Vol. 99, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 31, 1981 Pages 491-495

# ROLE OF CALMODULIN IN ANTIDIURETIC HORMONE MEDIATED WATER TRANSPORT

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Received February 11, 1981

## SUMMARY

Several classes of tricyclic antidepressants inhibit the action of antidiuretic hormone (ADH) and cyclic adenine monophosphate (cAMP) on osmotic water flow across toad urinary bladder without any effect on sodium transport. This finding suggests that calmodulin is involved in the hydroosmotic action of ADH (and of serosal hypertonicity), possibly in inducing exocytosis at the luminal border of vesicles rich in water channels.

## INTRODUCTION

It has been suggested that intracellular calcium could play the role of a "second messenger" in the cellular events that are triggered by antidiuretic hormone (ADH) and lead to a permeability change at the luminal border of responsive epithelia. The exact nature of this role is not clear and conflicting results have been reported using calcium ionophore agents (1,2). Since in many systems the influence of intracellular calcium is probably mediated by a calcium binding protein (3,4) - in most instances calmodulin - we have studied the effect of calmodulin inhibitors on ADH-induced water flow in the toad urinary bladder which represents a model of mammalian cortical collecting duct (5).

## MATERIAL AND METHODS

Bufo marinus toads were purchased from Lemberger, Inc. (Germantown, Wisconsin). Water flow out of paired hemibladders was measured gravi-

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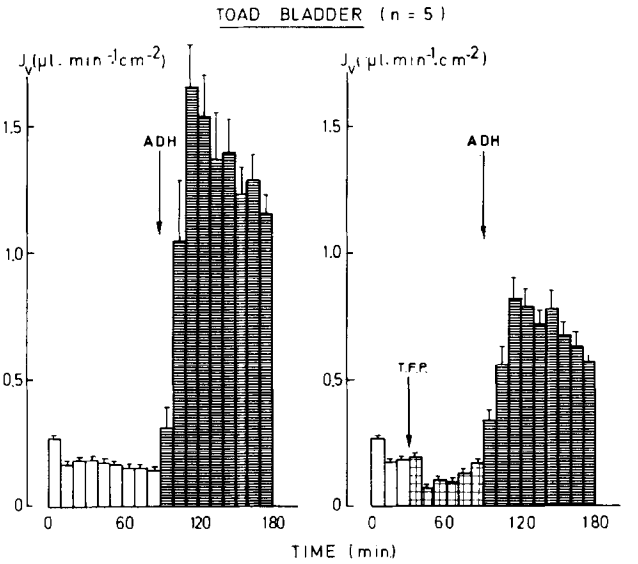


Figure 1: Paired hemibladders of Bufo marinus toads were mounted as bags. The osmotically induced water flow ($J_v$) out of the bladder was measured gravimetrically as a function of time according to Bentley (6). One hemibladder of each pair was treated with 25 µM trifluoperazine for one hour, and then both hemibladders were challenged with ADH (100 mU/ml).

metrically according to the technique of Bentley (6). The bladders were mounted as bags and filled with 5 ml of the latter Ringer solution containing: NaCl 11.5 mM, $KHCO_3$ 2.5 mM, $CaCl_2$ 1 mM. They were suspended in 15 ml of the usual aerated frog Ringer solution containing: NaCl 115 mM, $KHCO_3$ 2.5 mM, $CaCl_2$ 1 mM; pH 7.8-8.0 during aeration with atmospheric air.

Short circuit current (SCC) was measured in different preparations by the usual voltage-clamp method of Ussing and Zerhan (7). The bladders were mounted between the two halves of a lucite chamber with an incubation area of 3.14 $cm^2$. Each side was filled with 2.5 ml of identical Ringer solution (usual frog Ringer: composition given above).

cAMP (adenosine-3':5'-cyclic monophosphoric acid) was purchased from Sigma Chemical Company (St. Louis, Missouri); antidiuretic hormone (ADH; arginine vasopressin) was a gift of Dr. Maeck (Sandoz, Brussels, Belgium). The calmodulin inhibitors used were trifluoperazine (a gift of Smith, Kline and French, Brussels, Belgium), pimozide (a gift of Janssen Pharmaceutica, Beerse, Belgium) and chropromazine (a gift of Roussel, Paris, France). Data are expressed as mean ± standard error (SEM); differences were analyzed by the student paired t-test.

## RESULTS AND DISCUSSION

Additions of 25 µM trifluoperazine - an inhibitor of calmodulin (3,8) - did not affect basal water flow but reduced significantly the ADH induced

TABLE 1
WATER FLOW ($J_v$) ACROSS TOAD BLADDER
[$\mu l \cdot min^{-1} \cdot cm^{-2}$]

| Conditions | | Control | Experimental | Δ ± SEM | n |
|---|---|---|---|---|---|
| Trifluoperazine | ADH | 1.6 ± 0.2 | 0.8 ± 0.1 | $0.8 \pm 0.3^{++}$ | 5 |
| | cAMP | 4.0 ± 0.2 | 1.8 ± 0.4 | $2.2 \pm 0.4^{+++}$ | 4 |
| Pimozide | ADH | 2.7 ± 0.7 | 1.5 ± 0.3 | $1.2 \pm 0.4^{+}$ | 4 |
| | cAMP | 3.3 ± 0.3 | 2.0 ± 0.5 | $1.3 \pm 0.3^{++}$ | 5 |
| Chlorpromazine | ADH | 1.8 ± 0.3 | 0.8 ± 0.1 | $1.0 \pm 0.3^{+}$ | 4 |
| | cAMP | 3.6 ± 0.3 | 1.3 ± 0.4 | $2.3 \pm 0.4^{++}$ | 4 |

$^{+}p < 0.05$ ; $^{++}p < 0.025$ ; $^{+++}p < 0.01$

Water flow was compared in paired hemibladders, one of which has been previously treated for one hour with one of the following agents: Trifluoperazine 25 μM, Pimozide 250 μM or Chlorpromazine 50 μM, all added to the serosal solution. The above values were recorded 30 minutes following the addition of either ADH (100 mU/ml) or cAMP 5 mM.

increased in water flow (Fig. 1). Other calmodulin inhibitors have the same effect although at larger doses (Table 1); cAMP proved likewise less effective in the presence of the drug (Table 1). Trifluoperazine was also effective inhibiting a vasopressin-induced increase in water flow once already established; thus, in four experiments, trifluoperazine was added 30 minutes after vasopressin; 30 minutes later $J_v$ decreased to $0.4 \pm 0.1$ $\mu l \cdot min^{-1} \cdot cm^{-2}$ versus $1.10 \pm 0.2$ in paired hemibladders treated with vasopressin only ($\Delta = 0.7 \pm 0.1$, $p < 0.01$, n=4). Also trifluoperazine inhibited the increase in water flow brought about by serosal hypertonicity. Adding 240 mOsm mannitol to the serosal solution resulted in an increase in water flow that could be at least partially prevented by previous exposition to trifluoperazine, 25 μM; 60 minutes following addition of mannitol, $J_v$ averaged $1.6 \pm 0.2$ $\mu l \cdot min^{-1} \cdot cm^{-2}$ versus $0.6 \pm 0.15$ in paired tissues exposed also to trifluoperazine ($\Delta = 1.0 \pm 0.3$, $p < 0.05$, n=4).

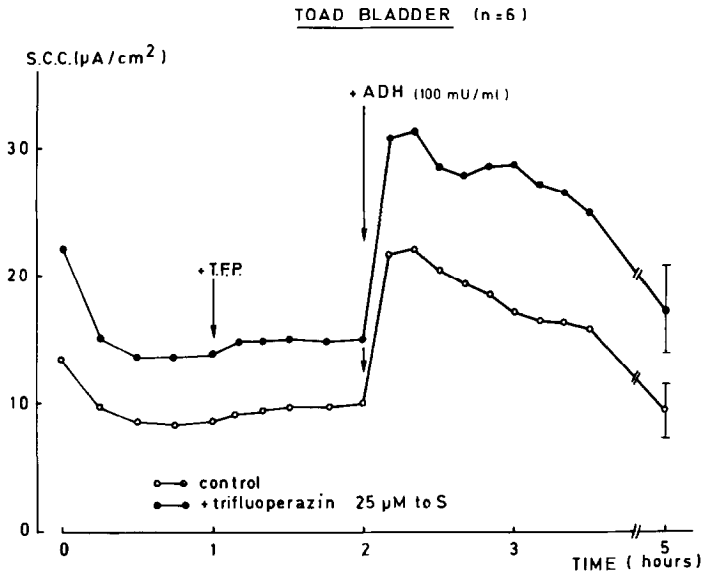


Figure 2: Short-circuit current (SCC) was measured as a function of time (t). One hemibladder of each pair served as a control, while the other was treated with 25 μM of trifluoperazine on the serosal solution for one hour. At t = 2 hours both bladders were challenged with ADH (100 mU/ml). There was no statistical difference at any time between the trifluoperazine treated bladder and the control. At t = 1 hour transepithelial resistances averaged 1185 ± 224 and 1509 ± 228 $\Omega.cm^2$ ($\Delta$ = 324 ± 181) in control and trifluoperazine treated tissue; at t = 5 hours they were respectively 1064 ± 164 and 1204 ± 188 ($\Delta$ = 140 ± 287).

Of interest, at the dose tested, trifluoperazine was devoid of effect on sodium transport, whether basal or ADH stimulated (Fig. 2). This, therefore, appears to be another way to block selectively the ADH induced increase in water flow. Phenothiazine derivatives have been shown to exhibit detergent-like properties (9) at high concentration, and the lack of effect on sodium transporting activity observed in the present study, therefore, suggests the absence of nonspecific toxicity on cell membrane at the concentration used and in this particular tissue at least. As this work was just completed, Ausiello and Hall (10) reported in abstract form similar conclusions regarding trifluoperazine inhibition of vasopressin-induced water flow, yet accompanied by partial inhibition of baseline sodium transport rate and eventual tissue death. Thus, the higher con-

centration ($10^{-4}$ M) used in those experiments apparently induced membrane damage. Many chemically or pharmacologically related compounds, exhibiting tranquilizer properties, have been shown to either increase or at higher doses to decrease sodium transport mainly in frog skin and toad bladder (11). The effect of chlorpromazine was already well characterized by Mamelak, Weissbluth and Maffly (12) at a time calmodulin was still unknown.

The present experiments established that calmodulin is specifically involved in ADH-induced water flow as well as in water flow induced by serosal hypertonicity. Calmodulin appears to affect a step distal to cAMP generation. Possible candidates could be 1) cAMP dependent protein kinase; 2) microtubules assembly and/or disassembly, and/or microfilaments; or 3) calcium-mediated vesicle exocytosis at the luminal border.

Although it is not yet possible to distinguish between these hypotheses, the similarities with the inhibition of exocytosis of cortical granules in sea-urchin eggs exposed to trifluoperazine (8) suggests the third mechanism.

ACKNOWLEDGEMENTS

We thank Drs. P.F. Baker, J. Crabbe and J.C. Henquin for helpful discussions. This work was partially supported by the Fonds National de la Recherche Scientifique, Brussels, Belgium.

REFERENCES

1. Taylor, A., and Eich, E. (1977) Kidney International 12:575A.
2. Hardy, M.A. (1978) J. Cell Biol. 76:787-791.
3. Cheung, W.Y. (1980) Science 207:19-27.
4. Means, A., and Dedman, J.R. (1980) Nature 285:73-77.
5. Leaf, A. (1965) Ergb. der Physiol. 56:216-263.
6. Bentley, P.J. (1958) J. Endocrin. 17:201-209.
7. Ussing, H.H., and Zerahn, K. (1951) Acta Physiol. Scand. 23:110-127.
8. Baker, P.F., and Whitaker, M.J. (1980) J. Physiol. (London) 298:55P.
9. Ros, M., Miller, A., and Glushko, V. (1980) Fed. Proc. 39:1917A.
10. Ausiello, D., and Hall, D. (1980) Clin. Res. 28:435A.
11. Grosso, A., and de Sousa, R.C. (1978) J. Membrane Biol. special issue:305-321.
12. Mamelak, M., Weissbluth, M., and Maffly, R.H. (1970) Biochem. Pharmacol. 19:2303-2315.