Control of Renal Hemodynamics and Glomerular Filtration Rate in Chronic Hypercalcemia

ROLE OF PROSTAGLANDINS, RENIN-ANGIOTENSIN SYSTEM, AND CALCIUM

MOSHE LEVI, MARILYN A. ELLIS, and TOMAS BERL, *Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado 80262*

ABSTRACT The role of prostaglandins (PG), renin-angiotensin system (RAS) and calcium (Ca) in the control of renal hemodynamics and glomerular filtration rate (GFR) in chronic hypercalcemia (serum Ca 12.8 mg%) was studied. Renal blood flow (RBF, 6.39 ml/min per gram kidney weight [gkw]) and GFR (0.52 ml/min per gkw) were significantly decreased in hypercalcemic rats when compared with normocalcemic rats (7.15, $P < 0.001$ and 0.74, $P < 0.05$, respectively). These changes in RBF and GFR occurred independent of any significant alterations in systemic hemodynamics, blood and plasma volume. Inhibition of the renal PG with indomethacin resulted in marked decrements in both RBF (6.39–4.12 ml/min per gkw, $P < 0.01$) and GFR (0.52–0.19 ml/min per gkw, $P < 0.01$) in hypercalcemic rats, whereas there was no significant alterations in normocalcemic rats. Inhibition of the RAS with captopril resulted in marked increments in both RBF (6.39–7.35 ml/min per gkw, $P < 0.05$) and GFR (0.52–0.74 ml/min per gkw, $P < 0.05$) in hypercalcemic rats. In fact, there was no significant difference from the RBF and GFR of similarly treated normocalcemic rats. Similar results were also obtained with the competitive angiotensin II (AII) antagonist (sarcosyl^1-isoleucyl^5-glycyl^8) AII. Since both the renal PG and the RAS are involved in the control of RBF and GFR in hypercalcemia, the role of each is best revealed in the absence of the other. Hence, comparison of the RBF and GFR in the PG-inhibited hypercalcemic rats in the presence of AII (4.12 and 0.19 ml/min per gkw, respectively) and absence of AII (5.99 and 0.53 ml/min per gkw, $P < 0.01$ for both) reveals the vasoconstrictive role for AII in hypercalcemia. On the other hand, comparison of the RBF and GFR in the AII-inhibited hypercalcemic rats in the presence of PG (7.35 and 0.74 ml/min per gkw, respectively) and absence of PG (5.99 and 0.53 ml/min per gkw, $P < 0.01$ and $P < 0.05$, respectively) reveals the vasodilatory role for PG in hypercalcemia. Finally, comparison of the RBF and GFR in both PG- and AII-inhibited hypercalcemic rats (5.99 and 0.53 ml/min per gkw, respectively) with similarly treated normocalcemic rats (7.30 and 0.94 ml/min per gkw, $P < 0.001$ and $P < 0.005$, respectively) reveals the vasoconstrictive role for Ca in chronic hypercalcemia. Our study therefore demonstrates that in chronic hypercalcemia the RBF and GFR are controlled by an active interplay of the vasoconstrictive effect of AII, the vasodilatory effect of renal PG, and the direct vasoconstrictive effect of Ca, independent of either AII or PG. The sum total of these forces produces a modest but significant decrease in RBF and GFR.

INTRODUCTION

The association of acute and chronic hypercalcemia with decrements in renal blood flow (RBF)[1] and glomerular filtration rate (GFR) has been widely recognized in both man and experimental animals (1–15). While the mechanisms responsible for the decrement in RBF and GFR have been studied in anesthetized, acutely hypercalcemic animals, there have been no such studies in the setting of chronic hypercalcemia. Since recent in vivo and in vitro studies have revealed

Received for publication 8 July 1982 and in revised form 2 March 1983.

[1] *Abbreviations used in this paper:* AII, angiotensin II; CI, cardiac index; GFR, glomerular filtration rate; gkw, gram kidney weight; MAP, mean arterial pressure; PG, prostaglandin(s); RAS, renin-angiotensin system; RBF, renal blood flow; RVR, renal vascular resistance; Sar^1-Ile^5-Gly^8 AII, sarcosyl^1-isoleucyl^5-glycyl^8; SVR, systemic vascular resistance.

an important role for calcium ion in the vasoconstrictive response of the vasculature to angiotensin II (AII) (16–18), it is possible that the decrement in RBF and GFR in chronic hypercalcemia is at least, in part, mediated by this potent vasoconstrictive hormone. Alternatively, calcium ion could directly, independent of AII, mediate the decrement in RBF and GFR in chronic hypercalcemia (19). On the other hand, the vasodilatory renal prostaglandins (PG), whose synthesis in vitro has been shown to be stimulated by calcium ion (20–22), could have an attenuating effect on the vasoconstrictor activity of AII and calcium ion. The present experiments were therefore performed to (*a*) assess the systemic and renal hemodynamic effect of chronic hypercalcemia in the conscious rat, and (*b*) determine the role of PG, AII, and calcium per se in the control of RBF and GFR in such a setting.

METHODS

All studies were performed on Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing between 225 and 325 g. Animals were fed either a commercially obtained normal diet (ICN Nutritional Biochemicals, Cleveland, OH) or an identical diet to which dihydrotachysterol (Philips Roxan, Columbus, OH), 4.25 mg/kg of diet was added. Water intake was allowed ad lib.

Since animals on vitamin D-supplemented diet eat less food than do animals on normal diet (23), in all the studies, the daily food intake of the animals on normal diet was limited to that of pair-fed animals on vitamin D-supplemented diet. Such supplementation with vitamin D increased serum calcium by the 3rd d. This level remained stable for the ensuing 5 d of the study (23). Serum sodium, potassium, chloride, bicarbonate, and phosphate were not altered by the diet. The following studies were undertaken.

Effect of chronic hypercalcemia on systemic and renal hemodynamics, GFR and blood volume (group 1)

Following 8 d on either control (n = 9) or vitamin D-supplemented diet (n = 9), rats were anesthetized with ether. Polyethylene catheters (PE50) were inserted into the jugular vein and femoral artery for infusion and blood sampling respectively, and the bladder was catheterized with a catheter (PE60) with care to eliminate dead space. Cannulation of the right carotid artery into the left ventricle was accomplished with tapered PE350 tubing. Ventricular cannulation was confirmed by pressure wave tracing. During surgery, isotonic saline equivalent to 0.5% of the body weight was infused to replace estimated fluid losses. Animals were then allowed to recover fully from anesthesia, while they were placed in individual restraining cages. Mean arterial pressure (MAP) was continuously monitored (Electronics for Medicine, White Plains, NY) and inulin in 0.45% saline was infused at a rate of 0.6 ml/100 g body wt per h, which produced constant plasma inulin levels of ~20 mg/dl. After a 60-min equilibration period, the animals' urine flow was stable and two 30-min urine collections were obtained. Blood (200 μl) samples were drawn in the middle of each collection period and the volume was replaced with equal volumes of 0.9% saline. The blood and urine were analyzed for inulin concentration and inulin clearance was calculated in the standard manner. Following the two 30-min urine collections, cardiac index (CI), systemic vascular resistance (SVR), RBF, and renal vascular resistance (RVR) were determined by a radioactive microsphere technique described by Hsu et al. (24) and adapted for use in our laboratory (25). The microspheres used were 8.8±0.9 μm in diam and were labeled with ^{85}Sr.

Calculations were performed as follows:

$$\text{GFR (ml/min per g)} = \frac{\text{urine inulin concentration}}{\text{plasma inulin concentration}} \times \frac{\text{urine flow rate}}{\text{kidney weight}};$$

$$\text{CI (ml/min per kg)} = \frac{\text{cpm injected}}{\text{femoral blood cpm}} \times \frac{\text{femoral blood flow rate}}{\text{body weight}};$$

$$\text{SVR (mmHg/ml per min per kg)} = \frac{\text{MAP}}{\text{CI}};$$

$$\text{RBF (ml/min per g)} = \frac{\text{kidney cpm}}{\text{femoral blood cpm}} \times \frac{\text{femoral blood flow rate}}{\text{kidney weight}};$$

$$\text{RVR (mmHg/ml per min per g)} = \frac{\text{MAP}}{\text{RBF}}.$$

Blood volume was determined in six hypercalcemic and six pair-fed normocalcemic rats utilizing the ^{125}I-albumin method of Thiel et al. (26) and Flamenbaum et al. (27) as described by our laboratory (28).

PG in chronic hypercalcemia

Effect of hypercalcemia on urinary PG excretion. To assess the effect of hypercalcemia on urinary PG excretion, 24-h urinary samples were collected during day 7 of the study, in normocalcemic (n = 8) and hypercalcemic (n = 8) rats. For these studies, rats were placed in metabolic cages that allow for separation of urine and feces (Hoeltege Co., Cincinnati, OH). Urine was collected in glass tubes to which sodium azide was added to achieve a final concentration of 4 mg/ml of urine. After measuring the volume, the urine was stored at −20°C and subsequently was assayed for prostaglandin E_2 (PGE_2) by radioimmunoassay using a modification for urine of the procedure of Dray et al. (29).

Effect of PG inhibition on GFR and renal hemodynamics in hypercalcemic rats (group 2). To determine the role of PG in the control of hemodynamics and GFR in hypercalcemia, the cyclooxygenase inhibitor, indomethacin, was administered to normocalcemic control and hypercalcemic rats. Following 7 d of on either control (n = 8) or vitamin D-supplemented diet (n = 8), animals were given indomethacin (3.33 mg/kg i.p., every 8 h for 24 h). To confirm the degree of cyclooxygenase blockade, urinary PGE_2 excretion was measured during the 24-h period. Measurements of renal hemodynamics and GFR were made on day 8 of the study as described above in group 1 animals.

The renin-angiotensin system (RAS) in chronic hypercalcemia

Effect of hypercalcemia on plasma renin activity. To assess the effect of hypercalcemia on circulating levels of

renin activity, plasma renin activity was measured in normocalcemic control (n = 8) and hypercalcemic (n = 8) rats on day 8 of the study. In these studies, blood was obtained as free-flowing blood issuing from the trunk of guillotined animals. Blood was collected in prechilled tubes containing sodium EDTA and was centrifuged within 15 min at 0°C. The plasma was stored at −20°C and subsequently assayed for renin activity by radioimmunoassay (30).

Furthermore, to determine the effect of high urinary PGE_2 excretion (see below) on circulating levels of renin activity, plasma renin activity was also measured in normocalcemic control (n = 8) and hypercalcemic rats following PG synthesis inhibition with indomethacin (n = 8).

Effect of AII inhibition on GFR and renal hemodynamics in chronic hypercalcemia (group 3). To determine the significance of AII in the control of RBF and GFR in hypercalcemia, inhibitors of the synthesis or action of AII were administered to normocalcemic control and hypercalcemic rats. In the first group of studies (group 3 A), following 4 d of on either control (n = 5) or vitamin D-supplemented diet (n = 8), animals were given the converting enzyme inhibitor, captopril (SQ 14,225, E. R. Squibb & Sous, Inc., Princeton, NJ) (13.33 mg/kg, orally, every 8 h for 4 d). This dose of captopril completely prevented the pressor response to 50 ng of exogenous AI in every animal studied. Measurements of hemodynamics and GFR were made on day 8 of the study.

Since the converting enzyme inhibitor, captopril, may have additional effects than AII formation inhibition, such as increased levels of kinins, a second group of studies (group 3 B) were undertaken with the competitive AII antagonist (sarcosyl^1-isoleucyl^5-glycyl^8, Sar^1-Ile^5-Gly^8) AII (Bachem, Torrance, CA). The AII antagonist was administered on day 8 of the study (2.5 μg/kg per min, i.v., for 90 min) to normocalcemic (n = 4) and hypercalcemic (n = 7) rats. This dose completely prevented the pressor response to 25 ng of exogenous AII in every animal studied. Measurement of hemodynamics was made following the 90-min infusion period. GFR was not measured in this subgroup of rats.

Effect of AII inhibition in PG-inhibited hypercalcemic rats (group 4)

The role of AII in the control of renal hemodynamics and GFR in hypercalcemia independent of PG was studied by administering inhibitors of the synthesis or action of AII to normocalcemic control and hypercalcemic rats with PG inhibition. In the first group of studies (group 4 A), following 4 d of on either control (n = 4) or vitamin D-supplemented (n = 9) diet, animals were given the converting enzyme inhibitor captopril. On day 7 of the study these animals were given indomethacin. Measurements of renal hemodynamics and GFR were made on day 8 of the study.

A second group of studies (group 4 B) used a competitive AII antagonist (Sar^1-Ile^5-Gly^8) AII. The AII antagonist and indomethacin were begun on day 7 of the study and given for 24 h to control (n = 5) and hypercalcemic (n = 6) rats. Measurement of renal hemodynamics was made on day 8 of the study.

Statistical analysis

A two-tailed unpaired Student's t test was used to compare results of each experimental group with its corresponding control group and a one-way analysis of variance (with Student-Newman-Keuls modification) (31) was used to compare results within the experimental or control groups. A P value <0.05 was considered significant. All data are expressed as mean±SE.

RESULTS

Effect of chronic hypercalcemia on systemic and renal hemodynamics, GFR and blood volume (group 1). Table I shows the hemodynamic consequences of 8 d of hypercalcemia in the conscious rat. There were no significant alterations in systemic hemodynamics as MAP, CI, and SVR were similar in normocalcemic control and hypercalcemic rats. Likewise, both blood and plasma volume were similar in the two groups of rats. However, there were significant changes in renal hemodynamics and GFR in the hypercalcemic rats. RBF decreased (7.15–6.39 ml/min per gkw, $P < 0.001$), RVR increased (15.76–17.86 mmHg/ml per min per gkw, $P < 0.005$), and GFR decreased

TABLE I

Effect of Chronic Hypercalcemia on Systemic and Renal Hemodynamics, GFR, and Blood Volume in the Conscious Rat

	S_{Ca}	MAP	CI	SVR	RBF	RVR	GFR	Blood volume	Plasma volume
	mg/dl	*mmHg*	*ml/min/kgbw*	*mmHg/ml/min/kgbw*	*ml/min/gkw*	*mmHg/ml/min/gkw*	*ml/min/gkw*	*ml/100 g*	
Normocalcemic	9.96 ±0.14	114 ±2	270 ±4	0.42 ±0.02	7.15 ±0.08	15.76 ±0.28	0.75 ±0.07	8.06 ±0.27	4.08 ±0.14
	n = 9	n = 9	n = 9	n = 9	n = 9	n = 9	n = 9	n = 6	n = 6
Hypercalcemic	12.79 ±0.15	113 ±2	261 ±5	0.43 ±0.01	6.39 ±0.14	17.86 ±0.58	0.52 ±0.07	8.93 ±0.57	4.49 ±0.29
	n = 9	n = 9	n = 9	n = 9	n = 9	n = 9	n = 9	n = 6	n = 6
P	<0.001	NS	NS	NS	<0.001	<0.005	<0.05	NS	NS

Data on RBF and RVR are expressed per kidney, i.e., n = 18. S_{Ca}, serum calcium.

(0.75–0.52 ml/min per gkw, $P < 0.05$) when compared with normocalcemic control rats. Our subsequent studies were directed at defining the mechanisms responsible for these perturbations in renal hemodynamics.

Effect of PG inhibition on renal hemodynamics in chronic hypercalcemia (group 2). Urinary PGE_2 excretion rate was markedly increased in hypercalcemic rats (105±9 vs. 21±1 ng/24 h in normocalcemic controls, $P < 0.001$). The role of these elevated PG in the control of renal hemodynamics and GFR in chronic hypercalcemia was then evaluated by the administration of the cyclooxygenase inhibitor indomethacin. This agent decreased urinary PG excretion in hypercalcemic rats to 18±4 ng/24 h, a value not significantly different from that of normocalcemic rats receiving indomethacin (10±1 ng/24 h). Following the administration of indomethacin, there was an 83% inhibition in PG excretion in hypercalcemic rats. As shown in the top panel of Table II, in normocalcemic rats, PG inhibition resulted in no changes in systemic pressure.

TABLE II

Systemic Pressure, Renal Hemodynamics, and GFR in Normocalcemic (Top) and Chronically Hypercalcemic Rats (Bottom) Receiving Indomethacin, Captopril, or Both

Experimental group	S_{Ca}	MAP	RBF	RVR	GFR
	mg/dl	*mmHg*	*ml/min/gkw*	*mmHg/ml/min/gkw*	*ml/min/gkw*
			Normocalcemia		
1. Normocalcemic (n = 9)	9.96 ±0.14	114 ±2	7.15 ±0.08	15.76 ±0.28	0.75 ±0.07
2. Normocalcemic + indomethacin (n = 8)	10.03 ±0.17	119 ±2	7.57 ±0.14	16.04 ±0.34	0.88 ±0.07
3 A. Normocalcemic + captopril (n = 5)	10.12 ±0.29	106 ±3	7.70 ±0.24	13.60 ±0.57	0.70 ±0.05
4 A. Normocalcemic + captopril + indomethacin (n = 4)	10.00 ±0.10	105 ±3	7.30 ±0.13	13.90 ±0.27	0.94 ±0.08
P (anova)					
Group 1 vs. 2	NS	NS	NS	NS	NS
Group 1 vs. 3 A	NS	<0.05	<0.05	<0.01	NS
Group 2 vs. 4 A	NS	<0.01	NS	<0.01	NS
Group 4 A vs. 3 A	NS	NS	NS	NS	NS
			Hypercalcemia		
1. Hypercalcemic (n = 9)	12.79* ±0.15	113 ±2	6.39* ±0.14	17.86‡ ±0.58	0.52§ ±0.07
2. Hypercalcemic + indomethacin (n = 8)	11.98* ±0.22	125 ±2	4.12* ±0.33	34.58* ±3.85	0.19* ±0.04
3 A. Hypercalcemic + captopril (n = 8)	12.49* ±0.10	110 ±3	7.35 ±0.35	15.38 ±0.80	0.74 ±0.07
4 A. Hypercalcemic + captopril + indomethacin (n = 9)	12.20* ±0.17	109 ±3	5.99* ±0.32	19.11* ±1.11	0.53‡ ±0.07
P (anova)					
Group 1 vs. 2	NS	<0.01	<0.01	<0.01	<0.01
Group 1 vs. 3 A	NS	NS	<0.05	NS	<0.05
Group 2 vs. 4 A	NS	<0.01	<0.01	<0.01	<0.01
Group 4 A vs. 3 A	NS	NS	<0.01	NS	<0.05

* $P < 0.001$, compared with its normocalcemic control. S_{Ca}, serum calcium.
‡ $P < 0.005$.
§ $P < 0.05$.

Likewise, in normocalcemic rats there were slight but insignificant increases in RBF, RVR, and GFR. In contrast, in hypercalcemic rats (bottom panel of Table II) PG inhibition resulted in a significant increase in MAP. This was due to a small increment in both peripheral vascular resistance and CI, neither of which were themselves statistically significant. Despite the increase in systemic pressure, there was a marked decrement in renal hemodynamics. Thus, RVR doubled causing a marked decrement in RBF from 6.39 to 4.12 ml/min per gkw, $P < 0.01$. Likewise, there was a profound and even more severe decrement in GFR from 0.52 to 0.19 ml/min per gkw, $P < 0.01$.

Effect of AII inhibition on GFR and renal hemodynamics in chronic hypercalcemia (group 3). Plasma renin activity was not different in hypercalcemic rats, 7.13±0.094 vs. 5.78±0.51 ng AI/ml per h in normocalcemic controls. Despite comparable plasma renin activity, since enhanced end-organ response to AII in the hypercalcemic state could not be excluded, the role of AII in the control of renal hemodynamics and GFR in chronic hypercalcemia was evaluated.

In normocalcemic rats (top of Table II) following the administration of captopril, there was a mild but significant decrease in MAP. This decrease was due to small, statistically not significant decrements in CI and SVR. Captopril administration also caused an increase in RBF as RVR decreased, but GFR remained unaltered. In the hypercalcemic rats (bottom of Table II), there were no significant changes in systemic pressure, but marked increment in renal hemodynamics and GFR. Specifically, as RVR decreased RBF increased (6.39–7.35 ml/min per gkw, $P < 0.05$) when compared to AII intact hypercalcemic rats. Likewise, GFR increased (0.52–0.74 ml/min per gkw, $P < 0.05$). Following the administration of captopril, the resultant renal hemodynamics and GFR in the hypercalcemic rats were not significantly different than in the normocalcemic control rats. In fact, a comparison of the captopril-treated hypercalcemic rats with normocalcemic rats also receiving captopril reveals no difference in GFR, RBF, or RVR. Since the converting enzyme inhibitor, captopril, is known to have effects other than inhibition of AII synthesis, to better define the role of AII, renal hemodynamics were also evaluated by the administration of a competitive AII antagonist (Sar^1-Ile^5-Gly^8) AII (group 3 B). As shown in the top panel of Table III, this agent caused no significant alterations in either blood pressure or renal hemodynamics in the control rat. In contrast in the chronic hypercalcemic rat (bottom panel), RBF increased (6.39–7.58 ml/min per gkw, $P < 0.01$) as RVR decreased without associated changes in systemic pressure. In fact, following the administration of (Sar^1-Ile^5-Gly^8) AII, the resultant renal hemodynamics were not significantly different from that of normocalcemic (Sar^1-Ile^5-Gly^8) AII rats. Our data therefore show that the inhibition of the synthesis or the antagonism of the action of AII normalize renal hemodynamics and GFR in chronic hypercalcemia.

Effect of AII inhibition or antagonism in hypercalcemia during PG inhibition (Group 4). Plasma renin activity following PG inhibition was not significantly different in hypercalcemic rats, 3.66±0.38 ng AI/ml per h when compared with normocalcemic controls, 4.55±0.13 ng AI/ml per h. However, since enhanced end-organ response to AII was noted in the hypercalcemic rats, an effect that could be further enhanced by PG inhibition (32, 33), the role of AII in the control of renal hemodynamics and GFR in chronic hypercalcemia during PG inhibition was evaluated (Tables II and III).

As shown in the top panel of Table II, normocalcemic control rats who received captopril and indomethacin (group 4 A) had significant decreases in MAP when compared with normocalcemic control rats who were treated with indomethacin alone. This was due to a significant decrease in SVR. Likewise, RVR decreased but neither GFR nor RBF were altered.

In hypercalcemic rats receiving both indomethacin and captopril (bottom panel of Table II) there was also a significant decrease in MAP when compared with animals treated with indomethacin alone. This decrease was due to small, statistically not significant decrements in CI and SVR. Despite this decrease in MAP, inhibition of AII synthesis during PG inhibition in the hypercalcemic rats resulted in marked increments in renal hemodynamics and GFR. RBF increased (4.12–5.99 ml/min per gkw, $P < 0.01$), RVR decreased (34.58–19.11 mmHg/ml per min per gkw, $P < 0.01$) and GFR increased (0.19–0.53 ml/min per gkw, $P < 0.01$).

The role of AII in the control of renal hemodynamics in hypercalcemia during PG inhibition was also evaluated by the administration of (Sar^1-Ile^5-Gly^8) to indomethacin-treated rats (group 4 B). As shown in Table III, when compared with AII intact PG-inhibited rats (group 2), this agent did not alter MAP in either normocalcemic or hypercalcemic rats. Although it did not affect renal hemodynamics in normocalcemic rats, its effects in the hypercalcemic rats were profound. Thus RBF increased (4.12–5.86 ml/m per gkw, $P < 0.01$) and RVR decreased markedly (34.6–23.4 mmHg/ml per min per gkw, $P < 0.01$). Therefore in chronic hypercalcemia the inhibition of the synthesis or the antagonism of the action of AII during PG synthesis inhibition is associated with marked and significant increment in renal hemodynamics and GFR.

TABLE III

Effect of Sar[1]-Ile[5]-Gly[8] AII in Normocalcemic (Top) and Hypercalcemic Rats (Bottom) with and without Indomethacin Administration

Experimental group	S_{Ca}	MAP	RBF	RVR
	mg/dl	*mmHg*	*ml/min/gkw*	*mmHg/ml/min/gkw*
	Normocalcemia			
1. Normocalcemic (n = 9)	9.96 ±0.14	114 ±2	7.15 ±0.08	15.76 ±0.28
2. Normocalcemic + indomethacin (n = 8)	10.03 ±0.17	119 ±2	7.57 ±0.14	16.04 ±0.34
3 B. Normocalcemic + (Sar[1]-Gly[8]) AII (n = 4)	9.9 ±0.09	114 ± 5	7.63 ±0.36	15.66 ±0.73
4 B. Normocalcemic + (Sar[1]-Gly[8]) AII + indomethacin (n = 5)	10.0 ±0.1	115 ±3	7.82 ±0.22	14.69 ±0.23
P(anova)				
Group 1 vs. 3 B	NS	NS	NS	NS
Group 2 vs. 4 B	NS	NS	NS	NS
Group 4 B vs. 3 B	NS	NS	NS	NS
	Hypercalcemia			
1. Hypercalcemic (n = 9)	12.79* ±0.15	113 ±2	6.39* ±0.14	17.86‡ ±0.58
2. Hypercalcemic + indomethacin (n = 8)	11.98* ±0.22	125 ±2	4.12* ±0.33	34.58* ±3.85
3 B. Hypercalcemic + (Sar[1]-Gly[8]) AII (n = 7)	12.02* ±0.11	114 ±3	7.58 ±0.18	15.12 ±0.38
4 B. Hypercalcemic + (Sar[1]-Gly[8]) AII + indomethacin (n = 6)	12.60* ±0.16	130‡ ±3	5.86* ±0.26	23.42* ±1.35
P(anova)				
Group 1 vs. 3 B	NS	NS	<0.01	NS
Group 2 vs. 4 B	NS	NS	<0.01	<0.01
Group 4 B vs. 3 B	NS	<0.01	<0.01	<0.05

* $P < 0.001$, compared with its normocalcemic control. S_{Ca}, serum calcium.
‡ $P < 0.005$.

DISCUSSION

The fact that acute and chronic hypercalcemia cause a decreased RBF and GFR in both man and experimental animals has been repeatedly reported (1–15). Studies performed by Humes et al. in the rat (13) and Okahara et al. in the dog (15) analyzed the factors responsible for the control of RBF and GFR in acute hypercalcemia in the anesthetized state, and clearly pointed to the functional nature of these changes as they were sensitive to hormonal and pharmacological manipulations. In contrast, there are no studies that analyze the mechanisms responsible for the changes in GFR and RBF in chronic hypercalcemia. Some of the renal perturbations that occur in chronic hypercalcemia, such as the renal concentrating defect, are not readily reversible and therefore probably related to the anatomic interstitial and tubular changes that supervene (5). Whether these anatomic changes contribute to the impairments in RBF and GFR or whether, as in acute hypercalcemia, these changes are purely functional is not known.

Our initial studies demonstrated that chronic hypercalcemia in the conscious rat causes a reduction in RBF and GFR independent of the alterations in systemic hemodynamics frequently seen in acute hyper-

calcemia (34). The present study does not address the distribution of the decrement in RBF. However, since in a recent study (23) we did not find a decrease in inner medullary plasma flow, which is derived from the juxtamedullary circulation, it is possible that the outer cortical circulation is the one primarily affected. Our subsequent studies were directed to the systematic elucidation of the roles of PG, AII, as well as calcium per se in these perturbations.

The marked elevation of urinary PG excretion in chronic hypercalcemia made it attractive to postulate that these substances may play an important role in the maintenance of renal hemodynamics in this state. The administration of the cyclooxygenase inhibitor indomethacin brought about a marked decrement in PG excretion. This caused a profound decrease in GFR as RVR doubled and RBF fell markedly. Similar deleterious effects of PG inhibition on RBF and GFR have also been reported in other states associated with high urinary PG production (35–38).

The plasma renin activity of the chronic hypercalcemic rats was not significantly different from normocalcemic controls. Despite this fact, our experiments reveal that the RAS plays a significant role in the impairment of RBF and GFR as the inhibition of either the synthesis of (captopril) or the action of (Sar^1-Ile^5-Gly^8 AII) lead to marked improvement of and in fact normalization of RBF and GFR. While calcium ion has been postulated to mediate the action of exogenous angiotensin on the vascular smooth muscle and glomerulus (17), such a consequence of endogenous AII inhibition on renal vascular tone or the glomerulus in hypercalcemia has not been previously described.

Although these studies reveal the deleterious effects of PG inhibition and the beneficial effects of AII inhibition on RBF and GFR in chronic hypercalcemia, they do not unveil the role of PG and AII in the control of RBF and GFR independent of each other. Thus, in the experiments using indometacin, AII was intact and in the experiments performed during AII inhibition the renal PG were intact. This led us to perform experiments in animals treated with both PG and AII inhibitors.

The role of AII in the control of RBF and GFR in chronic hypercalcemia is best revealed by comparing the RBF and GFR of PG-depleted hypercalcemic rats without (group 2) and with (group 4) inhibition of the RAS. As shown in the bottom of Tables II and III, AII inhibition in the PG-inhibited hypercalcemic rats resulted in a marked improvement in both RBF and GFR. It becomes apparent that a large component of the decrement in RBF and GFR observed in hypercalcemia during PG inhibition is due to the enhanced vasoconstrictive effect of AII. These comparisons, thus, unveil the very important vasoconstrictor effect of AII in hypercalcemia independent of PG. This vasoconstriction is observed despite the fact that plasma renin activity is not increased, reflecting the enhanced renal sensitivity to AII in the hypercalcemic state.

The role of PG is apparent in the comparison of RBF and GFR of AII-inhibited hypercalcemic rats in the presence (group 3) and absence (group 4) of PG (bottom of Tables II and III). The RBF and GFR of hypercalcemic AII-inhibited rats who have intact PG-synthetizing ability is significantly greater than that of AII-inhibited hypercalcemic rats who have no PG-synthetizing ability. In fact, the former group has renal hemodynamics and GFR that are indistinguishable from normocalcemic controls. These comparisons unveil the important vasodilating effect of PG in hypercalcemia independent of AII.

Finally, the comparison of the RBF and GFR of hypercalcemic rats with that of normocalcemic control rats in the absence of both PG and AII reveals the role of calcium per se, in the control of RBF and GFR. As shown by groups 4 A and 4 B in Tables II and III, hypercalcemic rats with AII and PG inhibition have significantly lower RBF and GFR than the normocalcemic rats with AII and PG inhibition, thus revealing the vasoconstrictor role of calcium in chronic hypercalcemia, independent of both AII and PG.

It is of note that in the development of hypercalcemia and particularly following the administration of indomethacin there was a marked decrease in filtration fraction reflecting a greater decrement in GFR than in RBF. It is attractive to postulate that this dissociation points to an effect on the ultrafiltration coefficient as was shown in the acute setting (13). It is thus possible that in hypercalcemia AII-mediated contraction of glomerular mesangial cells is accentuated (39), an effect that is even more marked when glomerular PG synthesis (40, 41) is inhibited.

The present study, therefore, clearly dissects the contributions of calcium, AII, and PG on RBF and GFR in the conscious rat with chronic hypercalcemia. The possibility that a decrease in extracellular fluid volume in hypercalcemic rats could account for the observed changes was considered. However, both plasma and blood volume were similar in hypercalcemic and control rats. The sensitivity of the method used could miss a difference in the 10% range, but as shown in a recent study from our laboratory (28), a larger change is readily demonstrable. In our measurements, in fact, the hypercalcemic rats had even slightly higher plasma volumes. Finally, the failure of captopril and Sar^1-Ile^5-Gly^8 AII to significantly lower MAP in hypercalcemic rats (Tables II and III) also

provides evidence against significant volume depletion. The plasma volume measurements are in concert with the very similar rate of sodium excretion measured in the course of an 8-d balance study of rats becoming hypercalcemic on vitamin D and their pair-fed controls (23) (2.34±0.06 μeq/d in rats developing hypercalcemia and 2.46±0.05 μeq/d in control rats). Our results are therefore not likely to be due to changes in extracellular fluid volume. Although we did not examine the role of other hormonal systems that affect renal hemodynamics, such as catecholamines and vasopressin, the fact that both GFR and RBF are completely normalized by inhibition of AII, provided PG synthesis is unperturbed, suggests that other hormonal systems probably do not play a central role in this state. However, in the absence of PG, a role for catecholamines and vasopressin cannot be totally excluded. Our observations demonstrate that hypercalcemia of 8-d duration causes hemodynamic changes of the kidney that are functional in nature, findings that contrast to the abnormality in renal concentrating ability, which is associated with structural renal changes. We conclude, therefore, that in the conscious rat with chronic hypercalcemia, RBF and GFR are controlled by an active interplay of the enhanced vasoconstrictive effect of AII, the enhanced synthesis and action of the vasodilatory renal PG and the direct vasoconstrictor effect of calcium, independent of either AII or PG. The sum total of these forces produces a modest but significant decrease in renal hemodynamics and GFR.

ACKNOWLEDGMENTS

The authors want to thank Dr. Stuart Linas for his assistance in the performance of the blood volume studies and Ms. Linda M. Benson for secretarial assistance.

This work was supported by a National Institutes of Health grant, AM 26469.

REFERENCES

1. Cohen, S. I., M. G. Fitzgerald, P. Fourman, W. J. Griffiths, and H. E. de Wardener. 1957. Polyuria in hyperparathyroidism. *Q. J. Med.* **26:** 423–431.
2. Poulou, P. P. 1957. The renal tubular reabsorption and urinary excretion of calcium by the dog. *J. Lab. Clin. Med.* **49:** 253–257.
3. Edvall, C. A. 1958. Renal function in hyperparathyroidism: a clinical study of 30 cases. *Acta Chir. Scand.* **229**(Suppl.): 1–56.
4. Epstein, F. H., D. Beck, F. A. Carone, H. Levitin, and A. Mannitius. 1959. Changes in renal concentrating ability produced by parathyroid extract. *J. Clin. Invest.* **38:** 1214–1221.
5. Carone, F. A., F. H. Epstein, D. Beck, and H. Levitin. 1960. The effects upon the kidney of transient hypercalcemia induced by parathyroid extract. *Am. J. Pathol.* **36:** 77–89.
6. Gill, J. R., Jr., and F. C. Bartter. 1961. On the impairment of renal concentrating ability in prolonged hypercalcemia and hypercalciuria in man. *J. Clin. Invest.* **40:** 716–722.
7. Bank, N., and H. S. Aynedjian. 1965. On the mechanism of hyposthenuria in hypercalcemia. *J. Clin. Invest.* **44:** 681–693.
8. Bennett, C. H. 1970. Urine concentrations and dilution in hypokalemic and hypercalcemic dogs. *J. Clin. Invest.* **49:** 1447–1457.
9. Brunette, M. G., J. Vary, and S. Carriere. 1974. Hyposthenuria in hypercalcemia. *Pfluegers Arch. Eur. Physiol.* **350:** 9–23.
10. Edwards, B. R., R. A. L. Sutton, and J. H. Dirks. 1974. Effect of calcium infusion on renal tubular reabsorption in the dog. *Am. J. Physiol.* **227:** 13–18.
11. Vanherweghem, J. L., J. Ducobu, A. D'Hollander, and C. Toussaint. 1976. Effects of hypercalcemia on water and sodium excretion by the isolated dog kidney. *Pfluegers Arch. Eur. Physiol.* **363:** 75–80.
12. Chomdej, B., P. D. Bell, and L. G. Navar. 1977. Renal hemodynamic and autoregulatory responses to acute hypercalcemia. *Am. J. Physiol.* **232:** F490–F496.
13. Humes, H. D., I. Ichikawa, J. L. Troy, and B. M. Brenner. 1978. Evidence for a parathyroid hormone-dependent influence of calcium on the glomerular ultrafiltration coefficient. *J. Clin. Invest.* **61:** 32–40.
14. Lins, L.-E. 1979. Renal function in hypercalcemia. *Acta Med. Scand.* (Suppl.): **632:** 1–46.
15. Okahara, T., Y. Abe, M. Imanishi, T. Yukimura, and K. Yamamoto. 1981. Effect of calcium on prostaglandin E_2 release in dogs. *Am. J. Physiol.* **241:** F77–F84.
16. Goldberg, J., G. Aisenbrey, M. Levi, T. Berl, and R. W. Schrier. 1981. Common pathway for vasoconstrictor properties of angiotensin II (AII), norepinephrine (NE), and vasopressin (VP). *Kidney Int.* **19:** 167*a*. (Abstr.)
17. Ichikawa, I., J. F. Miele, and B. M. Brenner. 1979. Reversal of renal cortical actions of angiotensin II by verapamil and manganese. *Kidney Int.* **16:** 137–147.
18. Paiva, A. C. M., T. B. Paiva, and M. E. Miyamoto. 1977. The role of calcium in the response of rabbit aorta to angiotensin. *Mayo Clin. Proc.* **52:** 427–429.
19. Frohlich, E. D., J. B. Scott, and F. J. Haddy. 1962. Effect of cations on resistance and responsiveness of renal and forelimb vascular beds. *Am. J. Physiol.* **203:** 583–587.
20. Zenser, T. V., and B. B. Davis. 1978. Effects of calcium on prostaglandin E_2 synthesis by rat inner medullary slices. *Am. J. Physiol.* **235:** F213–F218.
21. Zenser, T. V., C. A. Herman, and B. B. Davis. 1980. Effects of calcium and A23187 on renal inner medullary prostaglandin E_2 synthesis. *Am. J. Physiol.* **238:** E371–E376.
22. Craven, P. A., R. K. Studer, and F. R. DeRubertis. 1981. Renal inner medullary prostaglandin synthesis: a calcium-calmodulin-dependent process suppressed by urea. *J. Clin. Invest.* **68:** 722–732.
23. Levi, M., L. Peterson, and T. Berl. 1983. Mechanism of concentrating defect in chronic hypercalcemia: the role of polydipsia and prostaglandins. *Kidney Int.* **73:** 489–497.
24. Hsu, C. H., T. W. Kurtz, and T. P. Waldinger. 1977. Cardiac output and renal blood flow in glycerol-induced acute renal failure in the rat. *Circ. Res.* **40:** 178–182.
25. Linas, S. L., T. Berl, G. A. Aisenbrey, O. S. Better, and R. J. Anderson. 1980. The effect of anesthesia on hemodynamics and renal function in the rat. *Pfluegers Arch. Eur. Physiol.* **384:** 135–141.

26. Thiel, G., D. R. Wilson, M. L. Arce, and D. E. Oken. 1967. Glycerol-induced hemoglobinuric acute renal failure in the rat: II. The experimental model, predisposing factors, and pathophysiologic features. *Nephron.* **4:** 276–297.
27. Flamebaum, W., T. A. Kotchen, R. Nagle, and J. S. McNeil. 1973. Effect of potassium on the renin-angiotensin system and $HgCl_2$-induced acute renal failure. *Am. J. Physiol.* **224:** 305–311.
28. Linas, S. L., R. J. Anderson, S. J. Guggenheim, G. L. Robertson, and T. Berl. 1981. Role of vasopressin in impaired water excretion in conscious rats with experimental cirrhosis. *Kidney Int.* **20:** 173–180.
29. Dray, F., B. Charbonnel, and J. Maclouf. 1975. Radioimmunoassay of prostaglandins F, E_1, E_2 in human plasma. *Eur. J. Clin. Invest.* **5:** 311–318.
30. Stockigt, J. R., R. D. Collins, and E. P. Biglieri. 1971. Determination of plasma renin concentration by angiotensin I immunoassay. *Circ. Res.* **28,29**(Suppl. 2.): 175–191.
31. Sokal, R. R., and J. F. Rohlf. 1969. Biometry: The Principles and Practice of Statistics in Biological Research. W. H. Freeman & Co., publishers, San Francisco.
32. Aiken, J. W., and J. R. Vane. 1973. Intrarenal prostaglandin release attenuates the renal vasoconstrictor activity of angiotensin. *J. Pharmacol. Exp. Ther.* **184:** 678–687.
33. Baylis, C., and B. M. Brenner. 1978. Modulation by prostaglandin synthesis inhibitors of the action of exogenous angiotensin II on glomerular ultrafiltration in the rat. *Circ. Res.* **43:** 889–898.
34. Berl, T., M. Ellis, J. Goldberg, and M. Levi. 1981. Mechanism of hypertension in acute hypercalcemia. *Clin. Res.* **29:** 355*a*. (Abstr.)
35. Henrich, W. L., R. J. Anderson, A. S. Berns, K. M. McDonald, P. J. Paulsen, T. Berl, and R. W. Schrier. 1978. The role of renal nerves and prostaglandins in control of renal hemodynamics and plasma renin activity during hypotensive hemorrhage in the dog. *J. Clin. Invest.* **61:** 744–750.
36. Zipser, R. D., J. C. Hoefs, P. F. Speckart, P. K. Zia, and R. Horton. 1979. Prostaglandins: modulators of renal function and pressor resistance in chronic liver disease. *J. Clin. Endocrinol. Metab.* **48:** 895–900.
37. Oliver, J. A., R. R. Sciacca, J. Piuto, and P. J. Cannon. 1981. Participation of the prostaglandins in the control of renal blood flow during acute reduction of cardiac output in the dog. *J. Clin. Invest.* **67:** 229–237.
38. Arisz, L., A. J. M. Donker, J. R. H. Breutjens, and G. K. van der Hem. 1976. The effect of indomethacin on proteinuria and kidney function in the nephrotic syndrome. *Acta Med. Scand.* **199:** 121–125.
39. Foidart, J., J. Sraer, F. Delarue, P. Mahieu, and R. Ardaillou. 1980. Evidence for mesangial glomerular receptors for angiotensin II linked to mesangial cell contractility. *FEBS (Fed. Eur. Biol. Sci.) Lett.* **121:** 333–339.
40. Hassid, A., M. Konieczkowski, and M. J. Dunn. 1979. Prostaglandin synthesis in isolated rat kidney glomeruli. *Proc. Natl. Acad. Sci. USA.* **76:** 1155–1159.
41. Scharschmidt, L. A., M. J. Dunn, and A. Norris. 1983. Prostaglandins modulate angiotensin II stimulated glomerular contractility *Kidney Int.* **23:** 284*a*. (Abstr.)