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Effect of Monensin on Osmotic Water Flow across the Toad Bladder and Its Stimulation by Vasopressin and Cyclic AMP

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Summary. The effects of the sodium ionophore monensin on osmotic water flow across the urinary bladder of the toad *Bufo marinus* were studied. Monensin alone did not alter osmotic water flow; however, the ionophore inhibited the hydrosmotic response to vasopressin and cyclic AMP in a dose-dependent manner. The inhibitory effects of monensin were apparent when the ionophore was added to the serosal bathing solution but not when it was added to the mucosal bathing solution. The inhibitory effect of serosal monensin required the presence of sodium in the serosal bathing solution but not the presence of calcium in the bathing solutions. Thus, it appears that intracellular sodium concentration is a regulator of the magnitude of the hydrosmotic response to vasopressin and cyclic AMP.

Key words monensin · ionophore · osmotic water flow · toad bladder · vasopressin · cyclic AMP

Introduction

The fact that osmotic water flow across the urinary bladder of the toad is stimulated by vasopressin and its intracellular mediator, cyclic AMP, has been recognized for many years (Hays & Leaf, 1962; Orloff & Handler, 1962). The factors regulating this effect are less clear. It has been suggested that prostaglandins (Orloff, Handler & Bergstrom, 1965; Lipson, Hynie & Sharp, 1971; Zusman, Keiser & Handler, 1977), cytosolic calcium concentrations (Hardy, 1978; Taylor, Eich & Pearl, 1977), and intracellular pH (Taylor, Eich & Brem, 1980) all may play a role in modulating the hydrosmotic effect of vasopressin. In the present study, the effect of the sodium ionophore, monesin, on vasopressin-stimulated osmotic water flow was studied.

Materials and Methods

Texas toads (*Bufo marinus*) were kept on wood shavings moistened with tap water. The animals were double pithed and their bladders excised and placed in amphibian Ringer's solution containing (in mM): 90, NaCl; 25, $NaHCO_3$; 3, KCl; 1, $CaCl_2$; 0.5, $MgSO_4$; 0.5, KH_2PO_4; and 5.5, glucose. The solution was gassed with 97% O_2–3% CO_2 and had a final pH of 7.7 to 7.8. Osmolality was 220 mOsmol/kg H_2O.

Water flow along an osmotic gradient was measured by the technique of Bentley (1951). Each of the paired hemibladders was mounted as a sac, serosal side out, at the end of a hollow glass rod. The sac was filled with 3 ml of Ringer's solution diluted 1:5 and immersed in a bathing medium containing 20 ml of isotonic Ringer's solution. At the beginning of each experiment, one sac from each pair of hemibladders was designated the experimental sac, with the other member of the pair serving as the control. The sacs and their contents were weighed periodically. Weight loss was equal to the amount of water that had moved from the sac along the osmotic gradient. If a sac had an osmotic water flow greater than 150 μl/hr during the control period (Period 1), it was discarded along with its pair. After Period 1, monensin was added to the serosal or mucosal solution of the experimental sac and water flow measured for 1 hr (Period 2). At the end of Period 2, either antidiuretic hormone (ADH) or adenosine 3′,5′-monophosphate was added to the serosal bathing solution of both the experimental and the control sacs and water flow measured for 1 additional hour (Period 3).

Data were calculated using Student's paired *t*-test and are presented as the mean ± standard error. Since monesin is poorly soluble in water, the stock solution (10 mg/ml) was dissolved in ethanol. An equivalent volume of ethanol was added to the control hemibladders.

Results

Effect of Monensin on Basal Osmotic Water Flow (Table 1A)

When monensin was added to the serosal bathing solution at concentrations of 1 to 100 μg/ml, the drug did not significantly change osmotic water flow during Period 2. Monensin (10 μg/ml) in the mucosal bathing solution also had no effect on osmotic water flow.

Effect of Monensin on the Hydrosmotic Response to ADH (Table 1B and C)

The increase in osmotic water permeability caused by 2 mU/ml of vasopressin was inhibited by monensin

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Table 1. The effect of monensin on basal and stimulated osmotic water flow

	Osmotic water flow (μl/min/sac)			
	Control	Monensin	C−M	P
A. Effect on basal water flow				
1 μg/ml serosal monensin ($n=8$)	−0.7±0.2	−0.6±0.2	−0.1±0.2	>0.5
10 μg/ml serosal monensin ($n=8$)	−0.1±0.15	−0.5±0.14	0.4±0.3	>0.1
100 μg/ml serosal monensin ($n=8$)	−0.9±0.14	0.2±0.8	−1.1±0.8	>0.2
10 μg/ml mucosal monensin ($n=8$)	−0.6±0.3	−0.8±0.2	0.2±0.3	>0.5
B. Effect on H_2O flow response to ADH, 2 mU/ml				
1 μg/ml serosal monensin ($n=8$)	12.8±2.6	12.9±2.9	0±1.4	>0.5
10 μg/ml serosal monensin ($n=8$)	21.6±1.1	7.1±1.1	14.5±1.0	<0.001
100 μg/ml serosal monensin ($n=8$)	16.2±2.7	4.7±1.0	11.4±2.1	<0.001
10 μg/ml mucosal monensin ($n=8$)	13.0±2.1	11.3±1.8	1.7±2.2	>0.4
C. Effect on H_2O flow response to ADH, 100 mU/ml				
10 μg/ml serosal monensin ($n=8$)	19.8±3.2	19.9±2.2	−0.1±2.1	>0.5
100 μg/ml serosal monensin ($n=7$)	19.5±2.6	12.8±2.7	6.7±1.5	<0.005
D. Effect of monensin (10 μg/ml) on H_2O flow response to cAMP				
2.5 mM cAMP ($n=8$)	7.1±1.7	3.7±1.4	3.4±0.6	<0.001
5.0 mM cAMP ($n=8$)	15.3±2.4	17.2±1.9	−1.9±1.4	>0.2

(n = number of experiments)

(*A*): Following a basal period (Period 1) monensin was introduced into the mucosal or serosal bathing solutions of experimental hemibladders. Osmotic water flow was measured for 1 hr (Period 2). The effect of monensin on basal water flow was calculated by comparing (Period 2–Period 1)$_{control}$ with (Period 2–Period 1)$_{experimental}$. (*B, C,* and *D*): After Period 2, ADH or cyclic AMP was added to the serosal media of all hemibladders and water flow monitored for 1 hr (Period 3). To determine the effect of monensin on stimulated water flow (Period 3–Period 2)$_{control}$ was compared with (Period 3–Period 2)$_{monensin}$. Data were analyzed using Student's paired t test and are presented as means ±SE.

at concentrations of 10 μg/ml or greater in the serosal bathing medium. Monensin at a concentration of 1 μg/ml had no significant effect on the stimulation of osmotic water flow by ADH. The extent of the inhibition by monensin of the hydrosmotic response to 2 mU/ml of ADH was comparable in bladders treated with 10 or 100 μg/ml of serosal monesin (Table 1 *B*). When 100 mU/ml of ADH was used, a difference was noted between serosal monensin at 10 μg/ml and 100 μg/ml. At a concentration of 10 μg/ml, monensin had no significant effect on the stimulation of osmotic water flow by 100 mU/ml of ADH. In contrast, when the ionophore was added to the serosal bathing medium at a concentration of 100 μg/ml, significant inhibition of the hydrosmotic response to 100 mU/ml of ADH was noted (Table 1 *C*).

When monensin was added to the mucosal solution at a concentration of 10 μg/ml, there was no significant effect on the water flow response to 2 mU/ml of ADH (Table 1 *B*).

Effect of the Simultaneous Addition of Monensin and ADH

When monensin at a concentration of 10 μg/ml and ADH (2 mU/ml) were added simultaneously to the serosal bathing solution, the ionophore had no significant effect on the stimulation of osmotic water flow by ADH over a period of one hour (data not shown).

The Effect of Monensin on the Osmotic Water Flow Response to Cyclic AMP (Table 1 D)

The stimulation of osmotic water flow across the toad bladder by 2.5 mM cAMP was significantly inhibited by serosal monensin at a concentration of 10 μg/ml. When a higher concentration of cAMP (5 mM) was added, no significant inhibition by monensin was observed.

Effect of Ionic Substitution on ADH-Stimulated Osmotic Water Flow and Its Inhibition by Monensin (Table 2)

When calcium was removed from the bathing solutions, the osmotic water flow response to 2 mU/ml of vasopressin was comparable to control bladders in other experiments. In addition, the inhibition of vasopressin-stimulated osmotic water flow by 10 μg/ml of serosal monensin was unaffected by the absence of calcium in the bathing solutions. In contrast, when choline replaced sodium in the serosal bathing solu-

Table 2. The effect of monensin on ADH-stimulated osmotic water flow in the absence of calcium or sodium

	Response to ADH (μl/min/sac)			
	Control	Monensin	C−M	P
A. Calcium-free mucosal and serosal bathing solutions ($n=8$)	18.3±1.3	5.0±1.5	13.3±1.4	<0.001
B. Sodium-free serosal bathing solutions ($n=8$)	12.4±0.9	11.9±1.1	0.5±1.0	>0.5

In these experiments, bladders were incubated either in media containing no calcium in the mucosal and serosal bathing solutions (*A*) or in a serosal medium in which choline replaced sodium (*B*). After initial equilibration, monensin (10 μg/ml) was added to the serosal bathing solution of experimental hemibladders and osmotic water flow measured for 1 hr (Period 2). At this point, 2 mU/ml of ADH was added to the serosal bathing solutions of all hemibladders and osmotic water flow measured for an additional hour (Period 3). To determine the effect of monensin on ADH-stimulated water flow (Period 3–Period 2)$_{control}$ was compared with (Period 3–Period 2)$_{monensin}$. Data were analyzed using Student's paired *t*-test and are presented as means ± SE.

tion, the inhibition of ADH-stimulated osmotic water flow by serosal monensin was completely eliminated.

Discussion

Monensin is a monocarboxylic acid that forms a cage-like structure with monovalent cations. It complexes ten times more readily with sodium than with potassium. In red blood cells, monensin increases sodium transport about five times as much as potassium transport. The ionophore does not bind divalent cations. At physiologic pH, the ionophore carries a net negative charge but it is electrically neutral after binding a cation (Pressman, 1976).

Monensin inhibits the hydrosmotic response to ADH and cyclic AMP in the toad bladder. The inhibition is dependent upon both the concentration of monensin and the concentration of ADH or cyclic AMP. At a concentration of 1 μg/ml, monensin does not inhibit the response to 2 mU/ml of ADH. At a concentration of 10 μg/ml, monensin inhibits the hydrosmotic response to 2 mU/ml of ADH or 2.5 mM cyclic AMP but not the response to 100 mU/ml of ADH or 5 mM cyclic AMP. At a monensin concentration of 100 μg/ml, the response to 100 mU/ml of ADH was also inhibited.

A number of aspects of the effect of monensin are worthy of comment. First, monensin in the serosal but not the mucosal bathing solution inhibits the response to ADH. Monensin also alters sodium transport only when added to the serosal bathing medium (S.A. Mendoza and M. Thomas, *manuscript in preparation*). The simplest explanation for this difference is that monensin alters sodium transport across the basolateral membrane but not the apical membrane. Second, monensin only inhibited the hydrosmotic response to ADH when sodium was present in the bathing medium. Thus, the inhibition is due to the fact that monensin is a sodium ionophore and not a nonspecific effect of the drug. Third, the inhibition by monensin of the osmotic water flow response to ADH does not require the presence of calcium in the bathing solutions. It is known that elevated cytosolic calcium inhibits ADH and cyclic AMP-induced water flow (Taylor et al., 1977; Hardy, 1978) and that Na−Ca exchange across the basolateral membrane is a regulator of cytosolic Ca concentration (Taylor & Eich, 1978). Nevertheless, extracellular Ca plays no apparent role in the inhibitory effect of monensin. These experiments do not rule out a possible role of intracellular Ca on the effect of monensin. Fourth, in some systems, monensin stimulates Na−H exchange. If this occurs in the toad bladder, it would increase intracellular pH. Taylor et al. (1980) reported that lowering the pH of the bath from 8.5 to 6.5 inhibits the hydrosmotic response to ADH. Thus, it is unlikely that the effect of monensin can be attributed to changes in intracellular pH, although this question has not been studied directly.

In conclusion, it has been shown that monensin in the serosal bathing solution inhibits the hydrosmotic response to vasopressin and cyclic AMP. This inhibition requires the presence of sodium in the serosal bathing solution but does not require extracellular calcium. Thus it would appear that intracellular sodium concentration is a regulator of the hydrosmotic response to vasopressin. A variety of physiologic factors including the synthesis of prostaglandins (Orloff et al., 1965; Lipson et al., 1971; Zusman et al., 1977), intracellular calcium (Taylor et al., 1977; Hardy, 1978), intracellular pH (Taylor et al., 1980), and intracellular sodium all may play a role in the regulation of the hydrosmotic response to vasopressin and cyclic AMP. In addition, it is tempting to relate the observed effects of monensin to the fact that lithium also inhibits the hydrosmotic response to vasopressin (Singer & Franko, 1973). It is possible that lithium and sodium interfere with the hydrosmotic response to vasopressin and cyclic AMP in the same manner, although lithium is active only in the mucosal bathing solution and monensin is active only in the serosal bathing solution.

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