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INCREASED TUBULOGLOMERULAR FEED-BACK MEDIATED SUPPRESSION OF GLOMERULAR FILTRATION DURING ACUTE VOLUME EXPANSION IN RATS

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SUMMARY

1. Volume expansion is currently believed to change the intrinsic properties of the juxtaglomerular apparatus such that the sensitivity of the tubuloglomerular feed-back (TGF) mechanism is reduced, thus allowing glomerular filtration rate, and hence salt and water excretion, to rise. Recent studies conflict with this view and indeed the older literature reveals that the rise in glomerular filtration rate (GFR) under these conditions is far more modest than would be expected if TGF control were eliminated.

2. To investigate this problem, TGF control of filtration rate was examined by measuring single-nephron glomerular filtration rate (SNGFR) during loop of Henle perfusion at varying rates in rats under control conditions, after acute, moderate (4% of body weight), iso-oncotic volume expansion and in rats treated with antibodies to atrial natriuretic peptide (ANP) prior to the acute volume expansion.

3. With TGF control of filtration interrupted by filtrate collection from the proximal tubule, SNGFR in the expanded rats was massively increased compared with controls, although SNGFR measured in the distal tubule, and hence with TGF control intact, was only modestly increased, as was whole-kidney filtration rate. Loop perfusion at increasing rates up to 30 nl min^{-1} progressively decreased SNGFR in controls, and in the expanded rats the range over which control was exerted extended up to 60–80 nl min^{-1}. For changes in loop flow around the spontaneous operating point, the sensitivity of the TGF mechanism, defined as Δ SNGFR/Δ loop flow, was similar in both groups. Treatment of rats with ANP antibodies prior to volume expansion substantially blunted the changes in renal salt and water excretion and the increase in SNGFR seen in the absence of loop perfusion.

4. These results are not consistent with a diminution of TGF function after

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volume expansion, rather with an enhancement. The latter is best accounted for by vasodilatation of preglomerular resistance vessels on volume expansion, a result predicted by calculations from a model based on the serial arrangement of preglomerular and TGF-controlled vascular resistance elements and the established pharmacological actions of ANP.

INTRODUCTION

Renal sodium balance represents a steady state between the amount of sodium filtered by the glomerulus and the reabsorption processes along the nephron. When extracellular volume is increased, the kidney maintains an increased excretion rate of sodium and water, an increase which could be brought about both by a decrease in sodium reabsorption capacity along the nephron *and* an increase of glomerular filtration rate. The latter increase has been attributed to a resetting or attenuation of the tubuloglomerular feed-back mechanism (TGF), which usually depresses the glomerular filtration rate (GFR) under hydropenic conditions. It is believed that this resetting results from alterations in the behaviour of the juxtaglomerular apparatus, a view based mainly on the hypothesis that the chain of events initiated by the signal triggering the TGF mechanism from the tubular lumen at the macula densa, is, at some stage, mediated by components of the renin–angiotensin system (for literature see Schnermann & Briggs, 1985), and additionally, on the observation that the activity of the renal renin–angiotensin system is substantially depressed after expansion of extracellular volume (Thurau, 1975).

Two recent observations are, however, inconsistent with this concept. Firstly, in rats chronically volume-expanded by dietary salt loading, resetting of the TGF-mediated control of GFR was able to be attributed to the appearance of an inhibitory principle in tubular fluid, and not to any change in the intrinsic regulatory characteristics of the juxtaglomerular apparatus (Häberle & Davis, 1984). Secondly, a recent study by Moore & Mason (1986) in rats chronically expanded by deoxycorticosterone acetate (DOCA) and high-salt diet, confirmed earlier findings of Müller-Suur, Gutsche, Samwer, Oelkers & Hierholzer (1975) and suggested that the complete resetting of the regulatory characteristics of the juxtaglomerular apparatus, reported originally by Schnermann, Hermle, Schmidmeier & Dahlheim (1975) under similar conditions, apparently depends upon other (unknown) conditions, rather than on the expansion of the extracellular volume *per se* and the depression of renal renin activity. Furthermore, a critical re-examination of the literature reveals that even under conditions of acute extracellular volume expansion by NaCl infusion, GFR is, at most, modestly increased, a finding in contrast to that which might be expected if control of GFR by the tubuloglomerular feed-back mechanism were abolished. Notwithstanding this mild increase in GFR in such volume-expanded animals, single-nephron glomerular filtration rates (SNGFR) measured in the proximal tubule (i.e. in the absence of a TGF signal at the macula densa) are indeed very much higher than the SNGFR values in normal animals (for example Blantz, Rector & Seldin, 1974; Baylis, Ichikawa, Willis, Wilson & Brenner, 1977). These findings imply – contrary to the original concept – that under conditions of acute volume expansion, TGF suppresses GFR to an even *greater* extent than under control conditions. In addition, since the SNGFR values *without* TGF control

are substantially higher than in control animals, the findings also imply that volume expansion must have changed determinants of SNGFR apart from the tubuloglomerular feed-back mechanism. Of these determinants, the glomerular plasma flow exhibits the most striking changes (Baylis & Brenner, 1978), suggesting that a drastic fall in preglomerular resistance occurs.

In order to clarify this problem, we first undertook to establish as accurately as possible the extent and nature of the changes in GFR and TGF behaviour in rats acutely volume-expanded to an extent similar to that achieved by chronic salt loading (Häberle & Davis, 1982). In parallel, a theoretical model of renal haemodynamics was developed. Since earlier literature (for references see Baylis & Brenner, 1978) and initial findings from the above experiments strongly implied a substantial fall in preglomerular vascular resistance, the model was used to predict the form of the TGF curve under such circumstances. Since the predictions and the subsequent observations on the form of the TGF curve were remarkably consistent, and since it is currently believed that a substantial component of this fall in preglomerular resistance on acute volume expansion can be attributed to the release of atrial natriuretic peptide (ANP) (Hirth, Stasch, John, Kazda, Morich, Neuser & Wohlfeil, 1986), we tested the proposition that the observed changes in TGF behaviour were due to a fall in preglomerular resistance, by inactivating released ANP by the administration of monoclonal antibodies directed against ANP, prior to volume expansion.

METHODS

Experiments were performed in male Wistar rats (Savo-Ivanovas, Kisslegg, F.R.G.) weighing 200–310 g. For each experiment two animals were anaesthetized by intraperitoneal injection of Inactin, 100 mg kg body wt^{-1} (Byk-Gulden, Konstanz, F.R.G.). Body temperature was maintained at 37·5 °C by a feed-back-controlled heated operating table. Catheters were inserted into the femoral artery for blood pressure monitoring and blood sampling, and into the femoral vein for the adminstration of infusions. A solution of 154 mM-NaCl containing 6·25 g inulin dl^{-1} (Inutest, Laevosan, Linz, Austria) was infused at a rate of 0·3 ml h^{-1} 100 g body wt^{-1}. The bladder was catheterized via a suprapubic incision along the linea alba and the urethra and the left ureter ligated. The left kidney was exposed by a transverse flank incision, dissected free of its attachments, placed in a Lucite cup, embedded in agar (3 g% in 0·9% NaCl) and the surface bathed with mineral oil at 38 °C. One of the animals was then acutely volume-expanded by infusion, over 10 min, of a volume of Ringer solution equivalent to 4·0% of body weight and containing 25% by volume fresh rat plasma taken from another anaesthetized rat. On the assumption that plasma volume contributes approximately 25% of extracellular volume, this procedure should yield a uniform expansion of both plasma and interstitial volumes equivalent to that found in the chronic volume expansion study of Häberle & Davis (1982). In a separate subgroup of five rats an artificial 'plasma' was used instead of fresh rat plasma. This was prepared from Ringer solution by adding 5·4 g% bovine serum albumin (BSA N 67000, Standard, extrapure, Serva, Heidelberg, F.R.G.). Thereafter, the sustaining infusion was increased to 1·2 ml h^{-1} 100 g body wt^{-1} and the inulin concentration reduced to 1·5 g dl^{-1}.

In order to assess the role of an ANP-induced fall in preglomerular resistance after acute volume expansion, a further group of rats was injected with monoclonal antibodies directed against atriopeptide II (ANP II). The preparation of the antibodies is described elsewhere (John, Stasch, Neuser, Hirth & Morich, 1986). For injections into rats, 300 μl of mouse ascites fluid containing the antibodies were diluted with saline to a final volume of 1 ml and 0·1 g bovine serum albumin dl^{-1} was added. This solution was injected intravenously at a dose of 1 ml kg body wt^{-1} over a period of 1 min, 40 min before the acute volume expansion.

Clearance measurements

After completion of surgery, a 30 min equilibration period was allowed before commencing a 20 min control period. In the control animals this period was followed by further 60 min clearance periods. In the volume-expanded animals four further 60 min clearance periods were allowed. Urine was collected separately from left (ureter) and right (bladder) kidneys and an arterial blood sample taken at the middle of each period, in order to determine inulin clearance and sodium excretion.

Micropuncture

The response of the TGF mechanism to a standard challenge was assessed in control or expanded rats as follows: a late proximal tubular loop was identified by random puncture of a proximal loop with a micropipette (o.d. 2–4 μm) filled with Ringer solution stained by the addition of Food and Drug Commission (FD & C) Green (0·1 g dl^{-1}). Following injection of small volumes of this fluid, the course of the nephron could be easily traced. A microperfusion pipette containing FD & C Green-stained Ringer solution was then inserted into the last accessible proximal loop and the perfusion rate set to 0, 10, 15, 20, 40, 60 or 80 nl min^{-1}, although the last two rates were employed only in the volume-expanded animals. A paraffin wax block was then inserted into the next proximal loop and the upstream loops observed for dilatation, and, if at least seven loops proximal to the perfusion pipette could be identified, a collection pipette filled with Sudan Black-stained paraffin oil was inserted into the next loop proximal to the wax block. This procedure was chosen in order to be able to perform successive collections at different perfusion rates by puncturing the nephron progressively upstream. After insertion of the oil-filled pipette tubular fluid was collected quantitatively for at least 3 min. Subsequently, at least two further fluid collections at other perfusion rates were made. Between collections the filtrate was allowed to escape from the hole made for the previous collection. After changing the perfusion rate, an equilibration period of at least 2 min was allowed before commencing the next collection. In order to determine the extent of the suppression of SNGFR by the TGF mechanism *in vivo* (i.e. in 'free flowing' nephrons) in control and acutely volume-expanded rats, SNGFR was measured by distal collection and subsequently by very slow partial collection of late proximal tubular fluid of the same nephron under free flow. This procedure allowed the estimation of late proximal flow rate by dividing the distally measured SNGFR values by the corresponding late proximal free-flow tubular fluid-to-plasma inulin concentration ratio (TF/P_{in}) values. For these experiments the following protocol was applied. Using the identifying procedure described above, superficial loops of the distal convoluted tubule could be traced. If at least two such loops were present (about 10% of all nephrons) the most upstream one was regarded as 'early distal' and punctured with a second oil-filled micropipette (o.d. 8 μm) and a timed, quantitative fluid collection made for at least 5 min. Subsequently, the last accessible proximal loop of the same nephron was punctured with a further micropipette (o.d. 12 μm) and tubular fluid was collected from the free-flowing nephron at a rate of about 2–3 nl min^{-1} by gentle suction over a period of about 10 min.

Analyses

The volume of the tubular fluid samples was measured by injecting the sample into an oil-filled constant-bore glass microcapillary (Microcap, 0·5 μl, Drummond) and measuring the length of the column by means of an eyepiece micrometer. Flow rate was calculated and expressed in nanolitre per minute per gram kidney weight. Plasma protein concentration was measured using a commercial kit based on the Biuret method (Total Protein, Boehringer Mannheim, Mannheim, F.R.G.). Urinary sodium was measured by flame photometry.

Inulin (polyfructosan) was determined in plasma, urine and tubular fluid by perchloric acid hydrolysis to fructose and determination of the latter by the hexokinase–glucose-6-phosphate dehydrogenase method (Renschler, 1963; Bergmeyer, Bernt, Schmidt & Stork, 1974; Bernt & Bergmeyer, 1974). Reagents were obtained in kit form from a commercial supplier (Boehringer Mannheim, Glucose/Fructose Kit 139 106), and made up and employed according to the accompanying instructions.

For the determination of inulin in nanogram quantities in tubular fluid samples, an earlier version of the above method (Zwiebel, Höhmann, Frohnert & Baumann, 1969) was adapted and simplified. Since this new method offers substantial advantages over the commonly employed microanalyses for inulin and has not previously been described, it follows here in some detail.

Preparation of samples

Aliquots of 0·33 M-perchloric acid (450 nl) are pipetted onto the bottom of lightly siliconized glass dishes, filled with water-saturated paraffin oil. As many droplets of acid are required as tubular fluid samples and standard solutions of inulin. An aliquot of tubular fluid, of the order of 15–40 nl, is then pipetted into one of the perchloric acid droplets. Using the same nanolitre pipette, aliquots of appropriate standard concentrations of inulin are also pipetted into perchloric acid droplets. The glass dishes are covered and may be left at room temperature until the analysis is performed, either immediately or the next day.

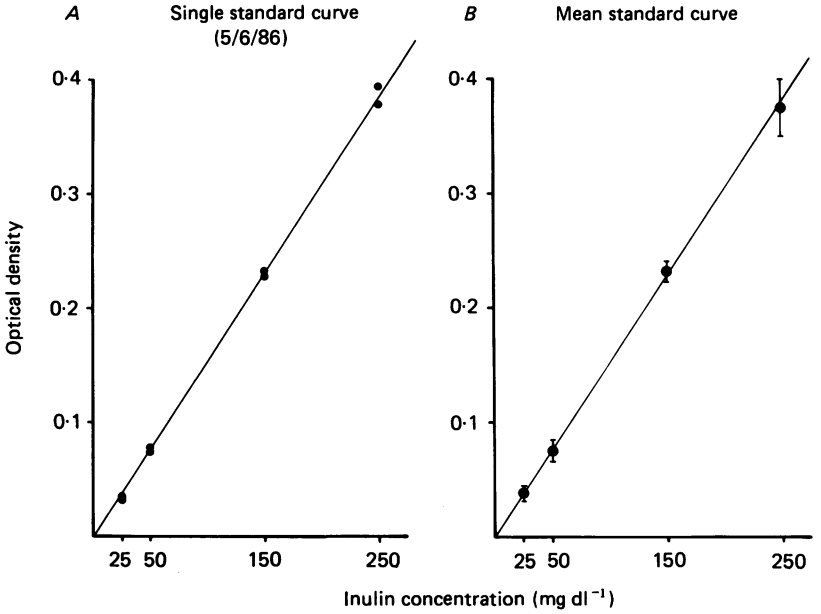

Fig. 1. *A*, standard curve from a single inulin analysis. *B*, mean optical densities (±S.D.) from twenty-six standard curves. The equations of the linear regression of optical density (E) against inulin concentration [In] are: *A*, [In] = 640·5 (ΔE) + 2·6, r = 0·9995; *B*, [In] = 658·6 (ΔE) − 0·3, r = 0·9999. Sample volume for both curves was 31·7 nl.

Analysis

The glass dishes containing the samples and standards under oil are incubated at 80 °C in an oven for 30 min. Two reagent mixtures are prepared from the same kit as used for the 'macro' analysis: R1, consisting of 1 ml buffer–nicotinamide-adenine dinucleotide phosphate–ATP solution, 5 ml distilled water and 40 μl Boehringer suspension 2 (hexokinase–glucose-6-phosphate dehydrogenase) and R2, consisting of 1 ml buffer solution, 5 ml water, 40 μl suspension 2 and 40 μl Boehringer suspension 3 (phosphoglucose isomerase).

The optical density of R1 and R2 is measured at 340 nm in a normal macrocuvette, with distilled water as reference. This should not exceed 0·08, and should not have increased from the previous analysis. This check is necessary because of the limited stability of the buffer solution. The remainder of the analysis is carried out in 'blocks' of four samples, to minimize evaporation and to enable identical handling of all samples. Eight aliquots (1·6 μl) of R1 are pipetted into small depressions in a Teflon disc and 100 nl of the perchloric acid–inulin mixture (four samples in duplicate) added and gently mixed by aspiration and expression of the mixture (three times). The entire volume in each depression is then aspirated into a glass capillary with a long-drawn-out tip, taking care that the fluid column remains in the thinnest part of the capillary to minimize exposure to air. The capillary is then transferred to a stand kept on ice and covered to minimize the exposure to light. When all eight capillaries are filled, the stand is removed from the ice, incubated for 10 min at room temperature, then returned to the ice and dark. While this group is incubating, the

procedure is repeated with reagent R2 and the same samples. Following the incubations, the optical density of the reaction mixtures is measured in a microcuvette (filling volume approximately 1·3 μl) (Greger & Schneider, 1969) in a suitable spectrophotometer at 340 nm, against water as the reference. The cuvette is rinsed and dried between measurements, as is the nanolitre pipette. The optical density measured with R1 represents the glucose in the sample, and that with R2 the glucose plus fructose (inulin); the difference thus yields the inulin contribution alone. A standard curve is prepared as usual and the unknown concentrations determined. The following characteristics were obtained for the assay: interassay variation 3·1% (100 mg dl^{-1} test solution, one sample in duplicate in six separate assays, mean value ± S.D., 100·6 ± 3·1 mg dl^{-1}); intraassay variation 3·7% (100 mg dl^{-1} test solution, ten samples in duplicate in one assay, mean concentration ± S.D., 97·3 ± 3·6 mg dl^{-1}); precision 98·6% (mean deviation ± S.D. from nominal concentration of 100 mg dl^{-1}, $n = 25$, $-1·4 \pm 3·7$ mg dl^{-1}); linearity of standard curve up to 500 ng dl^{-1} better than 99·6% (mean coefficient of correlation ± S.D. for the linear regression of optical density against inulin concentration for ten standard curves consisting of four concentrations, each measured in duplicate, 0·9962 ± 0·0047). A representative individual standard curve and the means from twenty-six standard curves are shown in Fig. 1. The limit of detectability, using the volumes and cuvette described here with the available photometer (a single-beam instrument, Model PM QII, Zeiss, Wetzlar, F.R.G.), was approximately 5 ng (1 pmol).

Statistics

The significance of differences between means was assessed by Student's t test for paired or unpaired samples as appropriate. A probability of 0·05 or less was regarded as significant.

RESULTS

Table 1 summarizes systemic and renal function in control and acutely volume-expanded rats. GFR, urine flow, plasma protein concentration, sodium excretion and blood pressure were similar in control rats and in the pre-expansion period of the experimental rats. Acute volume expansion raised GFR, urine flow and sodium excretion significantly compared with the control group whereas plasma protein concentration and blood pressure were not altered. The Table shows further that urine flow, sodium excretion and blood pressure declined over the subsequent 4 h. As can be seen from Table 2, although urine flow and sodium excretion after volume expansion were still significantly raised in rats treated with ANP II antibodies, these increases were much smaller than in the absence of the antibodies. The rise in GFR (Table 1) is completely abolished. The antibody infusion had no significant effect on any of the measured parameters.

Tables 3 and 4 and Fig. 2 show the results of micropuncture studies. In control rats, increasing loop of Henle perfusion rate above 10 nl min^{-1} induced a significant and progressive fall in SNGFR. The function relating SNGFR to loop of Henle perfusion rate is sigmoid. Acute volume expansion with fresh native or artificial rat plasma plus Ringer solution increases SNGFR significantly, compared with the control animals, at all loop of Henle perfusion rates. Again an S-shaped relationship between loop of Henle perfusion rate and SNGFR is obtained. However, whereas in control rats increasing loop of Henle perfusion rate from 20 to 40 nl min^{-1} did not result in any further significant decrease of SNGFR, in volume-expanded rats increasing loop of Henle perfusion rate in 20 nl min^{-1} steps up to 80 nl min^{-1} caused progressive, significant falls in SNGFR to a level comparable to that obtained in control rats at a perfusion rate of 20 nl min^{-1}. In the rats acutely volume-expanded by infusion of artificial plasma and Ringer solution, SNGFR was slightly, but

TABLE 1. Summary of renal function and systemic parameters in control rats and in rats before and at various stages after acute volume expansion (AVE)

	GFR (ml min^{-1} g KW^{-1})	$\dot{V}$ (μl min^{-1} g KW^{-1})	Plasma protein concentration (g%)		BP (mmHg)	$U\dot{V}_{Na}$ (nmol min^{-1} g KW^{-1})
			Start	Finish		
Control	1·18±0·22 (17)	3·4±0·9 (28)	5·45±0·66 (25)	5·03±0·52 (25)	119±9 (31)	48·0±19·3 (15)
Acute volume expansion						
Pre-expansion	1·23±0·26 (5)	3·7±2·3 (12)	5·34±0·56 (29)		124±13 (32)	73·0±35·9 (10)
Post-expansion						
0–60 min	1·56±0·49* (25)	43·0±20·0* (33)	5·18±0·47 (38)		119±13 (37)	6056±2762* (24)
60–120 min	1·45±0·39* (26)	24·5±15·3* (31)	5·00±0·55* (36)		118±12 (35)	2916±1632* (23)
120–180 min	1·43±0·40* (23)	16·1±14·0* (29)	4·79±0·46* (15)		114±12 (31)	2027±1494* (21)
180–240 min	1·46±0·40* (15)	13·2±10·4* (19)	4·66±0·45* (17)		111±13* (21)	1858±1329* (17)

GFR: glomerular filtration rate; $\dot{V}$: urine flow; BP: mean arterial blood pressure; $U\dot{V}_{Na}$: urinary sodium excretion. KW: kidney weight. Results in this Table and Tables 2–4 are given as the mean ± S.D., with the number of experiments in parentheses. * Indicates significant difference from control ($P \leqslant 0·05$).

TABLE 2. Summary of renal function and systemic parameters in six rats before (Control) and after (AB infusion) the administration of ANP II antibodies and at various times after the subsequent volume expansion (Post-expansion)

	GFR (ml min^{-1} g KW^{-1})	$\dot{V}$ (μl min^{-1} g KW^{-1})	Plasma protein concentration (g%)	BP (mmHg)	$U\dot{V}_{Na}$ (nmol min^{-1} g KW^{-1})
Control	1·30±0·22	3·5±1·0	5·20±0·08	130±9	78·2±41·6
AB infusion	1·33±0·20	4·0±1·1	4·88±0·17*	127±7	71·4±44·2
Post-expansion					
0–60 min	1·15±0·23‡	24·4±13·9†‡	4·56±0·37†‡	115±11†	1813±1048†‡
60–120 min	1·26±0·20	21·0±12·6†	4·62±0·39‡	115±11†	1603±1428†
120–180 min	1·15±0·19	11·4±6·2†	4·57±0·26†	115±9†	856±558†

Abbreviations as in Table 1.

* Indicates a significant difference of AB infusion values from control.

† Indicates a significant difference of post-expansion values from AB infusion period ($P \leqslant 0·05$).

‡ Indicates a significant difference of post-expansion values from the corresponding post-expansion values without AB infusion (Table 1) ($P \leqslant 0·05$).

TABLE 3. Nephron filtration rates (measured at a proximal site) at different loop of Henle perfusion rates in control rats and in rats after acute volume expansion (AVE) with Ringer solution to which either fresh rat plasma or bovine serum albumin had been added

	SNGFR (nl min^{-1} g kidney wt^{-1}) at loop of Henle perfusion rate (nl min^{-1})						
	0	10	15	20	40	60	80
Control	36·7±9·2 (54)	36·8±8·1 (19)	25·9±8·9 (37)	23·1±7·9 (34)	20·9±6·4 (30)	—	—
Acute volume expansion (plasma)	57·1±7·9* (62)	51·3±12·5* (6)	49·7±11·9* (37)	41·4±7·4* (10)	35·9±7·7* (32)	30·1±6·3 (10)	26·9±4·7 (14)
Acute volume expansion (albumin)	49·4±8·9*† (19)	—	—	—	29·6±4·9*† (11)	—	23·4±5·6 (15)

* Indicates a significant difference from control ($P \leqslant 0·05$).

† Indicates a significant difference between AVE with plasma and AVE with albumin ($P \leqslant 0·05$).

TABLE 4. Nephron filtration rates measured at a proximal site during loop of Henle perfusion at 0 or 40 nl min^{-1} in rats in a control period (Control), after administration of ANP II antibodies (AB infusion) and after subsequent acute volume expansion (AVE)

	SNGFR (nl min^{-1} g kidney wt^{-1}) at loop perfusion rate (nl min^{-1})		
	0	40	$\Delta SNGFR_{0-40}$
Control	33·2 ± 7·2 (11)	22·2 ± 3·4 (8)	10·6 ± 8·1 (8)
AB infusion	35·4 ± 6·7 (13)	21·7 ± 7·0 (11)	14·0 ± 8·5 (11)
AVE	43·2 ± 12·0*† (42)	31·4 ± 8·2* (16)	13·8 ± 8·6 (13)

* Indicates a significant difference of AVE values from the AB infusion period ($P \leqslant 0.05$).
† Indicates a significant difference of AVE values from the corresponding AVE values without AB infusion (Table 3) ($P \leqslant 0.05$).

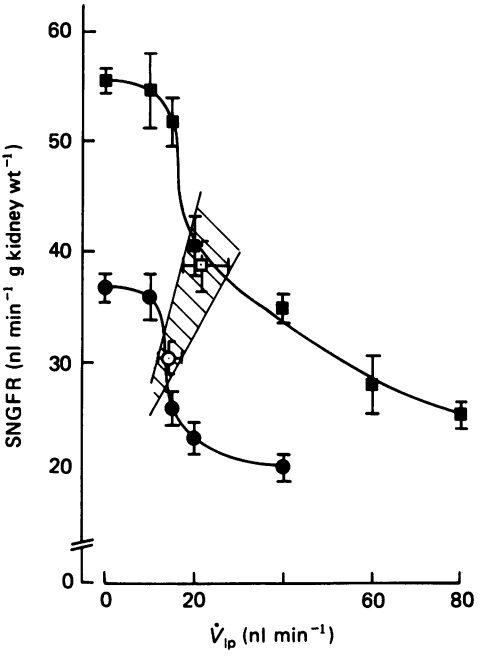

Fig. 2. The effect of late proximal flow rate ($\dot{V}_{lp}$) on nephron filtration rate (SNGFR) measured in the proximal tubule in control rats (●) and in rats after acute volume expansion (■). The open symbols represent the so-called operating point, i.e. those values of late proximal flow and SNGFR existing in the free-flowing nephron with the TGF control loop functioning. The shaded area shows the estimated range within which this value can lie.

significantly, lower at loop perfusion rates of 0 and 40 nl min^{-1}, but not at 80 nl min^{-1}. The mean arterial blood pressure ± S.D. in the 3 h after volume expansion in these animals was 107 ± 6 mmHg ($n = 5$), significantly less than the corresponding value in the animals expanded with native plasma (Table 1). In the group treated with ANP II antibody (Table 4), SNGFR at zero loop perfusion was still increased

significantly after acute volume expansion, but this rise was much less than in the untreated volume-expanded group. At a loop perfusion rate of 40 nl min^{-1} the values were comparable. Infusion of antibodies did not change SNGFR compared with control.

TABLE 5. Summary of correlation coefficients from the linear regression of nephron filtration rate (SNGFR) measured proximally during loop of Henle perfusion at rates of 0, 15–20 or 40 nl min^{-1}, against mean arterial blood pressure in control rats and in rats after acute volume expansion (AVE)

	$SNGFR_0$	$SNGFR_{15-20}$	$SNGFR_{40}$
Control	0·2747*	−0·1626	−0·2332
	(57)	(65)	(35)
Acute volume expansion	0·3396*	0·5205*	−0·0722
	(55)	(50)	(36)

The number of experiments is given in parentheses.
* Indicates a significant correlation ($P \leqslant 0{\cdot}05$).

SNGFR values were also measured at distal puncture sites ($SNGFR_{dist}$) in control and acutely volume-expanded rats. $SNGFR_{dist}$ was significantly higher at $38{\cdot}9 \pm 6{\cdot}8$ nl min^{-1} g kidney wt^{-1} ($n = 9$) in the volume-expanded group than in the control group ($30{\cdot}4 \pm 7{\cdot}5$ nl min^{-1} g kidney wt^{-1}, $n = 14$). The corresponding late proximal flow rates ($22{\cdot}5 \pm 11{\cdot}0$ and $14{\cdot}5 \pm 4{\cdot}0$ nl min^{-1} g kidney wt^{-1}, respectively) were also significantly different. These data are also shown in Fig. 2 and represent the 'operating points' of feed-back control for the two groups. It is obvious that in acutely volume-expanded rats, SNGFR is suppressed to a much greater extent by the tubuloglomerular feed-back mechanism than in control rats.

Table 5 shows the correlation coefficients between mean systemic blood pressure and SNGFR at different loop of Henle perfusion rates in both groups. In control rats the SNGFR with the loop of Henle unperfused correlated significantly with blood pressure; in the volume-expanded rats SNGFR at loop perfusion rates of 0 and 15–20 nl min^{-1} were significantly correlated with blood pressure.

DISCUSSION

The principal result emerging from this study is this: when plasma and interstitial volumes are acutely expanded, to an extent similar to that achieved by dietary salt loading, GFR increases due to changes to the characteristics of the tubuloglomerular feed-back mechanism. These changes are reflected in three distinct alterations in the so-called 'feed-back curve', i.e. in the function relating SNGFR to loop of Henle inflow (see Fig. 2). (i) There is an increase in the range of SNGFR which is controlled by the TGF mechanism. This change results in an *upward shift* of the feed-back curve. (ii) The range within which variation of the loop inflow affects the rate of glomerular filtration increases. This change results in *a shift to the right* of the feed-back curve. (iii) For a given blood pressure and a given loop inflow SNGFR is higher in the expanded than in control rats, although the sensitivity of the TGF mechanism (defined as $\Delta SNGFR/\Delta \dot{V}_{lp}$) at loop inflow rates below 30 nl min^{-1} is similar in both groups.

The following discussion of these changes will centre around three themes or propositions.

Firstly, it is contended that the changes reflected in the alterations to the feed-back curve are responsible for the increase of whole-kidney GFR. This proposition is the result of the following considerations: in a previous study, using the same protocol, a similar TGF response was observed in nephrons, the loops of Henle of which were perfused with Ringer solution, to that observed in nephrons perfused with endogenous tubular fluid (Häberle & Davis, 1986; J. M. Davis, T. Takabatake, T. Kawata & D. A. Häberle, unpublished observations). It can thus be assumed that the feed-back curve obtained with Ringer solution in the present study is a valid representation of the feed-back curves in nephrons perfused by their own tubular fluid. Further, the operating point of the control system *in vivo* (i.e. that combination of SNGFR and loop of Henle inflow which is obtained in the intact, undisturbed nephron and which is estimated from the distally measured SNGFR and the late proximal 'free flow', TF/P_{in}) in both control and volume-expanded animals lies on, or very close to, the experimentally determined 'open-loop' feed-back curve. Finally, the changes in whole-kidney GFR and distally measured SNGFR after volume expansion are entirely comparable. It is, thus, not unreasonable to assume that the functioning of the control system *in vivo* is adequately described by both the open-loop feed-back curve and operating point, experimentally determined in single superficial nephrons, and that the changes reflected in these parameters after volume expansion are responsible for the change in whole-kidney GFR.

The second contention is that the observed changes in feed-back regulation are directly related to the change in extracellular volume and not to any substances – hormones, for instance – contained in the expansion plasma. This is suggested by the fact that the changes in feed-back curves obtained after volume expansion with either Ringer solution–native plasma or Ringer solution–artificial plasma were not substantially different. The reason for the slight difference between the two groups is probably lower mean arterial blood pressure in the group expanded with 'artificial' plasma (see below and Appendix).

The final contention is that the changes in the feed-back regulation after volume expansion are consistent with the assumption that volume expansion dilates the preglomerular resistance vessels (for a description of the functional model underlying this assumption see Appendix). This contention may be deduced from the following observations. Firstly, the change in the feed-back curve cannot be explained by any change in the signal at the macula densa due to variations of fluid and salt reabsorption between the late proximal puncture site and the macula densa, since Persson, Schnermann & Wright (1979*b*) showed that even after massive isotonic volume expansion, fluid and electrolyte reabsorption in the loop of Henle were not different from that in control. It is thus reasonable to assume that, for a given loop of Henle perfusion rate, the NaCl concentration at the macula densa, and hence the stimulating signal, is similar in both groups. To avoid confusion, it should be noted that the following discussion will involve consideration of two 'signals': the first, mentioned above, is the 'tubular (or macula densa) signal', which might be thought as acting on the juxtaglomerular apparatus cells (JGA cells). The second signal is the response generated by these cells, and acting on the blood vessels. This signal might

be termed the 'mediating signal'. Secondly, although, in principle, the shift to the right of the feed-back curve could be due to a volume expansion-induced reduction in the sensitivity of the juxtaglomerular apparatus to a given tubular signal, i.e. a reduction in the mediating signal, or to a reduction of the vascular sensitivity to a constant mediating signal, or both, the following observations indicate that the latter is more likely to be the important mechanism. The key observation for such a proposition is that in unperfused nephrons, i.e. in the *absence* of feed-back control, SNGFR values are massively increased. If one accepts the conventional view that the juxtaglomerular apparatus controls the GFR *only* as suggested above, and not by any additional signals from the blood side, then, considering that the renal blood flow is increased under these circumstances (Ploth, Rudolph, Thomas & Navar, 1978), and that volume expansion is usually accompanied by a decrease of the filtration coefficient (Baylis & Brenner, 1978) rather than by an increase, the above finding implies that the acute expansion of extracellular space must have induced a predominantly preglomerular vasodilatation (see Appendix).

If this conclusion is considered together with the observations noted above, that, firstly, within the normal range of loop of Henle inflows (10–25 nl min^{-1}) a given change in loop inflow results in a similar change of SNGFR in both groups (for a given blood pressure), and secondly, in volume-expanded rats, SNGFR is higher for any given loop inflow than in control rats, the view that volume expansion modifies feed-back control predominantly by changes of the blood vessels is further substantiated. As shown in the Appendix, the feed-back curve obtained in volume-expanded animals can be calculated from the feed-back curve of the control animals, if it is assumed that for any loop of Henle inflow, the diameter of the afferent arteriole (the portion of which, closest to the glomeruli, is believed to be controlled by the TGF mechanism) is increased by a certain constant fraction similar to that calculated for the preglomerular autoregulated segments. Although primarily this could be interpreted in two ways, i.e. that either, for any given loop of Henle inflow, the mediating signal is reduced after acute volume expansion or that the mediating signal is normal, but acts on a dilatated vessel, the latter possibility appears to be more likely. This follows again from the observation that the pre- and postglomerular vessels are apparently massively dilatated in the absence of a tubuloglomerular feed-back signal (see also ANP effects). Consequently it is again suggested that the change in feed-back control and, hence, the increase of GFR, is the result of a vasodilatation mediated by extrarenal parameters and not the result of a change in the mediating feed-back signal produced by the juxtaglomerular apparatus.

This contention is entirely compatible with the changes in atrial natriuretic peptide (ANP) concentrations in blood during acute volume expansion and with the effects of this peptide on renal haemodynamics. When ANP is administered to rats, renal blood flow and GFR increase immediately and dose-dependently (Briggs, Steipe, Schubert & Schnermann, 1982). Indeed, the dose-dependent vasodilatation of the preglomerular vascular resistance segments implicit in that study has recently been observed directly (Marin-Grez, Fleming & Steinhausen, 1986). Furthermore, intravenous administration of ANP results in changes in the TGF response comparable to those seen in the present study, given comparable arterial blood pressures (Briggs *et al.* 1982; Huang & Cogan, 1987). Acute volume expansion is

known to result in an increase in circulation ANP levels (Lang, Thölken, Ganten, Luft, Rushkoaho & Unger, 1985; Hirth *et al.* 1986). If this increase is counteracted by injection of specific monoclonal antibodies against ANP (Hirth *et al.* 1986; John *et al.* 1986), the present study shows that whole-kidney GFR (which represents the GFR controlled by TGF) does not increase at all and the increases in proximally measured SNGFR, in the absence of loop perfusion (and hence in the absence of TGF control) and during maximal stimulation of feed-back (loop perfusion at 40 nl min^{-1}), are much attenuated. Since the antibodies have no effect *per se* on these parameters in non-expanded animals, it is not unreasonable to ascribe the attenuation of the increases in GFR and TGF to the inactivation of ANP by the antibodies and furthermore, since the increase in SNGFR in the absence of TGF control was also greatly attenuated, it is probable that the preglomerular vasodilatation has been prevented by the ANP antibodies. Finally, since ANP reportedly attenuates TGF (Briggs *et al.* 1982; Huang & Cogan, 1987) (probably because of the simultaneous fall in blood pressure, see Appendix) whereas the present study revealed an enhancement of TGF by acute volume expansion, the attenuation of the effects of volume expansion by ANP antibodies is more likely to result from the attenuation of ANP effects on vascular resistance rather than of some speculative effect of ANP on the juxtaglomerular apparatus. Also consistent with this hypothesis are findings in rats chronically volume expanded by dietary salt loading. Under these conditions in which ANP is *not* increased (Luft, Sterzel, Lang, Trabold, Veelken, Ruskoaho, Guo, Ganten & Unger, 1986), SNGFR at zero loop flow is not increased (Häberle, Davis & Kawata, 1986). Since, however, even the relatively high dose of ANP II antibodies did not completely abolish the effect of acute volume expansion on the feed-back curve or renal salt and water excretion, and since the clarification of this problem (i.e. questions of dosage, specificity, extent of interstitial pressure changes after vasodilatation, etc.) is quite beyond the scope of the present investigation, the question, whether or not ANP release in response to acute volume expansion is the sole vasodilatatory mechanism accounting for the observed changes in feed-back regulatory characteristics, must remain open. To summarize the working hypothesis: by increasing ANP secretion acute volume expansion results in a change in the nature of feed-back control which, in turn, leads to an increase in GFR. The change in ANP secretion presumably results from an increase in central venous hydrostatic pressure, and the resultant increase in arterial ANP concentration causes renal vasodilatation, which finally results in a change of the efficacy of the mediating feed-back signal.

As shown in Table 5, SNGFR at zero or at low loop perfusion rates is significantly correlated with mean arterial blood pressure. A similar relationship can be observed when blood pressure is experimentally reduced (Ploth, Schnermann, Dahlheim, Hermle & Schmidmeier, 1977). Since SNGFR values at high loop inflows no longer correlate with systemic blood pressure, a decrease of blood pressure *per se* will result in a decrease of $\Delta SNGFR/\Delta \dot{V}_{loop}$ (Ploth *et al.* 1978). It should be mentioned that such a decrease of blood pressure is one of the specific effects of ANP, and this consideration is particularly relevant when ANP secretion is massively enhanced by a rapid injection of a large saline volume. The apparent decrease of $\Delta SNGFR/\Delta \dot{V}_{loop}$ is further augmented by the shift to the right of the feed-back curve after volume

expansion, i.e. that SNGFR can be reduced still further by loop of Henle perfusion rates which, in normal rats, already maximally stimulate the feed-back mechanism. If the shift to the right and the fall in mean blood pressure are neglected, feed-back curves would be obtained in which TGF control over GFR appears, indeed, to be attenuated or even absent (see Fig. 4). In the experiments of Persson, Müller-Suur & Selén (1979*a*) the influence of net interstitial pressure was studied by perfusing capillary networks of nephrons with surface glomeruli with solutions containing zero, normal or increased concentrations of plasma protein. If, as pointed out in the Appendix, capillary perfusion with colloid-free solutions were to result in a higher capillary hydrostatic pressure (which was not measured in those experiments) than during perfusion with normal solutions, it might be expected that glomerular blood flow would be more depressed under these circumstances, resulting in a reduced feed-back response compared with control. The stimulating effect of 'remnant plasma' upon the TGF response is difficult to interpret in view of lack of information in that study on osmolarity of the remnant plasma (which changes considerably during ultrafiltration due to the Donnan effect).

Thus, although the present findings cannot exclude the possibility that volume expansion may also directly inhibit the juxtaglomerular apparatus, such that for a given loop inflow, the mediating feed-back signal is reduced, the present hypothesis of preglomerular vasodilatation may explain all the experimental results upon which the alternative hypothesis (Persson *et al.* 1979*a*, *b*; Persson, Boberg, Hahne, Müller-Suur, Norlén & Selén, 1982) is based, *plus* the observations in this and a number of other studies, that acute volume expansion massively increases SNGFR in the *absence* of any tubuloglomerular feed-back signal, and further, that this increase depends upon the mean arterial blood pressure. Since the latter phenomena cannot be explained by the alternative hypothesis, the present explanation for the increase of GFR after acute volume expansion in terms of preglomerular vasodilatation leading secondarily to a change in the expression of feed-back control appears more plausible.

APPENDIX

The proposition that acute volume expansion influences TGF control of GFR by dilatation of preglomerular resistance vessels necessarily presupposes that such vessels exist and that these resistance vessels control renal blood flow by a mechanism different from TGF. The following evidence strongly supports this view: (i) In nephrons with unperfused loops of Henle, glomerular capillary pressure, estimated from stop-flow pressure, is, at the most, about 60 mmHg (for literature see Schnermann & Briggs, 1985). This pressure is partly (Moore, 1984) or even completely (Gerz, Mangos, Braun & Pagel, 1966) autoregulated, demonstrating that preglomerular resistance vessel autoregulation indeed exists. (ii) In micropuncture studies on aberrant interlobular arteries on the renal surface (Tønder & Aukland, 1979; BokNam, Ericson, Åberg & Ulfendahl, 1981), autoregulated resistance control was found in vascular segments even further upstream from the interlobular artery. This finding is consistent with direct observations of the renal vascular bed in the hydronephrotic kidney (Steinhausen, Blum, Dussel, Endlich & Parekh, 1986) which show autoregulatory behaviour in the arcuate and interlobular arteries and afferent

arteriole. (iii) Although TGF in rats kept on a high-NaCl diet is completely inhibited by a factor in tubular fluid (Häberle & Davis, 1982, 1984; T. Kawata, J. M. Davis & D. A. Häberle, unpublished observations), essentially normal autoregulation of GFR and renal blood flow is observed (T. Kawata *et al.*, unpublished observations; Arendhorst & Finn, 1977).

If, however, an independently regulated (autoregulated) resistance system were to exist *in series* with the tubuloglomerular feed-back system, a degree of interdependence between both systems can be predicted from the elementary laws relating flow and pressures in serial resistances in the case of a perturbation in one or the other of the systems. In this Appendix, some of the regulatory characteristics of this system will be deduced from the elementary principles and the consistency of these predictions with experimental observations in the literature shown. In addition, it will be demonstrated that the conclusions drawn from our experimental observations are, at least semiquantitatively, entirely compatible with the predictions of this model for the case of an acute volume expansion dilatating the preglomerular vascular resistances.

Model calculations

(*a*) *Abbreviations*

P_{gc}	glomerular capillary pressure
P_t	hydrostatic pressure in the Bowmann space
P_{ra}	mean hydrostatic pressure in the renal artery
P_v	mean hydrostatic pressure in the renal vein
P_{int}	interstitial net pressure
P_{aa}	hydrostatic pressure in the afferent arteriole
C_{prot}	protein concentration in the arterial blood
Hct	haematocrit fraction
RBF	renal blood flow
SNGFR	single-nephron glomerular filtration rate
GFR	glomerular filtration rate (whole kidney)

(*b*) *Components of the model*

As shown by studies of Källskog, Lindbom, Ulfendahl & Wolgast (1976), Tønder & Aukland (1979) and BokNam *et al.* (1981), blood pressure in the interlobular artery is about 70 mmHg, and that in the glomerular capillaries some 45–50 mmHg; in the star vessels it is about 20 mmHg and in the peritubular capillaries 8–10 mmHg. As outlined above, the resistance vessels responsible for the reduction of blood pressure from that in the renal artery to that in the glomerular capillary, appear to be autoregulated. From these experimental observations the following scheme (Fig. 3) can be designed in which the resistors R_1–R_3 are autoregulated. Resistance R_4 represents that portion of the afferent arteriole under the control of the tubuloglomerular feed-back mechanism (Schnermann, Briggs, Kriz, Moore & Wright, 1980). The resistances R_5 and R_6 are assumed not to be specifically controlled (Andreucci, Dal Canton, Corradi, Stanziale & Migone, 1976). It must be noted that the model neglects the reduction inflow due to glomerular filtration and hence R_5 will

be underestimated. However, providing the changes in filtration fraction remain small, as appears to be the case for such GFR variations normally achieved by variations of the TGF, this error remains relatively constant and can be neglected. It is assumed that elimination of the tubular signal (no flow through the loop of Henle) causes a decrease of R_4, and that TGF stimulation increases that resistance

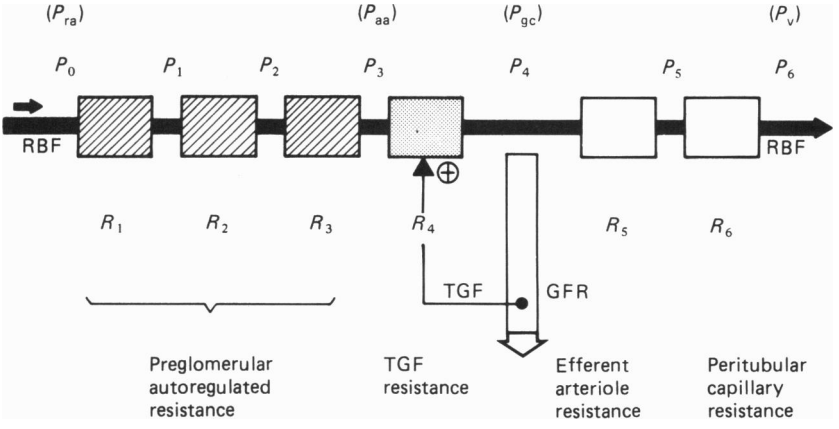

Fig. 3. Schematic representation of the model employed in the present study. The rectangles represent the various resistance segments between the renal artery and renal vein. Those hatched are considered to show myogenic autoregulatory behaviour, R_4 is the resistance under control of the tubuloglomerular feed-back mechanism, and R_5 and R_6 are considered not to show any active regulatory behaviour. P_{ra} and P_v represent the pressures in the renal artery and vein respectively. See text for detailed description.

(Schnermann *et al.* 1980). The magnitude of the different resistances (groups) can be calculated from normal renal blood flow from the relationship below, if the following assumptions are made: that the hydrostatic pressures reported by Källskog *et al.* (1976) and BokNam *et al.* (1981) are representative for the entire nephron population within a 'normal' kidney, that nephron density is 30000 g kidney wt^{-1} (Kittelson, 1917), and that the different pre- and postglomerular resistances, divided by the number of nephrons, represent the respective pre- and postglomerular resistances for an individual nephron.

$$\sum_{R_1}^{R_6} R\ \mathrm{RBF} = P_{ra} - P_v, \tag{1}$$

where $\Sigma_{R_1}^{R_6} R$ represents the total renal vascular resistance.

$$P_{ra} - \sum_{R_1}^{R_3} R\ \mathrm{RBF} = P_{aa}\ \text{(condition of preglomerular autoregulation)}, \tag{2}$$

where $\Sigma_{R_1}^{R_3} R$ is the preglomerular vascular resistance; excluding the TGF-controlled resistance:

$$\sum_{R_4}^{R_6} R\ \mathrm{RBF} = P_{aa} - P_v. \tag{3}$$

If the pressure gradient across the kidney ($P_{ra} - P_v$) and the RBF are known, the total renal resistance can be calculated (eqn (1)). Further, if the autoregulated pressure drop ($P_{ra} - P_{aa}$) is known, the total preglomerular resistance can be calculated. It is assumed, as suggested by the autoregulation theory of Johnson

(1980), that each of the autoregulating resistances tends to keep the pressure drop across itself stable. Thus the total preglomerular resistance can be arbitrarily divided into resistance segments R_1–R_3 with appropriate numbers. If, further, the glomerular capillary pressure under 'normal' free-flow conditions is known, the resistance R_4 can be calculated as:

$$P_{gc} = P_{aa} - (R_4 \text{ RBF}). \quad (4)$$

(c) *The effect of a variation of the tubuloglomerular feed-back mechanism*

Since changes in the P_{gc} on maximal activation or inactivation of TGF are known (Persson, Gushwa & Blantz, 1984) and since it is also known that even under such conditions, renal autoregulation is preserved, for instance during the administration of acetazolamide or furosemide (Duchin, Peterson & Burke, 1977), the values for the different resistances can be calculated.

Given the values below and assuming that the plasma flow in a single glomerulus is RBF $(1-\text{Hct})/30000$, SNGFR can be calculated from the filtration model of Deen, Robertson & Brenner (1972). Table 6 and Fig. 4*A* represent the results of such a calculation:

RBF	7 ml min^{-1} g kidney wt^{-1}
C_{prot}	5·45 g% (from Table 1)
P_{aa}	70 mmHg
P_{ra}	120 mmHg
P_v	5 mmHg
P_{gc}	48, 53 or 40 mmHg
Hct	0·48
P_{tub}	10 mmHg
P_{int}	0
K_f	0·035 nl s^{-1} $mmHg^{-1}$

TABLE 6. Renal haemodynamics in control rats with TGF unstimulated (TGF_0), maximally stimulated (TGF_{max}) or spontaneously regulating (TGF_{cl})

Pressures...		P_1		P_2		P_3		P_4		P_5				
Resistances...	R_1		R_2		R_3		R_4		R_5		R_6	RBF*	SNGFR†	GFR*
		80		80		70		48		8				
TGF_{cl}	4·30		1·43		1·43		3·15		5·71		0·43	7·00	32	0·96
		90		80		70		40		8				
TGF_{max}	5·26		1·75		1·27		5·26		5·71		0·43	5·70	17	0·51
		90		80		70		53		8				
TGF_0	3·82		1·27		1·27		2·14		5·71		0·43	7·85	40	1·21

* ml min^{-1}; † nl min^{-1}.

In this model, K_f is assigned the value of 0·035 nl s^{-1} $mmHg^{-1}$, i.e. about 50% of the value estimated by Brenner and colleagues in a series of studies (Baylis & Brenner, 1978). However, it is contended that that K_f value is overestimated because of a permanent systematic error inherent in its method of determination. In that method, P_{gc}, P_t and filtration fraction are determined in free-flowing nephrons (i.e. with the TGF control loop intact). SNGFR, however, is determined by quantitative proximal

collection, i.e. with the TGF control abolished. Thus, assuming TGF does, in fact, influence P_{gc}, K_f is calculated from a P_{gc} which is artifactually too low (or, conversely, from an SNGFR which is too high). The following example illustrates this. If the values from the above calculations of renal haemodynamics (in a free-flowing kidney, with a normal TGF control) are combined with a K_f value calculated as above for 'normal hydropenic' kidneys (0·07 nl s^{-1} $mmHg^{-1}$) an SNGFR of 43 nl min^{-1} is obtained, a value typical of conditions in which TGF control has been abolished.

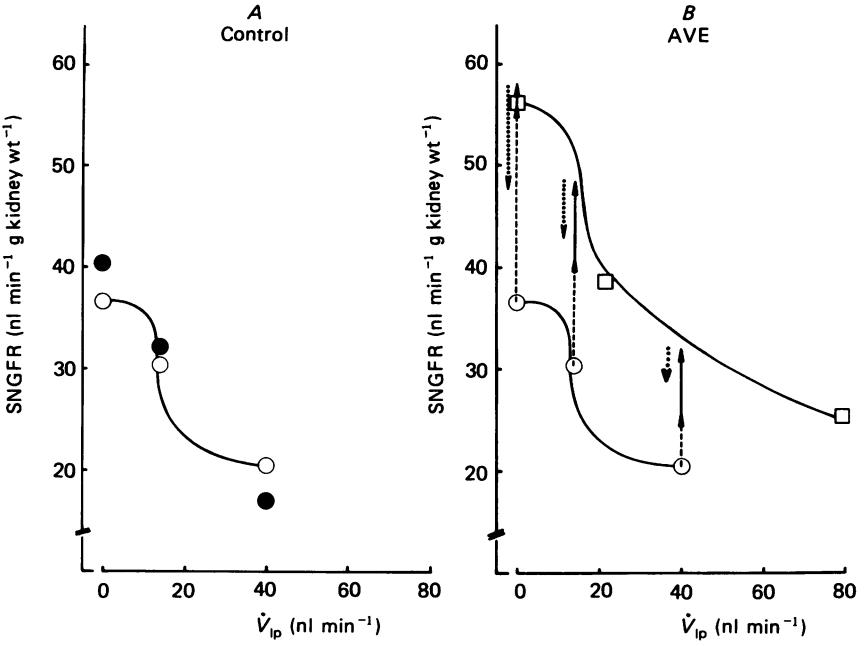

Fig. 4. Results of model calculation. *A*, the curve is the measured feed-back curve under control conditions (Fig. 2). The closed circles are, from left to right, the values for nephron filtration rate (SNGFR) predicted by the model for loop of Henle perfusion rates of 0 (open control loop), the normal *in vivo* rate and 40 nl min^{-1} (TGF maximally stimulated, respectively). *B*, the effect of acute volume expansion (AVE). The additional curve (□) is the feed-back curve after AVE (Fig. 2). The dashed lines show the predicted changes in SNGFR (at the given loop of Henle perfusion rates) from the measured control values assuming that AVE has resulted in a preglomerular vasodilatation which does *not* include the TGF-controlled resistance (i.e. R_1 to R_3 only). The continuous line shows the *additional* effect of assuming that the TGF-controlled resistance (R_4) also dilates after AVE. The dotted line shows the effect of a fall in mean arterial blood pressure from 120 to 100 mmHg, beginning from the values achieved after the foregoing manoeuvres.

(*d*) *Acute volume expansion*

Under conditions of acute volume expansion, renal interstitial hydrostatic pressure increases. Although in this study net interstitial pressure was not measured, the increase can be estimated to be of the order of 6 mmHg (Blantz & Tucker, 1975). According to autoregulation theory, the following equation (eqn (5)) describes the dependence of the wall tension T and the radius r of an autoregulated vessel on the

transmural pressure P. The subscripts 1 and 2 refer to the experimental conditions compared.

$$\frac{T_2}{T_1} = \frac{G(P_1 - P_2) + P_2}{P_1} = \frac{P_2 r_2}{P_1 r_1}. \tag{5}$$

G is a coefficient expressing the degree of 'completeness' of autoregulation (1 = complete autoregulation, 0 = no autoregulation). From this equation it is possible to calculate the normalized increase in the radius of the resistance vessels R_1, R_2 and R_3 (1·04, 1·06 and 1·07 respectively for the above change in interstitial pressure, if it

TABLE 7. Renal haemodynamics in acutely volume-expanded rats with preglomerular autoregulation of blood flow, increased interstitial hydrostatic pressure, and TGF either unstimulated (TGF_0), maximally stimulated (TGF_{max}) or spontaneously regulating (TGF_{cl})

Pressures...		P_1		P_2		P_3		P_4		P_5				
Resistances...	R_1		R_2		R_3		R_4		R_5		R_6	RBF*	SNGFR†	GFR*
		93		85		77		52		—				
TGF_{cl}	3·48		1·09		1·04		3·15		5·71		0·43	7·72	41	1·22
		93		85		77		58		—				
TFG_0	3·09		0·97		0·93		2·14		5·71		0·43	8·76	56	1·70
		93		85		77		44		—				
TGF_{max}	4·26		1·33		1·28		5·26		5·71		0·43	6·29	25	0·75

* ml min^{-1}; † nl min^{-1}.

is assumed that perfect autoregulation is maintained). Since the resistance of a vessel is related to its radius according to eqn (6),

$$R = K\frac{1}{r^4}, \tag{6}$$

the relative changes in the resistances R_1, R_2 and R_3 can be calculated to be 0·85, 0·79 and 0·76 of the control values respectively. Table 7 and Fig. 4*B* (dashed lines) give the results, assuming the following values:

P_{ra}	120 mmHg
P_v	5 mmHg
P_{int}	6 mmHg
P_t	13 mmHg
C_{prot}	5 g% (Table 1)
Hct	0·42 (see Results)

Although with these assumptions (increase of interstitial pressure and an appropriate dilatation in the autoregulated vessels) the model yields SNGFRs very close to those measured after acute volume expansion in the present study, these calculations do not provide a satisfactory explanation for the massive shift of the feed-back curve to the right (i.e. that to achieve a given SNGFR, a much higher loop inflow is required).

As pointed out in the discussion, ANP has been observed to dilatate not only the preglomerular autoregulated vascular segments but also the entire afferent arteriole

(Marin-Grez *et al.* 1986). In that study the fractional dilatation was similar in all preglomerular vessels studied. In the calculation shown in Table 8 and Fig. 4*B* (continuous lines) it is assumed that the TGF-controlled afferent arteriolar segment (R_4) is *also* dilatated similarly to R_3, and it is seen that the upward shift of SNGFR for a given loop inflow can indeed be imitated.

The remaining numerical assumptions for calculation of SNGFR are those in the previous calculations (Table 7).

TABLE 8. Renal haemodynamics in acutely volume-expanded rats with *all* preglomerular resistances dilated and TGF either inactivated (TGF_0) or activated by loop perfusion at 18 nl min^{-1} (TGF_{18}) or 40 nl min^{-1} (TGF_{40})

Pressures...		P_1		P_2		P_3		P_4		P_5				
Resistances...	R_1		R_2		R_3		R_4		R_5		R_6	RBF*	SNGFR†	GFR*
		93		85		77		62		—				
TGF_0	2·90		0·91		0·86		1·63		5·71		0·43	9·24	58	1·73
		93		85		77		58		—				
TGF_{18}	3·20		1·00		0·95		2·40		5·71		0·43	8·41	48	1·45
		93		85		77		48		—				
TGF_{40}	3·80		1·20		1·13		4·00		5·71		0·43	7·07	34	1·00

* ml min^{-1}; † nl min^{-1}.

(*e*) *Blood pressure dependence*

As predicted by Ohm's law and demonstrated by the above examples, any change of the TGF-controlled resistance (or of the other preglomerular resistances) will, because of its (their) contribution to total renal resistance, result in a change in RBF and thus in a co-operative change of the preglomerular autoregulated resistances. Thus any response to a change of the tubular signal results from a *local* resistance change in the TGF segment of the afferent arteriole and an additional change of the autoregulated preglomerular resistances. Since, on the other hand, variations of the systemic blood pressure affect the state of the autoregulated preglomerular resistances, it can be expected that the feed-back response will depend on blood pressure. For example, at low blood pressure elimination of the TGF signal should result in a smaller increase of SNGFR than at high blood pressure. This interdependence can also be simulated with the present model. Since it is known that, under 'normal' conditions, RBF and GFR in the rat fall when blood pressure is reduced below levels of about 90 mmHg or even 100 mmHg (Navar, Bell & Burke, 1982) it can be calculated that the ability of the preglomerular autoregulated resistance vessels to dilate is exhausted at a sum value of about 4·5 resistance units. If one assumes that acute volume expansion does not affect the intrinsic autoregulative properties of these vessels, one has to conclude that in a vasodilatated kidney, as modelled above, the autoregulatory capacity is exhausted at blood pressures higher than in control conditions and that this point of exhaustion is shifted to lower blood pressures when TGF is activated. This is demonstrated in Table 9 and Fig. 4*B*.

It should be noted that for the example given in Table 7, without an appropriate

feed-back adjustment the end-point of autoregulation would be 118 mmHg for the unperfused loop of Henle and 115 mmHg for a fixed loop perfusion (14 nl min^{-1}). If, in the latter case, TGF is allowed to compensate for the decrease of GFR due to the exhaustion of autoregulation, then that end-point would be shifted, assuming a high gain, to 108 mmHg.

TABLE 9. Renal haemodynamics in acutely volume-expanded rats at reduced systemic blood pressure (100 mmHg) with TGF inactive (TGF_0) or activated by loop of Henle perfusion rates of either 14 nl min^{-1} (TGF_{14}) or 40 nl min^{-1} (TGF_{40})

Pressures...	P_1	P_2	P_3	P_4	P_5				
Resistances...	R_1	R_2	R_3	R_4	R_5	R_6	RBF*	SNGFR†	GFR*
	4·50		65	1·63	53	5·71	—	0·43	7·74	43	1·27
TGF_0											
	4·50		67	2·40	50	5·71	—	0·43	7·30	37	1·12
TGF_{14}											
	4·50		71	4·00	45	5·71	—	0·43	6·50	29	0·86
TGF_{40}											

The numerical assumptions are those used for the example in Table 7. * ml min^{-1}; † nl min^{-1}.

TABLE 10. Haemodynamics in kidneys with increased postglomerular capillary pressure and TGF either unstimulated (TGF_0) or maximally stimulated (TGF_{max})

Pressures...	P_1	P_2	P_3	P_4	P_5	RBF
Resistances...	R_1	R_2	R_3	R_4	R_5	R_6 (ml min^{-1})
	7·83		70	2·14	56	5·71	20	—	6·40
TGF_0									
	8·00		—	5·26	50	5·71	20	—	5·27
TGF_{max}									

(f) Consistency of this model with the 'interstitial pressure' hypothesis

In the experiments of Persson *et al.* (1979*a*), peritubular capillaries were perfused at high rates with different solutions and the effect of this perfusion upon the tubuloglomerular feed-back, assessed by the measurement of stop-flow pressure, studied. If it were assumed that the upper autoregulating end-point in the rat is about 140 mmHg then, from the data in Table 6, it follows that the maximum value the autoregulated preglomerular resistances can achieve is 8 resistance units (at a maximum value of R_4 of 5·26 and an RBF of 7 ml min^{-1} g kidney wt^{-1}). If it were further assumed that, during the perfusion of the peritubular capillaries with Ringer solution, intracapillary pressures of 20 mmHg were achieved (Sato, 1974) the haemodynamic profile in Table 10 can be calculated for a systemic blood pressure of 120 mmHg.

This example shows clearly that a rise in 'backpressure' and the consequent effects of this manoeuvre upon RBF and the preglomerular autoregulated resistance can yield an apparent 'resetting' of the feed-back response (ΔP_{gc} of 6·2 mmHg compared with 13 mmHg under control conditions, Table 6). If the above haemodynamic data are used to calculate SNGFR in the absence of a TGF signal a value of about 71% of control is obtained, a finding consistent with observations in an early study

employing similar capillary perfusion (Bank, Aynedjian & Wada, 1972). If it were assumed that perfusion with colloid-free solutions required higher flows (due to the losses of fluid into the renal interstitial space) than with normal plasma, such differences might account for the differences in TGF response during the application of the different solutions.

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