Bentley, 1958). Into each bladder sac was instilled an appropriate volume of buffered Ringer's solution which had been diluted with four volumes of distilled water. Each sac was then suspended in a bath of oxygenated Ringer's solution at room temperature for 1 hour prior to the addition of test substances. The rate of net diffusion of water was determined by weighing each sac at timed intervals. If the rate of water loss for either member of a pair exceeded 1 mg per minute during this initial period, that pair was discarded. Test substances were then added to the fluid surrounding those bladders which proved satisfactory, and incubation was continued as indicated.

Results. In Table 1 are presented the results of experiments in which bladder sacs were initially incubated with DCI prior to the addition of vasopressin or theophylline. It is readily apparent that the stimulation of

water efflux resulting from the addition of either of these compounds was severely impaired by the presence of DCI.

TABLE I

The effect of preincubation with DCI on the stimulation by vasopressin and theophylline of water efflux.

Additions*	Pairs	Period I † water loss (mg/min)	Period II † water loss (mg/min ± S.D.)	Inhibition due to DCI ‡ (per cent)
Vasopressin (7.5×10^{-6})	2	0.8	24.6 ± 7.8	69
Vasopressin (7.5×10^{-6}) + DCI (0.25)		0.7	7.3 ± 0.9	$P < 0.01$
Vasopressin (7.5×10^{-6})	2	0.7	15.6 ± 6.9	59
Vasopressin (7.5×10^{-6}) + DCI (0.35)		0.9	6.7 ± 2.4	$P < 0.01$
Vasopressin (3.0×10^{-6})	3	0.5	20.2 ± 4.4	95
Vasopressin (3.0×10^{-6}) + DCI (0.5)		1.0	0.4 ± 0.8	$P < 0.01$
Vasopressin (7.5×10^{-6})	2	0.6	14.9 ± 8.1	86
Vasopressin (7.5×10^{-6}) + DCI (0.75)		0.5	2.5 ± 3.0	$P < 0.02$
Theophylline (4)	4	0.9	8.5 ± 2.1	70
Theophylline (4) + DCI (0.7)		0.9	2.7 ± 1.1	$P < 0.01$

* The final concentrations in milligrams per ml of the test materials added to the outer compartments are indicated in parentheses.

\+ During Period I (60 minutes), one of each pair of bladder sacs was incubated in the presence of DCI; the other received no additions. At the beginning of Period II, the other materials were added as indicated to both members of each pair and the incubation was continued 60 minutes.

‡ The per cent inhibition by DCI was calculated by assuming that the stimulated rate of water loss from the paired sacs would have been identical had no DCI been added. The "P" values are the probabilities that the observed differences are due to chance, as calculated from "student's t test".

The results shown in Table II reveal that DCI addition to bladders already stimulated by vasopressin or theophylline also inhibits water efflux. Although DCI also alters the stimulation of water translocation exerted by cyclic AMP or the combination of theophylline and cyclic AMP, it does this to an extent far less than that observed with vasopressin.

Discussion. The observations presented here have several interesting implications. In the first place, cyclic AMP, as previously reported by others (Orloff, 1962), enhances water translocation in a manner similar to

TABLE II

The effect of DCI addition on stimulation by vasopressin, theophylline, cyclic AMP, and cyclic AMP and theophylline on water efflux from toad bladders.

EXP. NO.	Additions*		Pairs	Period I † water loss (mg/min±S.D.)	Period II † water loss (mg/min±S.D.)	Inhibition due to DCI‡ (per cent)
1	Vasopressin	(4.0×10^{-6})	5	23.3 ± 6.9	18.7 ± 4.0	52.5
	Vasopressin + DCI	(4.0×10^{-6}) (0.7)		26.0 ± 4.2	10.0 ± 3.8	P < 0.01
2	Theophylline	(7.6)	6	9.1 ± 2.5	8.1 ± 0.8	21.6
	Theophylline + DCI	(7.6) (0.7)		8.6 ± 6.8	5.8 ± 1.6	P < 0.01
3	Theophylline + cyclic AMP	(7.6) (3.2)	5	20.2 ± 4.3	8.2 ± 1.0	9.4
	Theophylline + cyclic AMP + DCI	(7.6) (3.2) (0.7)		21.9 ± 3.4	7.5 ± 0.6	P < 0.14
4	Cyclic AMP	(3.2)	3	12.7 ± 6.4	10.6 ± 2.6	15
	Cyclic AMP + DCI	(3.2) (0.7)		10.5 ± 3.3	7.2 ± 2.3	P < 0.20
5	Vasopressin	(4×10^{-6})	3	14.8 ± 6.6	25. ± 4.7	64.9
	Vasopressin + DCI	(4×10^{-6}) (0.7)		21.0 ± 0.4	12.8 ± 4.4	P < 0.01
6	Theophylline	(7.6)	3	6.4 ± 0.5	16.2 ± 5.3	41.0
	Theophylline + DCI	(7.6) (0.7)		6.7 ± 1.5	9.4 ± 2.7	P < 0.01
7	Theophylline + Cyclic AMP	(7.6) (3.2)	9	26.7 ± 8.8	22.9 ± 11.6	26.8
	Theophylline + Cyclic AMP + DCI	(7.6) (3.2) (0.7)		26.8 ± 7.2	15.2 ± 3.9	P < 0.015

* The final concentrations of the materials added to the outer compartments are indicated in parentheses. The units are milligrams per ml.

† During Period I, the bladders were incubated with vasopressin, cyclic AMP, theophylline, or cyclic AMP plus theophylline as indicated for thirty minutes in experiments 1-4 and for ten minutes in experiments 5-7. At the beginning of Period II, DCI was added to one of each pair of bladder sacs and incubation was continued for an additional thirty minutes in experiments 1-4 and for twenty minutes in experiments 5-7.

‡ Per cent inhibition by DCI was calculated by assuming that if DCI had been omitted, the rate of water loss during Period II from those bladders to which DCI was added would have exhibited the same ratio to the water loss during Period I as was actually observed in the paired sacs to which no DCI was added. The "P" values indicate the probability that the observed differences in the DCI-treated bladders are due to chance as calculated by the method of paired variates. (Finney, 1955).

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vasopressin. Moreover, theophylline, a known inhibitor of the phosphodiesterase which hydrolyzes cyclic AMP, exerts a vasopressin-like effect; this suggests that endogenous synthesis and hydrolysis of cyclic AMP

occur in the untreated bladder, but that the relative rates of these two processes are such that the intracellular concentration of the cyclic nucleotide normally remains low. It might be expected that addition of an inhibitor of its hydrolysis, such as theophylline, would result in a net accumulation of the nucleotide. On the other hand, the presence of an inhibitor of adenyl cyclase such as DCI might be expected to decrease or abolish synthesis of cyclic AMP; if accumulation of this cyclic nucleotide were required for increased bladder permeability, then it would be predicted that DCI would severely impair the stimulation exerted by theophylline, but would influence to a lesser extent the effect of exogenously added cyclic AMP. If the action of vasopressin were mediated by either enhancement of cyclic AMP synthesis or inhibition of its hydrolysis, then DCI would be expected to decrease or abolish the effect of this hormone.

The results presented show that water flux is indeed stimulated either by added cyclic AMP or by theophylline, but that a combination of these agents is required to approach in magnitude the stimulatory action of vasopressin. This may imply that neither inhibition of hydrolysis of endogenously synthesized cyclic AMP nor permeation by the exogenously added nucleotide is alone sufficient to raise its intracellular concentration to a maximally effective level.

It is clear that DCI severely inhibits the activities of both vasopressin and theophylline. On the other hand, the stimulatory activity of cyclic AMP alone or in combination with theophylline is much less drastically impaired by DCI; it should be noted that some degree of inhibition would be anticipated if endogenous synthesis makes an appreciable contribution to the intracellular concentration of cyclic AMP.

Although the present results suggest that the increased bladder permeability caused by vasopressin may be due to an increased net production of cyclic AMP rather than to a sequence of thiol-disulfide interchanges, they do not exclude an initial thiol-disulfide reaction between the hormone and some tissue receptor as proposed by Schwartz and Rasmussen. (Schwartz, 1960).

Summary. Theophylline potentiates the vasopressin-like activity of cyclic AMP in toad bladders. Dichloroisoproterenol is highly inhibitory to the action of vasopressin and theophylline in this system; the activity of cyclic AMP is inhibited to a lesser extent. It is suggested that vasopressin action is mediated via increased net formation of cyclic AMP in this tissue.

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