Evidence That Prostacyclin Modulates the Vascular Actions of Calcium in Man

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Abstract

Increases in extracellular calcium (Ca^{++}) can alter vascular tone, and thus may result in increased blood pressure (Bp) and reduced renal blood flow (RBF). Ca^{++} can stimulate prostaglandin E_2 (PGE_2) and/or prostacyclin (PGI_2) release in vitro, which may modulate Ca^{++} vascular effects. However, in man, the effect of Ca^{++} on PG release is not known. To study this, 14 volunteers received low-dose (2 mg/kg Ca^{++} gluconate) or high-dose (8 mg/kg) Ca^{++} infusions. The low-dose Ca^{++} infusion did not alter systemic or renal hemodynamics, but selectively stimulated PGI_2, as reflected by the stable metabolite 6-keto-$PGF_1\alpha$ in urine (159±21–244±30 ng/g creatinine, $P < 0.02$). The same Ca^{++} infusion given during cyclooxygenase blockade with indomethacin or ibuprofen was not associated with a rise in PGI_2 and produced a rise in Bp and fall in RBF. However, sulindac, reported to be a weaker renal PG inhibitor, did not prevent the Ca^{++}-induced PGI_2 stimulation (129±33–283±90, $P < 0.02$), and RBF was maintained despite similar increases in Bp. The high-dose Ca^{++} infusion produced an increase in mean Bp without a change in cardiac output, and stimulated urinary 6-keto-$PGF_1\alpha$ to values greater than that produced by the 2-mg/kg Ca^{++} dose (330±45 vs. 244±30, $P < 0.05$). In contrast, urinary PGE_2 levels did not change. A Ca^{++} blocker, nifedipine, alone had no effect on Bp or urinary 6-keto-$PGF_1\alpha$ levels, but completely prevented the Ca^{++}-induced rise in Bp and 6-keto-$PGF_1\alpha$ excretion (158±30 vs. 182±38, $P > 0.2$). However, the rise in 6-keto-$PGF_1\alpha$ was not altered by the α_1 antagonist prazosin (159±21–258±23, $P < 0.02$), suggesting that calcium entry and not α_1 receptor activation mediates Ca^{++} pressor and PGI_2 stimulatory effects. These data indicate a new vascular regulatory system in which PGI_2 modulates the systemic and renal vascular actions of calcium in man.

Introduction

Acute and chronic hypercalcemia may increase blood pressure (Bp),[1] and pharmacological blockade of calcium entry into vascular smooth muscle can improve many forms of hypertension (1–4). Transcellular calcium flux also functions as the common in vivo pathway for vasoconstriction induced by arginine vasopressin (AVP), angiotensin II, and norepinephrine (5). Studies in man and experimental animals indicate that a rise in extracellular calcium increases renal vascular resistance, reduces renal blood flow (RBF), and impairs renal function (6–8). Although there is evidence suggesting that calcium can directly produce smooth muscle constriction (9), other studies indicate a more complex interaction with the renin-angiotensin system, catecholamines, and prostaglandins (PG) (10–12).

It has been suggested that vasodilatory PG, PGE_2, and prostacyclin (PGI_2) may be protective modulators of systemic and RBF. This is supported by experiments showing decrements in renal function after cyclooxygenase inhibition during states of ischaemia, hypovolemia, and sodium depletion (13–15).

Calcium can directly activate membrane phospholipases to release arachidonic acid from membrane phospholipids, which then can be converted into products including PGE_2 and/or PGI_2. This is supported by in vitro data suggesting that the ionophore A23187, in the presence of calcium, stimulates PGE_2 synthesis in the renal medulla and PGI_2 production in endothelial cells (16, 17). However, the interaction of calcium and PG in man has not been previously investigated.

The present study was designed to investigate (*a*) the effect of mild and moderate hypercalcemia on vasodilatory PG release; (*b*) whether PGE_2 and/or PGI_2 function as negative modulators of calcium's vasocontrictive actions; (*c*) the role of transcellular calcium flux for the hemodynamic and PG changes induced by calcium; and (*d*) since previous studies in man indicate that α_1 adrenergic activation is a potent stimulus for PGE_2 and PGI_2 release (18), the effect of α_1 blockade on the hemodynamic and PG effects of calcium. Our study suggests that PGI_2 is a modulator of the vascular effects of calcium.

Methods

Subjects. 14 healthy volunteers were studied at the LAC/USC Clinical Research Center after 5 d equilibration on a 80-meq Na^+, 60-meq K^+ diet. All patients gave informed consent and the protocol was approved by our Internal Review Board. Their ages ranged from 20 to 46. Eight men and six women were studied. All protocols were performed in the afternoon while the subjects were supine to minimize the effects of posture and diurnal variation (19).

Effect of calcium infusion on systemic and renal hemodynamics. To assess the effect of calcium on the systemic and renal circulation, low- and high-dose calcium infusions were administered over 3 h via constant intravenous infusion (IMED Pump; IMED Corp., San Diego, CA) at 2 mg/kg (n = 14), and 8 mg/kg calcium gluconate (n = 8). Bp and pulse rate were measured before and every 30 min during infusion using an automated Bp monitoring system (Dinamap Critikon, Inc., Dublin, CA). Cardiac output (CO) was determined noninvasively via a doppler computer technique (20) during some of the low- and high-dose calcium infusions. This doppler method has been validated via comparison with standard thermodilution methods. RBF was measured using para-aminohippurate (PAH) clearance normalized to 1.73 m^2 body surface area.

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1. *Abbreviations used in this paper:* AVP, arginine vasopressin; Bp, blood pressure; CO, cardiac output; GC, gas chromatography; HR, heart rate; MAP, mean arterial pressure; PAH, para-aminohippurate; PG, prostaglandin(s); PGB_2, PGD_2, PGE_1, $PGF_1\alpha$, and PGF_2, prostaglandins B_2, D_2, E_1, E_2, $F_1\alpha$, and F_2; PGI_2, prostacyclin; RBF, renal blood flow.

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PAH was given at a loading dose of 4 mg/kg and then maintained at a constant infusion. Four baseline plasma samples for PAH were obtained at 5-min intervals after a 2-h equilibration period. Plasma was subsequently obtained at 30-min intervals during the calcium infusions. PAH was measured via a standard spectrophotometric method (21). RBF was calculated as: (effective renal plasma flow)/1 − hematocrit). Serum was obtained for total and ionized calcium before and at hourly intervals during infusions (22).

Effect of PG inhibition on systemic and renal hemodynamics. To evaluate the role of vasodilatory PG in the control of systemic and renal vascular tone during mild and moderate hypercalcemia, cyclooxygenase inhibitors, indomethacin (50 mg) (Indocin; Merck Sharpe & Dohme, West Point, PA) or ibuprofen (600 mg) (Motrin; Upjohn Co., Kalamazoo, MI) were administered orally every 8 h for 2 d before the low-dose calcium infusions (n = 14). Another cyclooxygenase inhibitor, sulindac (200 mg) (Clinoril; Merck Sharp & Dohme), was also given orally twice a day for 3 d to some subjects (n = 7) before the low-dose calcium infusions.

Effect of calcium infusions on urinary PG excretion. To assess basal urinary PG levels, 3-h urines (1300–1600 h) were collected on two separate days during intake of 150 ml of water orally or dextrose in water intravenous infusions. The dose effect of calcium on urinary PG excretion was assessed by collecting 3-h urines during both low- (n = 14) and high (n = 8)-dose calcium infusions. Urine was collected in glass bottles, the volume measured, and aliquots were immediately frozen at −30°C for PGE_2 and 6-keto-$PGF_1\alpha$ assay. Urine samples were also analyzed for sodium, potassium, and creatinine by standard methods.

Effect of calcium channel blockade on calcium-induced Bp and PG changes. Nifedipine (Procardia; Pfizer Inc., New York, NY), a slow calcium channel antagonist (23), was given at a dosage of 20 mg sublingually 30 min before the high-dose calcium infusion (n = 8). The contents of two standard 10-mg nifedipine capsules were punctured and contents placed sublingually. 3-h urines were collected during calcium blockade alone and, on separate days, with the combination of the calcium antagonist and high calcium infusion.

Role of alpha adrenergic activation in calcium-induced hemodynamic and PG changes. Evidence from our lab suggests that catecholamines via α_1 activation stimulate renal PGE_2 and PGI_2 in man (18); therefore, the effect of α_1 adrenergic blockade on calcium-induced hemodynamic and PG alterations was evaluated. The selective α_1 adrenergic antagonist prazosin (5 mg) (24) (Minipress; Pfizer Inc.) was given orally 1 h before the high calcium infusions (n = 6). This dosage was previously shown to completely block the pressor and PGI_2 stimulatory effects of norepinephrine infusion (18). Systemic Bp and pulse rate were determined before prazosin administration and at 30-min intervals thereafter until completion of calcium infusion.

Radioimmunoassay (RIA) of PGE_2 and 6-keto-$PGF_1\alpha$ in urine. PGE_2 was measured by a previously described method (25) using specific antisera raised in our lab, ethyl acetate extraction, and LH-20 Sephadex chromatography. Antibody cross-reactivity is PGE_2, 100%, PGD_2, 4%, $PGF_1\alpha$, 1%, and 6-keto-$PGF_1\alpha$, PGB_2, 13,14-dihydro PGE_2, and 13,14-dihydro-15-keto-PGE_2, all <0.1%. Tracer recovery averages 65%, interassay variation 10%, and water blanks carried through the entire method average 3 pg. The sensitivity is 10 pg/sample and 50% displacement occurs at 30 pg. RIA validation procedures included assay of serially diluted urine (r = 0.94) and comparison with results using the PGE_2 antisera from the Institute Pasteur, Paris, France. Comparison revealed a correlation (r = 0.98) with slope of 0.93 (25).

6-keto-$PGF_1\alpha$ was also measured via our published RIA (26). Urine samples after authentic [^{3}H]6-keto-$PGF_1\alpha$ is added (New England Nuclear, Boston, MA) are brought to pH 3.5 with 1 N HCl, and extracted with ethyl acetate. The dried extract is chromatographed on Sephadex LH-20 columns (0.5 × 80 cm) using the solvent system dichloromethane/methanol (95:5). This system completely separates the 2,3-dinor 6-keto-$PGF_1\alpha$ metabolite. Antisera was generated in our lab in rabbits by injection of 6-keto-$PGF_1\alpha$ linked to bovine thyroglobulin using the carbiodiimide reaction in a manner described previously for PGE_2 (25). The antibody has a working titer of 10^3 dilution with 60–70% bound at Bo (zero standard added) and 50% displacement at 50 pg. Nonspecific binding is <5%. Crossreactivity is: 6-keto-$PGF_1\alpha$, 100%, 2,3-dinor 6-keto-$PGF_1\alpha$, 25%, PGF_2, 2%, and PGE_2, PGE_1, PGD_2, 13,14-dihydro 6,15-diketo $PGF_1\alpha$, 13,14-dihydro-15-keto 2,3-dinor-6-keto-$PGF_1\alpha$, all <0.1%. Recovery of added indicator averages 65%. The bound from free PG is separated using a second antibody technique. The method blank carried through the entire method is 4±2 (SD) pg. Intraassay variation is 5% and interassay variability is 12%. Hourly excretion values are similar to other values reported in the literature. Standard validation procedures have been performed including assay of serially diluted urine (r = 0.98) and addition of known amounts of cold standard to urine (r = 0.98). Values are unaltered by further chromatography (thin-layer chromatography or reverse-phase high performance liquid chromatography). Further validation was recently completed in collaboration with Dr. J. Vrbanac at the Mass Spectrometry Clinical Research Resource (CLINSPEC) Laboratory at the Department of Pharmacology, Medical University of South Carolina. Two gas chromatography mass spectrometric methods were used. Initially, we utilized a t-butyl dimethyl silyl ether derivative of 6-keto-$PGF_1\alpha$ (27) using a Hewlett-Packard HP5970A Mass Selective Dector. A 30-m DB-5 bonded phase-fused silica capillary column (J & W Scientific, 0.25 mm i.d., 0.25 μm film thickness) was interfaced directly with the ion source. Conditions of analysis were: electron impact at 70 eV; injection port temperature of 290°C; splitless injection; U = 35 cm/s; injection port surge after 3 min; column temperature during injection of 200°C; initial rapid ramp to 270°C, then programming at 3°C/min to 310°C. The M^+-57 ion was used for quantitation (698.5 protium, 702.5 deuterium). Deuterated 6-keto-$PGF_1\alpha$, used as internal standard, was kindly provided by Dr. John Pike (Upjohn Co.). Two gas chromatography (GC) peaks representing the syn- and antistereoismers for the methyl ester-methoxine t-butyl dimethyl ether derivative of 6-keto-$PGF_1\alpha$ eluted between 14.6 and 14.9 min.

For increased sensitivity, a negative ion chemical ionization procedure was utilized using a Finnigan 3200 GC/mass spectrometer modified for negative ion capabilities (28). The methoxine-pentafluorobenzyl-trimethyl silyl derivative of 6-keto-$PGF_1\alpha$ was prepared using the method of Blair (29). For GC a 30-min DB-I bonded phase-fused silica capillary column was directly interfaced with the ion source. Conditions of analysis were as follows: negative ion chemical ionization using methane as the reagent gas; 80 eV; on-column injection at 190°C; and temperature programming from 270° to 300°C at 4°C/min. Fragment ions at 618.5 ($^2H_4$) and 614.5 (^1H) were monitored [M-$C_7H_2F_5$].

The RIA gave a good correlation with the GC/mass spectrometric values obtained (r = 0.80).

Statistical analysis. All data was analyzed using a CLINFO computer. Paired student's t test was used to compare results. Each subject was used as his or her own control. All results are reported as the mean±SE. All urinary PG values are expressed in units of nanograms per gram of creatinine.

Results

Effects of low-dose calcium infusion on systemic and renal hemodynamics. The effect of the 2-mg/kg dose calcium infusion on systemic and renal hemodynamics is shown on Table I. This dose of calcium did not alter Bp, heart rate (HR), CO, or RBF. Mild hypercalcemia was produced with this dosage and ionized calcium concentration increased slightly (5.00±0.06–5.44±0.10 mg/dl, $P < 0.02$), while total serum calcium levels remained unchanged. The infusion did not produce changes in urinary potassium, creatinine, or volume (Table II). However, urinary sodium excretion increased (10.4±3.4–21.9±7.8 meq/liter, $P < 0.05$).

In marked contrast to the lack of hemodynamic effects of this calcium infusion, the same dose of calcium during cyclooxygenase inhibition produced a significant increase in systemic and renal vascular tone (Fig. 1). The calcium infusion with in-

Table I. Effect of Low-dose Calcium Infusion on Systemic and Renal Hemodynamics

	T Ca	I Ca	Systolic Bp	Diastolic Bp	MAP	CO	HR	RBF (ml/min/1.73 m²)
	mg/dl	*mg/dl*	*mmHg*	*mmHg*	*mmHg*	*liter/min*	*beats/min*	
Control	9.1±0.2	5.00±0.06	117±5	76±4	85±2	6.5±0.4	68±4	1,210±100
Calcium infusion	9.3±0.2	5.44±0.10*	115±5	77±2	86±2	6.9±0.6	66±3	1,180±80

T Ca, total serum calcium; I Ca, ionized calcium. * $P < 0.02$.

domethacin or ibuprofen produced a significant increase in mean arterial pressure (MAP) (14±3 mmHg, $P < 0.01$) and a 28% fall in RBF (1,210±100–860±60 ml/min per 1.73 m², $P < 0.01$) (Fig. 1). Similarly, another cyclooxygenase inhibitor, sulindac, produced an increase in MAP during the low-dose calcium infusion (Fig. 1). However, sulindac pretreatment did not alter RBF (1,160±42 vs. 1,180±80, $P > 0.3$). Cyclooxygenase inhibitors given alone did not alter basal MAP or RBF (84±3 mmHg and 1,190±70 ml/min per 1.73 m², respectively, both $P > 0.3$).

Effect of the low-dose calcium on PG release. The effect of the low-dose calcium infusion on vasodilatory PG excretion is shown in Fig. 2. The 2-mg/kg calcium dose significantly increased urinary 6-keto-$PGF_1\alpha$ release (159±21–244±30 ng/g creatinine, $P < 0.02$). In contrast, urinary PGE_2 excretion decreased. Indomethacin administration completely prevented the calcium-induced rise in 6-keto-$PGF_1\alpha$ excretion and resulted in levels below the control (102±18 ng/g creatinine). However, sulindac did not alter basal 6-keto-$PGF_1\alpha$ (130±33 vs. 159±21, $P > 0.3$) or the calcium-induced stimulation of PGI_2 (129±33–283±90, $P < 0.02$), Fig. 3.

Effect of high-dose calcium infusion on Bp and vasodilatory PG release. The 8-mg/kg calcium infusion increased MAP (87±3–108±4 mmHg, $P < 0.01$) and total serum calcium (9.2±0.1–12.4±0.4 mg/dl, $P < 0.001$). However, CO was not altered (6.9±0.6 vs. 6.5±0.4 liter/min, $P > 0.3$), suggesting MAP rose due to increases in peripheral vascular resistance. Urinary K^+ and creatinine did not change, but Na^+ excretion and volume increased (Table II).

Fig. 4 shows the effects of the high-dose calcium infusion on urinary PG excretion. PGE_2 levels were not altered by the calcium infusion. In contrast, urinary 6-keto-$PGF_1\alpha$ excretion was markedly stimulated and levels were greater than that produced by the low calcium infusion (330±45 vs. 244±30 ng/g creatinine, $P < 0.05$), suggesting a dose-response effect. The subsequent studies were designed to investigate the mechanism of this calcium-induced stimulation of PGI_2.

Effect of calcium channel inhibition and alpha adrenergic blockade on calcium-mediated PGI_2 release. Nifedipine completely prevented the rise in MAP produced by the high calcium infusion (87±2 vs. 82±2 mmHg, P = NS). The high-dose calcium infusion increased urinary Na^+ and volume, but these changes were significantly blocked by nifedipine (Table II). No changes in urinary K^+ or creatinine were observed during these maneuvers. The calcium blocker prevented the calcium-induced rise in 6-keto-$PGF_1\alpha$ and produced levels not significantly different than control (170±24 vs. 210±46 ng/g creatinine) (Fig. 5). Nifedipine given alone on a separate day to five subjects did not alter basal MAP (82±4 mmHg) or 6-keto-$PGF_1\alpha$ excretion (158±30 vs. 182±38).

Prazosin given before the calcium infusion resulted in urinary Na^+, K^+, creatinine, and volume that were similar to levels obtained during calcium channel blockade with nifedipine (Table

Table II. Effect of Calcium Infusions Alone or with Antagonists on Urinary Na^+, K^+, Creatinine, and Volume

Infusion	Na (meq/3 h)	K^+ (meq/3 h)	Creatinine (mg/3 h)	Volume (mg/3 h)
Control (n = 14)	10.4±3.4	17.2±2.8	293±31	402±111
Low dose Ca^{2+} (n = 14)	21.9±7.8*	16.3±1.7	269±26	425±114
Low dose Ca^{2+} + indomethacin (n = 14)	16.4±6.1	13.5±4.6	243±39	596±198
High dose Ca^{2+} (n = 8)	45.7±11.3‡	17.8±3.4	299±39	652±104*
High dose Ca^{2+} + nifedipine (n = 8)	21.5±5.1§	13.6±2.8	263±33	522±176
High dose Ca^{2+} + prazosin (n = 6)	20.7±14.3§	13.6±1.0	393±120	599±185

* $P < 0.05$ vs. control.
‡ $P < 0.01$ vs. control.
§ $P < 0.05$ vs. high Ca^{2+}.

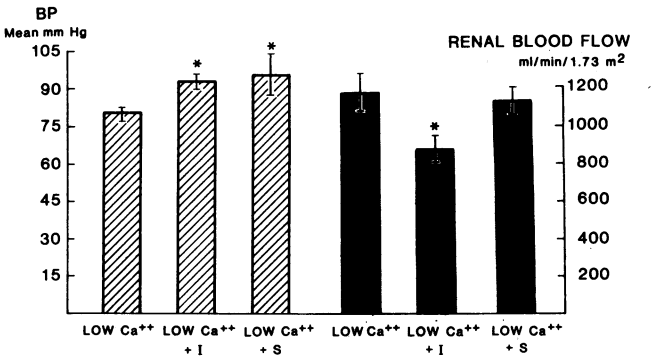

Figure 1. Effect of low dose calcium administration alone or with cyclooxygenase inhibitors on mean Bp (▨) and RBF (■). Bars represent mean±SEM. Low Ca alone (n = 14). I, indomethacin (n = 8) and ibuprofen (n = 6). S, sulindac (n = 7). *$P < 0.01$ (low Ca^{++} + inhibitor vs. low Ca^{++} alone).

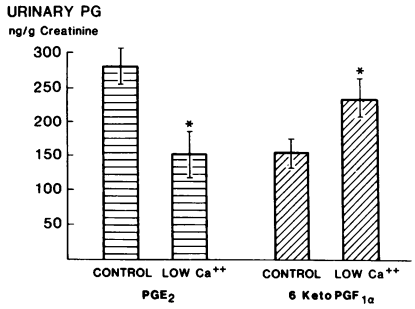

Figure 2. Effect of low dose calcium infusion on urinary PGE_2 (■) and 6-keto-$PGF_{1}\alpha$ (▬) excretion. Mean±SEM values are shown. $n = 14$, $P < 0.02$ (low Ca^{++} vs. control).

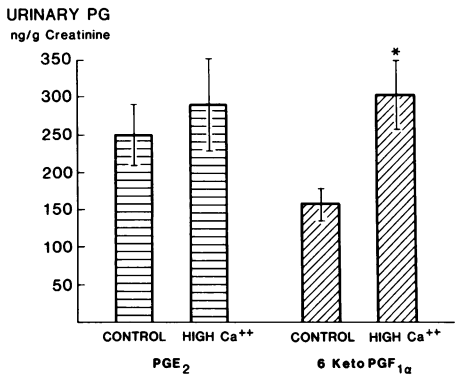

Figure 4. Effect of high dose calcium infusion on urinary PGE_2 (■) and 6-keto-$PGF_{1}\alpha$ (▬) excretion. $n = 8$, $^*P < 0.01$ (high Ca^{++} vs. control).

II). However, prazosin did not prevent the pressor effect of the calcium infusion (88±3–102±4 mmHg, $P < 0.02$), and did not alter the high calcium-induced rise in 6-keto-$PGF_{1}\alpha$ (159±21–258±23, $P < 0.02$) (Fig. 5).

Discussion

Considerable evidence suggests that vasodilatory PG play a key role as protective modulators of systemic and RBF during states of increased pressor activity (30–33). Studies in animals and man indicate that angiotensin II and norepinephrine stimulate PGE_2 and/or PGI_2 release (18, 26, 34–36), while cyclooxygenase blockade produces decrements in renal function during states of ischemia, heart failure, or sodium depletion (14, 15, 37, 38). Inhibition of renal PG synthesis results in unopposed renal vasoconstriction, which reduces blood flow and glomerular filtration rate.

Many reports suggest that an increase in extracellular calcium can alter systemic and renal vascular tone, thus increasing Bp and reducing RBF (1–4, 6–8, 10). The mechanisms of calcium-induced vascular effects are complex, since calcium can directly produce vascular smooth muscle constriction (9) or mediate the pressor actions of AVP and angiotensin II (5, 39). Other studies suggest a major role for catecholamines since calcium can stimulate norepinephrine release and mediate α_1 adrenergic receptor activity (4, 40–42).

The present results indicate that PGI_2 is a potent modulator of the systemic and renal vascular actions of calcium. The low-dose calcium infusion given alone did not alter systemic or renal vascular hemodynamics. In contrast, the same calcium infusion given during indomethacin or ibuprofen administration produced a significant increase in Bp and reduction in RBF. Both cyclooxygenase inhibitors totally prevented the calcium induced rise in 6-keto-$PGF_{1}\alpha$, suggesting that PGI_2 attenuates the vasoconstrictive effects of calcium.

Sulindac has been reported to differ from other cyclooxygenase inhibitors by sparing renal, but inhibiting systemic PG, as reflected by a lack of effect on urinary 6-keto-$PGF_{1}\alpha$ and PGE_2 excretion, while lowering systemic PG, as reflected by reduced platelet thromboxane formation (43, 44). However, a recent study in healthy men showed that although basal PGE_2 or 6-keto-$PGF_{1}\alpha$ were not altered, furosemide-stimulated PGE_2, but not 6-keto-$PGF_{1}\alpha$ release, was reduced by sulindac (45). In addition, other evidence in dogs indicates that the active sulfide form of sulindac when delivered in high concentrations into the renal artery inhibits renal PG production (46). Therefore, sulindac may partially spare renal PG synthesis due to the reduced levels of active drug that reach the site of renal PG synthesis.

In our study, the results obtained with sulindac were different than with indomethacin or ibuprofen. The infusion of calcium with sulindac did not change RBF despite similar rises in systemic Bp obtained with the other cyclooxygenase inhibitors. In addition, sulindac did not alter either basal or calcium-stimulated levels of urinary 6-keto-$PGF_{1}\alpha$. These results suggest that sulindac may partially spare renal PGI_2 production, and that urinary 6-keto-$PGF_{1}\alpha$ primarily reflects renal PGI_2 synthesis, a conclusion that is consistent with reports by others (47, 48).

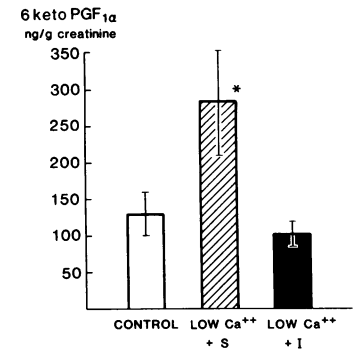

Figure 3. Effect of sulindac (S) and indomethacin (I) on calcium-stimulated 6-keto-$PGF_{1}\alpha$ release. Bars represent mean±SEM values. $n = 7$ for S, and $n = 18$ for I. $^*P < 0.01$ (low Ca^{++} + S vs. control).

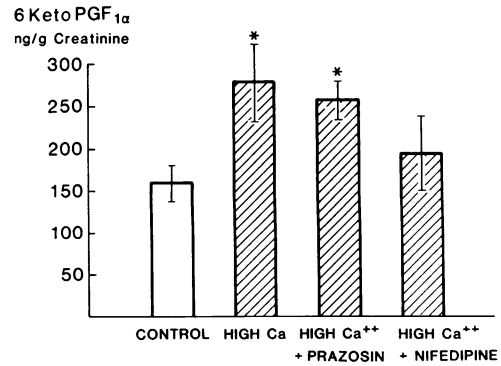

Figure 5. Effect of prazosin and nifedipine on high dose calcium-stimulated 6-keto-$PGF_{1}\alpha$ release. $n = 8$ for high Ca alone and high Ca + nifedipine. $n = 6$ for prazosin. $^*P < 0.01$.

The low-dose calcium infusion produced no rise in total calcium and only minimal increases in ionized calcium concentration. However, even this slight calcium increase stimulated PGI_2 synthesis, as reflected by an increase in 6-keto-$PGF_1\alpha$ excretion. This suggests that PGI_2 production is very sensitive to even small changes in extracellular calcium concentration.

Previous evidence in vitro suggests that calcium plays a key role in PG biosynthesis. The divalent cation ionophore A23187 in the presence of calcium stimulates PGE_2 synthesis in renal medullary tissue (16). More recent evidence indicates that the mechanism of calcium-induced PGE_2 synthesis is via phospholipase A_2 activation through a calmodulin-dependent mechanism (49, 50). A23187 in the presence of calcium also stimulates PGI_2 synthesis in endothelial cells and vascular smooth muscle in culture (17, 51, 52).

The source of the calcium needed for vasodilatory PG biosynthesis is not known. However, recent evidence indicates that PGI_2 and PGE_2 may respond to different cellular pools of calcium. Extracellular calcium is particularly important for PGI_2 production in vascular tissue. Increases in extracellular calcium directly stimulates PGI_2 synthesis in endothelial cells through a mechanism involving calcium entry (53). The resulting increase in intracellular calcium activates phospholipase A_2 via calmodulin. In contrast, other evidence suggests a key role for intracellular calcium for PGE_2 release in renal tissue. The ionophore A23187-mediated PGE_2 release is via an increase in intracellular calcium, since changes in extracellular calcium concentration alone do not alter PGE_2 release in renal medullary tissue (16, 54). In cultured medullary collecting tubules, basal PGE_2 synthesis requires the presence of extracellular calcium, but increasing extracellular calcium from 1.0 to 4.0 mM does not further alter PGE_2 release (54, 55). Therefore, changes in extracellular calcium in vivo may selectively alter renal PGI_2 synthesis.

The higher dose calcium infusion produced marked hypercalcemia and a clear pressor response. The mechanism of the Bp increase was through a change in peripheral vascular resistance, since CO did not increase. Some studies suggest that calcium can stimulate catecholamine release, which can then produce pressor effects through α_1 receptor activation (40, 42). To evaluate this potential interaction, the selective α_1 antagonist prazosin was administered before some high-dose calcium infusions. The results of these studies suggest that the pressor action of increased extracellular calcium is not secondary to catecholamine release and α_1 adrenergic activation, since prazosin did not alter the pressor response. In contrast, nifedipine completely prevented the calcium-induced Bp increase, suggesting that direct calcium entry is a major mechanism of altered vasomotor tone.

In comparison with the low dose of calcium, the higher dose infusion produced a greater rise in urinary 6-keto-$PGF_1\alpha$ excretion. These results suggest that the calcium stimulation of PGI_2 release is dose related.

In addition to its pressor actions, norepinephrine stimulates PGI_2 synthesis in vitro and in man via α_1 adrenergic receptor activation (18, 36). We therefore evaluated the effect of prazosin on the calcium-induced urinary 6-keto-$PGF_1\alpha$ increase. Prazosin did not alter the calcium stimulated of PGI_2 excretion, suggesting that this response was not via α_1 receptor activation. The dosage of prazosin used was previously shown to completely prevent norepinephrine-induced pressor and PGI_2 stimulatory effects (18). Therefore, if the calcium effect was secondary to catecholamine release, it would have been blocked by prazosin.

Nifedipine given alone did not alter urinary 6-keto-$PGF_1\alpha$ excretion. However, the calcium antagonist markedly blunted the calcium-induced PGI_2 release. These results indicate that increasing extracellular calcium concentration stimulates PGI_2 release through a mechanism involving calcium entry that is sensitive to calcium antagonists. This is the first evidence in man indicating the critical role of extracellular calcium and its entry for PGI_2 synthesis.

The low and higher dose calcium infusions failed to stimulate urinary PGE_2 excretion despite marked increases in 6-keto-$PGF_1\alpha$. The precise explanation for this selective action of extracellular calcium to stimulate only PGI_2 is not totally clear from this study. One explanation is that PGE_2 synthesis may be less sensitive to acute changes in extracellular calcium (54, 55). In addition, recent evidence in vitro suggests that PGE_2 synthesis produced by hormones such as angiotensin II is linked to specific pools of hormonally sensitive calcium that activate phospholipase A_2 (56). Other reports in the isolated rat kidney indicate that omission of calcium or addition of calcium blockers to the perfusion medium, which attenuate the effect of AVP to produce renal vasoconstriction, fail to alter the increase in renal PG elicited by the peptide (57). This evidence suggests that AVP-induced renal PG synthesis does not require extracellular calcium. Therefore, the selectivity in renal PG synthesis can be due to different local cellular responses, depending upon the pool of calcium involved in phospholipase A_2 activation and PG synthesis. Alternatively, the phospholipases may reside at different subcellular sites, making them more or less responsive to hormonal and/or calcium effects. The present results suggest that the arachidonic acid released in response to changes in extracellular calcium is primarily converted into PGI_2, resulting in unaltered or reduced levels of PGE_2.

The measurement of another PGI_2 metabolite in urine, 2,3 dinor 6-keto-$PGF_1\alpha$ has been reported to represent an index of systemic vascular PGI_2 production (58). Although an additional study measuring the dinor metabolite could further test our contention that Ca^{++} stimulates both renal and extrarenal PGI_2, we were unable to use this technique in the current investigation. Since urinary 6-keto-$PGF_1\alpha$ primarily reflects renal PGI_2 production, our conclusion that Ca^{++} stimulates renal and extrarenal PGI_2 synthesis is based upon indirect evidence. We have reviewed the extensive literature showing in vitro that Ca^{++} alters systemic vascular tone and PGI_2 synthesis. In addition, the current results reveal marked changes in systemic and renal hemodynamics during the low-dose Ca^{++} infusion, with general PG inhibition and only systemic changes with the more selective extrarenal cyclooxygenase inhibitors.

In summary, this study indicates a new vascular regulatory system in which PGI_2 plays a key role in modulating the systemic and renal vascular actions of calcium. PGI_2 synthesis is highly sensitive to changes in extracellular calcium and variations in calcium entry that provide support for its physiologic role in man.

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