EFFECTS OF NOREPINEPHRINE ON THE ACTION OF ANTIDIURETIC HORMONE IN THE DOG KIDNEY

A Thesis

Presented to

the Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirement for the Degree

Master of Science

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Peter Foster

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ABSTRACT

In general norepinephrine (NE) exhibits antagonistic effects to that of antidiuretic hormone (ADH) with respect to renal salt and water excretion. This was demonstrated by infusing ADH intravenously at rates of 0, 5, and 25 μU/kg/min in three different groups of mongrel dogs which were chemically sympathectomized with guanethidine sulfate. Within each group infused with constant levels of ADH, NE was infused at increasing rates from 0.05 to 3.2 μg/kg/min during eight consecutive thirty minute periods.

Under these conditions NE was diuretic, natriuretic, and increased both osmolar and free water clearance, while ADH had the opposite effects. These effects of NE appear to be the result of decreased permeability of the collecting duct first to solutes and second to water.

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INTRODUCTION

A review of the literature reveals many studies which cite the action of the antidiuretic hormone (ADH) as being antidiuretic and natriuretic (1,3), while norepinephrine (NE) is diuretic and antinatriuretic (2,4,12,27). However, depending upon the dose level of these hormones and the hydration state of the animal different effects can also be demonstrated (10).

The action of ADH in producing urine of low volume and high osmotic pressure is well known and based upon two factors: 1) an increase in the permeability of the collecting duct to water (29); and 2) an increase in the osmotic pressure gradient of the renal medullary and papillary areas. The increase in osmotic pressure is the result of either an increase in the active transport of sodium out of the thin ascending limb (29) and/or a decrease in medullary blood flow (32), thus reducing removal of medullary solutes.

The diuretic response to the catecholamines, NE and epinephrine, has been well documented by several investigators (2,27,33) in man. Fisher (12) concluded that NE inhibits the antidiuretic action of ADH and that NE effects were not the result of alterations in glomerular filtration rate (GFR), effective renal plasma flow (ERPF), or changes in medullary hypertonicity; however, the latter two parameters were not measured. It was therefore hypothesized by Fisher that

catecholamines exert their effect by decreasing tubular water reabsorption. The mechanism by which this is accomplished was not clear from the data; however two possibilities were offered: 1) NE interferred with the mechanism for solute free water reabsorption or 2) NE accelerates the clearance of ADH from the plasma. The first possibility is supported in part by the fact that NE and epinephrine inhibit the *in vitro* action of ADH on water transport by the toad bladder (28), which suggests that the *in vivo* effects of catecholamines on kidney water excretion might be mediated by a mechanism, in part, common to both ADH and NE.

Baldwin (2) noted from his studies that NE and epinephrine caused an increase in free water clearance (C_{H_2O} = Urine Flow - Osmolar Clearance) when control values for C_{H_2O} were negative, as when endogenous plasma ADH concentration $(ADH)_p$ is high. When control values for C_{H_2O} were positive, as is the case when $(ADH)_p$ is low, the catecholamines produced no consistent or appreciable change in free water clearance.

Ladd (17) showed that the intraveonus administration of ADH (5-17 μU/kg/min) reduced water excretion and enhanced sodium excretion (natriuresis) in previously hydrated subjects receiving saline intravenously. Satorius and Roberts (24) also observed a natriuretic effect in rats with 8 mU ADH/kg body weight. Several investigators (2,4,12,27) found a reduction in sodium excretion (antinatriuresis) with infusion of NE at dose levels of 0.20 μg/kg/min or more in both the dog and man.

ADH can be antidiuretic and natriuretic under some conditions and the catecholamines are diuretic and antinatriuretic as described above. Therefore, it was reasonable to postulate that NE and ADH may be mutually inhibitory on some aspect(s) of renal function. The purpose of this investigation was to investigate selected parameters of renal function which might underlie the mechanism involved in the hormonal effects of NE and ADH. The parameters which were investigated were 1) cardiovascular effects as reflected by systemic blood pressure, GFR, and ERPF, 2) the counter-current exchange multiplier system as reflected by tissue sodium concentration gradient, and 3) changes in permeability of the collecting duct as indicated by changes in urine flow, solute clearance (C_{osm}), and C_{H_2O}.

METHODS AND MATERIALS

Preparation of Animals

Mongrel dogs weighing 11 to 21 kg each were used. Dogs were dewormed with Dichlorvas (Shell), given a systemic antibiotic (penicillin), hepatitis and distemper shots. The animals were then maintained on a standard animal quarters regimen for at least six days prior to being used as experimental animals and were fasted for 24 hrs prior to the experiment.

Endogenous levels of NE were reduced by injecting 10 mg/kg body weight of guanethidine sulfate (Ismelin, CIBA) subcutaneously 12 hours before the experiment. The maximum action of guanethidine sulfate occurs 6 to 18 hours after injection (14). The use of guanethidine has the following advantages:

1) Guanethidine reduces endogenous NE to very low levels by preventing the release of transmitter NE in response to nerve stimulation as well as blocking the transport mechanism for the uptake and storage of NE (8,20).

2) Guanethidine does not modify the effects of exogenous NE, since guanethidine does not exhibit alpha-adrenergic blocking activity (6) and animals treated with guanethidine show increased sensitivity to injected catecholamines due to blockage of NE uptake by sympathetic nerve endings (23).

In order to reduce secretion of endogenous ADH, animals were gavaged with a 2.5% dextrose in water solution (2% of body weight) 12 and again 2 hrs prior to the experiment.

Inactin, a sodium salt of 5-ethyl-5-(1-methylpropyl)-thiobarbituric acid (Promonta), 50 mg/kg i.p., was used as an anesthetic because it permits the establishment of a water diuresis by hypotonic infusion (34).

All surgical incisions were made using a cautery knife. Both ureters were exposed by a small midline incision in the lower abdomen and exteriorizing the bladder. Ureters were then cannulated by making a small incision near the entrance of the ureters to the bladder. Polyethylene cannulae (PE 90) were then threaded up each ureter to the renal pelvis and tied in place. Both femoral arteries and one vein were exposed and cannulated with indwelling catheters (Jellco) for the purpose of obtaining arterial blood pressure (EM Physiograph), arterial blood samples, and infusing solutions respectively. Infusion of solutions at a rate of 1 ml/kg/min was accomplished by using a variable flow pump (Masterflex). The solutions were infused at a temperature of 38 C and the temperature was monitored by an electronic thermometer (Tri-R Instruments) at the point of infusion. Infusion of solutions at 1.5 ml/min was accomplished using a peristaltic pump (Process Instruments). The femoral blood supply remained patent throughout the experiment.

Experimental Protocol

The animals were allowed 30 min to recover from surgical trauma after surgical procedures were completed. This 30 min

recovery period was followed by a pre-experimental period in which a hypotonic Ringer solution (Table I) was infused at 1 ml/kg/min for 30 min. Following this pre-experimental period the infusion rate was reduced to 1.5 ml/min and a series of eight consecutive experimental periods of 30 min each were carried out. The hypotonic Ringer solution used during the experimental periods contained creatinine and ^{3}H-paraaminohippuric acid (^{3}H-PAH) (Table I) for measuring GFR and ERPF respectively. At the beginning of the first experimental period the animals were primed with creatinine and ^{3}H-PAH (see Table I).

In the periods in which the hormones ADH (8-lysine vasopressin) and NE (Sigma) were infused, a priming dose of each hormone was given. The amounts of the priming dose were calculated based on the respective half-life ($t_{\frac{1}{2}}$) and volume of distribution of ADH and NE, so that a constant plasma level could be reached promptly and maintained. The equation used to calculate priming dose (P.D.) was as follows:

$$\left(\frac{\text{P.D.}}{\mu g/kg}\right) \times \left(\frac{0.693}{t_{\frac{1}{2}}\ \text{min}}\right) = \frac{\text{Infusion rate}}{(\mu g/kg/min)}$$

A priming dose of NE (Table II) was given for periods 2 to 8. NE was infused at 0.05 µg/kg/min in the second period and at 0.10, 0.20, 0.40, 0.80, 1.60, and 3.20 µg/kg/min for the subsequent periods 3 to 8 respectively. NE was stored at 0 C in a 100 mM acetic acid stock solution and diluted just before each experiment. The constant infusion rates of ADH,

TABLE I

COMPOSITION OF INFUSION SOLUTIONS

HYPO-RINGER SOLUTION (mM)	(EXPERIMENTAL) HYPO-RINGER SOLUTION + TRACERS (g/l)	PRIMING DOSE* SOLUTION (g/100 ml)
NaCl = 70	Creatinine = 2.5	Creatinine = 3.4
KCl = 5.4	PAH = 0.46	PAH = 0.56
$CaCl_2$ = 3.8	^{3}H-PAH = 25 (uc)	^{3}H-PAH = 30 (uc)
$NaHCO_3$ = 10	Dextrose = 1.5	Mannitol = 3.6
	Mannitol = 4.0	

*Dosage = 1 ml/kg

TABLE II

PRIMING DOSE FOR NE INFUSION

PERIOD	CONCENTRATION OF NE (ug/kg)
2	0.14
3	0.14
4	0.28
5	0.56
6	1.12
7	2.24
8	4.48

beginning in period 1, were either 5 μU/kg/min or 25 μU/kg/min with a priming dose 140X the amount infused in one minute. The infusion rates of ADH used were in the physiological range (5). It should be pointed out that the plasma levels of NE and ADH will be constant and proportional to the infusion rate even if the volume of distribution is other than that estimated as long as the $t_{\frac{1}{2}}$ remains constant. The $t_{\frac{1}{2}}$ of ADH has been found to be 5.5 min and the volume of distribution 140 ml/kg (18). This finding would indicate that the ADH priming dose may have been too large for the sustaining infusion, but would have reached a steady state with respect to the infusion rate by the end of the first period. The $t_{\frac{1}{2}}$ of NE was 2 min (33) and the volume of distribution that of plasma and interstitial volume 200 ml/kg (33).

The first 15 min of each 30 min period was used to allow for equilibration and only urine and plasma obtained in the last 15 min was analyzed. Urine flow was collected for the first 15 min of each period and its flow was usually within 5% of the flow rate of the second 15 min period. Hence NE effects, at least on urine flow, could be considered to have reached a steady state within the first 15 min of a period.

Blood samples were taken during the middle of the last 15 min of each period. Blood pressure was monitored continuously.

At the end of the last period and while the infusion continued, both kidneys were exposed, excised, and frozen in

a mixture of acetone and dry ice to be analyzed later for tissue Na, K, and water.

Two groups of control animals were used; one group with guanethidine (G) and one group without guanethidine (NG) pretreatment. Neither control group was infused with ADH or NE. The average weight of the animals in each group was 17.3, 15.7, 16.3, 16.2, and 16.0 kg in the 0 ADH, 5 ADH, 25 ADH, G, and NG group, respectively.

Analytical Procedures

Kidney tissue was analyzed by cutting the two frozen kidneys transversely into thin slices of 3-4 mm in thickness each and then by free-hand slicing the sections into 4 parts consisting of an outer cortex, inner cortex, outer medulla and the inner medullary region. These tissue slices averaging about 500 mg each were then blotted and weighed on a torsion balance. One slice from each kidney was then used to measure tissue Na, K, and water content. Tissue water content was obtained by drying the tissue to a constant weight (24 hrs at 100 C) and subtracting the dry weight from the wet weight. Tissue Na and K were determined by digesting the dried slices in 2 ml of 0.1 M LiOH solution. Aliquots of the digested slices were then analyzed for Na and K by flame photometry. Lithium did not interfere with the determination of Na or K.

Blood and urine samples both were analyzed for creatinine, ^{3}H-PAH, osmotic pressure, Na, and K. Creatinine was measured

by a picric acid method of Jaffe and Folin as described by Smith (26). The activity of labeled PAH was measured by pipetting 0.05 ml of urine and 0.50 ml of plasma into counting vials containing 15 ml of a scintillation cocktail consisting of 850 ml of toluene, 300 ml of BBS-3 (Beckman solubilizer), 0.42 g of 2,5-diphenyloxazole, and 52.5 mg of p-Bis 2-(5-phenyloxazolyl)-benzene. The vials were then counted in a liquid scintillation counter (Beckman model LS 150). Osmotic pressure was determined by a osmometer (Precision Systems - Osmette model #2007) which ultilizes the freezing point depression principle. Na and K were measured using a flame photometer (Eppendorf). Plasma total solids were determined by refractometry (National).

Statistical analyses used, which included correlation, standard error of the mean, and two way analysis of variance, were done using the methods of Freund (13). Each kidney was treated as a separate observation. The standard error of the mean of each of the 8 periods were averaged for each group of dogs and are presented in the figure legends.

RESULTS

Urine flow was progressively increased when NE was infused at rates of 0.05 to 0.40 μg/kg/min (periods 1 to 5), but as infusion rates were increased further, from 0.40 to 3.20 μg/kg/min (periods 5 to 8), urine flow was decreased (Fig. 1). The diuretic effect of NE was significantly reduced ($p < .005$) by the constant infusion of ADH at either 5 or 25 μU/kg/min, the latter had the greatest effect in diminishing the diuretic action of NE. The non-guanethidized control animals (NG-controls) had a constant urine flow during all eight periods, which, except for the last two periods, remained lower than the experimental animals. The guanethidized control animals (G-controls) had a slightly higher urine flow than the NG-controls.

The effects of NE on C_{H_2O} were triphasic (Fig. 2). At low infusion rates (0.05 to 0.20 μg/kg/min, periods 1 to 4) NE decreased C_{H_2O}; at intermediate infusion rates (0.20 to 0.80 μg/kg/min, periods 4 to 6) NE increased C_{H_2O} until the effects of decreased GFR supervened and decreased C_{H_2O}. ADH infusion had a modifying effect on the action of NE upon C_{H_2O}, the more ADH infused the greater the modification. ADH infusion at 25 μU/kg/min tended to make C_{H_2O} a more linear function of NE infusion. Two way analysis of variance indicated a significant effect ($p < .001$) of NE on C_{H_2O} in all groups of ADH-infused animals but no significant differences were evident due to ADH infusion. A positive C_{H_2O} was achieved only in experimental animals in which no ADH or 5 μg/kg/min ADH was

Fig. 1. Effects of NE and ADH infusion on urine flow in dogs. NE infusion was initiated in period 2 at a rate of 0.05 μg/kg/min and doubled for every consecutive period in all experimental animals. ADH was infused at a constant rate of either 0, 5, or 25 μU/kg/min during all eight periods. G-control and NG-control animals represent the results of animals with and without guanethidine pretreatment respectively and no NE or ADH infusion. Hydration was the same in all groups. Each point represents the mean of six observations for the experimental and G-control animals, and eight observations for the NG-control animals. The average standard error for the 0 ADH, 5 ADH, 25 ADH, G-control, and NG-control animals were ± 0.16, 0.09, 0.07, 0.12, and 0.02 ml/min respectively. The 0 ADH animals had a significantly higher ($p < .005$) urine flow than the 5 ADH animals. There was no significant difference between the 5 and 25 ADH animals.

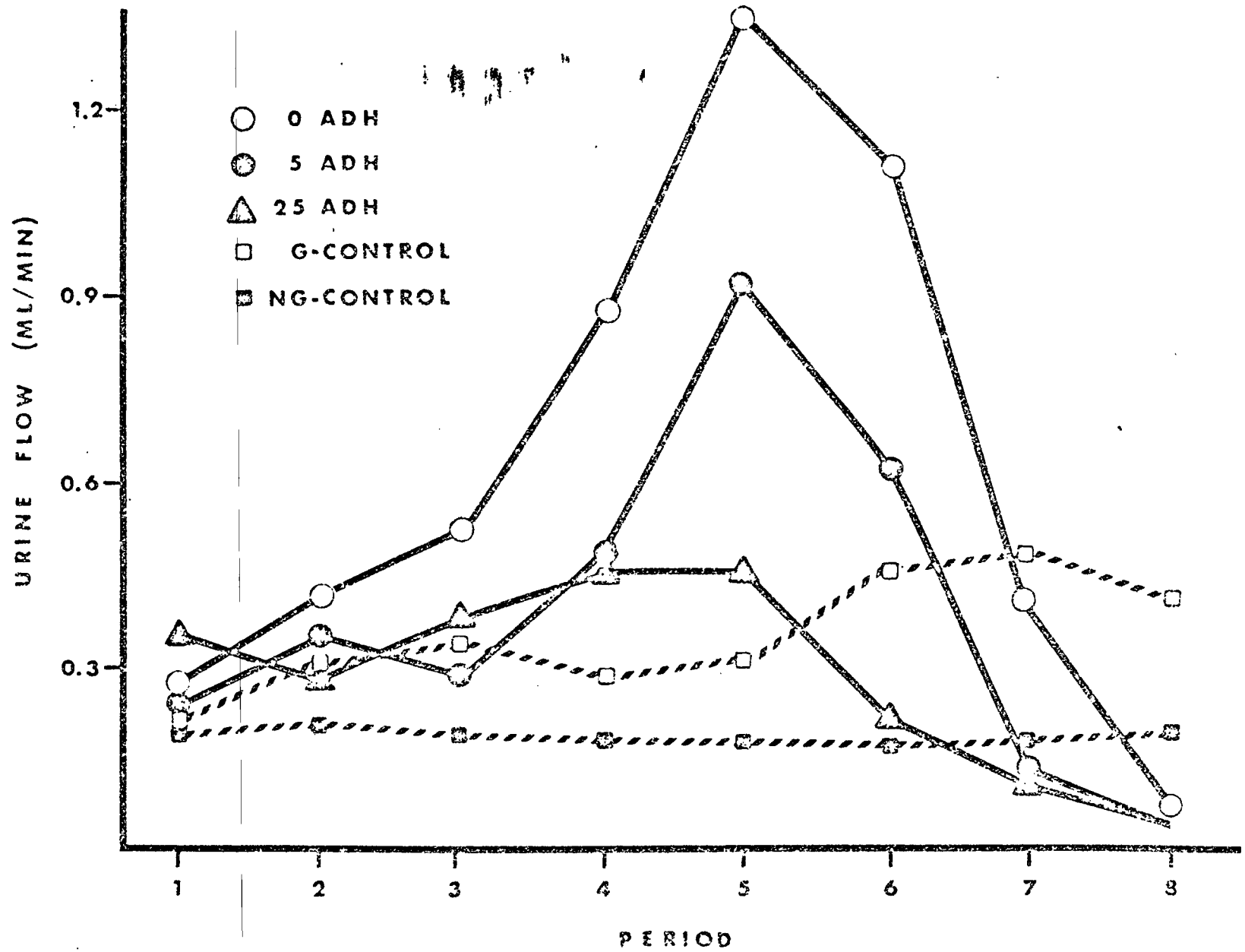
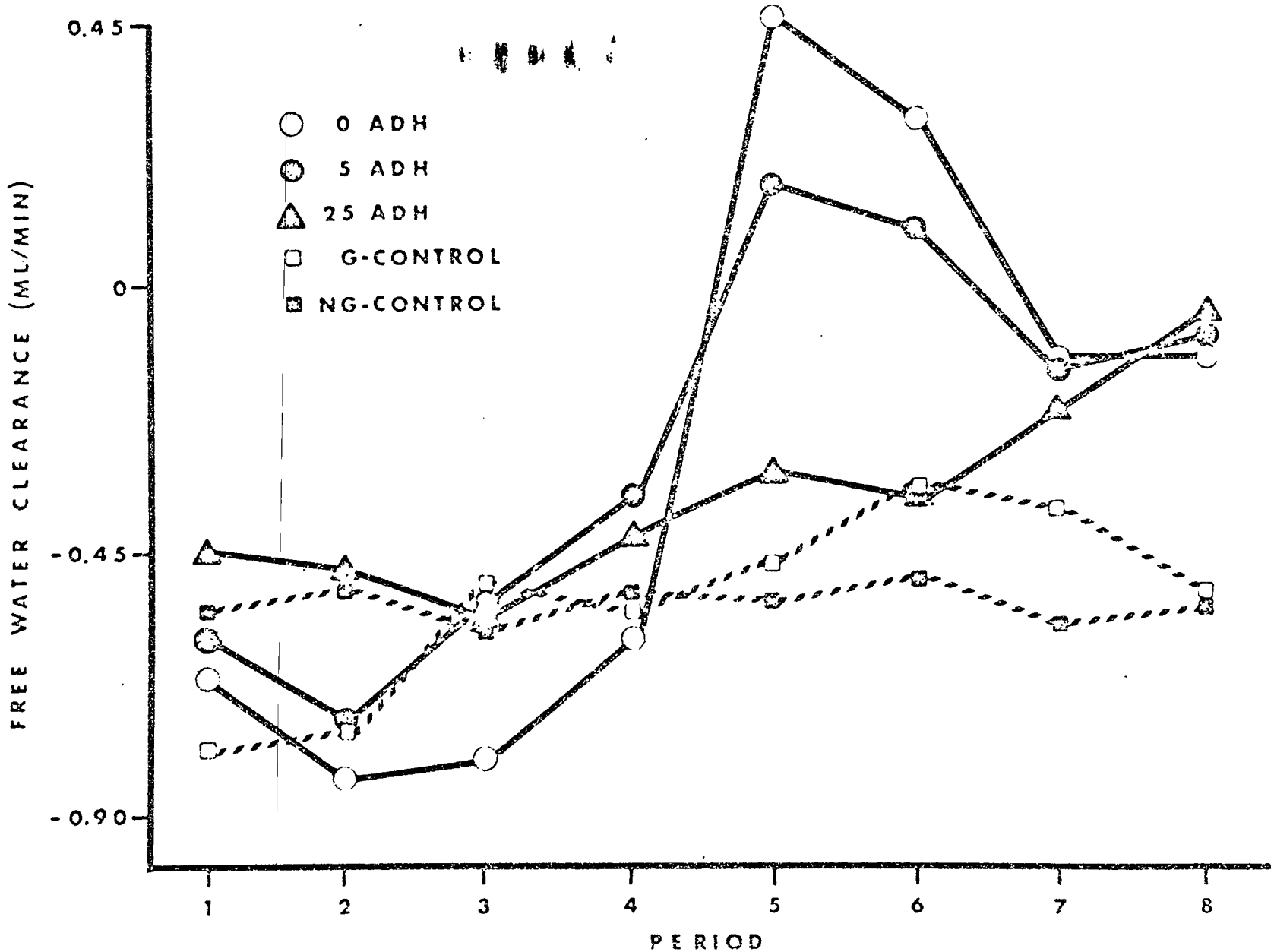

0 ADH
5 ADH
25 ADH
G-CONTROL
NG-CONTROL
URINE FLOW (ML/MIN)
1.2
0.9
0.6
0.3
1
2
3
4
5
6
7
8
PERIOD

Fig. 2. Effects of NE and ADH infusion on free water clearance in dogs. NE infusion was initiated in period 2 at a rate of 0.05 µg/kg/min and doubled for every consecutive period in all experimental animals. ADH was infused at a constant rate of either 0, 5 or 25 µU/kg/min during all eight periods. G-control and NG-control animals represent the results of animals with and without guanethidine pretreatment respectively and no NE or ADH infusion. All animals were hydrated in the same manner. Each point represents the mean of six observations for the G-control and experimental animals, and eight observations for the NG-control animals. The average standard error for the 0 ADH, 5 ADH, 25 ADH, G-control, and NG-control animals were $\pm$ 0.18, 0.08, 0.11, 0.16, and 0.04 ml/min respectively. NE infusion had a significant effect ($p < .001$) on C_{H_2O} in all experimental groups.


0.45
0
-0.45
-0.90
FREE WATER CLEARANCE (ML/MIN)
0 ADH
5 ADH
25 ADH
G-CONTROL
NG-CONTROL
1
2
3
4
5
6
7
8
PERIOD

infused and only when the infusion rate of NE was either 0.40 or 0.80 μg/kg/min (periods 5 and 6). The NG-control animals' C_{H_2O} remained unchanged during all eight periods. The G-control animals had a lower C_{H_2O} in periods 1 and 2, with a higher C_{H_2O} during the last four periods than the NG-controls.

NE infusion significantly increased ($p < .001$) C_{osm} in the 0 ADH-infused animals, as compared to those animals infused with ADH, during all eight periods (Fig. 3). The greatest increase in C_{osm} occurred during the first four periods in the group of animals infused with NE only and decreased during the last four periods. There was no significant difference between the 5 and 25 μU/kg/min ADH-infused animals. The C_{osm} remained constant during the first five periods in the two groups of ADH-infused animals and decreased during the last three periods. The C_{osm} in both groups of control animals remained constant during all eight periods. The G-control animals had a greater C_{osm} than the NG-control animals.

In plotting C_{H_2O} as a function of C_{osm} (Fig. 4) a significant negative correlation ($r = -.95$, $p < .01$) existed in the 25 μU/kg/min ADH-infused animals. No significant correlation existed in the 0 or 5 μU/kg/min ADH animals ($r = -.45$ and $-.55$ respectively). The lack of a significant correlation in the 0 and 5 ADH groups indicates that C_{H_2O} is not significantly related to C_{osm} in most periods. In both the NG and G-control groups C_{H_2O} was significantly correlated to C_{osm} with p values of $< .01$ and $< .05$ respectively.

Fig. 3. Effects of NE and ADH infusion on osmolar clearance in dogs. NE infusion was initiated in period 2 at a rate of 0.05 μg/kg/min and doubled for every consecutive period in all experimental animals. ADH was infused at a constant rate of either 0, 5 or 25 μU/kg/min during all eight periods. G-control and NG-control animals represent the results of animals with and without guanethidine pretreatment respectively and no NE or ADH infusion. All animals were hydrated in the same manner. Each point represents the mean of six observations for the G-control and experimental animals, and eight observations for the NG-controls. The average standard error for the 0 ADH, 5 ADH, 25 ADH, G-control, and NG-control animals were ± 0.16, 0.08, 0.12, 0.06, and 0.05 ml/min respectively. C_{osm} was significantly increased ($p < .001$) in the 0 ADH animals as compared to the 5 ADH animals with no significant difference between the 5 and 25 ADH animals.

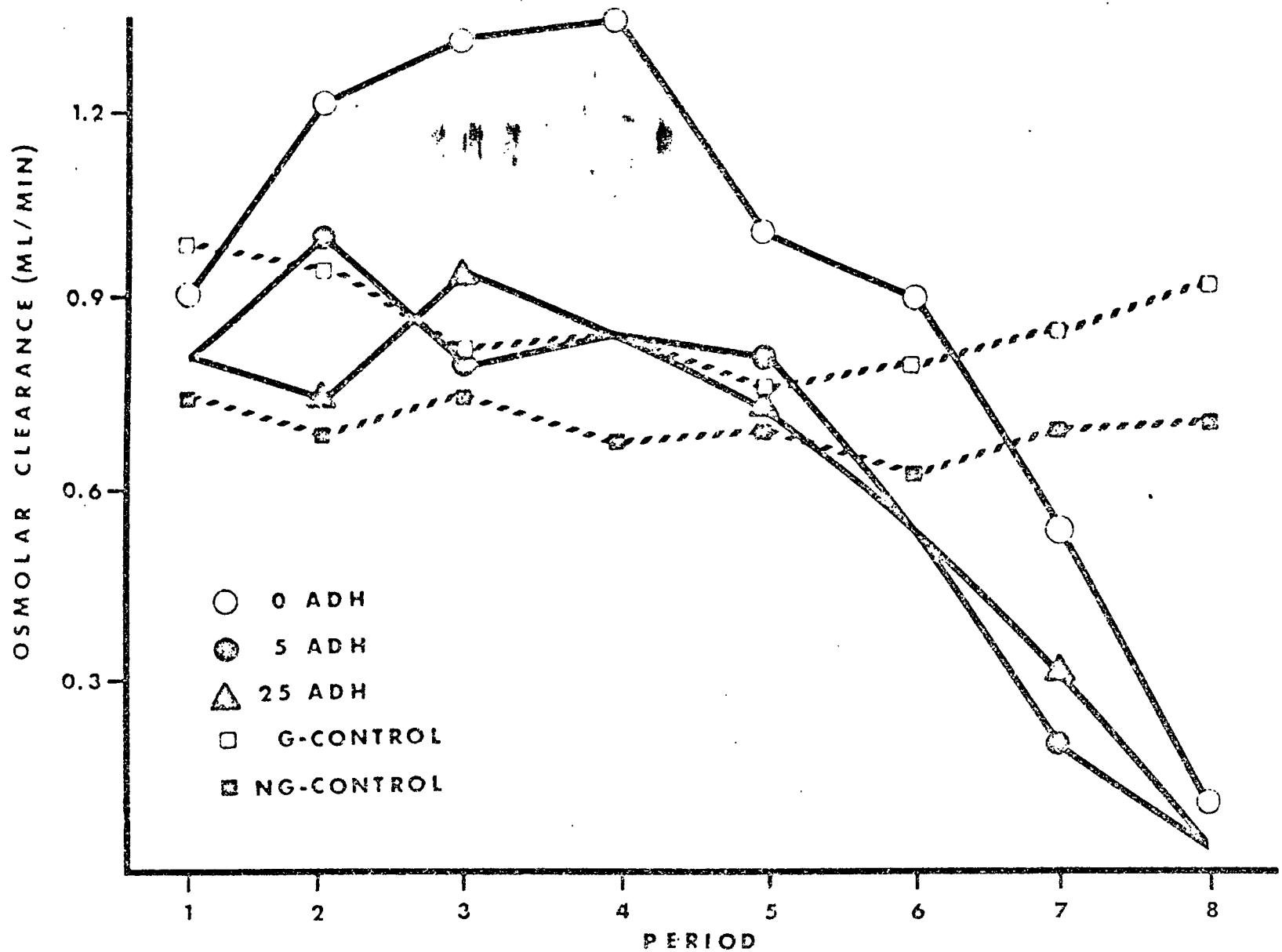
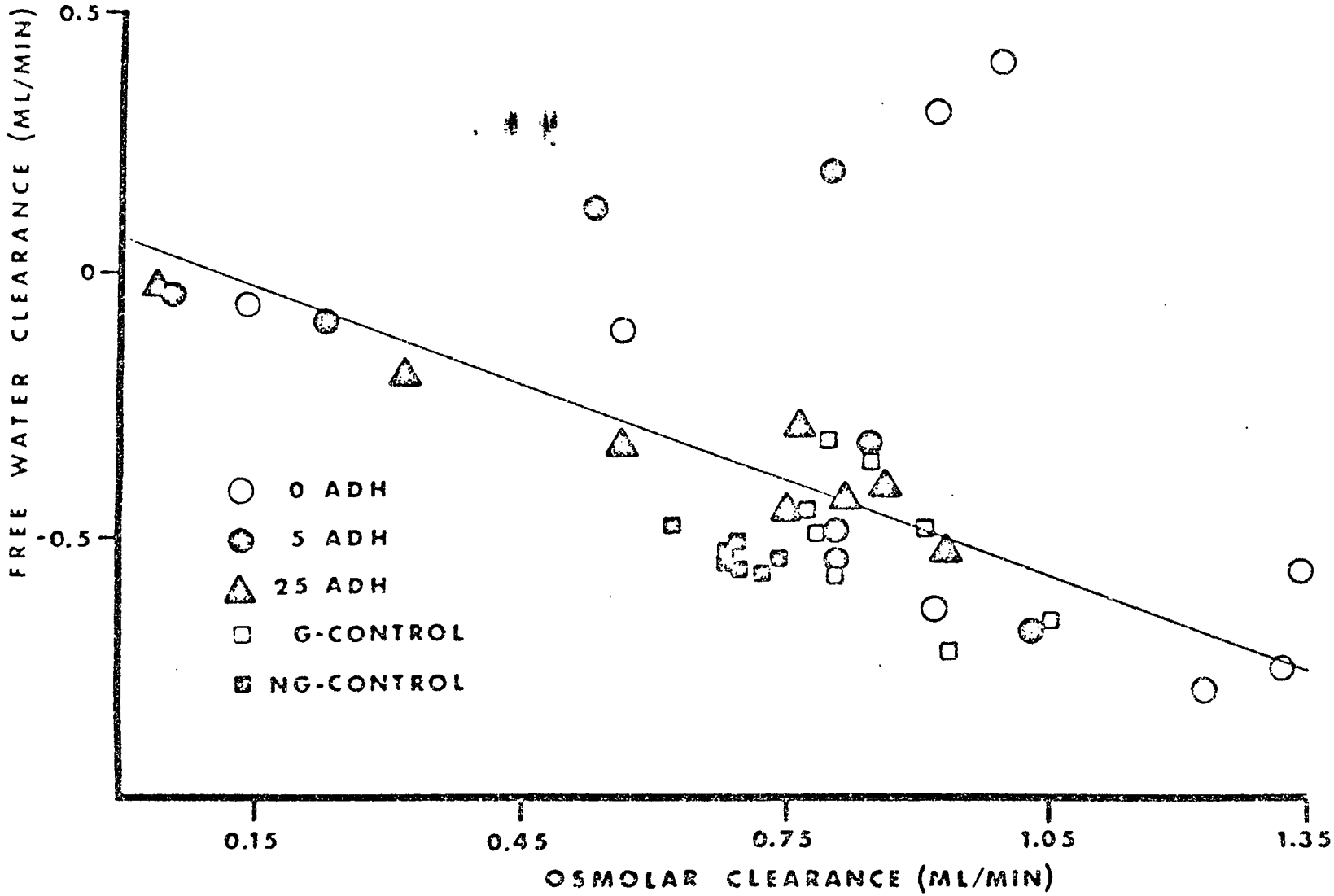

OSMOLAR CLEARANCE (ML/MIN)
1.2
0.9
0.6
0.3
0 ADH
5 ADH
25 ADH
G-CONTROL
NG-CONTROL
1
2
3
4
5
6
7
8
PERIOD

Fig. 4. Represents free water clearance plotted against osmolar clearance, where the straight line separates experimental points into "high" and "low" values. The "low" values correspond to periods in which there was an apparent maximum amount of solute free water reabsorption for a given amount of solute clearance, while "high" values correspond to periods in which there was an increased amount of solute free water for a given solute load. All animals were hydrated in the same manner. Each point represents the mean of six observations for G-control and experimental animals, and eight observations for the NG-control animals. A significantly negative correlation ($p < .01$, $< .05$, $< .01$) existed for the 25 ADH, G-control, and NG-control animals.


FREE WATER CLEARANCE (ML/MIN)
0.5
0
-0.5
0 ADH
5 ADH
25 ADH
G-CONTROL
NG-CONTROL
0.15
0.45
0.75
1.05
1.35
OSMOLAR CLEARANCE (ML/MIN)

NE had a significant ($p < .001$) natriuretic effect (Fig. 5) in the absence of ADH infusion, particularly during the first four periods, in which sodium excretion ($U_{Na}V$) was increased from 15 to 52 μEq/min. This effect was reduced by the infusion of ADH and no significant natriuretic effect was seen in the ADH-infused animals. The $U_{Na}V$ at both levels of ADH infusion did not differ significantly from each other, ranging from an average value of 19 μEq/min in period 1 to 0.6 μEq/min in period 8. The $U_{Na}V$ in both control groups were similar to that in the ADH-infused animals during the first six periods, but did not decrease in the last two periods as it did in all experimental groups.

There was no increase in the potassium excretion (U_KV) which remained between 10 and 20 μEq/min for the first six periods and dropped to 4-5 μEq/min and 1 μEq/min in periods 7 and 8 respectively (Fig. 6). U_KV decreased during the first five periods and increased during the last three periods in both control groups.

The GFR, as measured by creatinine clearance (C_{Cr}), remained constant during the first five periods and decreased sharply in all experimental groups in the last two periods (Fig. 7B). The ERPF, as measured by labeled PAH clearance (C_{PAH}), decreased slightly from period 1 through period 5. The last three periods showed a marked decrease in all experimental animals regardless of the level of ADH infusion (Fig. 7A). In the two control groups the GFR remained constant

Fig. 5. Effects of NE and ADH infusion on sodium excretion in dogs. NE infusion was initiated in period 2 at a rate of 0.05 μg/kg/min and doubled for every consecutive period in all experimental animals. ADH was infused at a constant rate of either 0, 5 or 25 μU/kg/min during all eight periods. G-control and NG-control animals represent the results of animals with and without guanethidine pretreatment respectively and no NE or ADH infusion. All animals were hydrated in the same manner. Each point represents the average of six observations for the G-control and experimental animals, and eight observations for the NG-control animals. The average standard error for the 0 ADH, 5 ADH, 25 ADH, G-control, and NG-control animals were ± 11.0, 1.5, 2.5, 2.3, and 2.6 μEq/min respectively. $U_{Na}V$ was increased significantly ($p < .005$) in the 0 ADH animals as compared to the 5 ADH animals with no significant difference between the 5 and 25 ADH animals.

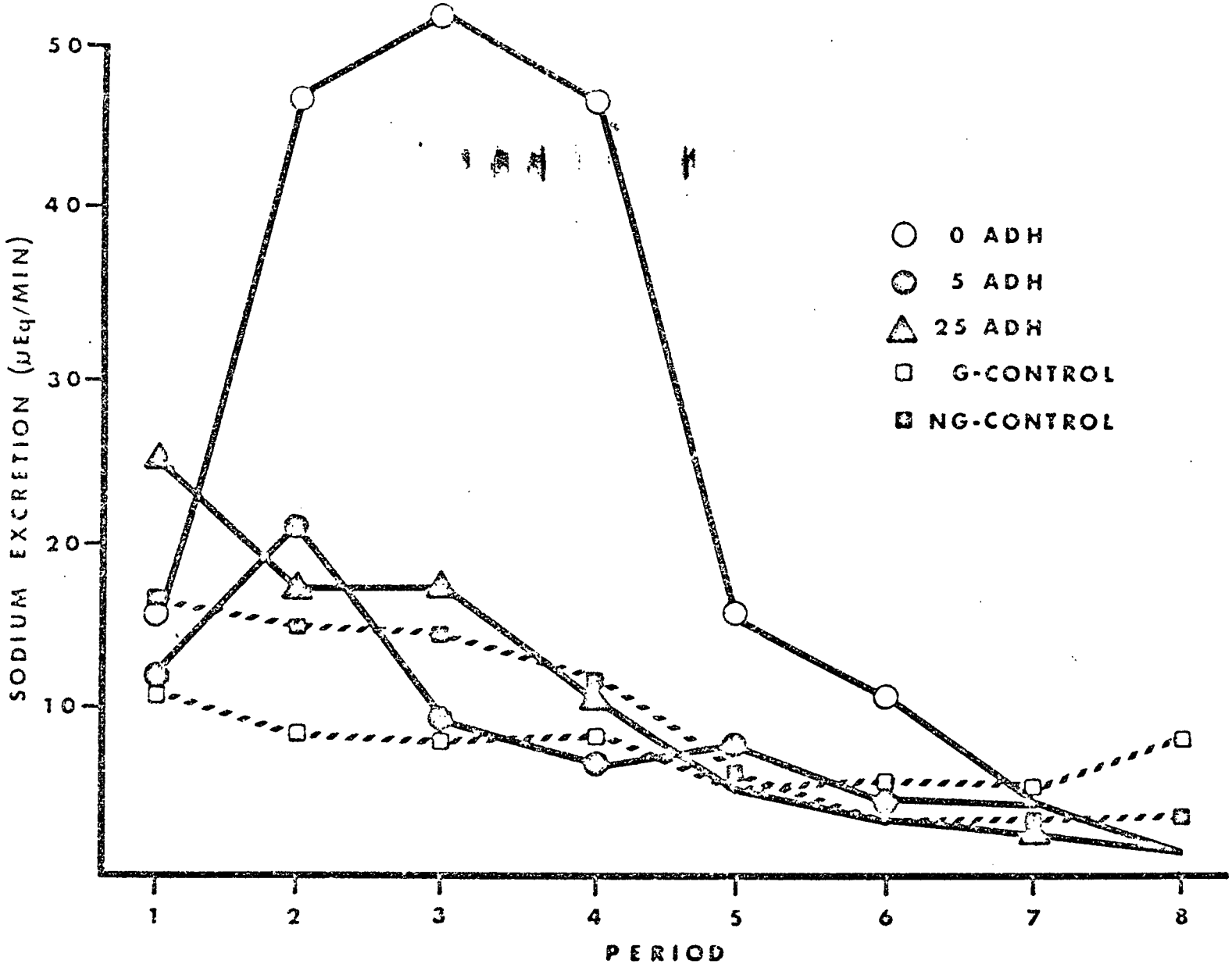

50
40
30
20
10
SODIUM EXCRETION (μEq/MIN)
0 ADH
5 ADH
25 ADH
G-CONTROL
NG-CONTROL
1
2
3
4
5
6
7
8
PERIOD

Fig. 6. Effects of NE and ADH infusion on potassium excretion in dogs. NE infusion was initiated in period 2 at a rate of 0.05 µg/kg/min and doubled for every consecutive period in all experimental animals. ADH was infused at a constant rate of either 0, 5 or 25 µU/kg/min during all eight periods. G-control and NG-control animals represent the results of animals with and without guanethidine pretreatment respectively and no NE or ADH infusion. All animals were hydrated in the same manner. Each point represents the mean of six observations for the G-control and experimental animals, and eight observations for the NG-control animals. The average standard error for the 0 ADH, 5 ADH, 25 ADH, G-control, and NG-control animals were ± 3.2, 0.8, 3.2, 2.6, and 1.7 µEq/min respectively.

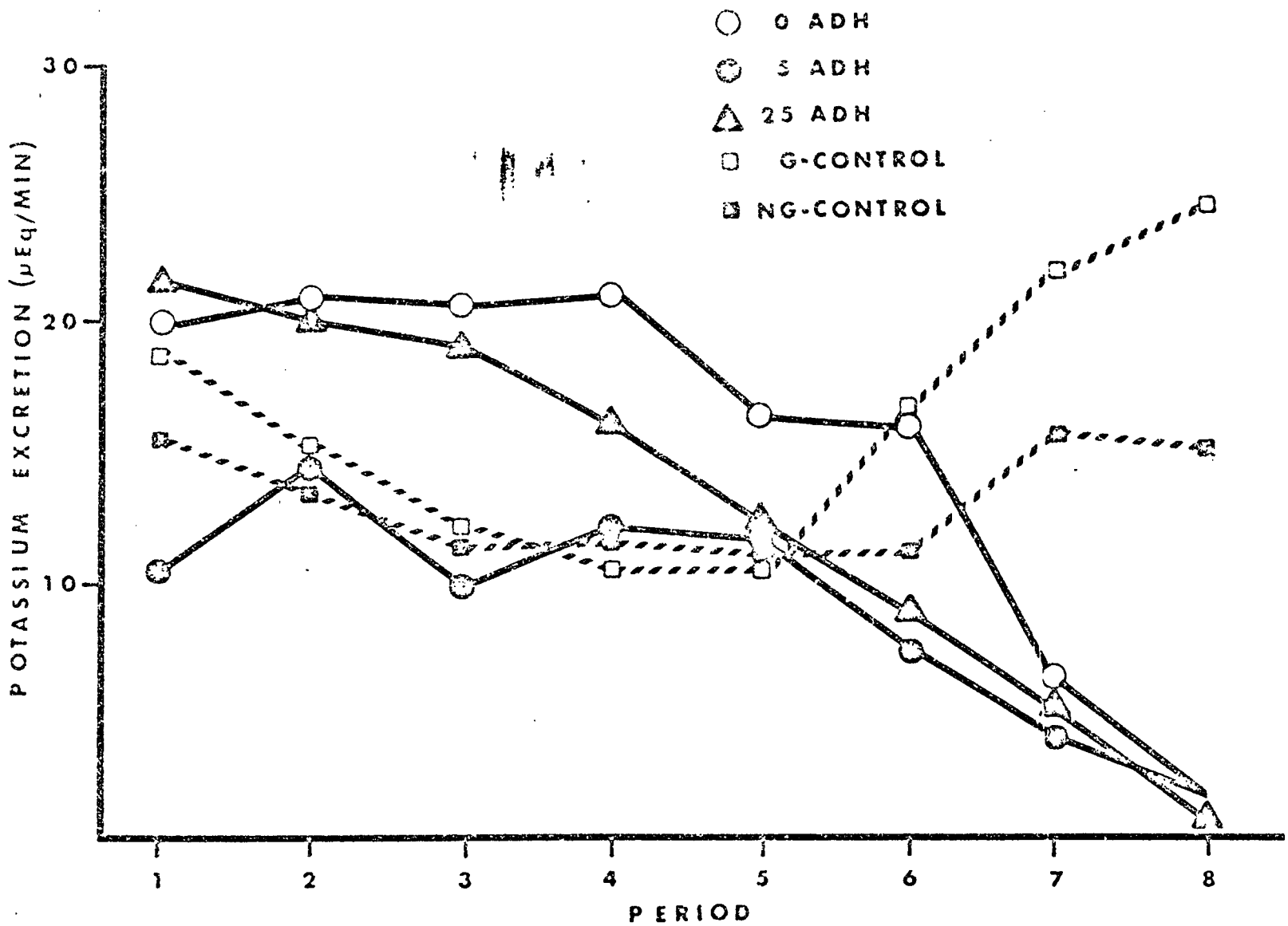
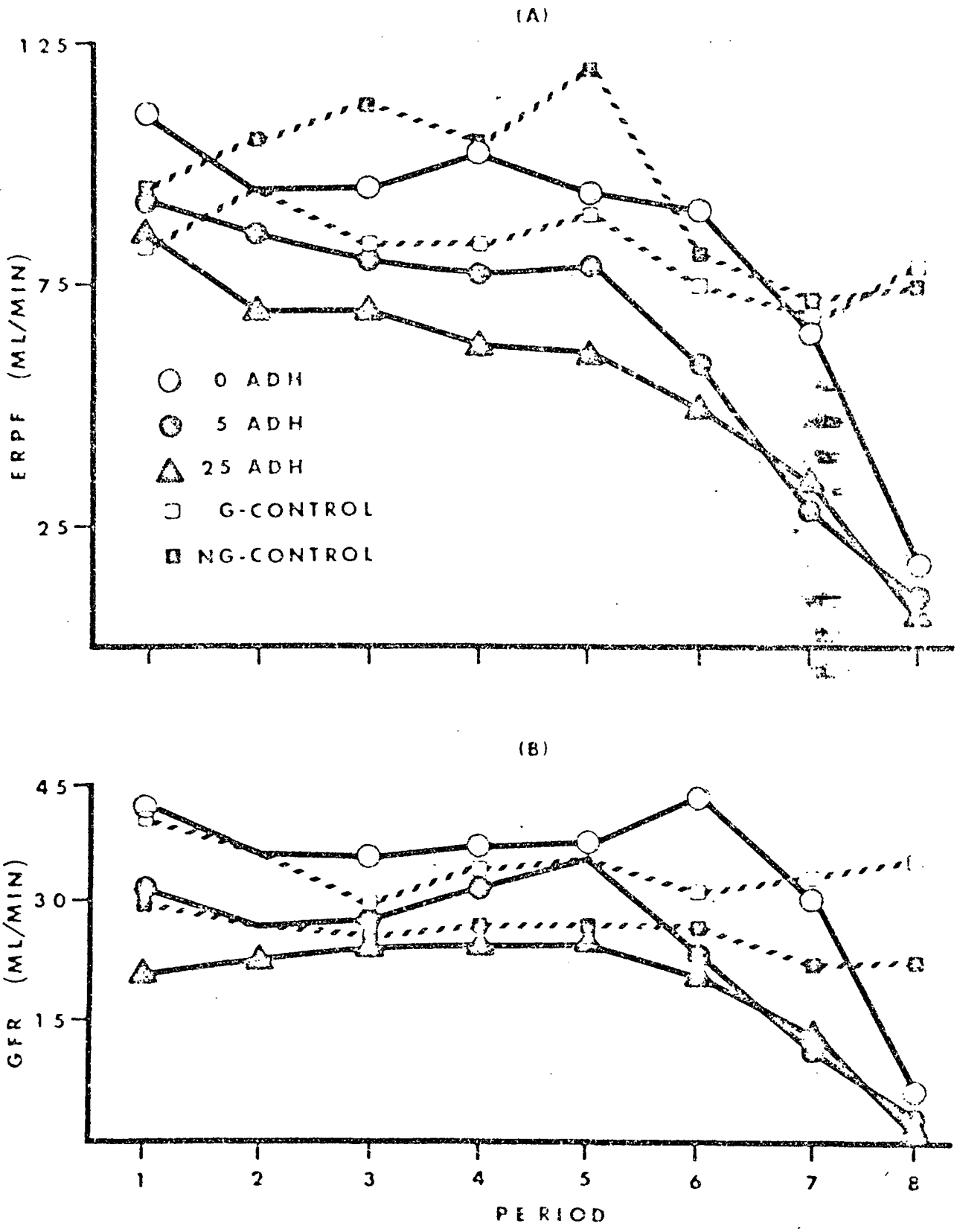

0 ADH
5 ADH
25 ADH
G-CONTROL
NG-CONTROL
POTASSIUM EXCRETION (μEq/MIN)
30
20
10
1
2
3
4
5
6
7
8
PERIOD

Fig. 7. Effects of NE and ADH infusion on renal plasma flow (ERPF) and glomerular filtration rate (GFR) in dogs. NE infusion was initiated in period 2 at a rate of 0.05 μg/kg/min and doubled for every consecutive period in all experimental animals. ADH was infused at a constant rate of either 0, 5 or 25 μU/kg/min during all eight periods. G-control and NG-control animals represent the results of animals with and without guanethidine pretreatment respectively and no NE or ADH infusion. All animals were hydrated in the same manner. Each point represents the mean of six observations for the G-control and experimental animals, and eight observations for the NG-control animals. The average standard error for the 0 ADH, 5 ADH, 25 ADH, G-control, and NG-control animals were ± 5.4, 7.7, 6.0, 9.4, and 6.3 ml/min with regard to ERPF, and ± 2.6, 2.2, 2.0, 2.0, and 2.1 ml/min for the GFR respectively. ADH infusion significantly depressed ($p < .001$) GFR for 0 vs 5 ADH and the 5 vs 25 ADH groups; and also depressed the ERPF ($p < .001$) for the 0 vs 5 ADH and ($p < .005$) for the 5 vs 25 ADH groups.


(A)
125
75
25
ERPF (ML/MIN)
0 ADH
5 ADH
25 ADH
G-CONTROL
NG-CONTROL
(B)
45
30
15
GFR (ML/MIN)
1
2
3
4
5
6
7
8
PERIOD

during all eight periods.

ADH infusion significantly depressed ($p < .001$) GFR for the 0 vs 5 and the 5 vs 25 μU/kg/min ADH groups; and depressed the ERPF ($p < .001$) for the 0 vs 5 and ($p < .005$) for the 5 vs 25 μU/kg/min groups. Animals with 0 ADH infusion had the highest GFR and ERPF, ranging from 36 to 42 ml/min and 95 to 115 ml/min respectively over the first five periods. Animals infused with 25 μU/kg/min ADH had the lowest GFR (20 to 22 ml/min) and ERPF (58 to 70 ml/min) over the first five periods.

The filtration fraction (C_{Cr}/C_{PAH}) increased from an average of 0.32 in period 1 to 0.44 in period 8 in the ADH-infused animals (Fig. 8). In animals infused only with NE, there was little or no change in the filtration fraction of 0.36 during the first four periods but filtration fraction increased linearly from 0.39 to 0.55 during the last four periods. There was no significant difference in filtration fraction due to ADH infusion. The filtration fraction decreased in the G-control animals from 0.49 in period 1 to 0.38 in period 2 and was similar to the experimental animals for the last six periods. The filtration fraction of the NG-control animals, except for the first period, was lower than both the G-control and experimental animals with an average value of 0.27.

Mean arterial blood pressure increased in all groups over the first four periods (Table III), with no consistent or significant difference between experimental treatments due to

Fig. 8. Changes in the filtration fraction as the result of NE and ADH infusion in dogs. NE infusion was initiated in period 2 at a rate of 0.05 µg/kg/min and doubled for every consecutive period in all experimental animals. ADH was infused at a constant rate of either 0, 5 or 25 µU/kg/min during all eight periods. G-control and NG-control animals represent the results of animals with and without guanethidine pretreatment respectively and no NE or ADH infusion. All animals were hydrated in the same manner. Each point represents the mean C_{Cr} divided by the mean C_{PAH} based on six observations for the G-control and experimental animals, and eight observations for the NG-control animals.

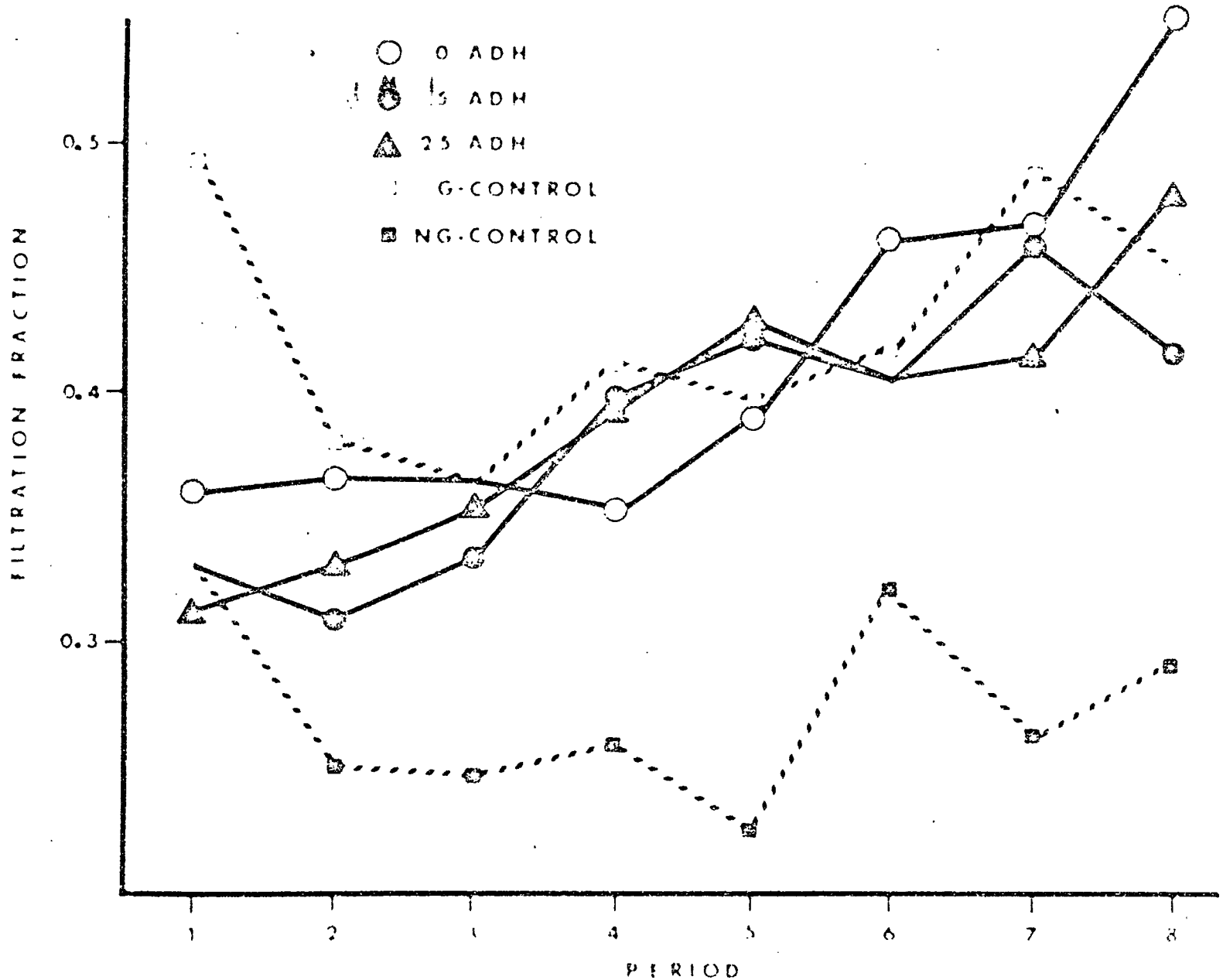

0 ADH
½ ADH
2.5 ADH
G-CONTROL
NG-CONTROL
FILTRATION FRACTION
0.5
0.4
0.3
1
2
3
4
5
6
7
8
PERIOD

TABLE III

MEAN

BLOOD PRESSURE
(mm Hg)

Treatment	Period 1	2	3	4	5	6	7	8
0 ADH	125* (±4.7)	138 (±5.6)	145 (±7.1)	156 (±9.7)	163 (±7.4)	173 (±5.5)	176 (±11.2)	179 (±5.4)
5 ADH	126 (±9.6)	142 (±5.8)	145 (±6.1)	162 (±5.0)	154 (±10.1)	140 (±13.8)	130 (±12.8)	118 (±10.5)
25 ADH	129 (±4.3)	134 (±6.0)	141 (±5.2)	159 (±8.4)	179 (±9.2)	179 (±7.5)	167 (±9.2)	163 (±13.7)
G-Control	121 (±18.7)	123 (±17.9)	126 (±18.8)	129 (±16.3)	134 (±11.3)	128 (±10.2)	126 (±5.5)	119 (±3.1)
NG-Control	129 (±6.0)	132 (±8.8)	135 (±11.7)	140 (±11.0)	142 (±11.2)	144 (±9.8)	139 (±10.0)	136 (±8.4)

*Each value represents the mean and standard error of 3 observations for the experimental and G-control dogs, and 4 observations for the NG-control dogs. A significant difference ($p < .005$) existed between the 0 and 5 ADH animals, and the 5 and 25 ADH animals, but not between the 0 and 25 ADH animals.

NE infusion. The G-control animals, however, had a consistently lower mean blood pressure than the NG-control group.

Plasma Na was not significantly altered by the infusion of NE or ADH at either the 5 or 25 μU/kg/min level (Table IV). Plasma K was, however, non-significantly increased in the experimental animals from a mean value of 3.5 mEq/l in period 1 to 4.3 mEq/l in period 8 (Table IV). This increase in plasma K was paralleled by a similar increase in both control treatments from a mean value of 3.4 to 3.8 mEq/l in period 1 and 8 respectively.

Plasma osmotic pressure was not significantly altered due to NE infusion or to the level of ADH infusion (Table IV). The plasma osmotic pressure ranged from 275 to 297 mOsm/kg H_2O.

Plasma total solids increased non-significantly from period 1 to period 8 in the 0 and 25 μU/kg/min ADH-infused groups and decreased non-significantly in the 5 μU/kg/min ADH animals (Table V). Changes in hematocrit paralleled those in plasma total solids. Both control groups had lower total solid and hematocrit levels than the experimental groups.

Analysis of kidney tissue slices for Na and K concentration showed a significant increase in the Na concentration gradient ($p < .001$) and a significant decrease ($p < .001$) in the K concentration gradient running from the outer cortex to the inner medulla in all groups (Table VI), although no significant differences existed between groups.

TABLE IV

PLASMA SODIUM, POTASSIUM, AND OSMOTIC PRESSURE

Treatment	Period 1	2	3	4	5	6	7	8
	PLASMA SODIUM (mEq/l)							
0 ADH	127* (±0.7)	129 (±3.8)	130 (±4.3)	130 (±3.5)	130 (±5.2)	129 (±4.0)	128 (±3.3)	128 (±6.2)
5 ADH	127 (±5.0)	124 (±3.6)	128 (±2.0)	132 (±3.2)	122 (±5.9)	131 (±1.9)	127 (±3.4)	122 (±4.5)
25 ADH	127 (±0.5)	132 (±0.7)	128 (±0.5)	126 (±0.8)	127 (±1.6)	128 (±1.2)	127 (±0.5)	124 (±0.7)
G-Control	131 (±5.2)	130 (±3.2)	132 (±3.6)	131 (±3.7)	133 (±3.3)	130 (±2.1)	129 (±3.2)	132 (±2.9)
NG-Control	127 (±2.3)	128 (±1.1)	129 (±2.7)	128 (±1.2)	125 (±1.3)	128 (±1.5)	123 (±5.1)	127 (±2.0)
	PLASMA POTASSIUM (mEq/l)							
0 ADH	3.8 (±.21)	3.6 (±.16)	3.6 (±.07)	3.7 (±.14)	3.7 (±.14)	3.7 (±.14)	4.1 (±.14)	4.5 (±.05)
5 ADH	3.7 (±.17)	3.5 (±.27)	3.6 (±.33)	3.9 (±.46)	3.9 (±.07)	4.2 (±.45)	4.2 (±.25)	4.3 (±.21)
25 ADH	3.1 (±.05)	3.3 (±.14)	3.3 (±.14)	3.2 (±.16)	3.4 (±.19)	3.6 (±.31)	4.0 (±.19)	4.1 (±.14)
G-Control	3.5 (±.07)	3.3 (±.09)	3.3 (±.12)	3.3 (±.12)	3.4 (±.15)	3.6 (±.10)	3.8 (±.12)	3.8 (±.15)
NG-Control	3.2 (±.10)	3.2 (±.15)	3.2 (±.10)	3.3 (±.13)	3.3 (±.17)	3.6 (±.20)	3.6 (±.18)	3.8 (±.25)
	PLASMA OSMOTIC PRESSURE (mOsm/kg H_2O)							
0 ADH	293 (±2.8)	286 (±2.1)	285 (±3.7)	284 (±3.6)	283 (±3.3)	282 (±3.7)	283 (±3.2)	288 (±5.8)
5 ADH	288 (±1.5)	284 (±2.4)	282 (±2.8)	280 (±6.5)	275 (±10.8)	282 (±2.4)	278 (±2.0)	285 (±4.9)
25 ADH	297 (±5.1)	294 (±5.3)	292 (±3.8)	293 (±3.5)	291 (±3.5)	292 (±4.1)	293 (±4.0)	294 (±5.8)
G-Control	282 (±3.5)	282 (±3.5)	282 (±2.8)	281 (±2.7)	279 (±2.4)	282 (±3.2)	280 (±3.9)	284 (±5.5)
NG-Control	282 (±3.5)	287 (±7.7)	279 (±5.5)	282 (±7.5)	280 (±6.5)	281 (±6.8)	277 (±9.2)	282 (±6.1)

*Each value represents the mean and standard error of 3 observations for the experimental and G-control dogs, and 4 observations for the NG-control dogs.

TABLE V

PLASMA TOTAL SOLIDS AND
% HEMATOCRIT

Treatment	Period 1	2	3	4	5	6	7	8
	PLASMA TOTAL SOLIDS (g/100 ml)							
0 ADH	5.9* (±.11)	5.9 (±.05)	5.9 (±.05)	6.2 (±.16)	6.3 (±.20)	6.7 (±.20)	6.7 (±.11)	6.4 (±.16)
5 ADH	5.8 (±.28)	5.8 (±.19)	5.9 (±.14)	5.8 (±.09)	5.7 (±.11)	5.8 (±.09)	5.5 (±.20)	5.3 (±.29)
25 ADH	6.3 (±.22)	5.9 (±.20)	5.8 (±.25)	5.9 (±.22)	6.1 (±.20)	6.3 (±.14)	6.2 (±.12)	6.2 (±.09)
G-Control	5.3 (±.47)	5.3 (±.33)	5.2 (±.20)	5.2 (±.23)	5.1 (±.17)	5.1 (±.17)	5.0 (±.12)	5.5 (±.53)
NG-Control	5.7 (±.37)	5.7 (±.43)	5.6 (±.45)	5.3 (±.65)	5.4 (±.52)	5.0 (±.80)	5.0 (±.65)	5.2 (±.45)
	% HEMATOCRIT							
0 ADH	42 (±1.8)	42 (±1.8)	42 (±2.1)	39 (±3.0)	45 (±3.0)	50 (±0.3)	52 (±0.5)	54 (±0.3)
5 ADH	44 (±0.5)	47 (±0.7)	47 (±0.5)	47 (±0.7)	48 (±1.2)	48 (±1.0)	50 (±2.7)	50 (±2.6)
25 ADH	44 (±1.9)	44 (±1.7)	44 (±1.8)	46 (±2.6)	49 (±2.9)	49 (±2.9)	51 (±2.5)	55 (±2.6)
G-Control	42 (±1.5)	44 (±1.2)	43 (±1.5)	43 (±2.3)	44 (±2.2)	44 (±2.2)	45 (±2.7)	44 (±2.9)
NG-Control	37 (±1.0)	39 (±2.5)	39 (±3.5)	41 (±1.0)	41 (±2.5)	45 (±0.5)	47 (±2.0)	47 (±2.0)

*Each value represents the mean and standard error of 3 observations for the experimental and G-control dogs, and 4 observations for the NG-control dogs.

TABLE VI

TISSUE SODIUM, POTASSIUM, AND % H_2O CONTENT

Treatment	Outer Cortex	Inner Cortex	Outer Medulla	Inner Medulla
		TISSUE SODIUM (mEq/l)		
0 ADH	72.3 ±9.5*	97.1 ±9.0	140.0 ±13.2	170.0 ±19.8
5 ADH	74.2 ±2.9	94.7 ±3.8	121.7 ±3.8	128.8 ±4.7
25 ADH	64.9 ±5.4	81.9 ±3.7	122.4 ±6.8	131.2 ±9.2
G-Control	71.4 ±3.2	79.8 ±8.8	102.7 ±11.0	133.1 ±6.8
		TISSUE POTASSIUM (mEq/l)		
0 ADH	59.0 ±5.2	48.2 ±2.7	39.4 ±1.4	44.2 ±3.7
5 ADH	58.2 ±5.1	53.9 ±3.4	37.9 ±2.0	40.3 ±1.5
25 ADH	61.2 ±3.7	48.4 ±2.7	36.3 ±1.2	37.0 ±1.1
G-Control	44.4 ±3.3	40.3 ±3.4	33.9 ±3.1	36.9 ±1.4
		TISSUE % H_2O CONTENT		
0 ADH	79.0 ±.78	83.0 ±.67	87.5 ±.71	86.0 ±.80
5 ADH	78.0 ±.26	81.8 ±.21	88.3 ±.76	88.2 ±.54
25 ADH	78.0 ±.58	81.0 ±.37	86.0 ±.26	86.0 ±.82
G-Control	80.9 ±.92	83.3 ±.70	87.1 ±.74	87.3 ±.82

*Each value represents the mean and standard error of 6 observations for the experimental and G-control dogs. A significant increase ($p < .001$) was noted in the tissue Na and % water content, and a significant decrease ($p < .001$) in the tissue K concentration gradient running from the outer cortex to the inner medulla in all groups.

Tissue water content (%) increased significantly ($p < .001$) from the outer cortex to the inner medulla in all groups (Table VI), averaging 79% in the outer cortex and increasing to 87% in the inner medulla. There was no significant differences in % tissue water content between groups.

DISCUSSION

The infusion of NE has a diuretic and natriuretic effect at low concentrations (0.05 to 0.40 μg/kg/min) and causes a decrease in both urine flow and $U_{Na}V$ at higher concentrations. The concurrent infusion of ADH inhibits both the diuretic and natriuretic effects of NE.

Possible mechanisms that can be offered to explain how NE could exert these renal effects include: a) cardiovascular changes, b) changes in the counter-current exchange system, and c) permeability changes in the nephron. Of these three, changes in permeability appear to be more likely.

In considering cardiovascular mechanisms involving vascular effects such as changes in mean arterial blood pressure, GFR, and ERPF, it has been shown that mean arterial blood pressure affects sodium and water reabsorption in the proximal tubule (9,10,15,22). This effect is dependent upon the transmission of pressure to the postglomerular capillary bed as a result of renal vasodilatation and/or an increase in arterial blood pressure (11,22). With an increase in capillary hydrostatic pressure it has been suggested that Starling forces which influence capillary uptake are operable (11,22), that is forces which would favor decreased renal capillary reabsorption should also favor increased urine flow and $U_{Na}V$ (9). These forces include increased capillary hydrostatic pressure (as reflected by increased arterial blood pressure) and decreased

plasma oncotic pressure (as measured by decreased plasma total solids and filtration fraction). However, the basis for such a mechanism is not supported by the data in this study, since mean arterial blood pressure increased to the same extent in all experimental groups during the first four periods (Table III, see p. 31), while urine flow and $U_{Na}V$ did not increase in the same manner in all groups. This would indicate that renal capillary hydrostatic pressure was not directly related to the mean arterial blood pressure. The lack of a direct relationship was probably due to intrarenal vasoconstriction caused by NE infusion (27), which would tend to decrease postglomerular capillary hydrostatic pressure. Plasma oncotic pressure (Table V, see p. 34) was not significantly increased in the groups of animals with both the highest and lowest diuretic and natriuretic response. Filtration fraction (Fig. 8, see p. 29), which could affect postglomerular plasma oncotic pressure, did not change in a manner which could explain the results. Urine flow increased despite an increase in filtration fraction which should have concentrated postglomerular colloids and thus increased reabsorption.

The lack of any significant correlation between mean arterial blood pressure and GFR or ERPF can be attributed to the fact that the canine kidney is capable of autoregulation (32) over the blood pressure range found in this study, thus eliminating possible effects of blood pressure on either filtration rate or on plasma flow.

The GFR and ERPF remained constant over the periods in which a diuretic and natriuretic response to NE infusion occurred. It should be pointed out, however, that although significant differences were found between the 0, 5, and 25 μU/kg/min ADH groups with regard to both ERPF and GFR (Fig. 7, see p. 27), these differences are not related temporally or quantitatively to the observed increase in urine flow (Fig. 1, see p. 13) or $U_{Na}V$ (Fig. 5, see p. 23) due to NE. The decreased ERPF and GFR due to ADH presence may have contributed to its antidiuretic action. This is consistent with the view that ADH brings about a reduction in renal medullary blood flow. The filtration fraction in both the 5 and 25 μU/kg/min ADH-infused animals were not significantly different. However, the respective GFR and ERPF were significantly lower ($p < .001$ and $< .005$ respectively) in the 25 ADH as compared to the 5 ADH animals. The similarity of the filtration fraction despite a reduction in both GFR and ERPF suggests that the afferent and efferent arterioles are constricting equally.

It has been proposed by Thurau and Deetjen (30) that blood flow through the renal medulla, as opposed to the renal arterial flow, is not autoregulated, but rather reacts in direct relation to changes in mean arterial blood pressure. Therefore NE, a potent peripheral vasoconstrictor, could function as a diuretic by increasing systemic blood pressure which in turn could have increased medullary blood flow.

An increase in medullary blood flow would tend to washout the solutes accumulated in the medullary interstitium and cause a reduction in the corticomedullary concentration gradient. A study by Selkurt *et al.* (25) showed that elevated renal arterial pressures (averaging 200 mm Hg) brought about a diuretic and natriuretic response which was related to a washout of the corticomedullary concentration gradient with no increase in GFR. However, analysis of the kidney tissue slices in this study indicated that the Na concentration gradient was maintained (Table VI, see p. 35), indirectly indicating no substantial increase in blood flow through the medullary and papillary regions as a result of NE infusion.

Of the three possible mechanisms considered originally to explain the changes in urine flow and $U_{Na}V$, the data suggest that the antagonistic effects of NE on ADH action can best be accounted for by a mechanism involving changes in tubule permeability. The effects of changes in permeability can be illustrated by plotting C_{H_2O} as a function of C_{osm} (Fig. 4, see p. 20). Under antidiuretic conditions the collecting duct becomes more permeable to H_2O (29). Thus, the solute-free water or C_{H_2O} should become negatively correlated to the osmotic load or C_{osm} presented to this portion of the tubule. This proved to be the case in those animals infused with ADH. Conversely, if the tubule becomes less permeable to water then the correlation between C_{H_2O} and C_{osm} should become more positive. Under these conditions the amount of solute-free water

should increase as the C_{osm} increases (Fig. 4). This proved to be the case for the two periods 5 and 6 in both the zero and 5 μU/kg/min ADH-infused animals. During these two periods the C_{H_2O} became positive and showed a positive correlation to C_{osm}.

Figure 4 (see p. 20) represents C_{H_2O} plotted against C_{osm} where the straight line separates experimental points into "high" and "low" values. The "low" values correspond to periods in which there is an apparent minimum amount of C_{H_2O} for a given solute load, while the "high" values correspond to periods in which there is an increased amount of C_{H_2O} for a given solute load. In this manner it is possible to show that points of high value correspond to periods 4, 5, 6, and 7 in the group of animals not infused with ADH, periods 4, 5, and 6 in the 5 μU/kg/min ADH-infused animals, and only periods 4 and 5 in the 25 μU/kg/min ADH animals. Such comparisons indicate that during each of the periods mentioned, NE was capable of exerting a permeability effect on water movement opposite to that of ADH.

Changes observed in C_{H_2O}, urine flow, $U_{Na}V$, and C_{osm} all can be attributed to decreased permeability of the collecting duct, first to solutes and later to water, as a result of increasing concentrations of NE during infusion. If one considers permeability to be directly related to the size of pores located in the tubular cell membrane as suggested by Koefoed-Johnsen and Ussing (16), progressive decreases in permeability

initially would decrease the rate of reabsorption of solutes more so than H_2O, since solutes have a larger effective diameter than H_2O. Therefore, a decrease in permeability resulted in an increase in C_{osm} and $U_{Na}V$ (natriuresis) over the first four periods. The initial increase in C_{osm} (Fig. 3, see p. 18) appears to be a general effect on all solutes and not just due to a natriuretic effect, since the concentration of Na accounts for 10% of the total osmotic pressure of the urine in period 1 and 15% in period 3. The bulk of the total osmotic pressure is probably due to urea, since urea is known to be reabsorbed in the collecting duct (7,34). As the permeability of the collecting duct decreases further the effect on water reabsorption becomes more pronounced. These permeability changes would account for the initial decrease in C_{H_2O} over the first three periods due to a more pronounced permeability effect on solutes and secondarily (periods 4 and 5) an increase in C_{H_2O} due to permeability effects principally on water reabsorption.

The lack of an increase in U_KV (Fig. 6, see p. 25) during the periods in which $U_{Na}V$ and C_{osm} increased cannot be explained on the basis of solute permeability changes in the collecting duct. This is consistent with the view that most of the K appearing in the urine is added to the tubular fluid in the distal tubule, and that little net movement of K (probably reabsorptive in nature) takes place in the collecting duct (34).

The decreases observed in ERPF, GFR, urine flow, C_{H_2O}, C_{osm}, $U_{Na}V$, and U_KV during the last four periods in the experimental animals, appears to have been the result of progressive intrarenal vasoconstriction, brought about by the infusion of NE at rates greater than 0.40 µg/kg/min (periods 5 to 8). By contrast the control animals, in which no NE was infused, showed only a slight decrease in ERPF and no change in GFR or other parameters during the same periods in which decreases were seen in the experimental animals. This would indicate that the intrarenal vasoconstriction that resulted from the infusion of NE at rates greater than 0.40 µg/kg/min, had a direct quantitative effect on renal function.

Seemingly conflicting reports between our finding of NE being natriuretic and the reports in the literature of antinatriuretic effects of NE (2,4,12, 27) are probably due to the dose response of $U_{Na}V$ to NE, since in all the studies cited the dosages were 0.20 µg/kg/min NE or greater. This was demonstrated in this study, which showed that at infusion rates below 0.20 µg/kg/min NE is natriuretic but antinatriuretic above 0.20 µg/kg/min.

The sigmoid nature of the C_{H_2O} curve (Fig. 2, see p. 15) is suggestive of an allosteric type of inhibition (21). This type of inhibition implies that the binding of NE or ADH to a receptor site on the tubular membrane changes the conformation of the receptor site thereby influencing the subsequent binding of NE or ADH. However, it is not clear from the literature

(15,19) as to what the characteristics of the receptor site on the tubular membrane are for NE or ADH. An allosteric type of inhibition, therefore, cannot be confirmed.

SUMMARY

NE is diuretic and natriuretic, when infused into dogs at rates of 0.05 to 0.40 μg/kg/min. This effect is independent of cardiovascular effects or changes in the countercurrent multiplier system, but is dependent upon decreased permeability of the kidney tubular collecting duct to solutes and water. The infusion of ADH at 5 and 25 μU/kg/min reduced both the diuretic and natriuretic effects of NE.

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