Vascular effects of parathyroid hormone and parathyroid hormone-related protein in the split hydronephrotic rat kidney

K. Endlich*, T. Massfelder†*, J. J. Helwig† and M. Steinhausen‡

I. Physiologisches Institut der Universität Heidelberg, D-69120 Heidelberg, Germany and † Laboratoire de Physiologie Cellulaire Rénale, Université Louis Pasteur, Strasbourg, France

1. The effects of locally applied parathyroid hormone-related protein (PTHRP), a putative autocrine/paracrine hormone, on vascular diameters and glomerular blood flow (GBF) in the split hydronephrotic rat kidney were studied. As PTHRP interacts with parathyroid hormone (PTH) receptors in all tissues tested so far, the effects of PTHRP were compared with those of PTH.

2. Preglomerular vessels dilated in a concentration- and time-dependent manner that was almost identical for PTH and PTHRP. A significant preglomerular vasodilatation (5–17%) occurred at a threshold concentration of 10^{-10} mol l^{-1} PTH or PTHRP, which raised GBF by 20 ± 2 and 31 ± 4%, respectively (means ± S.E.M., $n = 6$). PTH or PTHRP (10^{-7} mol l^{-1}) increased preglomerular diameters (11–36%) and GBF (60 ± 10 and 70 ± 8%, respectively) to near maximum. The most prominent dilatation was located at the interlobular artery and at the proximal afferent arteriole.

3. Efferent arterioles were not affected by either PTH or PTHRP.

4. Estimated concentrations of half-maximal response (EC_{50}) for preglomerular vasodilatation and GBF increase were in the nanomolar to subnanomolar range.

5. After inhibition of angiotensin I-converting enzyme by 2×10^{-6} mol kg^{-1} quinapril I.V. ($n = 6$), 10^{-8} mol l^{-1} PTHRP dilated preglomerular vessels and efferent arterioles (9 ± 1% proximal and 6 ± 1% distal).

6. We conclude that the renal vasculature of the hydronephrotic kidney is highly sensitive to vasodilatation by PTH and PTHRP, which, in addition, may constrict efferent arterioles by stimulating renin release. Thus, PTH and PTHRP could be potent modulators of renal haemodynamics and glomerular filtration rate.

Based upon a variety of pharmacological and physiological evidence, it has become increasingly clear that parathyroid hormone (PTH), beyond its hypercalcaemic properties, is a potent and efficacious agonist in the cardiovascular system. Indeed, PTH has hypotensive and vasodilator effects on various vascular beds in several animal species (for review see Mok, Nickols, Thompson & Cooper, 1989). These effects are caused by direct interaction with vascular smooth muscle (Pang, Yang, Shew & Tenner, 1985; Nickols, Metz & Cline, 1986). The recently discovered humoral hypercalcaemia of malignancy factor, PTH-related protein (PTHRP), has been shown to interact with PTH receptors in bone and kidney (Nissenson, Diep & Strewler, 1988). This interaction has been found despite the fact that PTH and PTHRP are coded by different genes (Mangin, Ikeda, Dreyer & Broadus, 1989), are immunologically distinct (Godsall, Burtis, Insogna, Broadus & Stewart, 1986), and share sequence homology limited to the first thirteen amino acids (Strewler *et al.* 1987). Like PTH, PTHRP also interacts with cardiovascular tissues (Winquist, Baskin & Vlasuk, 1987; Roca-Cusachs, Dipette & Nickols, 1991). Both peptides increase regional blood flow and/or decrease vascular resistance (Roca-Cusachs *et al.* 1991). While PTH is secreted from specific glands and is dependent upon the circulation for transport to target sites, PTHRP and the mRNA transcripts for PTHRP are localized in various normal tissues including vascular smooth muscle cells (Hongo *et al.* 1991; Kramer, Reynolds,

‡ To whom correspondence should be addressed.

* K. Endlich and T. Massfelder contributed equally to this work.

Castillo, Valenzuela, Thorikay & Sorvillo, 1991). Although the function of PTHRP has not been established, the kinetics of regulation of PTHRP production in response to physiological stimuli strongly implicate this protein as a locally active paracrine or autocrine factor. For instance, PTHRP could be involved in relaxation of smooth muscle (Thiede *et al.* 1990; Pirola *et al.* 1993).

At the renal level, PTH and PTHRP induce a concentration-related dilatation of the renal artery (Winquist *et al.* 1987) of the vascular bed of the preconstricted isolated rat kidney (Musso, Barthelmebs, Imbs, Plante, Bollack & Helwig, 1989*a*; Musso, Plante, Judes, Barthelmebs & Helwig, 1989*b*), and of microdissected afferent and efferent arterioles (Trizna & Edwards, 1991). The adenylate cyclase system could be involved in this renal vascular effect since PTH and PTHRP have been shown to stimulate this system in a rabbit renal microvessel preparation enriched with glomerular arterioles (Helwig, Yang, Bollack, Judes & Pang, 1987; Musso *et al.* 1989*b*). In addition, both peptides bind with high affinity to those vessels in a specific and saturable manner (Nickols, Nickols & Helwig, 1990). Since these vessels are an important site of renal resistance to blood flow, these observations suggest that PTH and PTHRP have the ability to regulate renal haemodynamics. Studies using either electromagnetic flow probes (Crass, Jayaseelan & Darter, 1987) or radioactive microsphere injections (Pang, Janssen & Yee, 1980) have indicated that PTH extracts or pure synthetic PTH increase renal blood flow. However, no attempts have been made to determine the specific localization of these actions along the renal arterial tree, since direct observation of vascular responses is not possible in most experimental models.

The aim of the present study was to observe specifically the *in vivo* effects of PTHRP on the diameters of pre- and postglomerular vessels in the split hydronephrotic kidney (Steinhausen, Snoei, Parekh, Baker & Johnson, 1983) and to compare them with the well-characterized effects of PTH. This model also enables us to obtain a direct assessment of single glomerulus blood flow. The suspension of the kidney in a controlled bath permitted the topical application of peptides without influencing systemic blood pressure, thus avoiding indirect autoregulatory responses of the vessels to changes in blood pressure. Whereas the tubular system atrophies during the development of hydronephrosis, the vascular system remains remarkably intact, as evidenced by histological, ultrastructural, and electrophysiological studies (Nobiling *et al.* 1986). Furthermore, responses to various vasoactive substances (Steinhausen, Endlich & Wiegman, 1990), the ability to autoregulate blood flow (Steinhausen, Blum, Fleming, Holz, Parekh & Wiegman, 1989), and a functionally intact vascular endothelium (Gulbins *et al.* 1993; Hoffend, Cavarape, Endlich & Steinhausen, 1993) are maintained. In our experiments, locally applied PTH and PTHRP both induced similar concentration-related increases of both glomerular blood flow and diameters of preglomerular vessels, with effects starting at a peptide level which may be physiologically relevant for PTHRP. Furthermore, after angiotensin I-converting enzyme inhibition (ACEI), PTHRP was also able to dilate efferent arterioles.

METHODS

Preparation of the hydronephrotic kidney

Experiments were performed on eighteen female Munich–Wistar rats weighing 226 ± 15 g (mean ± S.D.) in accordance with official guidelines for animal protection. The technique of splitting the rat kidney has been described in detail previously (Steinhausen *et al.* 1983, 1990). In brief, ligation of the ureter via a flank incision was performed during pentobarbitone sodium anaesthesia (Nembutal®, 60 mg kg^{-1} I.P.; Ceva, Bad Segeberg, Germany). The final experiments were performed under thiobutabarbitone anaesthesia (Inactin®, 100 mg kg^{-1} I.P.; Byk Gulden, Konstanz, Germany) 72 ± 26 days (mean ± S.D.) after the induction of hydronephrosis. Body temperature was maintained at 37·0–37·5 °C by means of a heating table, systemic blood pressure was monitored via a cannula in the left femoral artery, and isotonic saline (0·05 ml min^{-1}) was continuously infused via a cannula placed in the jugular vein. After exposure of the left hydronephroptic kidney by a flank incision, the kidney was split along the greater curvature with a thermal cautery. The ventral half of the kidney was sutured onto a semicircular wire frame which was attached to the bottom of a plexiglass bath. The entry of the renal hilus into the bath was sealed with silicone grease, and the bath was filled with an isotonic, isocolloidal solution (Haemaccel®, Behringwerke, Marburg/Lahn, Germany) maintained at 37 °C. A Leitz Ultropac water immersion objective (UO-55) was combined with a television and video-recording system for intravital microscopy. Kidney preparations were allowed to equilibrate in the tissue bath for at least 1 h after the surgical procedure. The microcirculatory parameters of this preparation have been demonstrated to be stable for more than 3 h (Steinhausen *et al.* 1989).

Renal vascular segments

Lumen diameter measurements of the following vessel segments were carried out, the vessels being identified according to their branching pattern from the selected cortical glomerulus (Fig. 1): (*a*) proximal arcuate artery (near the interlobar artery), (*b*) distal arcuate artery (near the interlobular artery), (*c*) proximal interlobular artery (near the arcuate artery), (*d*) distal interlobular artery (near the afferent arteriole), (*e*) proximal afferent arteriole (near the interlobular artery), (*f*) distal afferent arteriole (at the narrowest segment before entering the glomerulus), (*g*) proximal efferent arteriole (within 50 μm of the glomerulus), and (*h*) distal efferent arteriole (near the welling point). Lumen diameters were directly measured on the calibrated video screen.

Glomerular blood flow

Red blood cell velocity was determined in the efferent arteriole using a velocity tracking correlator (Model 102B; IPM Inc., San Diego, CA, USA) (Wayland & Johnson, 1967). In order to obtain glomerular blood flow (GBF), the measured red cell velocity was

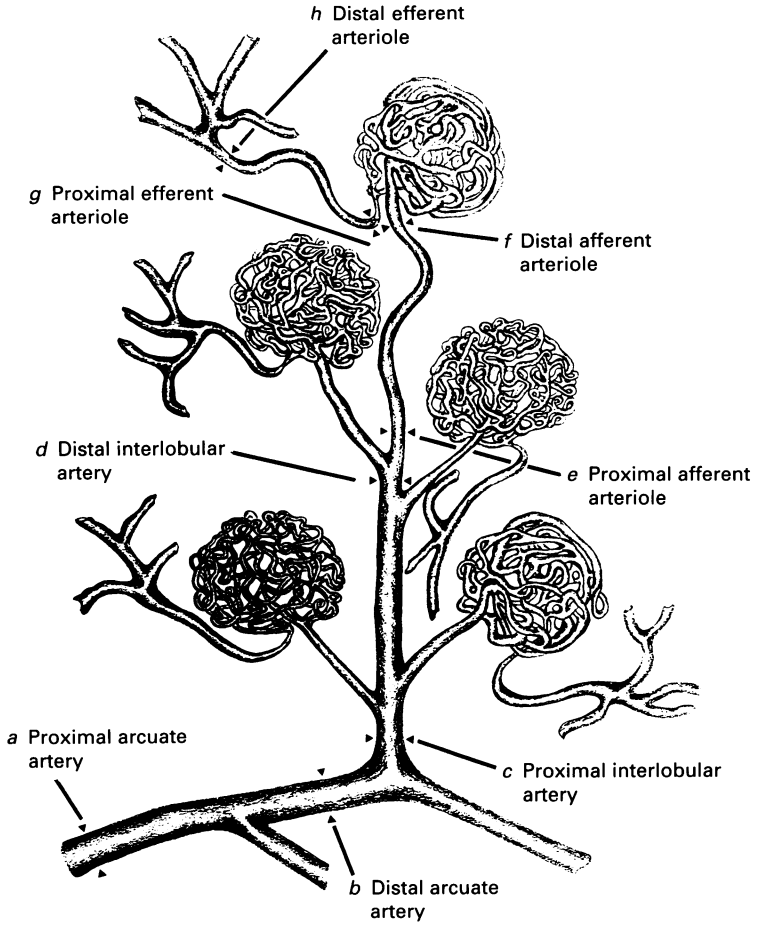

Figure 1. Schematic drawing of the renal vascular tree
The sites at which vessel diameters were measured are indicated by arrowheads. See text for details.

multiplied by the luminal cross-section of the efferent arteriole and corrected for the Fahraeus effect (Gaehtgens, Benner, Schickendantz & Albrecht, 1976).

Experimental protocol

The effects of PTH on renal microcirculation were studied in one group of animals. The effects of PTHRP alone and after ACEI were studied in a second and a third group, respectively. rPTH-(1–34) was purchased from Neosystem Laboratories (Strasbourg, France), and hPTHRP-(1–34) from Cambridge Research Biochemicals (Northwich, UK). Each group consisted of six animals.

The protocol started with two sets of control measurements (diameters, GBF and blood pressure) separated by 10 min. In the first and second group, the last control measurement was followed by the addition of five progressively increasing concentrations (10^{-11}, 10^{-10}, 10^{-9}, 10^{-8} and 10^{-7} mol l^{-1}) of PTH or PTHRP to the tissue bath. Specified concentrations refer to the concentration of the drug in the tissue bath. The hydronephrotic kidney was exposed to each concentration for 30 min. During each 30 min interval three measurements of blood pressure, GBF, and vascular diameters were performed at 5, 15 and 30 min after the addition of the peptide. At the highest concentration (10^{-7} mol l^{-1}) two additional measurements were performed 45 and 60 min after increasing the bath concentration.

In the third group, the last control measurement was followed by an intravenous bolus injection of 2×10^{-6} mol kg^{-1} quinapril (kindly provided by Goedecke-Parke-Davis, Freiburg, Germany) to block angiotensin I-converting enzyme. Measurements were performed 30, 45 and 60 min after the bolus injection. Thereafter, 10^{-8} mol l^{-1} PTHRP was added to the tissue bath, and measurements were performed 15, 30 and 45 min later.

At the end of each experimental protocol, 10^{-6} mol l^{-1} acetylcholine (ACh) and, 10 min later, 10^{-5} mol l^{-1} sodium nitroprusside (SNP) were locally applied to achieve maximal vasodilatation. One measurement was performed 10 min after the application of ACh, and another 10 min after the application of SNP. Rats were killed by an overdose of the anaesthetic at the end of the experiments.

Data analysis

All values are presented as means ± S.E.M. Changes in vascular diameters and GBF are expressed as percentage changes from the first control values, unless otherwise stated. Analysis of variance and the Bonferroni method for multiple comparisons were used to test for statistical significance of the concentration-dependent effects. To decide whether the effects of PTH and

Table 1. Control values of vessel lumen diameters, GBF, blood pressure and body weight

	PTH ($n = 6$)	PTHRP ($n = 6$)	PTHRP after ACEI ($n = 6$)
Diameter (μm)			
Arcuate artery			
Proximal	61·3 ± 3·2	56·9 ± 2·5	61·3 ± 1·9
Distal	43·2 ± 3·2	42·1 ± 3·1	46·1 ± 1·6
Interlobular artery			
Proximal	23·9 ± 0·4	24·2 ± 0·7	26·7 ± 1·0
Distal	11·7 ± 0·7	13·1 ± 1·6	16·2 ± 1·3
Afferent arteriole			
Proximal	8·6 ± 0·8	9·4 ± 0·6	11·2 ± 0·8
Distal	7·0 ± 0·6	8·3 ± 0·7	8·7 ± 0·6
Efferent arteriole			
Proximal	11·7 ± 1·1	13·3 ± 1·3	14·6 ± 1·4
Distal	18·6 ± 0·5	18·7 ± 0·8	20·4 ± 0·8
GBF (nl min^{-1})	55·8 ± 11·2	38·5 ± 3·2	54·5 ± 6·8
Blood pressure (mmHg)	106·7 ± 1·7	104·2 ± 2·4	107·5 ± 3·1
Body wt (g)	218 ± 7	228 ± 6	232 ± 13

Values are presented as means ± S.E.M.

PTHRP differed significantly from each other, mean values of all measurements ($n = 17$) during local application of the peptide were calculated for GBF and each vascular segment. These values were compared using Student's unpaired t test. The significance level was set at $P < 0·05$. Concentration–response curves were constructed from the percentage changes of the preglomerular diameters and GBF measured after 30 min exposure to each bath concentration. EC_{50} values (defined as the concentration at which the half-maximal response is obtained) for the preglomerular vessels and GBF were calculated as the geometric means of the individual EC_{50} values from each animal. The individual EC_{50} values were estimated by a logistic curve fit (SigmaPlot®; Jandel Scientific, Erkrath, Germany). Since in about one-fifth of the concentration–response curves a maximal response was not clearly reached at 10^{-7} mol l^{-1}, the maximal responses of the fitted curves were not allowed to exceed those found with SNP.

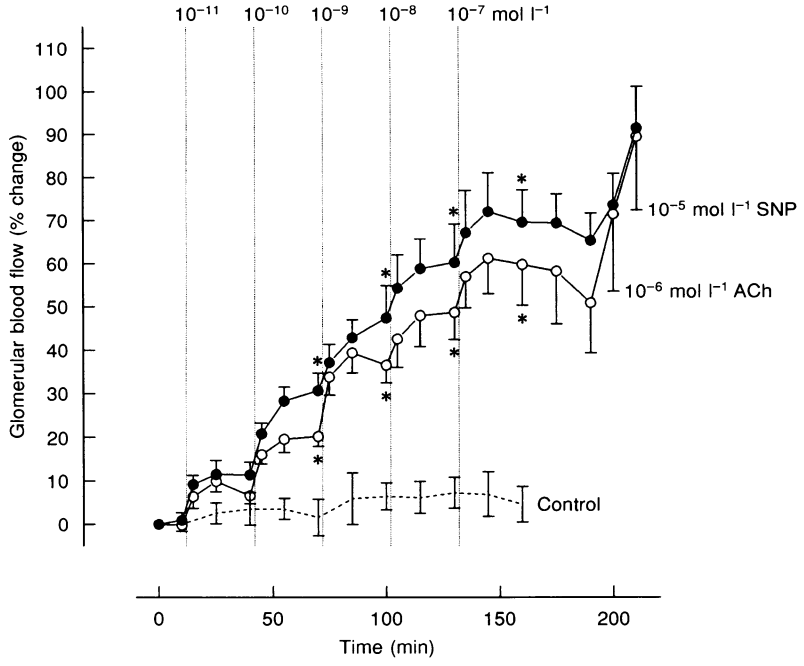

Figure 2. PTH and PTHRP effects on glomerular blood flow

Concentration- and time-dependent percentage changes of glomerular blood flow (means ± S.E.M.) in response to local application of PTH (○; $n = 6$) and PTHRP (●; $n = 6$). A time control is represented by the dashed line (data redrawn from Steinhausen *et al.* 1989). * $P < 0·05$ *versus* control.

RESULTS

Mean control values for the diameters of the measured renal vessels, for GBF, body weight, and mean arterial blood pressure are summarized in Table 1. Blood pressure did not change significantly during the experimental protocol in any group. Average blood pressure variations (S.D./mean) during the protocol were 2·6 ± 0·4% in the three series of experiments.

Glomerular blood flow increased in a concentration- and time-dependent manner during local application of PTH and PTHRP, respectively (Fig. 2). At each concentration step, GBF showed a measurable increase 5 min after the addition of PTH or PTHRP to the tissue bath. Within 30 min, a stable plateau was reached. After 30 min at a threshold concentration of 10^{-10} mol l^{-1}, GBF was significantly elevated above baseline (by 20 ± 2 and 31 ± 4% for PTH and PTHRP, respectively). After 30 min at the highest concentration of 10^{-7} mol l^{-1}, GBF had increased to near maximum levels (60 ± 10 and 70 ± 8% for PTH and PTHRP, respectively). ACh (10^{-6} mol l^{-1}) and SNP (10^{-5} mol l^{-1}) in the tissue bath raised GBF by an additional 18 ± 6% compared to the value 30 min after application of 10^{-7} mol l^{-1} of the peptide.

The increase of GBF was paralleled by a concentration- and time-dependent vasodilatation of the preglomerular

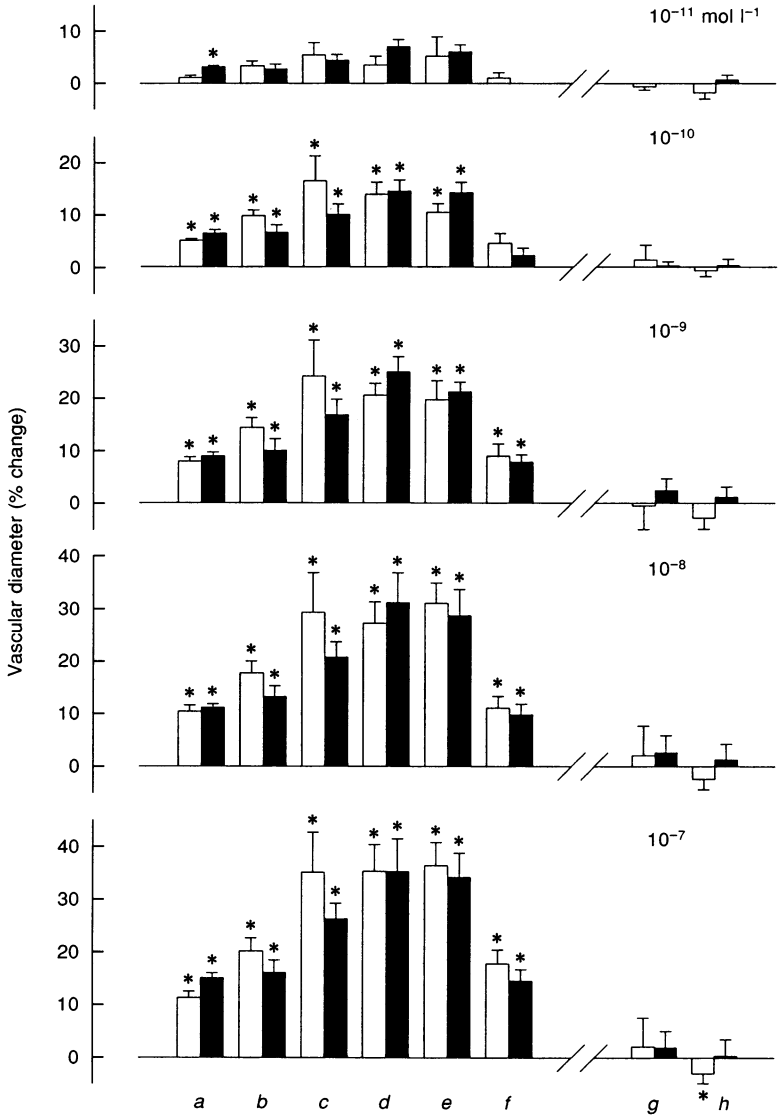

Figure 3. PTH and PTHRP effects on renal vessels

Concentration-dependent percentage changes of vascular diameters (means ± S.E.M.) in response to local application of PTH (□; $n = 6$) and PTHRP (■; $n = 6$). The vascular diameters were measured after 30 min exposure to each concentration. * $P < 0·05$ *versus* control. See Fig. 1 for vessel labelling.

Table 2. Estimated EC_{50} values (nmol l^{-1}) for PTH and PTHRP

	n	PTH	n	PTHRP
Arcuate artery				
Proximal	4	0·19 ± 0·06	3	0·3 ± 0·1
Distal	5	0·19 ± 0·04	5	0·7 ± 0·3
Interlobular artery				
Proximal	6	0·4 ± 0·1	6	1·0 ± 0·3
Distal	6	1·2 ± 0·5	6	0·24 ± 0·08
Afferent arteriole				
Proximal	6	1·6 ± 0·5	6	0·6 ± 0·2
Distal	6	7 ± 4	6	6 ± 3
Mean of preglomerular vessels		0·8 ± 0·5		0·8 ± 0·4
GBF	6	0·6 ± 0·1	6	0·5 ± 0·1

Values are presented as means ± S.E.M.

vessels. At each concentration step the vasodilatation reached a stable state within 30 min. Figure 3 shows the vascular diameter responses to the five concentrations of PTH and PTHRP (10^{-11}–10^{-7} mol l^{-1}). Preglomerular vessels significantly dilated by 7–17% at a concentration of 10^{-10} mol l^{-1} (except for the proximal arcuate artery which had a threshold concentration of 10^{-11} mol l^{-1} for PTHRP and the afferent arteriole near the glomerulus which had a threshold concentration of 10^{-9} mol l^{-1} for both peptides). At the highest concentration (10^{-7} mol l^{-1}) preglomerular vessels were dilated by 11–36%. The largest dilatation occurred at the interlobular artery (35 ± 5 and 36 ± 6% for PTH and PTHRP, respectively) and at the proximal afferent arteriole (36 ± 4 and 34 ± 5%, respectively). Local application of 10^{-6} mol l^{-1} ACh and 10^{-5} mol l^{-1} SNP at the end of the protocol induced an additional preglomerular vasodilatation of 3% (proximal arcuate artery) to 20% (proximal afferent arteriole) compared with the value 30 min after the application of 10^{-7} mol l^{-1} of the peptide. PTH as well as PTHRP had no effect on efferent arterioles, except for a slight diameter reduction (−3 ± 2%) of the efferent arterioles near their welling points during application of the highest concentration of PTH (10^{-7} mol l^{-1}). Local application of 10^{-6} mol l^{-1} ACh after the highest concentration of PTH and PTHRP significantly dilated proximal efferent arterioles by 10 ± 3 and 10 ± 2%, and distal efferent arterioles by 10 ± 2 and 7 ± 2%, respectively. A significant difference in the vascular effects of PTH and PTHRP was observed only in the proximal arcuate artery (3 ± 1% at the highest concentration of 10^{-7} mol l^{-1}).

The concentration–response curves for GBF and the diameter of proximal afferent arterioles, which are similar to those of the other preglomerular vessels, are given in Fig. 4. Estimated EC_{50} values are compiled in Table 2. There was no significant difference between the EC_{50}

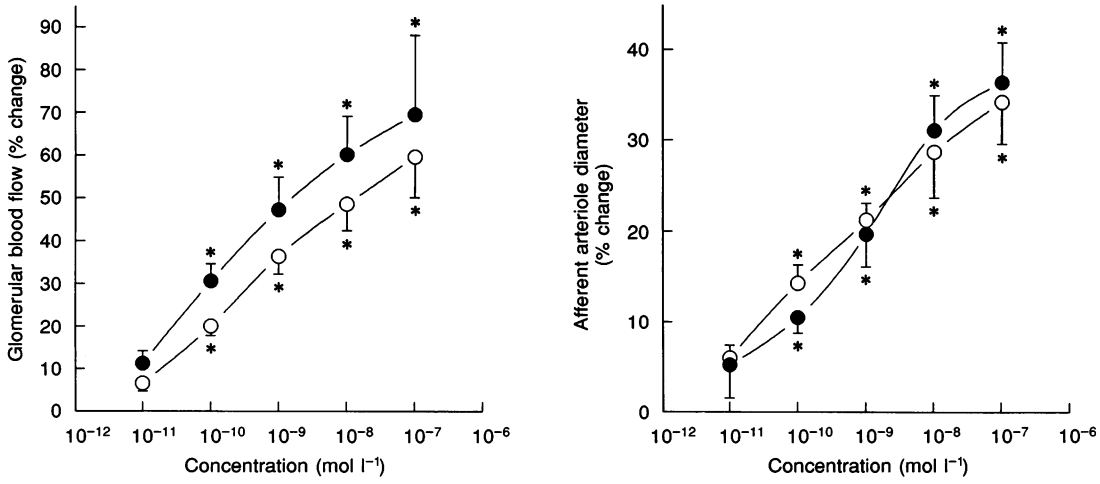

Figure 4. PTH and PTHRP concentration–response curves

Concentration–response curves for PTH- and PTHRP-induced increase of glomerular blood flow and proximal afferent arteriole diameter (means ± S.E.M.; $n = 6$ for each curve). ○, PTH; ●, PTHRP. * $P < 0·05$ *versus* control.

values for PTH and PTHRP. The estimated EC_{50} values for the vasodilatation of the preglomerular vessels were in the range 0·19–7 nmol l^{-1} with means of 0·8 ± 0·5 and 0·8 ± 0·4 nmol l^{-1} for PTH and PTHRP, respectively. The EC_{50} values of GBF increase were 0·6 ± 0·1 and 0·5 ± 0·1 nmol l^{-1} for PTH and PTHRP, respectively.

Inhibition of angiotensin I-converting enzyme by quinapril (2×10^{-6} mol kg^{-1}, I.V.) resulted in a stable increase in GBF of 27 ± 7, 31 ± 5 and 30 ± 4% after 30, 45 and 60 min, respectively. Preglomerular vessels were dilated by 4% (distal afferent arteriole) to 13% (distal interlobular artery) after 60 min (Fig. 5). Efferent arterioles remained unaffected by ACEI. After ACEI, 10^{-8} mol l^{-1} PTHRP further increased GBF to 43 ± 6% above control values after 30 min. At this time, PTHRP further dilated preglomerular vessels and now also dilated efferent arterioles by 9 ± 1% (proximal) and 6 ± 1% (distal) compared to 60 min after ACEI (Fig. 5).

DISCUSSION

Previous studies have shown that PTH and PTHRP exhibit similar renal vasodilating (Musso *et al.* 1989*a*,*b*; Trizna & Edwards, 1991), arteriolar adenylate cyclase-stimulating (Helwig *et al.* 1987; Musso *et al.* 1989*b*) and receptor-binding properties (Nickols *et al.* 1990). It is important to note that PTHRP probably acts by interacting with renovascular PTH receptors (Pang *et al.* 1985). However, no data are available concerning the localization of the *in vivo* vasodilating effects of PTH and PTHRP along the renal arteriolar tree. In the present study, we used the split hydronephrotic rat kidney preparation to localize and to compare the effects of both peptides on the *in vivo* diameter of preglomerular vessels (corresponding to arcuate and interlobular arteries and afferent arterioles) and efferent arterioles. The split hydronephrotic kidney preparation allows the study of microvascular function during perfusion with systemic blood. Drugs may be added locally in the tissue bath at defined concentrations or infused intravenously. In the present study, we chose to apply PTH and PTHRP directly in the tissue bath rather than intravenously because of the known potent cardiac and hypotensive effects of both peptides (Roca-Cusachs *et al.* 1991), thereby avoiding the indirect autoregulatory responses of the renal vessels to changes in systemic blood pressure.

During local application of PTH and PTHRP, we observed a similar pattern of reactivity of the renal arteriolar tree to both peptides, consisting of: (1) vasodilatation of all preglomerular vascular segments with the largest effects being on the interlobular artery and the proximal afferent arteriole, (2) no effect on the efferent arteriole, and (3) an increase of GBF. These effects were concentration dependent and highly sensitive with overall EC_{50} values in the subnanomolar range, and started to be significant at a peptide level, which may be physiologically relevant for PTHRP, and which may also be relevant for PTH in perturbations of normal parathyroid function.

Since PTH and PTHRP stimulate renin release in the nanomolar to subnanomolar range (Helwig, Musso, Judes & Nickols, 1991; Saussine *et al.* 1993*a*; Saussine, Massfelder, Parnin, Judes, Simeoni & Helwig, 1993*b*), we speculated that direct efferent dilatation by these peptides and indirect efferent constriction mediated by locally formed angiotensin II may cancel each other out. Intrarenal angiotensin II formation secondary to infusion of PTH was noted by Brenner and co-workers (Brenner, Schor & Ichikawa, 1982). Indeed, after ACEI, PTHRP was able to dilate not only preglomerular vessels but also efferent arterioles. Thus, pre- and postglomerular vessels are able to dilate in response to PTHRP, the dilatation of the latter being counteracted by vasoconstriction as a result of locally formed angiotensin II. However, effects of PTHRP on efferent arterioles were less prominent compared with those on afferent arterioles. Similarly, the vasodilatation of efferent arterioles is about one-half that of afferent arterioles in response to acetylcholine (Dietrich, Fretschner, Nobiling, Persson & Steinhausen, 1991). This probably reflects a lower basal tone of efferent arterioles in the hydronephrotic kidney model. Angiotensin I-converting enzyme inhibition induced vasodilatation of

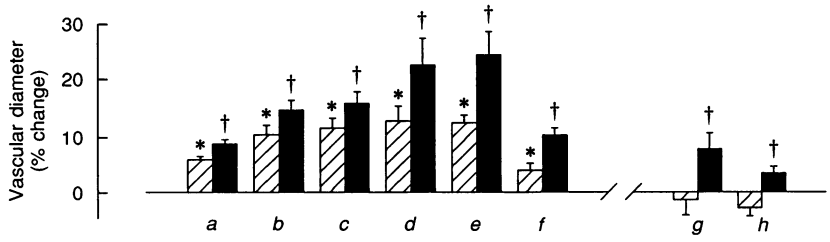

Figure 5. PTHRP effects on renal vessels after ACEI

Percentage changes of vascular diameters (means ± S.E.M.) *versus* control in response to ACEI (▨) by 2×10^{-6} mol kg^{-1} quinapril (60 min after I.V. bolus) and to subsequent local application of 10^{-8} mol l^{-1} PTHRP (■; 30 min exposure). $n = 6$; $*P < 0{\cdot}05$ *versus* control, $\dagger P < 0{\cdot}05$ *versus* ACEI. See Fig. 1 for vessel labelling.

preglomerular vessels, which may be related to a decreased level of angiotensin II. In addition, ACEI has been shown to elicit an endothelium-dependent vasodilatation by interacting with bradykinin (Hecker, Pörsti, Bara & Busse, 1994; Moroi *et al.* 1994).

The pattern of distribution of the vasodilating effects of PTH and PTHRP along the renal arteriolar tree obtained from the present experiments has to be considered in parallel with the experiments of Trizna & Edwards (1991) performed on microdissected pressurized afferent and efferent arterioles of the rabbit. In this preparation, PTH and PTHRP relaxed afferent and efferent arterioles in a concentration-dependent manner, as would be expected from our results. However, Trizna & Edwards (1991) reported somewhat higher EC_{50} values (in the range 6–10 nmol l^{-1}). On the one hand, EC_{50} values may depend on the site of measurement at the afferent arteriole, since EC_{50} values increased towards the glomerulus in our experiments (Table 2). On the other hand, it may reflect the difference between *in vivo versus in vitro* approaches. In the isolated rabbit renal arterioles (Trizna & Edwards, 1991), PTH and PTHRP were tested for their capacity to reverse a submaximal noradrenaline-induced tone, constricting afferent arterioles by more than 50% (Edwards, 1983). In the present study, the peptides were tested for their ability to relax the spontaneous *in vivo* vascular tone resulting from a number of vasoactive compounds. Moreover, in isolated arterioles, the interactions among the various segments of the vascular tree are excluded.

In previous studies, the vasodilating response of the isolated perfused rat kidney to PTH and PTHRP was rapid (1–2 min) and transient (10 min) in spite of the continuous presence of the peptides (Musso *et al.* 1989*a*,*b*). Furthermore, single doses of PTH or PTHRP gave higher vasodilating responses than sequential cumulative doses into the same kidney (Musso *et al.* 1989*a*,*b*). These results were reminiscent of tachyphylaxis of the renal vascular bed to both peptides. In support of this, PTH- and PTHRP-responsive adenylate cyclase of microvessels isolated from rabbit kidney cortex rapidly desensitizes to the action of both peptides (T. Massfelder & J. J. Helwig, unpublished results). These results contrast with the present findings showing that vasodilatation starts a few minutes after application of the peptides and that 15–30 min are necessary to develop a stable plateau for each peptide concentration. It is possible that the delayed vasodilating response to peptides applied from outside reflects the time needed to reach receptors by diffusion. In addition, differences in the mode of application of PTH and PTHRP could be responsible for the differences in the time dependence of the peptide effects, since substances applied intraluminally *versus* externally approach vascular smooth muscle and endothelial cells in a different order and with different concentration gradients. Evidence that the vascular endothelium might be involved in the vasodilating action of PTH and PTHRP has been recently obtained in the isolated perfused rabbit kidney in which the vasodilating action of PTHRP is partially blunted by a nitric oxide synthase inhibitor (Simeoni, Massfelder, Saussine, Judes, Geisert & Helwig, 1994).

The observation that PTH, like PTHRP, vasodilates all preglomerular renal segments is in accordance with the rise in GBF. This effect would also imply an increase in glomerular filtration rate (GFR). On the contrary, in micropuncture studies in plasma-expanded rats, intravenously infused PTH decreased GFR of superficial glomeruli by reducing the glomerular capillary ultrafiltration coefficient without affecting GBF (Ichikawa, Humes, Dousa & Brenner, 1978). However, considerable reduction of mean arterial blood pressure due to intravenous injection of PTH/PTHRP and hypotension-induced reflexes may interfere with the direct renal vasodilating effects of these peptides. A clear increase in renal blood flow has been demonstrated in anaesthetized dogs by diminishing systemic side effects with intra-arterial injection of PTH (Crass *et al.* 1987).

In conclusion, the split hydronephrotic rat kidney allowed us to localize, *in vivo*, the vasodilating actions of PTH and PTHRP in pre- and postglomerular vascular segments. We observed that pre- and postglomerular vessels show reactivity to these peptides. The effects of PTH and PTHRP were virtually identical with EC_{50} values for preglomerular vessels and GBF in the nanomolar to subnanomolar range. The *in vivo* effect of PTH/PTHRP on efferent arterioles may depend on the net effect of direct dilatation and indirect constriction via stimulation of renin release. These results together with the large rise in GBF strongly suggest the potential of PTH and PTHRP to regulate renal haemodynamics. Unlike PTH, PTHRP and the mRNA transcripts for PTHRP have been localized in various normal tissues including vascular smooth muscle cells (Hongo *et al.* 1991; Kramer *et al.* 1991). In addition, the presence of specific receptors for PTH and PTHRP on renal arterioles (Nickols *et al.* 1990), the direct potent stimulatory effects of PTH (Helwig *et al.* 1991; Saussine *et al.* 1993*a*) and PTHRP (Saussine *et al.* 1993*b*) on renin release, together with the regulation of PTHRP production in vascular smooth muscle cells by growth factors and vasoconstrictors including angiotensin II (Pirola *et al.* 1993), strongly implicate this protein as a locally active autocrine/paracrine factor. Nevertheless, whether the vascular tree of the kidney is able to produce and secrete PTHRP and whether the renovascular properties of PTHRP are physiologically relevant is actually unknown.

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Acknowledgements

We thank Mr R. Dussel and Ms H. Adler for their excellent technical assistance. This work was supported by the German Research Foundation (DFG, Ste 107/12-2) and by the French National Institute of Health (INSERM) grant CRE920203 and by the French Ministry of Higher Education and Research (MESR; recipient J.J.H.). This work is part of the Thesis of Sciences of T. M.

Received 28 October 1993; accepted 16 August 1994.