Modulating role for thromboxane in the tubuloglomerular feedback response in the rat.

W J Welch, C S Wilcox

J Clin Invest. 1988;81(6):1843-1849. https://doi.org/10.1172/JCI113529.

Research Article

Some studies have indicated that PGs can modulate the single nephron tubuloglomerular feedback (TGF) response. The aim of this study was to define the specific role of the vasoconstrictor PG, TX, by administration to rats of either vehicle (group 1; n = 20) or drugs that inhibit either cyclooxygenase (indomethacin [indo], 5 mg.kg-1, group 2, n = 17), TX synthetase (UK-38,485 [UK], 100 mg.kg-1, group 3, n = 19), or TX receptors (SQ-29,548 [SQ], 8 mg.kg-1, group 4, n = 14, or L-641,953 [L], 50 mg.kg-1, group 5, n = 8). Indo reduced excretion of the prostacyclin derivative 6-keto-PGF1 alpha and TXB2 and lowered whole kidney GFR and renal plasma flow, whereas UK lowered excretion of TXB2 only and did not change basal renal hemodynamics. The TGF response (assessed from reduction in proximal tubule stop-flow pressure (Psf, mmHg) during increases in perfusion of the loop of Henle (LH) from 0 to 40 nl.min-1) was unchanged after vehicle (9.8 +/- 0.5-10.9 +/- 1.0, NS) but blunted (P less than 0.001) by 40-65% in rats of groups 2-5 (indo, 11.1 +/- 1.0-4.4 +/- 0.7; UK, 9.0 +/- 0.8-4.8 +/- 0.7; SQ, 10.3 +/- 0.6-4.8 +/- 0.6; L, 10.7 +/- 0.5-6.7 +/- 1.3). This blunting was due to lower values for Psf at zero LH flow after indo, SQ, and L, and higher […]

Modulating Role for Thromboxane in the Tubuloglomerular Feedback Response in the Rat

William J. Welch and Christopher S. Wilcox

Division of Nephrology, Hypertension and Transplantation, Departments of Medicine, Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Florida 32610; and Veterans Administration Medical Center, Gainesville, Florida 32602

Abstract

Some studies have indicated that PGs can modulate the single nephron tubuloglomerular feedback (TGF) response. The aim of this study was to define the specific role of the vasoconstrictor PG, TX, by administration to rats of either vehicle (group 1; n = 20) or drugs that inhibit either cyclooxygenase (indomethacin [indo], 5 mg·kg^{-1}, group 2, n = 17), TX synthetase (UK-38,485 [UK], 100 mg·kg^{-1}, group 3, n = 19), or TX receptors (SQ-29,548 [SQ], 8 mg·kg^{-1}, group 4, n = 14, or L-641,953 [L], 50 mg·kg^{-1}, group 5, n = 8). Indo reduced excretion of the prostacyclin derivative 6-keto-PGF$_{1\alpha}$ and TXB$_2$ and lowered whole kidney GFR and renal plasma flow, whereas UK lowered excretion of TXB$_2$ only and did not change basal renal hemodynamics. The TGF response (assessed from reduction in proximal tubule stop-flow pressure (P$_{sf}$, mmHg) during increases in perfusion of the loop of Henle (LH) from 0 to 40 nl·min^{-1}) was unchanged after vehicle (9.8±0.5–10.9±1.0, NS) but blunted ($P < 0.001$) by 40–65% in rats of groups 2–5 (indo, 11.1±1.0–4.4±0.7; UK, 9.0±0.8–4.8±0.7; SQ, 10.3±0.6–4.8±0.6; L, 10.7±0.5–6.7±1.3). This blunting was due to lower values for P$_{sf}$ at zero LH flow after indo, SQ, and L, and higher values of P$_{sf}$ at 40 nl·min^{-1} LH flow after indo and UK. The fall in single nephron GFR (SNGFR, nl·min^{-1}) with increasing LH perfusion was unchanged after vehicle (10.9±2.8–11.2±0.8) but was blunted ($P < 0.05$) by 45–55% in rats given indo (13.9±1.2–6.2±2.2) or UK (12.8±2.1–7.0±1.5). UK produced dose-dependent reductions in TXB$_2$ excretion (IC$_{50}$, 15 mg·kg^{-1}) and inhibition of the TGF response (IC$_{50}$: 30 mg·kg^{-1}). After blockade of TX receptors by SQ, UK had no further affect on the TGF response. The fall in P$_{sf}$ at high LH flow was blunted ($P < 0.05$) by indo and UK, whereas the rise in P$_{sf}$ at zero LH flow was blunted by indo, SQ, and L. In conclusion, endogenous TX generation can modulate the reductions in P$_{sf}$ and SNGFR during increased delivery of NaCl to the LH.

Introduction

The tubuloglomerular feedback (TGF)[1] response is an important intrarenal process regulating GFR. The response is initiated by an increased delivery of sodium and chloride to the macula densa segment (1). The resulting changes in GFR are due to changes in plasma flow rate and glomerular capillary hydrostatic pressure, and perhaps also to changes in the glomerular capillary filtration coefficient (2). The mediators of these hemodynamic adjustments are not fully understood. Thus, the TGF response is blunted, but not abolished, by the blockade of either angiotensin-converting enzyme with captopril or angiotensin II (AII) receptors with saralasin (3). Likewise, the response is blunted, but not abolished, by blockade of cyclooxygenase with indomethacin (indo) or meclofenamate (4–6). Simultaneous blockade of AII generation and PG production with captopril and meclofenamate abolishes TGF-induced changes in glomerular capillary hydrostatic pressure although some changes in single nephron GFR (SNGFR) persist (7). The cyclooxygenase metabolite(s) that modulate the TGF response have not been elucidated.

TX is a cyclooxygenase metabolite that is produced by normal kidneys (8) or isolated glomeruli (9–11) and might be a vasoconstrictor agent regulating GFR. Indeed, activation of TGF in a whole-kidney model in which vasoconstriction was induced by intrarenal infusion of hypertonic chloride-containing solutions was accompanied by release of thromboxane B$_2$ (TXB$_2$) (8). The aim of the present study was to investigate the role of TX in a single-nephron model of the TGF response. We measured changes in SNGFR and proximal tubule stop-flow pressure (P$_{sf}$), which is an index of glomerular capillary hydrostatic pressure, during changes in the rate of perfusion of the loop of Henle (LH). The role of TX was investigated by administration of drugs inhibiting cyclooxygenase (indo), TX synthetase (UK-38,485 [UK]), or TX receptors (SQ-29,548 [SQ] and L-641,953 [L]). These drugs were selected because previous studies with inhibitors of cyclooxygenase had not discriminated between the roles of the vasoconstrictor PG, TXA$_2$, and vasodilator PGs such as prostacyclin (PGI$_2$) or PGE$_2$. The drugs used in the present studies inhibit the formation or action of TX at three distinct points. The specificity of action of these drugs was assessed first by measurements of the excretion of PGs or TXB$_2$; second, by comparison of the dose-response relationship for UK on TXB$_2$ excretion and TGF response; and third, by determining whether UK (a TX synthesis inhibitor) had any residual action on TGF after TX receptors were blocked by SQ.

Methods

Preparation and protocol. Experiments were performed on male Sprague-Dawley rats maintained on a rat chow diet (rodent laboratory chow 5001; Ralston-Purina Co., St. Louis, MO). Animals were anes-

Address reprint requests to Dr. Wilcox, Division of Nephrology and Hypertension, Veterans Administration Medical Center, Gainesville, FL 32602.

Received for publication 7 May 1987 and in revised form 10 December 1987.

1. *Abbreviations used in this paper:* ANOVA, analysis of variance; AII, angiotensin II; GCMS, gas chromatography/mass spectroscopy; indo, indomethacin; 6kPGF$_{1\alpha}$, 6-keto-PGF$_{1\alpha}$; L, L-641,953; LH, loop of Henle; MBP, mean blood pressure; PAH, para-aminohippuric acid; PGI$_2$, prostacyclin; P$_{sf}$, proximal tubule stop-flow pressure; RPF, renal plasma flow; SQ, SQ-29,548; TGF, tubuloglomerular feedback; U$_{Cl}$V, renal chloride excretion; UK, UK-38,485; U$_K$V, renal potassium excretion.

J. Clin. Invest.

0021-9738/88/06/1843/07 $2.00
Volume 81, June 1988, 1843–1849

thetized by intraperitoneal injection of inactin (100 mg·kg^{-1}; BYK, Gulden Konstanz, FRG) and prepared for micropuncture. Both external jugular veins were cannulated (PE-50), one for continuous infusion of inulin and para-aminohippuric acid (PAH), the other for administration of solutions. After tracheostomy, the right femoral artery was cannulated (PE-50) for blood sampling and pressure monitoring. Arterial blood pressure (BP) and heart rate were measured with a pressure transducer (Statham model P-23; Gould, Inc., Oxnard, CA) and recorded on a polygraph (model 5; Grass Instruments Co., Quincy, MA). The bladder was catheterized for urine collection from the right kidney. The left kidney was exposed by a lateral flank incision. Its ureter was cannulated (PE-10) and the kidney was mounted and stabilized in a plastic cup. The kidney was superfused with 0.15 M NaCl maintained at 37°C and illuminated via a quartz rod light. The animal was kept on a heated plexiglass surgical table and its body temperature was maintained by a servo heating device (model 73A; YSI, Yellow Springs, OH) at 37°C.

Inulin (2 g·dl^{-1}), PAH (0.2 g·dl^{-1}, Merck, Sharp and Dohme, West Point, PA), and albumin (3 g·dl^{-1}, Sigma Chemical Co., St. Louis, MO) were dissolved in 0.15 M NaCl and the solution infused at 0.5 ml·100 g body wt (bw)$^{-1}$·h^{-1}, after a priming dose of 0.5 ml to maintain a euvolemic state. Experiments followed a standard protocol. After surgery, there was a 30-min equilibration period followed by a control period of 90 min (period 1) during which clearance and micropuncture measurements were made. Thereafter, test solutions or vehicle were infused over a 5-min period, followed by a 15-min period for equilibration. There were six experimental groups. Group 1 received vehicle, group 2 received indo (5 mg·kg^{-1}), group 3 received UK (100 mg·kg^{-1}), group 4 received SQ (8 mg·kg^{-1}), and group 5 received L (50 mg·kg^{-1}). 20 min after administration of vehicle or drugs, there was a 90-min experimental period (period 2) during which similar measurements and collections were made as in period 1. Urine was collected during periods 1 and 2, and a 0.6-ml blood sample was drawn at the end of each. The different treatment groups are summarized in Table I. The preliminary results showed that the effects of SQ (group 4) and L on the TGF response were similar, and therefore a complete analysis of the response to TX receptor blockade was undertaken in the former group only.

Group 6 rats received SQ (8 mg·kg^{-1}) during period 2. 15 min thereafter, clearance and micropuncture measurements were made, after which a second dose of SQ (8 mg·kg^{-1}) was given with UK (100 mg·kg^{-1}). After another 15 min, clearance and micropuncture measurements were repeated. Thus, in this group the effects of the blockade of TX synthesis with UK could be studied in animals whose TX receptors had been blocked by SQ.

The dose-response relationships for the effect of UK on excretion of TXB_2 and on the TGF response were studied in rats receiving the following doses (mg·kg^{-1}) of UK: 1, 10, 50, 100, and 500. Clearance and micropuncture studies were performed as described above. One rat given the highest dose showed signs of toxicity (reduced BP) and its data were discarded.

Drugs. Indo inhibits cyclooxygenase and reduces the production of all PGs including PGI_2 and TX (4, 6, 7). Indo (2.5 mg·ml^{-1}, Sigma Chemical Co.) was dissolved in 1 M Na_2CO_3 solution and given to group 2 rats to assess the effects of nonselective inhibition of production of both vasodilator and vasoconstrictor PGs on the TGF response. UK (Pfizer-Central Research, Groton, CT) is a selective inhibitor of TX synthesis (12–14, 15) with the formula: 3-(1H-imidazol-1-yl-methyl)-2-methyl-1H-indole-1-propanoic acid. It was dissolved in 1 N NaOH at pH 12.5 and titrated with 1 N HCl to pH 8.5 to a concentration of 100 mg·ml^{-1}. It was given to group 3 rats to assess the effects of inhibition of TX synthesis on the TGF response. However, inhibition of TX synthesis in vitro in mononuclear cells can shunt endoperoxide metabolites of arachidonate towards synthesis of vasodilator PGs (16). Therefore, we also studied the action of two TX receptor antagonists to assess the role of TX receptor activation on the TGF response. SQ (Squibb Institute for Medical Research, Princeton, NJ) is a specific antagonist of TXA_2 and stable endoperoxide analogues with a relatively short duration of action (17). It is a chemical analogue of TXA_2 with the formula: [1*S*-[1α, 2β (5Z), 3β, 4α]]-7-[3-[[2-[(phenylamino) carboxyl] hydrazino] methyl]-7-oxabicyclo [2.2.1] hept-2-Y1]-5-heptenoic acid. It was given to group 4 rats. L (Merk-Frosst-Canada Inc., Pointe-Claire Dorval, Quebec, Canada) is a specific antagonist of TXA_2 but has a longer duration of action (18) and is chemically distinct from TXA_2. It has the formula: R-8-fluoro-dibenzo [b,f] thiepin-3-carboxylic acid-5-oxide. It was given to group 5 rats to assess the effects of a chemically dissimilar TX receptor antagonist on the TGF response.

Chemical methods. Inulin was measured by an anthrone method and GFR was calculated from its clearance. PAH was measured by the Bratten-Marshall reaction, and renal plasma flow (RPF) was calculated from its clearance (19). Urine sodium (Na^+) and potassium (K^+) concentrations were measured with a flame photometer (model 443; Instrumentation Laboratory, Inc., Lexington, MA). Urine chloride (Cl^-) concentration was measured on a chloride meter (model 920M; Corning Medical, Medfield, MA).

Assay for TXB_2 and 6-keto-$PGF_{1\alpha}$ ($6kPGF_{1\alpha}$)

The purification and assay of the PGI_2 derivative $6kPGF_{1\alpha}$ (Seragen Inc., Boston, MA) and TXB_2 in urine was developed from a previous method (8). Samples (0.5 ml) of urine were spiked with ~ 1,000 cpm of [^{3}H] TXB_2 (New England Nuclear, Boston MA) as a tracer to assess the individual recovery of each sample assayed and diluted with 0.5 ml of deionized water to facilitate organic extraction. The pH was adjusted from 3.0 to 3.5 with 1.0 N HCl and samples were extracted with 8-ml ethyl acetate. The organic phase was dried under nitrogen and dissolved in 50 μl of acetone. Each 50-μl sample was placed as a single spot on a 20 × 20-cm silica gel C-plate (Fisher Scientific Co., Pittsburgh, PA) at nine spots per plate. Standard TXB_2 and $6kPGF_{1\alpha}$ were spotted individually on one plate. Plates were developed in $CHCl_3$/MEOH/HOAC (90:10:5). The standards were visualized with anisealdehyde reagent spray; TXB_2 appeared at reference point 0.65 and $6kPGF_{1\alpha}$ at reference point 0.70. Corresponding sample areas were scraped, extracted with 8 ml of methyl formate, centrifuged at 2,600 rpm, dried under nitrogen, and dissolved in 500 μl of phosphate buffer (pH 7.4). A 50-μl aliquot was used for calculation of individual sample recoveries, and the remainder was used for radioimmunoassay.

Radioimmunoassay was carried out in a 4°C ice bath in 12 × 75-cm disposable glass tubes to which were added 200 μl of phosphate buffer (pH 7.4) and 100 μl of either standard or sample. Antisera (100 μl, Seragen, Inc.) was added and the samples vortexed. After 15 min, 100 μl of ~ 100 cpm·μl^{-1} of [^{3}H] antigen (New England Nuclear) was added to each tube and vortexed. The tubes were covered with parafilm and incubated at 4°C for 18 h. Samples were precipitated with 500 μl dextran-coated charcoal for 15 min and centrifuged at 2,650 rpm for 10 min. We recovered 500 μl of each sample immediately and placed it in a scintillation vial for counting in a liquid scintillation counter (model LS7000; Beckman Instruments, Inc., Fullerton, CA).

The assay had an interassay coefficient of variation (n = 31) for TXB_2 of 7.6% and for $6kPGF_{1\alpha}$ of 8.3%. The sensitivity was 5–10 pg·ml^{-1}. The accuracy was assessed each week by addition of 1,000 pg of TXB_2 and $6kPGF_{1\alpha}$ to 1 ml of rat urine. The results were +975±22 pg·ml^{-1} (n = 23) for $6KPGF_{1\alpha}$ and +1,001±26 pg·ml^{-1} (n = 26) for TXB_2. Dr. Garret A. Fitzgerald (Dept. of Clinical Pharmacology, Vanderbilt University Medical School, Nashville, TN) assayed three samples of urine from a rat given a large dose of indo using gas chromatography/mass spectroscopy (GCMS) methods. The results obtained (pg·ml^{-1}) in our radioimmunoassay, compared with the GCMS, were as follows: 1,292 vs. 430, 292 vs. 90, and 36 vs. 7. Therefore, our method overestimated TXB_2 by three- to fivefold, perhaps because it also assayed the di- and tetra-nor derivatives of TXB_2 (Fitzgerald, G., personal communication), but provided a close correlation with the pattern of changes in TXB_2 excretion, as assessed by GCMS.

Micropuncture preparation. The kidney was viewed with a stereomicroscope (model 5A Wild/Heerbrugg; E. Leitz Co., Rockleigh, NJ).

For microperfusion, a pipette (3–5 μm o.d.) containing 0.15 M saline stained with fast green number 5 (Sigma Chemical Co.) was inserted into a proximal tubule. After a 5–10-nl injection, several proximal loops of the same nephron were identified. A microperfusion pipette (4–6 μm o.d.) containing artificial tubular fluid and driven by a nanoliter perfusion pump (model A1400; World Precision Instruments, New Haven, CT) was inserted into the end proximal superficial loop to perfuse the LH. Artificial tubular fluid solution contained 132 mM NaCl, 4 mM $NaHCO_3$, 5 mM KCl, 2 mM $CaCl_2$, 7 mM urea, and 2 mM $MgCl_2$. The nanoliter perfusion pump was calibrated in vitro with isotopic techniques and in vivo volumetrically. An immobile wax block was inserted into the nephron proximal to the site of perfusion by a pipette (6–8 μm o.d.) containing bone wax stained with sudan black and connected to a hydraulic microdrive unit (model 5; Trent Wells, Inc., South Gate, CA). A pressure-measuring pipette (1–2 μm o.d.) was inserted into the nephron proximal to the wax block to measure P_{sf}. This pipette was filled with 2 M NaCl and connected to a servo-null micropressure device (model 4A; Instrumentation for Physiology and Medicine, La Jolla, CA). The pressure system was calibrated during each experiment. P_{sf} was recorded at each perfusion interval when it had reached a stable value after 1 to 3 min. The loop was perfused at 0, 10, 20, 30, and 40 nl · min^{-1} in random order.

In separate animals of groups 1–3, SNGFR was measured in addition to P_{sf} in response to perfusion of the LH at zero and 40 nl · min^{-1} after administration of vehicle, indo, or UK. After completion of P_{sf} measurements, the tubule was vented and a collection pipette (8–12 μm o.d.) filled with mineral oil stained with sudan black was inserted upstream. A timed fluid collection was made during perfusion of the LH at 0 or 40 nl · min^{-1}. A column of oil three to four times the width of the tubule lumen was injected downstream from the pipette, and tubular fluid was aspirated with sufficient negative pressure to maintain the position of the oil column. A second collection of tubular fluid was made after changing the rate of LH perfusion to 0 or 40 nl · min^{-1}. This collection was taken from a more proximal site of the same nephron after venting of the first collection site. Tubular fluid volumes were measured in a constant-bore glass capillary by a filar micrometer. Rats used for SNGFR measurements received an infusion of [^{3}H] inulin (50 μCi · 100 g $bw^{-1} \cdot h^{-1}$) and [^{14}C] PAH (0.4 μCi · 100 g $bw^{-1} \cdot h^{-1}$) delivered in similar volumes as the animals receiving unlabeled inulin and PAH. Aliquots of tubular fluid, urine, and plasma were counted in a liquid scintillation counter (model LS7800; Beckman Instruments, Inc.).

P_{sf} was measured at zero loop perfusion at the beginning and end of each series. If these values differed by > 1 mmHg, the data were discarded. It was technically harder to undertake recovery measurements of SNGFR. In 32 preliminary studies, SNGFR was measured first at zero loop perfusion; the SNGFRs recorded in this group at 0 and 40 nl · min^{-1} of LH perfusion were 35±2 and 21±2 nl · min^{-1}, respectively. In 13 rats, SNGFR was measured first at 40 nl · min^{-1} loop perfusion; the SNGFRs recorded in this group at 0 and 40 nl · min^{-1} of LH perfusion were similar at 37±2 and 20±2 nl · min^{-1}. Therefore, the order in which measurements were made did not appear to influence the response. Nevertheless, the order of studies was randomized between rats.

Values are reported as mean±1 SEM. Differences between groups were assessed by analysis of variance (ANOVA) with a Student's t test to assess posthoc differences. Values were taken as statistically significant at $P < 0.05$.

Results

The effect of each drug treatment on mean blood pressure (MBP) and whole kidney function is shown in Table I. The body weights were not different between the groups. The data obtained in the basal state (period 1) did not differ between groups. The results of an ANOVA indicated that, compared with vehicle, indo reduced urine flow, whereas UK increased urine flow and chloride excretion.

The effects of drugs on renal excretion of TXB_2 and $6kPGF_{1\alpha}$ are shown in Fig. 1. Urine volumes were insufficient for measuring PG or TXB_2 excretion in ~ 10% of the rats. During period 1, there were no differences in excretion between the three groups. Administration of vehicle (group 1) did not change excretion of TXB_2 or $6kPGF_{1\alpha}$ (data not shown). Compared with vehicle, treatment with indo (group 2) or UK (group 3) decreased the excretion of TXB_2 consistently, whereas the excretion of $6kPGF_{1\alpha}$ was decreased only by indo.

Group mean changes in P_{sf} during increases in the rate of

Table I. Body Weight, MBP, and Renal Function before (Period I) and after (Period II) Administration of Vehicle or Drug

		MBP		GFR		RPF		Filtration fraction	
	Body weight	1	2	1	2	1	2	1	2
	g	*mmHg*		*ml · min⁻¹*		*ml · min⁻¹*			
Vehicle (n = 20)	234±10	116±2	114±2	1.82±0.11	1.68±0.09	5.38±0.34	4.76±0.36	0.35±0.02	0.33±0.02
indo (n = 17)	234±12	115±3	112±3	2.02±0.16	1.66±0.11	6.22±0.61	4.74±0.44	0.34±0.02	0.37±0.02
UK (n = 19)	239±12	117±2	115±2	1.95±0.11	1.76±0.14	5.73±0.41	4.93±0.48	0.35±0.02	0.38±0.02
SQ (n = 14)	251±14	115±2	111±3	1.97±0.21	1.69±0.15	6.21±0.56	6.03±0.78	0.32±0.03	0.31±0.02

	UV		$U_{Na}V$		U_KV		$U_{Cl}V$	
	1	2	1	2	1	2	1	2
	μl · min⁻¹		*μmol · min⁻¹*		*μmol · min⁻¹*		*μmol · min⁻¹*	
Vehicle	8.0±0.6	8.9±0.9	0.194±0.024	0.191±0.031	1.821±0.291	2.131±0.366	1.30±0.23	1.57±0.33
indo	8.6±1.0	6.9±1.2*	0.247±0.1085	0.182±0.085	1.763±0.516	0.980±0.278	1.38±0.48	1.17±0.48
UK	7.6±0.7	10.5±1.2*	0.212±0.036	0.283±0.052	1.715±0.366	3.053±0.363	1.28±0.30	2.30±0.31*
SQ	7.4±1.2	8.3±1.9	0.194±0.033	0.233±0.051	1.494±0.236	1.469±0.235	1.07±0.20	0.90±0.24

Mean±SEM values. n, Number of rats studied. UV, urine flow; $U_{Na}V$, renal sodium excretion. Significance of differences compared with vehicle, using ANOVA and posthoc t tests: * $P < 0.05$.

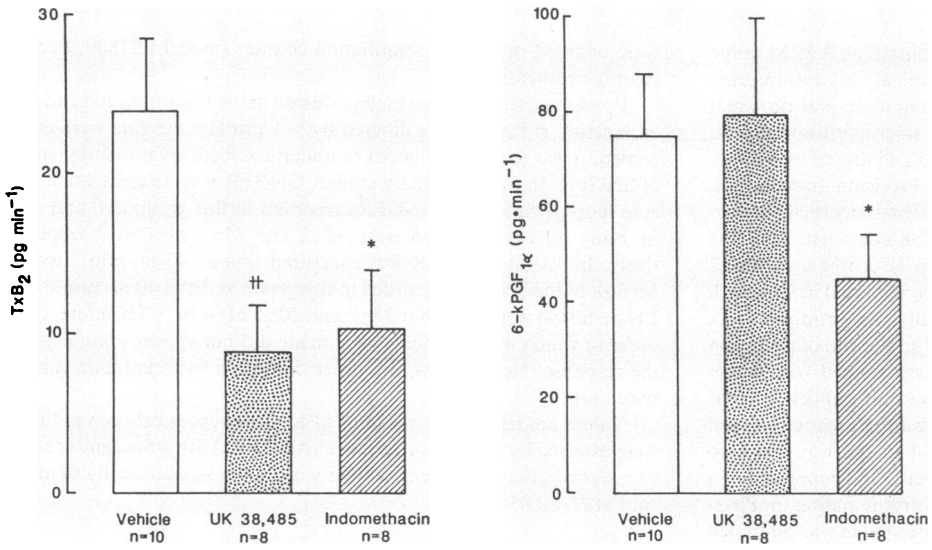

Figure 1. Mean (±SEM) values for renal excretion of TXB_2 and $6kPGF_{1\alpha}$ during period 2 in groups 1–3. Significance of difference from vehicle-treated rats of group 1: $*P < 0.05$. ‡ $P < 0.01$.

perfusion of the LH from 0 to 40 nl · min^{-1} are shown in Fig. 2. In the basal state during period 1, there were no differences between the groups. Administration of vehicle (group 1) did not change the response. In contrast, significant reductions in P_{sf} responses were seen after administration of indo, UK, SQ, or L. Compared with vehicle-treated group 1 rats, the P_{sf} responses to increasing perfusion of the LH were blunted ($P < 0.005$) in groups 2–5.

Table II contains more detailed data relating to the effect of drugs on the TGF response. This table shows group mean values for P_{sf} and SNGFR recorded during perfusion of the LH at 0 and 40 nl · min^{-1}. In the basal state (period 1) the values of P_{sf} at 0 and 40 nl · min^{-1} were not different among groups. However, the results of ANOVA and posthoc t tests demonstrate that after treatment (period 2), the P_{sf} at zero perfusion of the LH was lower in animals pretreated with indo ($P < 0.005$), SQ ($P < 0.01$), or L ($P < 0.05$) compared with those receiving vehicle, whereas the P_{sf} during perfusion of the LH at 40 nl · min^{-1} was greater ($P < 0.05$) in animals that had received indo or UK. During period 1, there were no differences between the groups for the changes in P_{sf} during increase in LH perfusion from 0 to 40 nl · min^{-1}. During period 2, administration of indo, UK, SQ, and L all reduced ($P < 0.005$) the TGF response compared with group 1 rats that had received vehicle. During period 1, the values of SNGFR recorded at perfusion of the LH at 0 and 40 nl · min^{-1} and the differences in SNGFR between these two perfusion rates did not differ between groups (Table II). After administration of vehicle (group 1), the SNGFR response to changes in LH perfusion were comparable. In contrast, after administration of indo (group 2) or UK (group 3) there were significant reductions in SNGFR responses to increasing LH perfusion. After indo, the overall blunting of the TGF response assessed from changes in P_{sf} was comparable with that assessed from changes in SNGFR and averaged −60 and −58%, respectively. After UK, the blunting was also comparable, −47 and −45%, respectively. However, there was more variability in the SNGFR data, and no significant changes in SNGFR at 0 or 40 nl · min^{-1} of LH perfusion were detected after either drug. Inspection of the data indicated that the order in which measurements of SNGFR were

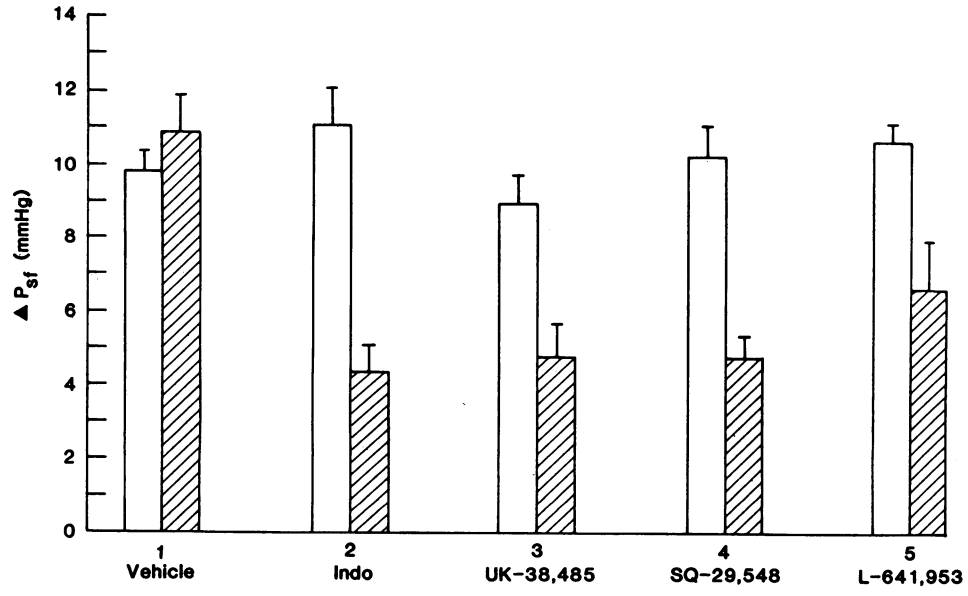

Figure 2. Mean (±SEM) values are shown for changes in P_{sf} during increases in LH perfusion from 0 to 40 nl · min^{-1} during periods 1 and 2 in groups 1–5. Data were analyzed by ANOVA with posthoc t test. During period 1, there were no differences between groups. However, during period 2, the values in groups 2–5 were all lower than those in group 1 ($P < 0.005$).

Table II. P_{sf} and SNGFR during LH Perfusion at 0 and 40 nl · min

	Period I			Period II		
	LH perfusion rate ($nl \cdot min^{-1}$)					
	0	40	Δ (0 to 40)	0	40	Δ (0 to 40)
P_{sf} (*mmHg*)						
Vehicle (n = 19)	31.9±0.9	22.8±0.9	9.8±0.8	32.7±1.1	22.6±0.8	10.0±0.7
indo (n = 15)	32.1±1.2	21.0±1.1	11.1±1.0	28.8±1.3*	24.4±1.1*	4.4±0.7§
UK (n = 19)	32.5±1.4	23.5±1.0	9.0±0.8	30.8±1.6	26.0±1.1*	4.8±0.7§
SQ (n = 14)	31.8±0.9	21.1±0.8	10.3±0.6	28.4±0.9‡	23.6±0.6	4.8±0.6§
L (n = 8)	33.6±1.1	22.5±1.1	10.9±0.5	30.6±1.7*	24.5±1.2	6.1±0.7§
SNGFR ($nl \cdot min^{-1}$)						
Vehicle (n = 7)	27.0±4.3	16.0±2.0	10.9±2.8	27.4±2.4	16.1±2.3	11.2±0.8
indo (n = 6)	36.0±3.9	22.0±3.6	13.9±1.2	30.4±4.0	24.2±4.3	6.2±2.2*
UK (n = 17)	28.9±2.7	16.1±2.1	12.8±2.1	25.7±2.7	18.6±2.1	7.0±1.5*

Mean±SEM values. n, Number of rats studied. Significance of changes compared with vehicle using ANOVA and posthoc t tests: * $P < 0.05$. ‡ $P < 0.01$. § $P < 0.005$.

made did not affect the results obtained. Thus, after vehicle administration, eight rats were studied first at zero loop perfusion; their values for SNGFR at 0 and 40 nl · min^{-1} of loop perfusion were 29±4 and 19±3 nl · min^{-1}, compared with values of 30±4 and 17±4 nl · min^{-1} in five rats studied first at 40 nl · min^{-1}. After indo, nine rats were studied first at zero loop perfusion; their values for SNGFR at 0 and 40 were 27±3 and 20±4 compared with values of 24±5 and 21±3 in three rats studied first at 40 nl · min^{-1}. After UK, seven rats were studied first at zero loop perfusion; their values for SNGFR at 0 and 40 nl · min^{-1} were 22±3 and 16±2 nl · min^{-1} compared with values of 25±1 and 18±3 in six rats studied first at 40 nl · min^{-1}.

The dose-response relationship for UK on renal TXB_2 excretion is compared with that on the TGF response in Fig. 3. The fractional changes in TXB_2 excretion and TGF during period 2 (after administration of the drug) are compared with the control period 1. TXB_2 excretion was maximally suppressed by 100 mg · kg^{-1}; the IC_{50} was 15 mg · kg^{-1}. The TGF response was maximally suppressed by 100 mg · kg^{-1}; the IC_{50} was 30 mg · kg^{-1}.

The mean values of P_{sf} at incremental rates of LH perfusion are shown in Fig. 4. During period 1, there was a sigmoidal reduction in P_{sf} with increasing LH perfusion in each group. The rate of LH perfusion associated with a 50% reduction in P_{sf} averaged 15–20 nl · min^{-1}. After administration of vehicle, there were no consistent changes. Indo and SQ flattened the response by reducing P_{sf} at low and high rates of LH perfusion, whereas the effects of UK were significant only at the high rates of LH perfusion.

The P_{sf} at graded rates of LH perfusion was studied in seven rats before administration of drugs, after administration of SQ, and after UK plus SQ (Fig. 5). SQ reduced P_{sf} at zero perfusion of the LH and increased it at 40 nl · min^{-1} of LH perfusion, as in the previous series (Fig. 4). After administration of SQ, UK had no further effect on P_{sf} at any rate of LH perfusion, although the value at zero LH perfusion was now not quite significantly lower than during period 1.

Discussion

The main finding of our study was that drugs acting at three distinct sites in the pathway leading to TXA_2 synthesis or action all suppressed changes in SNGFR and/or P_{sf} during increased delivery of filtrate to the LH. Thus, TGF was blunted by ~ 40–65% after inhibition of cyclooxygenase with indo, after inhibition of TX synthesis with UK, or after inhibition of TX receptors with SQ or L. The blunting of TGF-induced changes in glomerular capillary pressure by indo, SQ, and L were due to lower values at zero LH perfusion, whereas indo and UK increased the values at 40 nl · min^{-1} of LH perfusion.

The conclusions of this study depend critically upon the specificity of the drugs used. Indo is a noncompetitive inhibitor of cyclooxygenase that inhibited the renal synthesis of TXA_2 and PGI_2 (as assessed from excretion of their metabo-

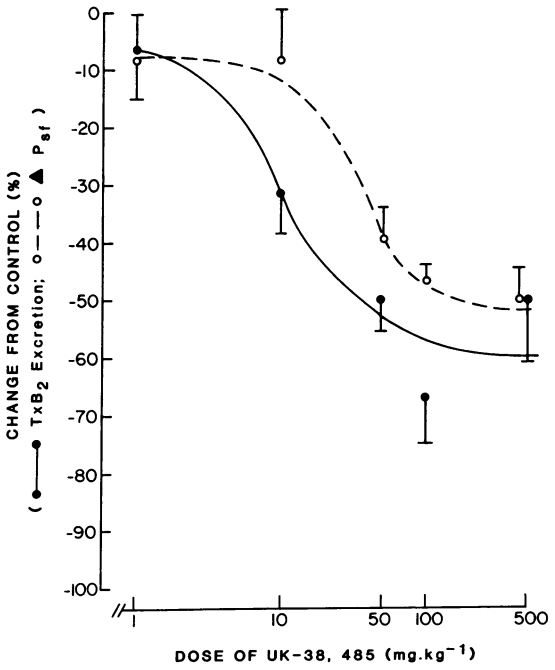

Figure 3. Mean±SEM values for the dose-response effects of UK on the excretion of TXB_2 and the TGF response (from change in P_{sf} during LH perfusion from 0 to 40 nl · min^{-1}). Data shown are for fractional changes in responses during period 2 compared with period 1.

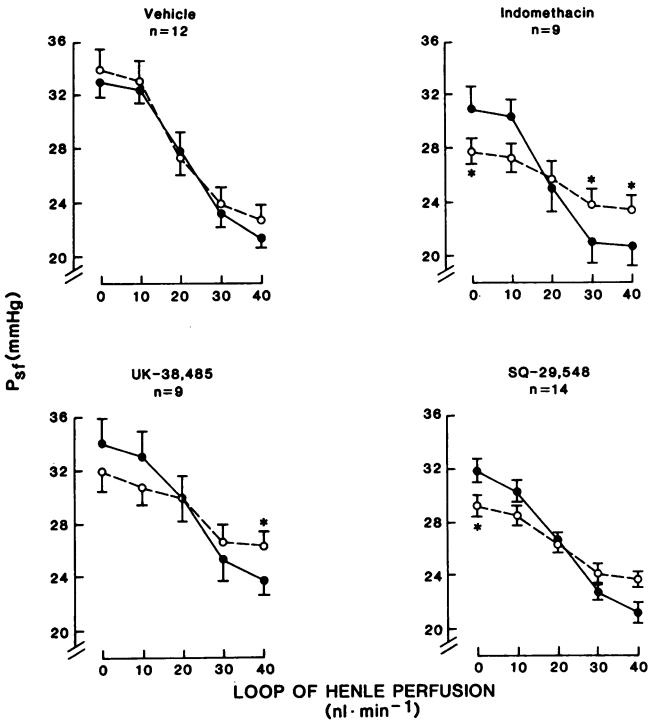

Figure 4. Mean (±SEM) values for P_{sf} at graded LH perfusion for period 1 (●) and period 2 (○) in groups 1–4. Compared with period 1: $*P < 0.05$, using ANOVA and posthoc *t* tests.

lites) over the time at which it blunted the TGF response. In contrast, UK is a competitive inhibitor of TX synthetase (15, 16). It produced dose-dependent inhibition of the TGF response and excretion of TXB_2 without affecting $6kPGF_{1\alpha}$. This confirms previous studies using GCMS (14), thin-layer chromatography (13), or radioimmunoassay (15) of samples of urine or superfusates from isolated glomeruli, which showed

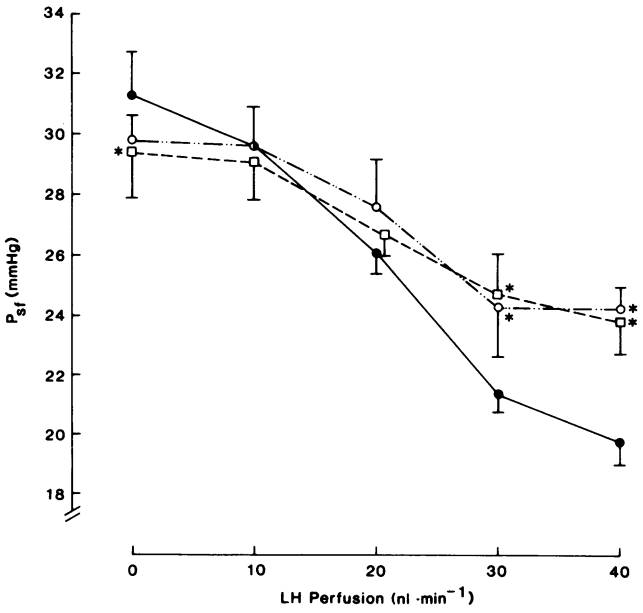

Figure 5. Mean±SEM values of P_{sf} at graded LH perfusion for period 1 (control [●]), period 2 (SQ [□]), and period 3 (SQ and UK [○]) in rats of group 6. Compared with period 1: $*P < 0.05$.

that UK is a specific inhibitor of renal TX synthesis without demonstrable effects on PGI_2 or PGE_2. However, maximal doses of UK reduced TGF responses and TXB_2 excretion by only 50–60%. The IC_{50} for TXB_2 excretion (15 mg · kg^{-1}) was similar to that for TGF (30 mg · kg^{-1}), which implies that the action of UK in inhibiting TGF could indeed relate to inhibition of renal TXA_2 generation. UK might have blunted TGF by shunting PG endoperoxides towards synthesis of some unmeasured PGs. To test this hypothesis, rats were pretreated with a TX receptor antagonist, after which there was no additional effect of a full dose of UK. We therefore concluded that the blunting of TGF by UK was indeed best attributed to a blunting of TXA_2 synthesis. The results also showed that two structurally quite dissimilar TX receptor antagonists produced a comparable degree of inhibition of TGF response to that produced by a TX synthetase antagonist. SQ is a relatively short-acting competitive antagonist of TXA_2-induced arteriolar vasoconstriction or platelet aggregation; it is chemically related to TX (17). L is a highly specific antagonist of TX- and PG endoperoxide–mediated platelet aggregation, smooth muscle contraction, or vasoconstriction (18); it has a prolonged duration of action and is structurally distinct from TX.

TX is a vasoconstrictor, and therefore the effects of drugs blocking its synthesis or receptors should be apparent during the vasoconstrictor phase of the TGF response at high LH perfusion. Indeed, indo and UK blunted the fall in P_{sf} during perfusion of the LH at 40 nl · min^{-1}, which is consistent with blunting of vasoconstriction. However, SQ and L did not have consistent effects at 40 nl · min^{-1}. Moreover, data were more variable for SNGFR, where no clear effects of indo or UK were seen at 40 nl · min^{-1} of LH perfusion, although both drugs blunted the overall TGF-induced changes in SNGFR by 45–55%. Indo, SQ, and L reduced P_{sf} significantly during perfusion of the LH at zero flow, implying that they blunted the vasodilator arm of the TGF response. Previously, indo has been shown to blunt the overall TGF response assessed from changes in early proximal flow rate without changing significantly the early proximal flow rate at zero LH perfusion (4). A blunting of the vasodilator and vasoconstrictor arms of the TGF-induced changes in P_{sf} might relate to diminished production by indo of both vasodilator PGs and TXA_2. However, it is hard to reconcile this conclusion with results of previous studies (4), which showed the time course of inhibition of the TGF response by indo did not correspond with that for inhibition of PG excretion. Moreover, this would not explain why administration of SQ and L also reduced P_{sf} during perfusion of the LH at zero flow. The values for P_{sf} and SNGFR at zero loop perfusion were rather lower than in some previous studies. TX receptor antagonists can be partial agonists at vascular sites (20), but SQ and L do not have significant agonist activity on smooth muscle or rat aortic strips (17, 18). In recent preliminary studies, we have shown that SQ and L release renin and AII, which might have led to vasoconstriction and therefore to a lower P_{sf} at zero LH flow (21).

The kidney releases TXB_2 into renal lymph and urine (8). TXA_2 is produced in the vascular pole of the kidney in the vicinity of the changes in vascular resistance induced by the TGF response (9–11). In a preliminary account, Franco, Bell, and Navar (22) report no effect of either UK or a TX receptor antagonist (EP 092) on TGF responses when these drugs were added directly to the tubular perfusate. Moreover, in our study, SQ had no demonstrable effects on whole kidney excretion of fluid, Na^+, K^+, or Cl^-, although there was a small

increase in $U_{Cl}V$ with UK. These data suggest that the effects of these drugs were not primarily on the sensing site at the macula densa (tubular reabsorption) but on the effector site, i.e., that they modulated the sensitivity of the responses to signals from the macula densa cells. Our conclusions differ from those of Franco et al. (22) who found no effect of systemic administration of EP092 (a TX-receptor antagonist) in four rats at a dose of 1.2 $mg \cdot kg^{-1} \cdot h^{-1}$ on the TGF response. The reasons for this difference are not clear, but their preparation might not have the same TX dependency as ours. Alteratively, the dose of the antagonist used by Franco et al. (22) may have been inadequate.

Persson, Gushwa, and Blantz (7) observed that the combined administration of a cyclooxygenase inhibitor (meclofenamate) and an angiotensin-converting enzyme inhibitor (MK-421) abolished TGF-induced changes in P_{sf}, while only blunting changes in SNGFR. They proposed that the changes in SNGFR at constant glomerular capillary pressure reflected parallel alterations in pre- and postglomerular vascular resistances. We observed quite comparable degrees of inhibition of the overall TGF response assessed from measurements of SNGFR or P_{sf} in rats treated with either a cyclooxygenase inhibitor, a TX synthetase inhibitor, or TX receptor antagonists. This is consistent with the effects of TX or stable mimetics in increasing vascular resistance, especially at preglomerular sites, when added to the perfusate of isolated kidneys and in constricting isolated glomeruli (23). These data indicate that TXA_2 modulates TGF-induced changes in SNGFR, at least in part by regulating the glomerular pressure.

Acknowledgments

We are grateful to J. Mehta, M.D., for helpful advice in planning these studies, to P. Urquilla, M.D., of Pfizer Central Research, Groton, CT, for supplying UK-38,485, to A. W. Ford-Hutchinson, Ph.D., and G. Letts, Ph.D., of Merck-Frosst Canada, Inc., Point-Claire, Dorval, Quebec, Canada, for supplying L-641,953, and to Martin L. Ogletree, Ph.D., of the Squibb Institute for Medical Research, Princeton, NJ, for supplying SQ-29,548. We are grateful to Garrett Fitzgerald, M.D., of the Department of Clinical Pharmacology, Vanderbilt University Medical School, Nashville, TN, for the GCMS validation of the TXB_2 assay. Harold Snellen provided expert technical assistance in the PG analyses.

This work was supported by grants from National Institutes of Health (ROI-AM36079 and 2ROIDK-HL36079).

References

1. Schnermann, J., D. W. Ploth, and M. Hermle. 1970. Activation of tubuloglomerular feedback by chloride transport. *Pfluegers Arch. Eur. J. Physiol.* 318:147–175.

2. Ichikawa, I. 1982. Direct analysis of the effector mechanism of the tubuloglomerular feedback system. *Am. J. Physiol.* 243:F447–F455.

3. Ploth, D. W., and R. N. Roy. 1982. Renin-angiotensin influences on tubuloglomerular feedback activity in the rat. *Kidney Int.* 22(Suppl. 12):S114–S121.

4. Schnermann, J., G. Schubert, M. Hermle, R. Herbst, R. T. Stowe, S. Yarimizu, and P. C. Weber. The effect of inhibition of prostaglandin synthesis on tubuloglomerular feedback in the rat kidney. *Pfluegers Arch. Eur. J. Physiol.* 379:269–279.

5. Persson, A. E. G., U. Boberg, B. Hahme, R. Muller-Suur, B. J. Norben, and G. Selen. 1982. Interstitial pressure as a modulator of tubuloglomerular feedback control. *Kidney Int.* 22(Suppl. 12):S122–S128.

6. Hahne, B., G. Selen, and A. E. G. Persson. 1984. Indomethacin inhibits renal functional adaptation to nephron loss. *Renal Physiol.* 7:13–21.

7. Persson, A. E. G., L. C. Gushwa, and R. C. Blantz. 1984. Feedback pressure-flow responses in normal and angiotensin-prostaglandin-blocked rats. *Am. J. Physiol.* 247:F925–F931.

8. Wilcox, C. S., S. Roddis, W. S. Peart, D. Gordon, and G. P. Lewis. 1985. Intrarenal prostaglandin release: effects of arachidonic acid and hyperchloremia. *Kidney Int.* 28:43–45.

9. Hassid, A., M. Konieczkowski, and M. J. Dunn. 1979. Prostaglandin synthesis in isolated rat kidney glomeruli. *Proc. Natl. Acad. Sci. USA.* 76:1155–1159.

10. Sraer, J., W. Siess, L. Moulonguet-Doleris, J. Oudinet, F. Dray, and R. Ardaillou. *In vitro* prostaglandin synthesis by various rat renal preparations. *Biochim. Biophys. Acta.* 710:45–52.

11. Farman, N., P. Pradelles, and J. P. Bonvalet. 1987. PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and TxB_2 synthesis along the rabbit nephron. *Am. J. Physiol.* 252:F53–F59.

12. Klotman, P. E., S. R. Smith, B. D. Volpp, T. M. Coffman, and W. E. Yarger. 1986. Thromboxane synthetase inhibition improves function of hydronephrotic rat kidneys. *Am. J. Physiol.* 250:F282–F287.

13. Remuzzi, G., L. Imberti, M. Rossini, C. Morelli, C. Carminati, G. M. Cattaneo, and T. Bertani. 1985. Increased glomerular thromboxane synthesis as a possible cause of proteinuria in experimental nephrosis. *J. Clin. Invest.* 75:94–101.

14. Udermann, H. D., E. K. Jackson, D. Puett, and R. J. Workman. 1984. Thromboxane synthetase inhibitor UK 38,485 lowers blood pressure in the adult spontaneously hypertensive rat. *J. Cardiovasc. Pharmacol.* 6:969–972.

15. Zipser, R. D., I. Dronborg, W. Rector, T. Reynolds, and G. Daskalpoulos. 1984. Therapeutic trial of thromboxane synthesis inhibition in the hepatorenal syndrome. *Gastroenterology.* 87:1228–1232.

16. Gordon, D., A. M. E. Nouri, and R. U. Thomas. 1981. Selective inhibition of thromboxane biosynthesis in human blood mononuclear cells and the effects on mitogen-stimulated lymphocyte proliferation. *Br. J. Pharmacol.* 74:469–475.

17. Ogletree, M. L., D. N. Harris, R. Greenberg, M. F. Haslanger, and M. Nakone. 1984. Pharmacological actions of SQ 29,548, a novel selective thromboxane antagonist. *J. Pharmacol. Exp. Ther.* 224:435–441.

18. Hall, R. A., J. R. Rokach, P. Belanger, L. Bianchi, D. Ethier, A. W. Ford-Hutchinson, Y. Girard, P. Hamel, R. Hamel, A. Lord, and P. Yusko. 1987. L641-953, (R-8-fluoro-dibenzo[b,f]thiepin-3-carboxylic acid-5-oxide): a novel thromboxane-prostaglandin endoperoxide antagonist. *Can. J. Physiol. Pharmacol.* 65:509–514.

19. Wilcox, C. S. 1978. The effect of increasing the plasma magnesium concentration on renin release from the dog's kidney: interactions with calcium and sodium. *J. Physiol.* 284:203–217.

20. Humphrey, P. P. A., P. Lumley, and B. P. White. 1987. The agonist action of AH23848 at guinea-pig vascular and airway smooth muscle Tx-receptors *in vivo*. *Br. J. Pharmacol.* 90:820–826.

21. Wilcox, C. S., and W. J. Welch. 1987. Modulation of renin release by thromboxane in the anesthetized rat. *Fed. Proc.* 46:1066. (Abstr.)

22. Franco, M., P. D. Bell, L. G. Navar. 1987. Evaluation of prostaglandins as mediators of tubuloglomerular feedback. *Kidney Int.* 31:423. (Abstr.)

23. Loutzenhiser, R., M. Epstein, C. Horton, and P. Sonke. 1986. Reversal of renal and smooth muscle actions of the thromboxane mimetic U-44069 by diltiazem. *Am. J. Physiol.* 250:F619–F626.