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Urinary kallikrein excretion during inhibition of endogenous angiotensin II in the pig

Bernd R. Binder, Manfred Maier[1], Hemanta Rana, Michael Starlinger* & Zydi Zhegu[2]

Laboratory for Clinical-Experimental Physiology, Department of Medical Physiology and I. Department of Surgery*, University of Vienna, Austria

1 This study was performed to assess the possible contribution of endogenous angiotensin II (AII) to the regulation of urinary kallikrein excretion. The AII antagonist saralasin or the saline vehicle was infused into the aorta above the renal arteries of pigs under halothane-O_2/N_2O anaesthesia. Systemic and renal functional parameters were followed for 140 min and during stimulation of the renin-angiotensin system by haemorrhage.

2 Urinary kallikrein excretion, determined as kininogenase activity, was increased immediately upon both initiation and termination of the 2 h saralasin infusion into pigs not subjected to haemorrhage. Renal cortical blood flow (RCBF) was maintained in both saline and saralasin-treated animals at blood pressures as low as 70 mm Hg, while glomerular filtration rate was dissociated during saralasin infusion. As long as RCBF was maintained, urinary kallikrein excretion rate was elevated during the progressive hypotension in both saline and saralasin-treated animals.

3 These findings confirm a close relationship between the maintenance of RCBF and increased activity of the kallikrein-kinin system whether or not AII is antagonized, and indicate that during haemorrhage the kallikrein-kinin system is stimulated by a mechanism not involving AII.

Introduction

The enzyme urinary kallikrein belongs to a group of serine proteases, termed glandular kallikreins, that release potent vasodilator peptides, kinins, from plasma kininogens (Maier *et al.*, 1983). Glandular kallikreins (tissue kallikreins) are found in a number of organs including the kidney, pancreas, and salivary glands and in their secretions. They are structurally, functionally and antigenically different from plasma kallikrein but related to each other. A glandular kallikrein has also been identified in human and pig plasma, suggesting its release into the circulation *in vivo* (Fink *et al.*, 1980; Scicli *et al.*, 1983). Most of the effects of renal kallikrein seem to be mediated via kinin release, although recent evidence suggests that the tissue-specific role of glandular kallikreins might be unrelated to their kininogenase activities and instead determined by their different substrate specificities (Mason *et al.*, 1983).

Renal kallikrein is synthesized by the kidney (Nustad *et al.*, 1975) and has been found to be released into the urine and perfusion fluid of isolated perfused kidneys of the rat (Misumi *et al.*, 1983; Roblero *et al.*, 1976). Urinary kallikrein output has been shown to reflect kallikrein release from the kidney (Van Leeuwen *et al.*, 1984). However, the mechanisms regulating renal kallikrein release are not well understood and appear to be different from control mechanisms in other organs (Van Leeuwen *et al.*, 1984).

Kallikrein excretion can be stimulated by a variety of manoeuvres such as increased perfusion pressure in the rat isolated perfused kidney (Misumi *et al.*, 1983), noradrenaline infusion (Mills *et al.*, 1978), aldosterone administration (Colina-Chourio *et al.*, 1978), infusion of vasodilating and vasoconstricting substances including angiotensin II (AII; Mills *et al.*, 1978) and also by haemorrhage (Maier *et al.*, 1981). Kallikrein activates prorenin *in vitro* (Sealey *et al.*, 1978) and angiotensin converting enzyme was found to be identical to kininase II, which efficiently inactivates kinins (Yang *et al.*, 1971). Consequently, inhibition of the converting enzyme has effects on the renal vasculature which are independent of the renin-angiotensin-system (RAS) and are probably mediated through changes in tissue kallikrein-kinin levels in the kidney (Johnston *et al.*, 1980).

[1]Author for correspondence.
[2]Present address: University of Tirana, Albania.

The vasoconstricting RAS is, therefore, in several ways linked to the vasodilator kallikrein-kinin-system and it has even been suggested that these two vasoactive systems might be regulated simultaneously (Misumi *et al.*, 1983). In our previous studies on haemorrhagic hypotension in pigs we found increased excretion of urinary kallikrein within the arterial pressure range of 100 to 70 mm Hg, which corresponded to the relative maintenance of renal cortical blood flow (RCBF) and glomerular filtration rate (GFR) (Maier *et al.*, 1981). Since the increase in kallikrein excretion during haemorrhagic hypotension was always accompanied by increased plasma renin activity, it is possible that the observed kallikrein increase was a consequence of elevated endogenous AII induced by haemorrhage. Based upon these facts it was the aim of the present study to assess the possible contribution of AII to the stimulation of kallikrein release (Mills *et al.*, 1978) during haemorrhagic hypotension by directly inhibiting the effects of AII with saralasin.

Preliminary results were presented in part at the Meeting of the Federation of American Societies for Experimental Biology, Atlanta, Georgia, 1981 and at the Symposium on Renal Blood Flow, Sodium and Hypertension, Dunedin, New Zealand, 1983.

Methods

Experimental protocol

Experiments were carried out on 28 pigs (4 groups, each of 7 animals; 24–31 kg body weight) fed a normal diet as described previously (Maier *et al.*, 1985). Anaesthesia was induced with sodium thiopentone (Pentothal, 30 mg kg^{-1}, i.v.). An endotracheal tube was inserted and the animals were allowed to breathe spontaneously a halothane-O_2/N_2O-mixture (0.7%, 29.3%, 70%). Catheters were inserted via the left carotid artery into the left ventricle (French No. 5, pig tail), into the left femoral artery, into the left femoral vein, and into the right femoral artery (polyethylene catheters i.d. 1.5 mm, o.d. 2 mm). The tip of the latter was placed above the renal arteries and the correct position of the catheter was checked after each experiment. The catheter from the left femoral artery was connected to a Statham transducer (Statham Instruments, Inc., Oxnard, CA.) for continuous recording of the mean arterial blood pressure (MAP; Gould Brush polygraph, Gould Medicals GesmbH., Düsseldorf, FRG). Polyethylene catheters (i.d. 0.9 mm) were also inserted via a small suprapubic incision into both ureters and 5 min combined urine samples were collected; the overall dead space in both catheters was less than 150 µl. The surgical procedure was followed by a 60 min stabilization period.

Experimental groups

Infusion of saralasin ((Sar^1-Val^5-Ala^8)-AII)or the saline vehicle combined with haemorrhagic hypotension After the stabilization period, the two groups of seven animals each received a continuous infusion of either saralasin or the saline vehicle. Saralasin (Sarenin, Röhm Pharma, Darmstadt) was dissolved in physiological saline (2 mg saralasin in 50 ml of 0.9% w/v NaCl solution). The freshly prepared solution or the saline vehicle alone was infused through the right femoral artery catheter into the aorta above the renal arteries at a constant rate of 0.8 ml min^{-1} (approx. 1 µg kg^{-1} min^{-1}) by means of a Harvard pump (Harvard Apparatus Co., Inc., Millis, MA.). No agonistic vasoconstrictor effects on systemic or renal haemodynamics were observed and the amount of saralasin used was sufficient, in separate experiments, to block completely the effects on systemic and renal hemodynamics of a bolus injection of AII (Hypertensin-Ciba, Ciba-Geigy Ltd Basle, Switzerland; 0.5 µg in 0.1 ml saline) into the aorta. Sixty minutes after the infusion was started (control period) hypovolaemia was induced by continuous bleeding from the carotid artery at a rate of 1 ml kg^{-1} min^{-1} until the death of the animal. MAP was allowed to stabilize at 100, 90, 80, 70 and 60 mm Hg for 2 to 3 min to obtain blood samples and to inject microspheres as outlined below.

Infusion of saralasin or the saline vehicle without haemorrhagic hypotension After the stabilization period two groups of seven animals were infused with either saralasin or the saline vehicle for 120 min as described above except that haemorrhagic hypotension was not induced. The infusions were then stopped and after an additional 20 min (total duration of experiment 140 min) the animals were killed.

Measurements

Twenty minutes before the end of the stabilization period 0.5 ml [^{3}H]-inulin (The Radiochemical Centre, Amersham, Buckinghamshire, U.K) dissolved in isotonic saline (approx. 5.5×10^7 d.p.m.) was given as a single bolus injection into the left femoral vein. The first blood sample was taken and divided urine collection begun at the end of the stabilization period. The infusion of either saralasin or the saline vehicle was then started and samples (5 ml) from the left femoral vein were taken every 20 min. In haemorrhaged animals the first blood sample was taken and divided urine collection was started after 60 min infusion of either saralasin or vehicle (at the end of the control period). Further samples were taken after every 10 mm Hg decrease in mean arterial pressure during bleeding. Plasma sodium (Eppendorf flame photometer, Eppendorf Gerätebau, Hamburg, FRG),

[^{3}H]-inulin (Beckman liquid scintillation counter LS 7500, Beckman Instruments, Inc., Irvine, CA.) and plasma renin activity were determined as indicated. Urine volumes were measured and concentrations of sodium, [^{3}H]-inulin and urinary kallikrein determined. From these data excretion rates of Na^+ and urinary kallikrein as well as values for GFR could be calculated.

Urinary kallakrein was determined as kinin generating activity (KGA) after incubation of urine samples with an excess of heat inactivated pig plasma for 5 min and assayed on the guinea-pig ileum preparation, as described in detail elsewhere (Maier *et al.*, 1985). The contractile response of the smooth muscle preparation was quantitated by comparison with a synthetic bradykinin standard (Bk; Sigma Chemical Co., St. Louis, MO.). Since 98.5% to 102.1% of bradykinin standards incubated at 37°C for 5 min with urine samples obtained at five different time points from each experiment could be recovered, no inhibitors of kininases were used in the bioassay. Only substrate-dependent activities were measured and expressed as μg Bk equivalents. Plasma renin activity (PRA) was measured by radioimmunoassay of angiotensin I generated after incubation of 0.1 ml of plasma sample for one hour at 37°C using an assay kit from New England Nuclear (Boston, MA.).

Renal cortical blood flow (RCBF) was evaluated in all pigs by means of radioactively labelled microspheres (15μm size, 3M Company, St. Paul, MN) as previously described in detail (Maier *et al.*, 1981). RCBF was determined at the end of the control period and at 100, 90, 80, 70, and 60 mm Hg during bleeding experiments and is expressed as ml g^{-1} kidney wet weight min^{-1}. In pigs not subjected to haemorrhagic hypotension the measurements were done at the end of the stabilization period and every 20 min thereafter. In each individual experiment the particular time point for the three possible injections of ^{51}Cr, ^{141}Ce and ^{85}Sr labelled microspheres was selected so that in each experimental group of seven animals a minimum of 3 measurements per observation period (control period, 100, 90, 80, 70, 60 mmHg or stabilization period, 20, 40, 60, 120, 140 min) could be used to calculate mean values for RCBF. At the end of the experiments both kidneys were removed and the renal cortex was macroscopically dissected from the medulla. Six specimens from the renal cortex were cut out, weighed and counted separately for their respective radioactivities.

Calculations

Both kidneys from each animal were weighed and GFR expressed as ml g^{-1} kidney min^{-1}. The values obtained were used to calculate the ratio of GFR/RCBF as an indication of the respective filtration fractions (FF). Renal cortical resistance was determined as MAP/RCBF (mm Hg ml^{-1} min^{-1}) and the blood loss of each animal was calculated as ml per kg body weight.

Statistical methods

All values for a parameter obtained during one particular observation period of each experimental group were used to calculate the mean and s.e.mean for that period. The data presented are from 14 saralasin-treated animals compared to 14 saline vehicle-treated animals. The data on saline-treated animals have been published previously, since the same saline vehicle was used under identical conditions for the serine protease inhibitor aprotinin (Maier *et al.*, 1985). Both studies were conducted at the same time as part of a large research programme and the number of experiments as well as the experimental procedure in every detail was the same. However, for convenient comparison the data are again presented in Tables 1 and 2 and in the legend to Figure 1. Statistical significance was evaluated by comparing saralasin versus vehicle using analysis of variance and by comparing saralasin versus vehicle at points indicated in the tables and figures with the Bonferroni-test (Glantz, 1981) using a FCM computer programme (Vienna, Austria). Significance was assumed at P values of less than 0.05.

Results

Infusion of saralasin or the saline vehicle without haemorrhagic hypotension

Compared to the control group of animals receiving an infusion of saline (Maier *et al.*, 1985), infusion of saralasin induced significant changes which are shown in Figure 1 and Table 1. Although urine flow was significantly reduced, the rate of kallikrein excretion more than doubled within the first 20 min of saralasin infusion from 7.8 ± 0.88 to 18.7 ± 4.16 μg Bk equivalents and returned to control values thereafter. PRA was slightly but significantly increased at 40 min and also returned to control levels thereafter. During the first 40 min GFR decreased significantly despite only small variations in RCBF, resulting in a reduced filtration fraction. While GFR as well as FF returned towards control values thereafter, a tendency for RCBF to increase and for renal cortical resistance to decrease was observed. In addition, infusion of saralasin caused a reduction in MAP and in urinary sodium excretion and an increase in heart rate from 135 ± 6 to 152 ± 9 beats min^{-1}. After the infusion of saralasin was stopped, heart rate, MAP, RCBF and renal resistance returned towards control values

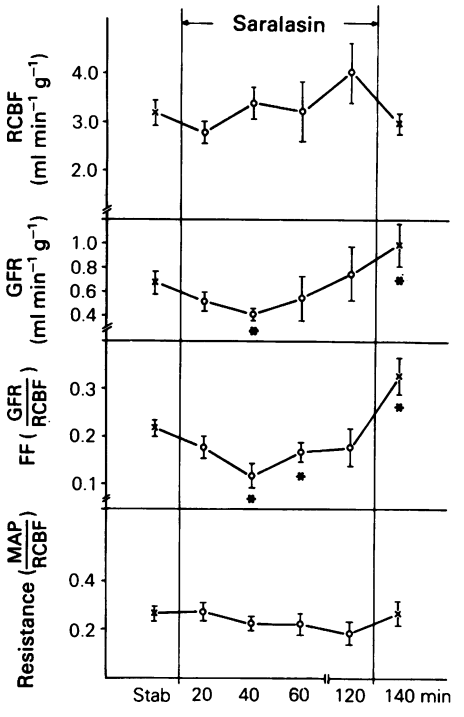

Figure 1 Pattern of renal haemodynamic parameters during infusion of saralasin in pigs without haemorrhagic hypotension ($n = 7$). Mean values for renal cortical blood flow (RCBF), glomerular filtration rate (GFR, §), filtration fraction (FF, §) and renal resistance are shown with vertical lines indicating s.e.mean. Significant differences as compared to the control group receiving an infusion of saline (Maier *et al.*, 1985) are indicated by an asterisk (*; Bonferroni-test) and by § (analysis of variance). Stab: stabilization period. During infusion of saline RCBF remained constant at 3.3 ± 0.5 ml min^{-1} and GFR at 0.7 ± 0.08 ml min^{-1} g^{-1}.

within 20 min while GFR, FF, urinary sodium excretion, urine flow, PRA and urinary kallikrein were significantly increased.

Infusion of saralasin or the saline vehicle during haemorrhagic hypotension

Similar to the bleeding experiments with infusion of saline (Maier *et al.*, 1985), the rate of urinary kallikrein excretion was elevated during infusion of saralasin at a MAP of 90 and 80 mm Hg (Table 2). However, it did not show the peak increase observed in saline treated animals. As seen in Figure 2b, infusion of saralasin during the bleeding experiments did not influence the pattern of RCBF between 100 and 70 mm Hg. At a MAP of 60 mm Hg RCBF was significantly higher than with infusion of saline (Figure 2a; Maier *et al.*, 1985). Consequently, renal resistance slightly declined between 100 and 70 mm Hg and did not show the dramatic increase between 70 and 60 mm Hg observed in saline-treated animals. In contrast, GFR and FF continuously declined despite the maintenance of RCBF. Sodium excretion in saralasin-treated animals was different only in the control period and at a MAP of 60 mm Hg from that in saline-treated pigs. Heart rate increased from a mean value of 150.6 to 178.0 beats min^{-1} and PRA continuously increased throughout the period of haemorrhagic hypotension, similar to saline-treated animals. The mean blood loss per kg body weight was significantly lower at each level of MAP in saralasin-treated animals and amounted to 19.6 ± 1.8 ml kg^{-1} at the end of the experiments as compared to 28.38 ± 2.09 ml kg^{-1} in saline-treated animals.

Discussion

We have investigated whether the increase in urinary kallikrein excretion during haemorrhagic hypotension previously observed (Maier *et al.*, 1981) is a consequence of the simultaneous increase in endogenous AII. Therefore, we repeated the experiments in the presence of the AII antagonist saralasin. After equilibration with saralasin, haemorrhagic hypotension was again followed by an increase in kallikrein excretion similar to bleeding experiments without infusion of saralasin (Table 2). Although PRA also increases in saralasin-treated haemorrhaged animals, the elevation of endogenous AII should not have an additional effect on kallikrein excretion since the dose of saralasin used completely blocked the pressure and renal haemodynamic effects of a bolus injection of AII. Therefore, a direct effect of either AII or saralasin which results in the stimulation of kallikrein excretion during haemorrhagic hypotension is highly unlikely. However, it is possible that this particular effect is mediated by binding to a different AII receptor or to the high affinity binding sites for AIII, which are less effectively occupied by saralasin (Devynck & Meyer, 1978). The fact that the peak in kallikrein excretion precedes that of PRA, as seen previously (Maier *et al.*, 1981; 1985), indicates that such a mechanism is also unlikely.

In control animals, urinary kallikrein excretion increased when the infusuion of saralasin ceased (Table 1). Thus, since at the same time PRA was also increased, a direct relationship between urinary kallikrein and endogenous AII seems possible in this situation. Urinary kallikrein excretion also increased temporarily after the initiation of saralasin infusion (Table 1). This finding might be explained by agonistic

Table 1 Systemic and renal functional parameters before, during, and after infusion of saralasin

	Stabilization	*20 min*	*Infusion of saline over 120 min* *40 min*	*60 min*	*120 min*	*140 min*
MAP (mm Hg)	126.7 ± 2.8	127.1 ± 3.0	125.8 ± 2.7	126.5 ± 3.8	127.5 ± 3.5	127.0 ± 3.1
KGA (μg Bk min^{-1})	8.1 ± 1.3	8.5 ± 2.3	9.0 ± 1.8	8.7 ± 2.7	7.9 ± 3.0	8.3 ± 3.2
$U_{Na}V$ (μEq min^{-1})	13.5 ± 2.8	13.2 ± 1.9	14.0 ± 2.7	14.3 ± 2.5	14.3 ± 2.4	14.5 ± 2.1
V (ml min^{-1})	0.3 ± 0.03	0.31 ± 0.02	0.3 ± 0.02	0.29 ± 0.02	0.31 ± 0.03	0.3 ± 0.03
PRA (ng ml^{-1} h^{-1})	2.01 ± 0.51	2.1 ± 0.5	2.3 ± 0.45	2.4 ± 0.39	2.27 ± 0.42	2.13 ± 0.37
	Stabilization	*20 min*	*Infusion of saralasin over 120 min* *40 min*	*60 min*	*120 min*	*140 min*
MAP (mm Hg)§	128.63 ± 3.57	119.37 ± 5.28	118.88 ± 3.28*	115.77 ± 2.94*	114.2 ± 4.2*	125.25 ± 4.0
KGA (μg Bk min^{-1})§	7.8 ± 0.88	18.7 ± 4.16*	10.52 ± 1.79	13.85 ± 4.06	10.1 ± 2.5	32.0 ± 12.0*
$U_{Na}V$ (μEq min^{-1})§	15.59 ± 3.76	10.86 ± 2.21	7.23 ± 1.66*	3.69 ± 0.57*	7.0 ± 2.1*	28.2 ± 8.3*
V (ml min^{-1})§	0.29 ± 0.02	0.23 ± 0.03*	0.2 ± 0.01*	0.2 ± 0.02*	0.2 ± 0.05*	1.04 ± 0.1*
PRA (ng ml^{-1} h^{-1})§	2.31 ± 0.41	2.08 ± 0.54	3.86 ± 0.58*	3.47 ± 0.71	1.84 ± 0.24	5.3 ± 0.4*

Results show mean ± s.e.mean for mean arterial blood pressure (MAP), kinin generating activity (KGA), sodium excretion rate ($U_{Na}V$), urine flow (V), and plasma renin activity (PRA), $n = 7$. Significant difference as compared to the control group receiving an infusion of saline (Maier *et al.*, 1985) is indicated by an asterisk (Bonferroni-test) and by a § (analysis of variance).

properties of saralasin (Arendhorst & Finn, 1977) which like AII may thereby cause an increase in urinary kallikrein excretion. Although we could not demonstrate any agonistic effect of saralasin, as assessed by changes of renal and systemic haemodynamics, we cannot exclude such a mechanism.

A reduction in renal artery pressure induced by constriction of the renal artery or by haemorrhagic hypotension is followed by a variety of haemodynamic, hormonal and neuronal changes. This includes an increase in sympathetic nervous activity (Walton *et al.*, 1959), an increase in catecholamines, prostaglandins (Jakschik *et al.*, 1974), vasopressin (Pullan *et al.*, 1980) and an increase in plasma renin activity with a subsequent increase in vasoconstrictor AII (Jakschik *et al.*, 1974); these could all affect kallikrein excretion.

Table 2 Systemic and renal functional parameters during haemorrhagic hypotension in anaesthetized pigs and the effect of an infusion of saralasin

			Infusion of saline			
MAP (mm Hg)	*125.0 ± 5.0*	*100.0 ± 2.5*	*90.2 ± 2.3*	*80.5 ± 2.7*	*70.1 ± 1.9*	*60.6 ± 2.9*
KGA (μg Bk min^{-1})	10.89 ± 3.88	18.51 ± 4.52	39.39 ± 11.51	20.51 ± 5.59	19.1 ± 2.6	1.07 ± 0.36
$U_{Na}V$ (μEq min^{-1})	14.06 ± 4.6	4.92 ± 2.44	8.33 ± 1.25	3.73 ± 0.75	1.05 ± 0.28	0.28 ± 0.12
V (ml min^{-1})	0.32 ± 0.03	0.34 ± 0.02	0.33 ± 0.05	0.2 ± 0.03	0.12 ± 0.02	0.02 ± 0.007
PRA (ng ml^{-1} h^{-1})	1.87 ± 0.65	6.8 ± 0.69	7.65 ± 1.32	8.46 ± 2.06	10.84 ± 1.99	12.22 ± 1.0
Blood loss (ml kg^{-1})	—	10.0 ± 1.0	14.3 ± 2.8	17.4 ± 1.0	22.2 ± 1.3	28.3 ± 2.0
			Infusion of saralasin			
MAP (mm Hg)	*120.0 ± 4.8*	*100.0 ± 2.9*	*90.2 ± 3.0*	*80.5 ± 2.8*	*70.0 ± 1.8*	*60.5 ± 2.5*
KGA (μg Bk min^{-1})§	12.0 ± 3.50	14.33 ± 0.86	20.72 ± 4.53*	20.34 ± 5.38	7.2 ± 2.03*	2.88 ± 0.76
$U_{Na}V$ (μEq min^{-1})§	7.58 ± 2.50*	8.96 ± 4.95	7.24 ± 2.43	4.62 ± 3.28	3.54 ± 2.46	1.82 ± 0.77*
V (ml min^{-1})§	0.23 ± 0.02*	0.15 ± 0.01*	0.25 ± 0.01*	0.14 ± 0.01*	0.08 ± 0.02	0.04 ± 0.002
PRA (ng ml^{-1} h^{-1})§	3.27 ± 0.47	3.6 ± 1.0 *	4.58 ± 1.6	7.05 ± 1.81	10.26 ± 3.61	9.16 ± 1.83
Blood loss (ml kg^{-1})§	–	4.5 ± 1.0 *	7.0 ± 1.3*	8.4 ± 1.5*	13.1 ± 2.4*	19.6 ± 1.8*

Results show mean ± s.e.mean for mean arterial blood pressure (MAP), kinin generating activity (KGA), sodium excretion rate ($U_{Na}V$), urine flow (V), and plasma renin activity (PRA), $n = 7$. Significant difference as compared to the haemorrhaged animals receiving an infusion of saline (Maier *et al.*, 1985) is indicated by an asterisk (Bonferroni-test) and by a § (analysis of variance).

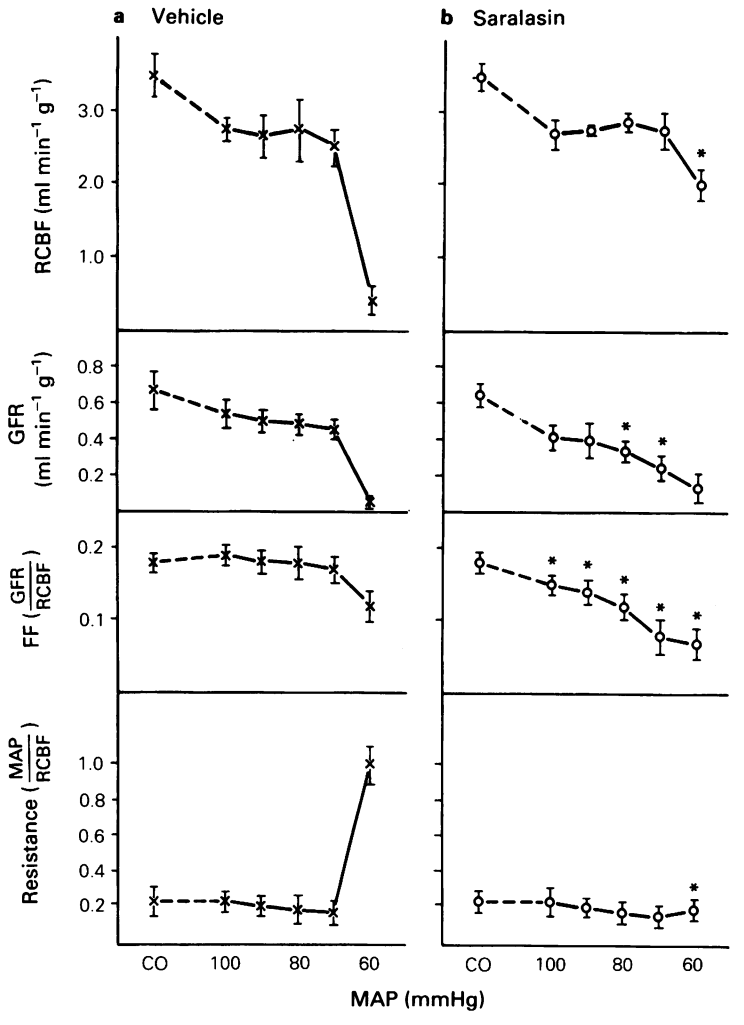

Figure 2 Pattern of renal haemodynamic parameters during an infusion of either saline (a, $n = 7$; Maier *et al.*, 1985) or saralasin (b, $n = 7$) in pigs subjected to haemorrhagic hypotension. Mean values are shown with vertical lines indicating s.e.mean and the asterisks (*) indicate significant differences (Bonferroni-test). The pattern of all parameters during infusion of saralasin is significantly different from that with infusion of saline (analysis of variance, $P < 0.001$). CO = control period, MAP = mean arterial pressure, other abbreviations are explained in legend to Figure 1.

In addition, alterations in salt and water metabolism could induce changes in the concentrations of cations in the urine, which may affect the activity of urinary kallikrein (Lieberthal *et al.*, 1982). For example, the decrease in urinary sodium concentration at a MAP of 60 mm Hg could be responsible for the decrease of urinary kallikrein at that pressure. Changes in pH may also influence urinary kinin excretion (Diaz *et al.*, 1980), although such an effect on urinary kallikrein has not been described and the enzyme appears to be stable over a wide range of pH (Maier *et al.*, 1983). Further, it is not known whether the distribution of kallikrein secreted into the local circulation and into the urine changes during haemorrhagic hypotension. According to our experiments AII does not appear to be a stimulus for kallikrein excretion in haemorrhagic hypotension. However, an AII receptor-mediated effect on kallikrein excretion upon initiation and termination of saralasin infusion can not be excluded.

Therefore, several different mechanisms might be responsible for an increase in kallikrein excretion. To identify a possible common local stimulus of kallikrein

excretion, the pattern of renal functional parameters with special regard to renal haemodynamics associated with increased kallikrein excretion during haemorrhage as well as upon saralasin infusion in control animals was analysed. This procedure can be justified because tissue kallikreins have been implicated in the regulation of local blood flow for 30 years (Hilton & Lewis, 1955).

Changes in renal haemodynamic parameters observed upon saralasin infusion in normal pigs (Figure 1) included a tendency of RCBF to increase and of calculated resistance to decrease. GFR and FF (GFR/RCBF) only temporarily but significantly declined and returned to or towards control values within 60 to 120 min. These results are similar to those found in normal dogs (Hall *et al.*, 1977a) and can generally be interpreted as renal vasodilatation due to inhibition of AII-induced vasoconstriction (Hall *et al.*, 1977b). Furthermore, when the infusion of saralasin was stopped RCBF and calculated resistance returned to baseline values while GFR and FF increased significantly above control values, suggesting that replacement of saralasin by endogenous AII at the receptor site leads to marked vasoconstriction, presumably of the efferent arterioles (Hall *et al.*, 1977b; 1981). In both situations kallikrein excretion was increased (Table 1). During haemorrhagic hypotension and infusion of saralasin, RCBF was maintained but GFR declined concomitant with the decline in MAP, most evident at a MAP of 80 and 70 mm Hg. Such dissociation of GFR from maintenance of RCBF at reduced arterial pressure is a phenomenon which has been described previously (Hall *et al.*, 1977b; 1981) and has been attributed to a reduced efferent arteriolar resistance due to inhibition of AII. Again this situation was accompanied by increased kallikrein excretion similar to our previous studies (Maier *et al.*, 1981; 1985). The maintenance of RCBF during haemorrhage despite inhibition of AII by saralasin (Figure 2b) further indicates that the vasodilator response of the renal afferent arterioles to reduced arterial pressure (Robertson *et al.*, 1972) is unaltered whether or not AII is inhibited. Therefore, upon initiation and termination of saralasin infusion as well as during haemorrhagic hypotension and infusion of saralasin a close relationship between increased urinary kallikrein excretion and alterations of glomerular resistance is evident.

Based upon these facts and the close relationship between kallikrein activity and adjustment of afferent arteriolar tonus (Maier *et al.*, 1985), for which an anatomical basis has recently been provided (Barajas *et al.*, 1985), we would like to offer the following hypothesis as a common mechanism for stimulation of the renal kallikrein kinin system. Most manoeuvres known so far to stimulate kallikrein excretion, including AII infusion (Mills *et al.*, 1978), haemorrhagic hypotension and initiation as well as termination of saralasin infusion, appear to influence the balance between pre- and post-glomerular resistance. This balance in turn ultimately determines glomerular filtration rate (Robertson *et al.*, 1972). Consequently, stimulation or inhibition or an endogenous substance intimately involved in the regulation and maintenance of postglomerular resistance results in a dissociation of this balance and might activate compensatory mechanisms in an attempt to re-establish functional integrity or to maintain residual function. If the kallikrein-kinin system is part of such a mechanism, it would increase or maintain renal blood flow by decreasing primarily the preglomerular resistance (Maier *et al.*, 1985, Baylis *et al.*, 1976). We would, therefore, like to suggest that a limited impairment of renal blood flow or a disproportion of pre- and postglomerular resistance might be a possible common stimulus for kallikrein release. The kinins generated locally could then act directly or via prostaglandins (McGiff *et al.*, 1972) to maintain renal blood flow and GFR.

In conclusion, the increase in urinary kallikrein excretion during haemorrhagic hypotension with and without inhibition of endogenous AII indicates that the renal kallikrein-kinin system is stimulated by a mechanism that does not involve AII. The temporary increase in kallikrein excretion upon saralasin infusion in control animals suggests that the renal haemodynamic changes induced by haemorrhage or by inhibition of the endogenous renin-angiotensin system stimulate the kallikrein-kinin system. This raises the possibility that increased generation of a vasodilator kinin within the renal circulation could contribute to the pharmacological effects induced by saralasin.

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