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Cellular calcium uptake in the action of prostaglandins on renal water excretion

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Cellular calcium uptake in the action of prostaglandins on renal water excretion. The role of cellular calcium uptake in the antidiuretic response to vasopressin was studied in anesthetized dogs undergoing water diuresis. In prostaglandin (PG) intact animals, an intrarenal infusion of verapamil caused only a modest blunting of the response to antidiuretic hormone (ADH), because the urinary osmolality (U_{Osm}) achieved in the contralateral control kidney was 338 ± 40 mOsm/kg H_2O but was only 270 ± 23 mOsm/kg H_2O in the verapamil-infused kidney. The possibility was then studied that PG inhibit the action of ADH by impairing cellular calcium uptake. If so, verapamil would be expected to abolish the effect of PG inhibition to enhance the action of ADH. In eight PG-inhibited dogs, the control kidney's U_{Osm} increased to a mean of 650 ± 103 mOsm/kg H_2O but only to 280 ± 22 mOsm/kg H_2O in the infused side. Thus, verapamil abolished the effect of PG inhibition to enhance the action of ADH. Likewise, in five dogs a second chemically dissimilar inhibitor of calcium transport, proadifen, also abolished the effect of PG inhibitors as U_{Osm} rose to 590 ± 78 mOsm/kg H_2O in the control kidney but only to 278 ± 11 mOsm/kg H_2O in the proadifen-infused kidney. Neither prior vasodilatation nor an increased solute excretion with mannitol of a degree observed with verapamil mimicked the effect of the calcium uptake blockers to inhibit the action of ADH. The present in vivo studies therefore demonstrate that the effect of PG inhibitors to enhance the hydroosmotic effect of vasopressin involve cellular calcium transport.

La captation cellulaire de calcium dans l'action des prostaglandines sur l'excrétion rénale d'eau. Le rôle de la captation cellulaire de potassium sur la réponse antidiurétique à la vasopressine a été étudiée chez les chiens anesthésiés et soumis à une diurèse aqueuse. Une perfusion intrarénale de vérapamil détermine une atténuation discrète de la réponse à d'hormone antidiurétique (ADH) puisque l'osmolalité urinaire (U_{Osm}) obtenue dans le rein controlatéral est de 338 ± 40 mOsm/kg H_2O mais seulement de 270 ± 23 mOsm/kg H_2O ($P < 0,05$) du côté perfusé avec du vérapamil. La possibilité que les prostaglandines (PG) inhibent l'action de l'ADH en empêchant la captation cellulaire du calcium a été étudiée. Si cela était le cas, on devrait s'attendre à ce que le vérapamil abolisse l'effet de l'inhibition de PG qui augmente l'action de l'ADH. Chez huit chiens dont PG était inhibé U_{Osm} des reins contrôles a augmenté jusqu'à une valeur de 650 ± 103 mOsm/kg H_2O, mais seulement à 280 ± 22 mOsm/kg H_2O du côté perfusé. Ainsi le vérapamil abolit l'effet de l'inhibition de PG. De la même façon, chez cinq chiens, un autre inhibiteur du transport du calcium chimiquement différent, le proadifen (0,375 mg/kg/min), abolit aussi l'effet des inhibiteurs de PG puisque U_{Osm} augmente à 590 ± 78 mOsm/kg H_2O dans les reins contrôles, mais seulement à 278 ± 11 mOsm/kg H_2O dans les reins perfusés avec le proadifen. Ni la vasodilatation préalable, ni l'augmentation de l'excrétion de substances dissoutes par le mannitol à un niveau comparable à celui observé avec le vérapamil ne reproduisent l'effet des inhibiteurs de la captation de calcium. Les résultats obtenus in vivo démontrent donc que l'effet des inhibiteurs de PG, l'augmentation de l'effet hydroosmotique de la vasopressine, implique le transport cellulaire du calcium.

It has become increasingly evident that the ability of vasopressin to augment the water permeability of various epithelial surfaces can be modified by a number of physiologic and pharmacologic factors [1, 2]. Among these, the role of calcium has been the subject of considerable investigation. It has thus been amply recognized that both man [3] and experimental animals [4] with chronic elevations in serum calcium exhibit an impairment in maximal urinary concentration. Likewise, acute elevations of serum calcium also diminish the hydroosmotic effect of exogenous vasopressin [5].

More recently, attention has been directed to the possibility that alterations in intracellular calcium concentration could also play a critical role in the action of vasopressin. The studies that support such a view, however, have been performed exclusively in vitro. Moreover, the results have not been entirely consistent. For example, calcium ionophores have been reported to both inhibit [6] and to enhance [7] the in vitro hydroosmotic effect of vasopressin. The latter observation is more compatible with the preliminary results of Humes et al [8] who found that verapamil, a blocker of cellular calcium transport, inhibits the hydroosmotic action of vaso-

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pressin on the toad bladder. In addition, the possibility that the effect of prostaglandins (PG) to antagonize the action of vasopressin [9–11] could also involve cellular calcium transport has been suggested in a preliminary in vitro study [12]. This possibility, however, has not been examined in vivo. The present experiments were therefore designed to assess in vivo whether inhibition of cellular calcium transport alters the renal response to vasopressin in both PG-replete and -depleted dogs. The results demonstrate that the hydroosmotic action of vasopressin in vivo is partially attenuated by blockade of cellular calcium uptake. Even more pronounced, the effect of inhibition of PG synthesis to potentiate the in vivo action of the vasopressin was totally abolished by inhibition of cellular calcium uptake. Thus, not only is cellular calcium uptake involved in the in vivo hydroosmotic effect of vasopressin, but PG appears to antagonize the in vivo action of vasopressin primarily by blocking cellular calcium transport.

Methods

Studies were performed in 30 mongrel dogs weighing between 15 and 30 kg. Food was withheld for 18 hours, and water was allowed ad lib. All animals were anesthetized with pentobarbital (30 ml/kg), intubated, and ventilated with a Harvard respirator (Harvard Apparatus Co., Inc., Millis, Massachusetts). Light anesthesia was maintained throughout by administration of supplemental pentobarbital. Polyethylene catheters were placed in both ureters and renal veins through bilateral flank infusions by a retroperitoneal approach. Systemic pressure was continuously monitored by a brachial artery catheter connected to a Statham transducer (Statham Instruments Inc., Oxnard, California). In animals whose renal perfusion pressure was decreased prior to the experiment, a Blalock vascular clamp was placed around the aorta above the renal arteries. In these animals, a femoral artery catheter was also placed so as to monitor pressure below the clamp, and 60 min before the experiment the renal perfusion pressure was decreased to between 100 and 110 mm Hg. In animals that received intrarenal infusions, a 25-gauge needle was placed in the renal artery. After completion of surgery, a solution of 0.9% sodium chloride containing sufficient inulin and para-aminohippurate (PAH) was infused (0.5 ml/min) into a foreleg vein to maintain plasma levels of these substances at 15 to 20 and 1 to 3 mg/dl, respectively. At the same time, all animals received a solution of 2.5% glucose and water infused in the same foreleg vein at a rate of 20 ml/min for 60 min. After the administration of this water load, the infusion rate was decreased to 2 to 4 ml/min above urine flow rate. One to two hours were allowed for recovery from surgery. Only animals that achieved a stable water diuresis (U_{Osm} below 100 mOsm/kg H_2O) were studied. The periods of urine collection during the experiments were between 5 and 10 min in duration. Arterial and renal venous blood samples were obtained at the midpoint of alternate urine collections. The following experiments were undertaken.

Studies in dogs with intact prostaglandin synthetizing ability (N = 7). These studies were designed to determine whether inhibition of transmembrane influx of extracellular calcium inhibits or alters the antidiuretic response to vasopressin. To this end, we used verapamil (α-iso propyl α[N-methyl N-homoveratyl-γ-aminopropyl]-3,4 dimethoxyphenylacetonitrite hydrochloride Knoll Pharmaceutical Co., Whippany, New Jersey). The primary action of this drug involves the inhibition of slow inward calcium current [13, 14], an effect that is demonstrable by calcium 45 uptake studies in bladder epithelial cells [8].

Because in these preliminary studies the i.v. infusion of verapamil was noted to cause a marked decrease in systemic pressure, an intrarenal dose of the drug was sought. An intrarenal infusion of 0.005 mg/kg/min produced unilateral without systemic effects as evidenced by a failure to alter systemic pressure and a lack of natriuresis in the uninfused contralateral kidney when compared with the ipsilateral-infused kidney [15]. After a stable water diuresis was established and control collections obtained, intrarenal verapamil was started. Following these clearance measurements, 100 mU of vasopressin (Aqueous Pitressin, Parke, Davis and Co., Detroit, Michigan) was given i.v., and experimental measurements were begun 10 min later. When the effect of vasopressin had dissipated, postcontrol collections were obtained. This protocol allowed for the comparison of the antidiuretic response to vasopressin in the verapamil-infused kidney versus the contralateral control kidney.

Studies in dogs whose prostaglandin synthesis is inhibited. These experiments were designed to determine whether the effect of PG to inhibit the action of vasopressin involves the transcellular influx of extracellular calcium. If so, agents that block such a pathway would be expected to abolish the effect of PG inhibitors to enhance the action of vasopressin. The following protocols were undertaken.

(a) Intrarenal infusion of verapamil in PG-inhibited dogs (N = 13). The experimental protocol for 8 of these animals was the same as the one described above, except that the animals were pretreated with indomethacin (10 mg/kg), a dose that is known to inhibit PG synthesis in the anesthetized dog [16]. To ascertain that PG inhibition was achieved, we measured the urinary PG excretion in 7 of these dogs before indomethacin administration and during the intrarenal infusion of verapamil. A urine modification of the procedure of Dray, Charbonnel, and Maclouf [17] was used. Because the infusion of verapamil caused an ipsilateral increase in renal blood flow, an additional 5 animals were studied. The protocol was the same except that in these dogs a suprarenal aortic clamp was used to reduce renal perfusion pressure to between 100 and 110 mm Hg 60 min before the experiment to vasodilate the kidneys by autoregulation prior to verapamil administration.

(b) Intrarenal infusion of proadifen in PG-inhibited dogs (N = 5). To ensure that the effects observed with verapamil were indeed related to its effects on membrane calcium permeability, we used a second chemically dissimilar drug that also blocks cellular calcium uptake. Proadifen (SKF #525A) (2 diethylaminoethyl 2,2 diphenylvalerate hydrochloride) [18, 19] was obtained from Smith Kline & French, Philadelphia, Pennsylvania. Because the supply of this agent was limited, these five studies were performed in dogs that were somewhat smaller (15 to 20 kg). After preliminary studies, a dose of 0.375 mg/kg/min intrarenally was found to produce unilateral natriuresis and diuresis without systemic hemodynamic changes [15]. The protocol was again the same as described for the above intrarenal studies except that proadifen was given instead of verapamil.

(c) Effect of increased solute excretion on the response to vasopressin in PG-inhibited animals (N = 5). The administration of both verapamil and proadifen caused a significant increase in solute clearance. This effect was also seen in the 5 animals whose renal perfusion pressure was reduced prior to verapamil administration. Because the increase in solute clearance could have modified the response to vasopressin, solute excretion was increased in 5 PG-inhibited dogs by the infusion of 5% mannitol, 10 ml/kg, over 60 min. The response of these animals undergoing a solute diuresis to the 100 mU bolus of vasopressin was then tested.

Analytic measurements and the calculations used for clearance measurements were performed as previously described [20]. Statistics were performed using Scheffe's [21] analysis of variance between experimental periods. Comparison between infused and uninfused kidney in the same experimental period were done with the Student's t test. A P value of less than 0.05 was considered significant. All data were expressed as the means ± SEM.

Results

Effect of verapamil on the renal response to vasopressin in dogs with normal prostaglandin synthetizing ability (Table 1). These experiments were undertaken to determine whether the administration of a blocker of cellular calcium uptake inhibits the renal response to vasopressin. The intrarenal administration of verapamil caused no detectable alterations in extracellular calcium concentration. In view of the profound effect of the verapamil infusion on systemic pressure, attempts were made to find a dose that would produce unilateral renal effects of the drug. This was achieved with the ipsilateral infusion of 0.005 mg/kg/min of the drug. This dose caused no significant changes in systemic pressure, as mean arterial pressure was 143 ± 10 before and 138 ± 7.5 mm Hg after verapamil. Likewise, as is shown in Table 1, there were no alterations in the function of the noninfused contralateral kidney. In these PG-replete dogs, the increased renal blood flow in the infused kidney did not achieve statistical significance. The slight but significant increase in basal U_{Osm} reflects the increment in solute excretion rather than presence of vasopressin in the circulation, because the contralateral urine remained maximally dilute. The maximal urinary osmolality achieved after the administration of 100 mU of vasopressin was slightly but significantly greater in the contralateral kidney (337 ± 45 mOsm/kg H_2O) than it was in the verapamil-treated kidney (270 ± 23 mOsm/kg H_2O, $P < 0.05$). A similar significant difference ($P < 0.05$) was obtained when the increment in U_{Osm} for each kidney was analyzed.

Studies in dogs whose prostaglandin synthesis is inhibited: (a) Effect of verapamil on the action of vasopressin in PG-inhibited dogs (Table 2, Fig. 1). These studies were undertaken to test the possibility that PG antagonize the response to vasopressin by inhibiting the transcellular movement of calcium. If so, the potentiating effect of PG inhibition on the hydroosmotic effect of vasopressin would be diminished by agents that block this cellular pathway. As shown in Fig. 1, the maximal U_{Osm} achieved after vasopressin administration was markedly different in the kidney receiving verapamil (infused kidney) than in the contralateral con-

Table 1. Effect of intrarenal verapamil on renal hemodynamics and solute and water excretion in prostaglandin-replete dogs[a]

	Glomerular filtration rate *ml/min*				Renal blood flow *ml/min*			
	Control	Verapamil	ADH	Postcontrol	Control	Verapamil	ADH	Postcontrol
Infused	43.0 ±3.5	46.0 ±3.5	48.0 ±3.5	47.0 ±4.1	337 ±52	372 ±51	385 ±39	344 ±42
P		NS	NS	NS		NS	NS	NS
Noninfused	43.0 ±5.1	44.0 ±4.8	43.0 ±3.6	45.0 ±4.0	398 ±52	348 ±38	310 ±35	304 ±26
P		NS	NS	NS		NS	NS	NS

[a] Values are the means ± SEM (N = 8 dogs).
[b] $P < 0.05$, compared with noninfused kidney in same experimental period.
[c] $P < 0.005$, compared with noninfused kidney in same experimental period.

Table 2. Effect of intrarenal verapamil on renal hemodynamics and solute and water excretion in prostaglandin-inhibited dogs[a]

	Glomerular filtration rate *ml/min*				Renal blood flow *ml/min*			
	Control	Verapamil	ADH	Postcontrol	Control	Verapamil	ADH	Postcontrol
Infused	44.0 ±4.6	43.0 ±4.0	48.0 ±5.1	43.0 ±5.4	380 ±62	435[b] ±73	368[f] ±55	346 ±61
P		NS	NS	NS		NS	NS	NS
Noninfused	37.0 ±2.8	37.0 ±3.2	44.0 ±5.2	45.0 ±4.8	423 ±45	367 ±68	292 ±51	312 ±49
P		NS	NS	NS		NS	NS	NS

[a] Values are the means ± SEM (N = 8 dogs).
[b] $P < 0.05$, compared with noninfused kidney in same experimental period.
[c] $P < 0.01$, compared with noninfused kidney in same experimental period.
[d] $P < 0.001$, compared with noninfused kidney in same experimental period.
[e] $P < 0.02$, compared with noninfused kidney in same experimental period.
[f] $P < 0.005$, compared with noninfused kidney in same experimental period.

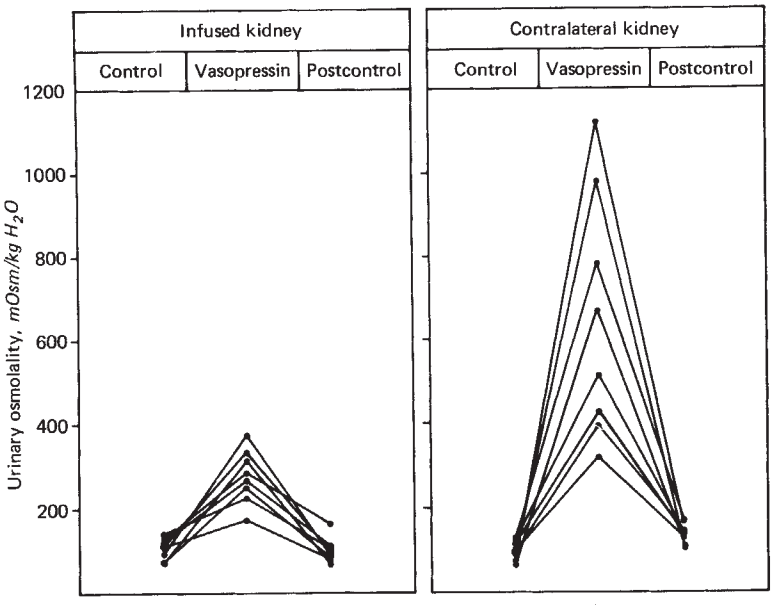

Fig. 1. *Effect of 100 mU of vasopressin on urinary osmolality in verapamil-infused kidneys* (left panel) *when compared with contralateral uninfused controls* (right panel) *in prostaglandin-depleted dogs.*

Table 1. *(continued)*

Solute clearance *ml/min*				Free water clearance *ml/min*				Urinary osmolality *mOsm/kg H_2O*			
Control	Verapamil	ADH	Postcontrol	Control	Verapamil	ADH	Postcontrol	Control	Verapamil	ADH	Postcontrol
1.51	2.18[c]	3.80[c]	1.96[c]	3.16	3.70[c]	−0.19	3.29[b]	85.0	117.0	270.0[b]	96.0
±0.32	±0.42	±0.70	±0.20	±0.32	±0.33	±0.36	±0.55	±11.3	±14.5	±22.7	±12.1
<0.01	NS	<0.005		NS	<0.001	<0.001		<0.05	<0.001	<0.001	
1.26	1.19	1.56	0.96	2.79	2.35	−0.35	1.88	82.0	90.0	337.0	92.0
±0.26	±0.27	±0.32	±0.12	±0.30	±0.25	±0.22	±0.25	±7.0	±11.8	±45.5	±7.2
NS	NS	NS		NS	<0.001	<0.001		NS	<0.001	<0.001	

Table 2. *(continued)*

Solute clearance *ml/min*				Free water clearance *ml/min*				Urinary osmolality *mOsm/kg H_2O*			
Control	Verapamil	ADH	Postcontrol	Control	Verapamil	ADH	Postcontrol	Control	Verapamil	ADH	Postcontrol
1.54	3.8[f]	4.52[f]	1.47	3.73	4.36[d]	−0.29[e]	2.89	79.0	104.0	280.0[c]	91.0
±0.25	±0.5	±0.39	±0.27	±0.47	±0.56	±0.20	±0.46	±10.7	±13.3	±21.9	±8.8
<0.001	NS	<0.001		NS	<0.001	<0.001		NS	<0.001		
1.10	1.14	1.96	1.14	2.64	2.14	−1.05	1.75	80.0	87.0	650.0	115.0
±0.14	±0.11	±0.46	±0.13	±0.45	±0.37	±0.18	±0.39	±9.5	±10.3	±103.5	±13.0
NS	NS	NS		NS	<0.001	<0.001		NS	<0.001	<0.001	

trol. The latter achieved a mean U_{Osm} of 650 mOsm/kg H_2O, reflecting the enhanced response to vasopressin in the PG-depleted state. In every animal, the U_{Osm} achieved in the contralateral control kidney was higher than that of the ipsilateral infused one, which achieved only a mean U_{Osm} of 280 mOsm/kg H_2O ($P < 0.01$). This latter response is almost identical to that observed in infused kidneys of animals not pretreated with PG inhibitors (280 vs. 270 mOsm/kg H_2O). Because a failure to suppress PG synthesis in verapamil-treated kidneys could explain these results, PG excretion was measured in 7 of these dogs. Mean PG excretion was 4127 ± 832 pg/min before indomethacin administration. This drug suppressed PG excretion equally in verapamil-treated kidneys (567 ± 67 pg/min) and contralateral control (598 ± 186 pg/min).

As was the case in the animals not treated with indomethacin, systemic pressure was not significantly altered in these studies. In these PG-inhibited animals, however, the verapamil-infused kidney had a somewhat higher renal blood flow than its contralateral control (Table 2). Because increases in renal blood flow, particularly in the renal medullary, could alter the response to vasopressin, 5 additional animals whose kidneys were vasodilated by decreased renal perfusion pressure prior to the experiments were studied. In these animals, verapamil did not increase renal blood flow, and the infused and contralateral kidneys had comparable flows, 223 ± 40 and 201 ± 38 ml/min, respectively. In spite of these comparable renal blood flows, the response to 100 mU of vasopressin was very different as the control uninfused kidney achieved a maximal U_{Osm} of 683 ± 142 mOsm/kg H_2O and the verapamil-infused kidney only 253 ± 25 mOsm/kg H_2O ($P < 0.02$).

(b) Effect of proadifen on the action of vasopressin in PG-inhibited dogs (Table 3 and Fig. 2). To ascertain that the profound effects exhibited by the infusion of verapamil in the PG-depleted state was not due to a nonspecific effect of this drug, we used another chemically dissimilar agent that also blocks slow channel calcium uptake. The intrarenal in-

Table 3. Effect of intrarenal proadifen (SKF #525-A) on renal hemodynamics and solute and water excretion in prostaglandin-inhibited dogs[a]

	Glomerular filtration rate *ml/min*				Renal blood flow *ml/min*			
	Control	Proadifen	ADH	Postcontrol	Control	Proadifen	ADH	Postcontrol
Infused	33.0 ±3.5	38.0 ±4.4	35.0 ±5.2	32.0 ±4.8	155 ±24	222[b] ±39	235[b] ±43	162 ±18
P		NS	NS	NS		NS	NS	NS
Noninfused	36.0 ±3.9	36.0 ±5.2	31.0 ±8.7	27.0 ±5.2	162 ±24	169 ±24	150 ±35	140 ±14
P		NS	NS	NS		NS	NS	NS

[a] Values are the means ± SEM ($N = 5$ dogs).
[b] $P < 0.05$, compared with noninfused kidney in same experimental period.
[c] $P < 0.01$, compared with noninfused kidney in same experimental period.
[d] $P < 0.02$, compared with noninfused kidney in same experimental period.
[e] $P < 0.005$, compared with noninfused kidney in same experimental period.

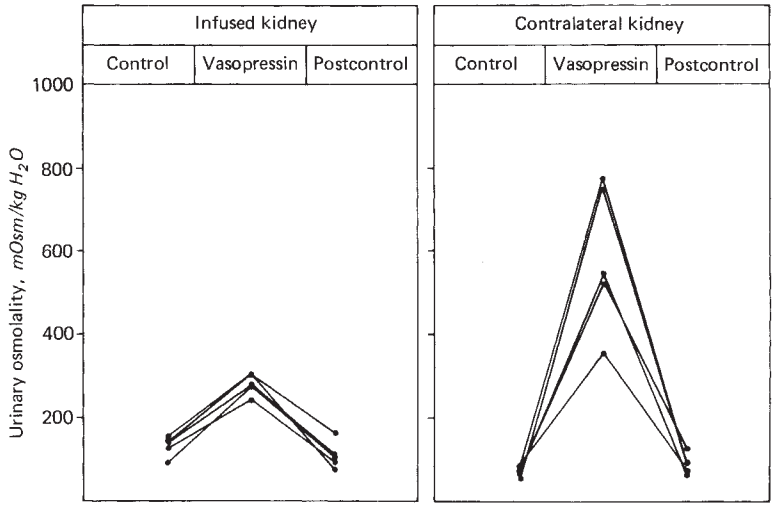

Fig. 2. *Effect of 100 mU of vasopressin on urinary osmolality in proadifen-infused kidneys* (left panel) *when compared with contralateral uninfused controls* (right panel) *in prostaglandin-depleted dogs.*

fusion of 0.375 mg/kg/min caused no significant alterations in serum calcium or in systemic pressure. As shown in Table 3, the infusion caused changes in renal blood flow and solute clearance but no alteration in the function of the contralateral kidney. As was the case with verapamil, in each animal the maximal U_{Osm} achieved was greater in the control than it was in the infused kidney (Fig. 2). Although the former achieved a mean maximal U_{Osm} of 590 mOsm/kg H_2O, the proadifen-infused kidney reached only a mean U_{Osm} of 278 mOsm/kg H_2O ($P < 0.005$).

(c) Effect of increased solute excretion on the response to vasopressin in PG-inhibited dogs (Fig. 3). Because both verapamil and proadifen caused an increase in ipsilateral solute excretion (Tables 2 and 3), the possibility that the blunted response to vasopressin observed in these kidneys as a consequence of this effect was considered. In 5 animals, an increment in solute excretion was achieved that was comparable or even exceeded that observed in dogs given the calcium uptake inhibitors. Thus, the mean solute excretion in the mannitol-treated animals was 5.32 ± 0.56 ml/min with a mean U_{Osm} of 171 ± 6.9 mOsm/kg H_2O. Despite the very high solute excretion (Fig. 3), the administration of 100 mU of vasopressin increased maximal U_{Osm} to a mean of 590 ± 32 mOsm/kg H_2O, which is significantly greater than observed in the verapamil-($P < 0.005$) or proadifen-infused kidneys ($P < 0.02$) and not different from that achieved by the contralateral uninfused kidneys in those experiments.

Discussion

The effect of an increase in extracellular fluid calcium concentration to impair the renal concen-

Solute clearance *ml/min*				Free water clearance *ml/min*				Urinary osmolality *mOsm/kg H_2O*			
Control	Proadifen	ADH	Postcontrol	Control	Proadifen	ADH	Postcontrol	Control	Proadifen	ADH	Postcontrol
0.91	5.09[c]	4.86[c]	1.11	2.56	3.70[b]	−0.17[d]	2.33	71.0	146.0[b]	278.0[e]	99.0
±0.15	±1.40	±1.06	±0.13	±0.32	±0.60	±0.24	±0.34	±7.7	±19.8	±11.5	±16.9
<0.01	NS	<0.05		NS	<0.001	<0.005		NS	<0.001	<0.001	
0.95	0.82	1.16	0.83	2.67	2.68	−1.01	1.90	70.0	64.0	590.0	83.0
±0.23	±0.12	±0.41	±0.07	±0.40	±0.32	±0.27	±0.33	±9.5	±7.6	±77.7	±12.9
NS	NS	NS		NS	<0.001	<0.005		NS	<0.001	<0.001	

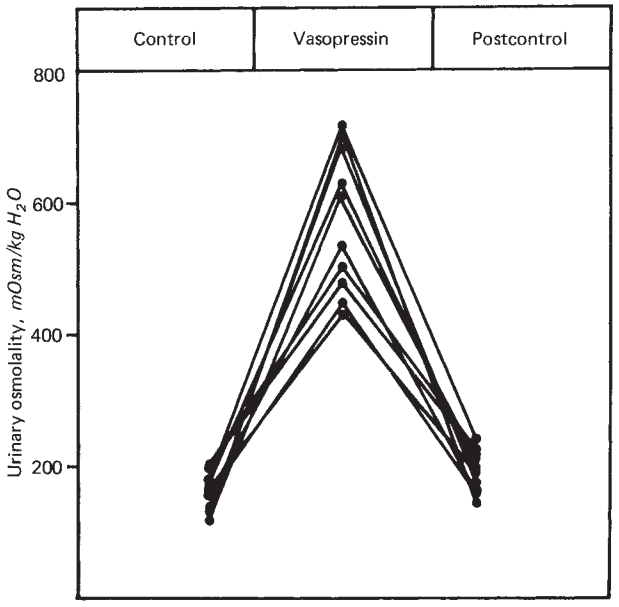

Fig. 3. *Effect of increased solute excretion with mannitol on the response to vasopressin in prostaglandin-depleted dogs.* Note that this response is comparable with that of the contralateral kidneys and distinctly greater than that of the infused kidneys in Figs. 1 and 2.

trating mechanism has been recognized for quite some time [3, 4]. Only recently, however, has a role of increased intracellular calcium concentration been suggested to be a determinant of the action of vasopressin [8]. The recent availability of agents that either promote or block the cellular uptake of calcium has provided experimental tools to study the effects of intracellular calcium concentration on water transport. Although these agents have been widely used in vitro, their in vivo effect on renal water excretion has not been investigated. In the present study, two separate blockers of cellular calcium uptake were used to investigate the in vivo effect of blockers of calcium membrane transport on the hydroosmotic response to vasopressin.

In the initial group of studies, intrarenal verapamil, a blocker of cellular membrane transport, was found to produce blunting of the hydroosmotic effect of submaximal doses of vasopressin. This effect was, however, very modest, and it should be emphasized that in none of the animals did verapamil prevent an antidiuresis. The small observed difference could well be due to an in vivo effect of verapamil to increase solute excretion rather than to block cellular calcium uptake. Moreover, the possibility existed that endogenous renal prostaglandins may inhibit calcium membrane transport and thus obscure a more profound effect of calcium transport inhibition on vasopressin-induced antidiuresis. If this were the situation, then the effect of inhibitors of calcium blockade on vasopressin-induced water transport would be more readily detectable in PG-deplete animals.

To test this possibility, we examined the effect of intrarenal verapamil on the hydroosmotic effect of vasopressin in animals pretreated with indomethacin, a potent inhibitor of PG synthesis. In these studies, a much greater inhibition of the antidiuretic effect of vasopressin was observed in the verapamil-infused kidneys (maximal U_{Osm}, 650 vs. 280 mOsm/kg H_2O, $P < 0.01$). Because the U_{Osm} in verapamil-treated kidneys of PG-deplete animals was no higher than untreated kidneys in PG-replete animals (270 vs. 280 mOsm/kg H_2O), these results suggest that verapamil totally abolished the known effect of PG inhibition to enhance the hydroosmotic effect of vasopressin. This finding is compatible with the interpretation that renal PG's normally block calcium membrane transport, thus accounting

for their inhibition of the action of vasopressin. In this regard, there is in vitro evidence that the action of vasopressin is dependent on membrane calcium transport because calcium ionophores enhance [7] and verapamil [8] or a calcium-free medium [22] impairs the action of vasopressin to enhance water movement across the toad bladder. It therefore followed that in the presence of diminished PG activity it might be easier to demonstrate the effect of calcium transport inhibition with verapamil to attenuate the hydroosmotic response to vasopressin. Such was clearly the case in the present study.

Because verapamil itself appears to be a mild inhibitor of PG synthesis [23], it is not likely that our results can be explained by enhanced PG synthesis on the infused kidney. In fact, our direct measurements of PG excretion reveal that the observed hydroosmotic effect is not due to disparate PG excretion in control and infused kidneys during indomethacin administration. It also seems clear from the present results that neither verapamil-induced increases in renal blood flow nor solute excretion could explain the effect of diminution in vasopressin response. Specifically, prior renal vasodilatation that abolished the increase in renal blood flow with verapamil did not alter the blocking effect of verapamil on the antidiuretic effect of vasopressin. Moreover, increases in solute excretion with mannitol to levels in excess of those observed with verapamil did not similarly impair the antidiuretic effect of vasopressin. Although these studies provide adequate individual controls for each of these two variables, they do not combine them. It must be noted, however, that the renal blood flow in the mannitol studies was 305 ± 45 ml/min during antidiuretic hormone administration. This flow is not as high as that of the verapamil-treated kidneys. It is, however, actually higher than proadifen-infused kidneys, providing a simultaneous combined control for these experiments.

Last, the possibility was considered in the present study that verapamil inhibited the action of vasopressin, particularly in the PG-inhibited animals, by an action other than blockade of cellular calcium uptake. To test this possibility, we examined the effect of another inhibitor of cell membrane transport, proadifen, on the antidiuretic action of vasopressin in PG-deplete animals. This cellular blocker produced an inhibition of the hydroosmotic effect of vasopressin that was comparable to that observed with verapamil (590 vs. 278 mOsm/kg H_2O, $P < 0.005$).

The mechanism whereby PG's alter the hydroosmotic response to vasopressin has been a subject of some controversy. There is considerable experimental evidence from both in vivo [24] and in vitro [25] studies to support the view that the effect involves an alteration in the water permeability of the collecting duct. But because both vasopressin and PG [26] affect solute transport in the ascending limb of Henle's loop, and both may well be involved in the control of intrarenal blood flow distribution, it is possible that their interaction also occurs by one or both of these mechanisms. The above sited in vitro data pointing to a role of cellular calcium on vasopressin action [7, 8] would suggest that our in vivo observations are mediated by a mechanism involving alterations in the hydraulic permeability of the collecting duct. The possibility, however, that the calcium-blocking agents alter the PG-antidiuretic hormone interaction by alternative pathways involving either changes in solute transport in the ascending limb of Henle or intrarenal blood flow distribution that could alter interstitial tonicity cannot be entirely excluded. These possibilities should be subject of future investigation.

Summary. Two chemically dissimilar blockers of calcium membrane transport were demonstrated to block the in vivo antidiuretic effect of vasopressin. This effect was most profound in the PG-deplete state. Evidence was presented that the effect of these inhibitors was unrelated to either the increase in renal blood flow or solute excretion. The present results thus are compatible with preliminary in vitro data that suggest that intracellular calcium is an important determinant of the action of vasopressin. Moreover, the ability of the two blockers to mimic a PG-replete vasopressin response in a setting of PG inhibition strongly suggests that PG's inhibit the in vivo action of vasopressin primarily by impairing calcium membrane transport.

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