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Effect of Vasopressin Administration on Sodium Excretion and Plasma Phosphate Concentration (39796)

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Introduction. It has been well established that arginine vasopressin (AVP) administration increases renal excretion of sodium and other electrolytes (1-3). Recent studies have demonstrated that AVP, when administered in pharmacologic doses, is a potent diuretic (4-7), although the mechanism whereby pharmacologic doses of AVP causes natriuresis is unknown.

It is well established, however, that the antidiuretic effect of AVP is mediated by stimulation of the medullary adenylate cyclase-cyclic adenosine 3',5'-monophosphate system (8, 9). Li^+ administration results in inhibition of medullary adenylate cyclase *in vitro* and *in vivo;* this inhibition of medullary adenylate cyclase is thought to account, at least in part, for the nephrogenic diabetes insipidus induced by Li^+ (10-15). Since Li^+ blocks the antidiuretic effect of physiologic doses of AVP we sought to determine whether or not Li administration also blocks the natriuretic effect of large doses of AVP.

In the course of these experiments we observed a novel and hitherto undescribed effect of AVP, i.e., AVP administration resulted in a dramatic increase in plasma phosphate concentration. In order to exclude that renal effects of AVP were due to an increase in parathyroid hormone (PTH) release secondary to the hyperphosphatemia, we also studied thyroparathyroidectomized dogs.

Methods. Forty-six experiments were performed on forty-six female mongrel dogs weighing between 10 and 20 kg. Anesthesia was induced with pentobarbital, 30 mg/kg intravenously, and maintained with subsequent small doses. An endotracheal tube was inserted and connected to a Bird respirator; plasma pCO_2 was maintained between 35 and 45 mm Hg by appropriate manipulation of the respirator. Saline 0.9% containing [^{125}I]iothalamate (100 μCi/liter) was infused through a femoral vein catheter at a rate of 0.5 ml/min as a marker of GFR. Blood was sampled from an arterial catheter. An equilibration period of at least 40 min was allowed before urine collections were started. Clearance periods were of 10-min duration. The following groups of animals were studied.

Group I. After three control clearance collections, aqueous vasopressin, in these experiments administered as aqueous Pitressin (Parke, Davis and Company), was infused at a rate of 50 mU/kg/min for 60 min. This protocol was used in the following subgroups.

(A) Seven normal dogs.

(B) Six Li^+-treated dogs. These dogs were prepared by daily intraperitoneal injections of LiCl, 3 mEq/kg body weight, in 30 ml of sterile water for 3 days, inclusive of the day of the study.

(C) Thyroparathyroidectomized (TPTX) dogs. Six normal dogs underwent TPTX 24 hr before the study. TPTX was considered complete if the serum Ca^{2+} dropped at least 2 mg%. In this group AVP infusion was continued for an additional 60 min.

Group II. In this group, Pitressin was administered at a rate of 50 mU/kg/hr after three control urine collections were obtained. The animals were prepared for clearance study as described above.

(A) Four normal dogs. In these dogs a solution of sodium nitroprusside was infused

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at varying rates in order to prevent the blood pressure from rising. The nitroprusside solution was prepared by dissolving 120 mg of the salt into 250 ml of 5% dextrose in water. The total amount of nitroprusside solution infused in each experiment was always less than 5 ml.

(B) Five Li^+-treated dogs.

(C) Nine TPTX dogs.

In Groups I and II it was observed that AVP caused a significant increase in plasma phosphate concentration. In order to exclude the possibility that this effect was caused by either impurities in the commercial AVP or diurnal variation, additional experiments were performed.

(A) In four normal dogs plasma phosphate was measured before and after administration of synthetic AVP [prepared in this laboratory and used in previous studies, see Ref. (16)] at a rate of 10 mU/kg/min. The natriuretic effect of this synthetic AVP was not measured since we have previously established that there is no difference between synthetic AVP and Pitressin with respect to their natriuretic effect (16).

(B) Four Li^+-treated dogs were used as "time controls." In these dogs clearance collections were obtained during 60 min but no AVP was infused.

Specimen collection, glomerular filtration rate (GFR), plasma and urinary electrolytes, and statistical analyses were performed as previously described (5, 17). Li^+ levels were measured with a flame photometer. In the table and figures the data presented are the means ± standard errors of the means of the first three control and last three experimental periods.

Results. AVP administration to normal dogs at a dose of 50 mU/kg/min ("high dose") resulted in a significant increase in urine flow and fractional sodium excretion (FENa) (Table I, Fig. 1). Plasma phosphate also increased significantly (Fig. 2) leading to an increase in the filtered load of phosphate; fractional phosphate excretion increased significantly from 4.0 ± 1.55 to 20.0 ± 5.21%, $P < 0.01$. Plasma calcium levels were unchanged after AVP administration.

Li^+-treated dogs had a plasma Li^+ concentration of 4.9 ± 0.64 mEq/liter. In Li^+-treated dogs AVP also caused a significant increase in urine flow, FENa, and plasma phosphate. The increase in FENa observed

TABLE I. EFFECT OF ARGININE VASOPRESSIN IN NORMAL, Li^+-TREATED AND TPTX DOGS.

Treatment		GFR[a] (ml/min)	V (ml/min)	FENa (%)	P_{PO_4} (mmole/liter)
		Group I, AVP (50 mU/kg/min)			
N	C	36.6 ± 5.05	0.2 ± 0.09	0.4 ± 0.23	1.6 ± 0.11
$n = 7$	*P*	NS	<0.05	<0.02	<0.02
	E	44.0 ± 2.07	2.8 ± 0.96	6.2 ± 1.81	2.2 ± 0.16
Li	C	31.1 ± 5.64	1.5 ± 0.41	1.5 ± 0.31	1.4 ± 0.10
$n = 6$	*P*	NS	<0.05	<0.05	<0.02
	E	37.4 ± 4.49	5.7 ± 1.69	7.9 ± 2.22	1.8 ± 0.18
TPTX	C	65.6 ± 7.87	1.0 ± 0.27	0.5 ± 0.15	1.7 ± 0.09
$n = 6$	*P*	NS	NS	<0.05	<0.05
	E	72.4 ± 14.67	2.9 ± 0.74	3.1 ± 0.82	2.3 ± 0.22
		Group II, AVP (50 mU/kg/hr)			
N + NaNP	C	17.3 ± 4.10	0.3 ± 0.10	1.5 ± 0.36	2.0 ± 0.19
$n = 4$	*P*	NS	<0.05	<0.05	<0.01
	E	21.4 ± 5.95	0.7 ± 0.13	3.0 ± 0.70	2.2 ± 0.18
Li	C	27.0 ± 5.87	0.5 ± 0.11	0.9 ± 0.18	1.3 ± 0.17
$n = 5$	*P*	NS	<0.05	<0.02	<0.01
	E	28.3 ± 5.02	1.2 ± 0.28	3.0 ± 0.47	1.7 ± 0.17
TPTX	C	61.4 ± 11.07	0.4 ± 0.13	0.4 ± 0.08	1.9 ± 0.14
$n = 9$	*P*	NS	<0.05	<0.02	<0.02
	E	59.1 ± 10.89	1.0 ± 0.28	1.3 ± 0.28	2.1 ± 0.13

[a] Abbreviations used: GFR = glomerular filtration rate; V = urine flow; FENa = fractional sodium excretion; P_{PO_4} = plasma phosphate; AVP = arginine vasopressin; N = normal; Li = lithium treated; TPTX = thyroparathyroidectomized; C = control; E = experimental.

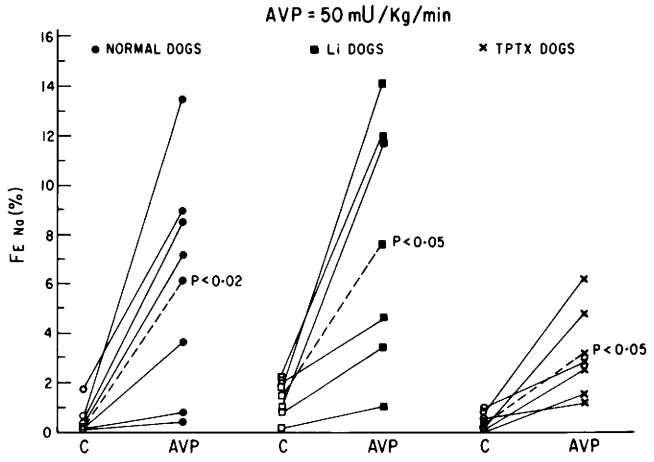

FIG. 1. Depicts fractional sodium excretion (FENa) in normal, Li^+-treated, and TPTX dogs before and during AVP administration at a dose of 50 mU/kg/min. The dashed lines represent the means.

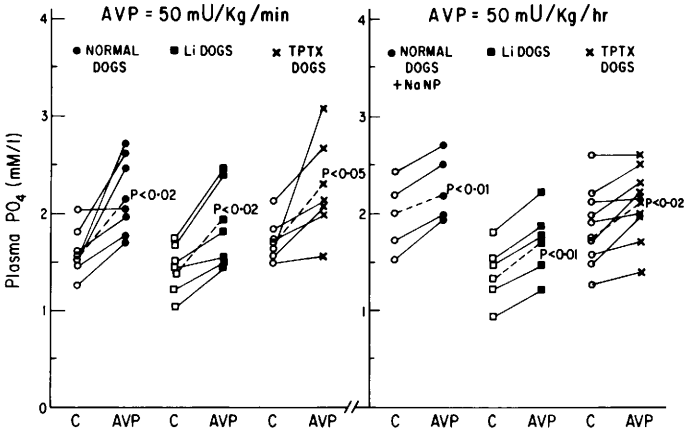

FIG. 2. Shows plasma phosphate concentration before and during AVP administration at doses of 50 mU/kg/min (left panel) and 50 mU/kg/hr (right panel). The dashed lines represent the means.

in these dogs was not statistically different from that seen in normal dogs. Thus AVP caused natriuresis in both normal and Li^+-treated dogs.

In TPTX dogs, AVP also caused an increase in FENa and plasma phosphate (Figs. 1 and 2). Administration of AVP for an additional 60 min did not result in a further increase in FENa; plasma phosphate increased slightly in the second hour without achieving significance (2.6 ± 0.16 vs 2.3 ± 0.22 m*M*/liter). The increase in FENa following AVP infusion in these dogs was somewhat lower than that observed in the normal dogs, but this difference did not achieve statistical significance.

The results obtained in Group II were almost identical to those observed in Group I. In the normal dogs, prevention of the rise in blood pressure (BP) with sodium nitroprusside (mean BP 143 ± 14.5 vs 148 ± 7.5 mm Hg) did not prevent the increase in FENa (Fig. 3) or the increase in plasma phosphate (Fig. 2).

In Li^+-treated and TPTX dogs AVP did not cause any increase in BP, but FENa and plasma phosphate increased significantly (Figs. 2 and 3).

Administration of synthetic AVP to normal dogs resulted in a significant increase in plasma phosphate from 1.5 ± 0.13 to 2.0 ± 0.04 m*M*/liter; $P < 0.05$ (Fig. 4). In Li^+-treated dogs not receiving AVP, plasma phosphate did not change significantly dur-

ing the same time period (Fig. 4). FENa in these dogs was unchanged (0.4 ± 0.08 vs 0.4 ± 0.11%). Plasma K^+ levels were unchanged in all groups after AVP administration.

Discussion. These data show an unexpected and previously undescribed effect of large doses of arginine vasopressin, i.e., hyperphosphatemia. The rise in plasma phosphate occurred within the first 30 min of AVP administration and tended to remain stable thereafter, or to increase slightly with prolonged infusion (at least 120 min). The hyperphosphatemia was observed with "high" and "low" doses of AVP and was unrelated to the presence of parathyroid glands.

In order to exclude the possibility that impurities in the commercial preparation of AVP (especially growth hormone) could have been responsible for the hyperphosphatemic effect, we also studied the effect of synthetic AVP on plasma phosphate. As can be seen in Fig. 4, synthetic AVP caused hyperphosphatemia of the same degree as that observed with the commercial preparation.

Other vasoconstrictive agents, such as angiotensin II, also have been shown to cause hyperphosphatemia (18); this effect may be mediated by vasoconstriction with consequent tissue ischemia which results in phosphate leakage from intracellular compartments. Two facts argue against tissue ischemia as being the mechanism whereby AVP causes hyperphosphatemia. First, serum potassium was unchanged when hyperphosphatemia was observed, and, second, the increase in serum phosphate was observed even when the blood pressure remained normal or was prevented from rising. It is thus highly unlikely that the increase in serum phosphate caused by AVP is mediated by its vasoconstrictive effect. We have no explanation for this phenomenon of hyperphosphatemia but it may be related to the release of phosphate from bone and/or muscle independent of the action of PTH. Further studies are in order to clarify the mechanism of hyperphosphatemia of AVP and to determine whether analogs of AVP can also cause hyperphosphatemia.

It is possible that AVP caused an increase in PTH levels secondary to the elevation in

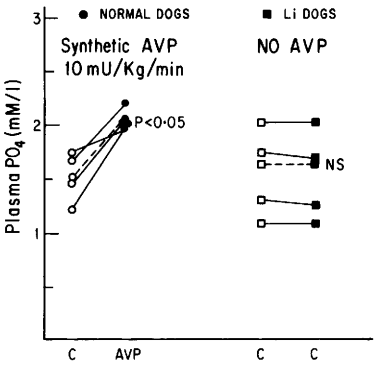

FIG. 4. Left panel: plasma phosphate concentration in normal dogs treated with synthetic AVP (10 mU/kg/min). Right panel: plasma phosphate concentration in Li^+-treated dogs that did not receive AVP. The dashed lines represent the means.

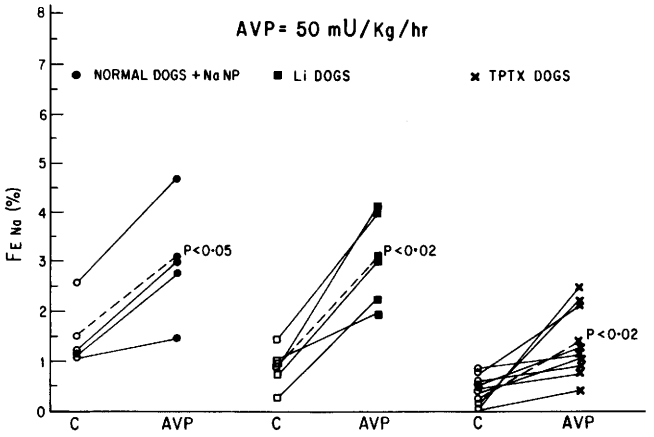

FIG. 3. Fractional sodium excretion in normal, Li^+-treated, and TPTX dogs before and during AVP administration at a dose of 50 mU/kg/hr. The dashed lines represent the means.

plasma phosphate which then led to a decrease in ionizable calcium. Although total serum calcium remained unchanged in our experiments ionized calcium could have decreased during AVP administration. PTH has been shown to reduce reabsorption of sodium, water, phosphate, and bicarbonate in the proximal tubule (19). The combination of decreased proximal reabsorption of water and electrolytes by PTH, in addition to the distal effect AVP might exert, could be the cause of the natriuresis observed during AVP administration. In order to exclude that a rise in endogenous PTH may account for the natriuretic effect of AVP, experiments were performed in thyroparathyroidectomized dogs. The natriuretic effect of AVP in thyroparathyroidectomized dogs tended to be of smaller magnitude than that in normal dogs (Figs. 1 and 3), but the difference did not achieve statistical significance. These observations suggest that the release of endogenous PTH does not play a significant role in the natriuresis induced by AVP.

The hyperphosphatemic effect of AVP may contribute to the natriuretic effect of this hormone. Phosphate is a poorly reabsorbable anion, and the increase in phosphate excretion secondary to the increase in the filtered load of phosphate enhances excretion of sodium and potassium.

Our data also demonstrate that the natriuretic effect of AVP was unaffected by Li^+ administration. Dousa and Barnes have recently demonstrated that small doses of AVP are natriuretic in Li^+-treated rats but not in normal rats (20). These data are in sharp contrast to the fact that the antidiuretic effect of AVP is always blunted by Li^+ administration (11–15). These observations suggest that the mechanism whereby AVP causes natriuresis is different than that whereby it causes water retention. It is tempting to speculate that the natriuretic effect of AVP is not mediated through the adenylate cyclase–cyclic AMP system. Unfortunately no measurement of renal adenylate cyclase was done in this study in order to confirm this suggestion. It should be pointed out, however, that, in addition to the inhibition of adenylate cyclase, Li^+ also has an inhibitory effect at a step beyond the generation of cyclic AMP since the administration of cyclic AMP fails to induce antidiuresis in Li^+-treated rats (13). This observation indicates that *in vitro* measurement of adenylate cyclase and cyclic AMP does not fully assess the inhibitory effect of Li^+ on this system. The assumption that renal adenylate cyclase was inhibited by Li^+ in our study is not unwarranted since previous studies have demonstrated that plasma Li^+ levels comparable to those seen in this study were accompanied by inhibition of the renal adenylate cyclase–cyclic AMP system in the rat (14). We have previously demonstrated that Li^+ administration, using an identical protocol to the present study, blunts the phosphaturic effect of PTH and cyclic AMP; this observation provides evidence of functional inhibition of cortical adenylate cyclase (21). Other investigators have also found evidence of inhibition of cortical adenylate cyclase in the rat both *in vitro* and *in vivo* (22, 23). These observations indicate that Li^+ administration is virtually always accompanied by inhibition of renal adenylate cyclase.

In conclusion, these data demonstrate that AVP administration has a striking hyperphosphatemic effect in normal, thyroparathyroidectomized and lithium-treated dogs. Our data also demonstrate that the natriuretic effect of AVP is not influenced by lithium administration or by lack of parathyroid glands.

Summary. Lithium administration blocks the antidiuretic effect of arginine vasopressin (AVP). In order to determine whether or not Li^+ administration also blocks the natriuretic effect of AVP, we administered AVP at 50 mU/kg/min ("high dose") or 50 mU/kg/hr ("low dose") to normal and Li^+-treated dogs. AVP administration at both doses resulted in a significant increase in urine flow and sodium excretion in both normal and Li^+-treated dogs. In addition, AVP administration resulted in a dramatic increase in plasma phosphate concentration. Studies were therefore performed in thyroparathyroidectomized (TPTX) dogs in order to determine whether an increase in parathyroid hormone (PTH) release could account for the natriuretic effect of AVP. AVP administration to TPTX dogs also resulted in the same increase in sodium excretion and plasma phosphate concentration as

in normal dogs. The hyperphosphatemia of AVP was unrelated to the vasoconstrictor-pressor effect of the hormone since it was present when blood pressure was prevented from rising by sodium nitroprusside administration. These data suggest a difference in the mechanism of action whereby AVP elicits its natriuretic and antidiuretic effects. The hyperphosphatemic effect of AVP administration is described for the first time.

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1. Chan, W. Y., and Sawyer, W. H., Proc. Soc. Exp. Biol. Med. **110,** 697 (1962).
2. Humphreys, M. H., Friedler, R. M., and Earley, L. E., Amer. J. Physiol. **219,** 658 (1970).
3. Sawyer, W. H., Amer. J. Physiol. **169,** 583 (1952).
4. Martinez-Maldonado, M., Eknoyan, G., and Suki, W. N., Amer. J. Physiol. **220,** 2013 (1971).
5. Kurtzman, N. A., Rogers, P. W., Boonjarern, S., and Arruda, J. A. L., Amer. J. Physiol. **228,** 890 (1975).
6. Wen, S. F., J. Clin. Invest. **53,** 660 (1974).
7. Knight, T., and Weinman, E. J., Clin. Res. **24,** 404 (1976).
8. Chase, L. R., and Aurbach, G. D., Science **9,** 545 (1968).
9. Dousa, T., Mayo Clinic Proc. **49,** 188 (1974).
10. Dousa, T., and Hetcher, O., Life Sci. **9,** 765 (1970).
11. Singer, I., and Rotenberg, D., N. Engl. J. Med. **289,** 254 (1973).
12. Singer, I., Rotenberg, D., and Puschett, J. B., Jr., J. Clin. Invest. **51,** 1081, (1972).
13. Forrest, J. N., Cohen, A. D., Torretti, J., Himmelhoch, J. M., and Epstein, F., J. Clin. Invest. **53,** 1115 (1974).
14. Geisler, A., Wraac, O., and Andersen, O. V., Acta Pharmacol. Toxicol. **31,** 203 (1972).
15. Martinez-Maldonado, M., Taspara, A. S., Taspara, N., Suki, W. N., and Eknoyan, G. R., J. Lab. Clin. Med. **86,** 445 (1975).
16. Walter, R., Smith, C. W., Mehta, P. K., Boonjarern, S., Arruda, J. A. L., and Kurtzman, N. A., *in* "Disturbances in Body Fluid Osmolality" (T. Andreoli, J. Grantham and F. Rector, Jr., eds.), pp. 1-36. American Physiological Society, Bethesda (1977).
17. Kurtzman, N. A., J. Clin. Invest. **48,** 586 (1970).
18. Gekle, D., and von Carlowitz, B., Pfluegers Arch. **342,** 73 (1973).
19. Agus, Z. S., Puschett, J. B., Senesky, D., and Goldberg, M., J. Clin. Invest. **50,** 617 (1971).
20. Dousa, T. P., and Barnes, L. D., Amer. J. Physiol. **231,** 1754 (1976).
21. Arruda, J. A. L., Richardson, J. M., Wolfson, J. A., Nascimento, L., Rademacher, D. R., and Kurtzman, N. A., Amer. J. Physiol. **231,** 1140 (1976).
22. Marcus, R., and Aurbach, G. D., Biochem. Biophys. Acta **242,** 410 (1971).
23. Steele, T., J. Pharmacol. Exp. Ther. **197,** 206 (1976).

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