British Journal of Pharmacology (1996) 118, 1995-2000

Effect of intrarenally infused parathyroid hormone-related protein on renal blood flow and glomerular filtration rate in the anaesthetized rat

Thierry Massfelder, *Niranjan Parekh, *Karlhans Endlich, Christian Saussine, *Michael Steinhausen & [1]Jean-Jacques Helwig

Laboratoire de Physiologie Cellulaire Rénale, Faculté de Médecine, Université Louis Pasteur, CJF INSERM 9409, Strasbourg, France and *I. Physiologisches Institut der Universität Heidelberg, Heidelberg, Germany

1 Parathyroid hormone-related protein (PTHrP) is expressed in the kidney and acts on vascular PTH/PTHrP receptors to vasodilate the isolated kidney and to stimulate renin release. However, effects of PTHrP on renal blood flow (RBF) and glomerular filtration rate (GFR) *in vivo* have not been assessed in the absence of its cardiac, peripheral and central effects. We investigated the renal effects of PTH and PTHrP infused into the left renal artery of anaesthetized rats.

2 Intrarenal infusions, adjusted to generate increasing concentrations of human PTHrP(1-34) and rat PTH(1-34) in renal plasma (2×10^{-11} to 6×10^{-9} M) produced a comparable dose-dependent increase in RBF. The rise was 4% at the lowest and 34% at the highest concentrations of peptides. Up to a concentration of 2×10^{-9} M, mean arterial pressure (MAP) and heart rate were not affected, but at 6×10^{-9} M, intrarenally infused peptides reached the peripheral circulation, and caused a fall in MAP within a few minutes. While MAP returned to basal value after the last peptide infusion, RBF remained more than 10% above control for at least 30 min.

3 Two competitive PTH/PTHrP receptor antagonists, [$Nle^{8,18}$, Tyr^{34}]-bPTH(3-34)amide and [Leu^{11}, D-Trp^{12}]-hPTHrP(7-34)amide (2×10^{-8} M) were devoid of agonist activity, but markedly antagonized the dose-dependent increase in RBF elicited by PTHrP.

4 GFR and urine flow were measured in left PTHrP-infused experimental kidney and right control kidney. Renal PTHrP concentration of 10^{-10} M elevated left RBF by 10%, and GFR by 20% without significantly increasing filtration fraction, and increased urine flow by 57%. In the right control kidney GFR and diuresis did not change.

5 The results indicate that PTHrP has similar renal haemodynamic effects as PTH and increases RBF, GFR and diuresis in anaesthetized rats.

Keywords: Parathyroid hormone; parathyroid hormone-related protein; receptor blockade; blood pressure; vasodilatation; renal blood flow; glomerular filtration rate; diuresis

Introduction

Parathyroid hormone-related protein (PTHrP), initially identified as the main factor responsible for the syndrome of humoral hypercalcemia of malignancy (Burtis *et al.*, 1987; Moseley *et al.*, 1987; Strewler *et al.*, 1987), has subsequently been shown to be present in normal human and animal tissues including lactating breast, pituitary gland, placenta, bone, epidermis, parathyroid gland, vascular endothelial cells and smooth muscle cells of non-vascular and vascular origin (Hongo *et al.*, 1991; Kramer *et al.*, 1991; Halloran & Nissenson, 1992; Burton *et al.*, 1994; Ishikawa *et al.*, 1994). It has been recently shown that aortic smooth muscle cells abundantly express PTHrP mRNA and that vasoconstrictor agents such as angiotensin II induce PTHrP-gene expression by effects on both gene transcription and mRNA stabilisation (Pirola *et al.*, 1993). Moreover, balloon distention of rat aorta is associated with a rapid and persistent induction of PTHrP mRNA *in vivo* (Pirola *et al.*, 1994). In primary rat aortic smooth muscle cells, the creation of a flowing motion of the culture medium is also associated with PTHrP gene expression (Pirola *et al.*, 1994). Further, hPTHrP(1-34) exhibits all the biological effects of PTH(1-34) on the cardiovascular system including hypotensive and inotropic cardiac actions (Nickols *et al.*, 1989; Roca-Cusachs *et al.*, 1991). For these and other reasons, the role of PTHrP is increasingly recognized as an important autocrine/paracrine hormone in regulating physiological functions, such as local modulation of microcirculation, whereas PTH only mimics the vascular action of PTHrP (for review see Philbrick *et al.*, 1996). However, a clear-cut physiological role for PTHrP in the renovascular system has not yet been identified.

In the kidney, a growing body of evidence argues for a role of PTHrP in controlling renovascular tone. The renal vasculature has been shown to be highly sensitive to the dilator action of PTHrP. PTH(1-34) and PTHrP(1-34) bind with high affinity to a single-class of receptors in isolated renal arterioles (Nickols *et al.*, 1990) and stimulate adenylyl cyclase activity in both renal arterioles (Musso *et al.*, 1989b) and glomeruli (Massfelder *et al.*, 1993). PTHrP also relaxes the renal artery (Winquist *et al.*, 1987), the microdissected afferent and efferent arterioles (Trizna & Edwards, 1991) and preconstricted isolated perfused kidney of the rat (Musso *et al.*, 1989a,b). Nitric oxide-synthase as well as adenylyl cyclase signalling pathways have been shown to be stimulated during the vasodilatation induced by hPTHrP(1-34) in the isolated perfused kidney (Musso *et al.*, 1989b; Simeoni *et al.*, 1994). The dilator effect of hPTHrP(1-34) on vessel diameters along the intrarenal arterial tree and on single glomerular blood flow has been directly visualised in the split hydronephrotic rat

[1] Author for correspondence at: Laboratoire de Physiologie Cellulaire Rénale, Pavillon Poincaré, Hospices Civils, B.P. 426, 67091 Strasbourg Cedex, France.
T.M. and N.P. contributed equally to this work.

kidney (Endlich *et al.*, 1995). In this model, the peptide selectively dilated the preglomerular vessels and increased glomerular blood flow in individual glomeruli with EC_{50} values in the nanomolar range. The latter observation strongly suggests that the peptide has the potential to regulate glomerular filtration rate (GFR) and hence renal excretory functions. Moreover, immunoreactive PTHrP has been identified within the vascular tree (unpublished observations) as well as in glomerular epithelial and in proximal and distal tubules (Soifer *et al.*, 1993). In this work, immunohistochemical staining intensity increased in proximal tubular cells after ischaemia, changing its location from diffusely cytoplasmic to subapical by 24 h after reperfusion thus indicating its potential role during the recovery of kidney functions. Furthermore, like PTH, PTHrP has been shown to stimulate renal renin release independent of baroreceptor- and macula densa-related mechanisms at physiologically relevant peptide levels (Helwig *et al.*, 1991; Saussine *et al.*, 1993).

Though the effect of PTHrP on renal haemodynamics has been established in *in vitro* kidney preparations and in the hydronephrotic kidney, it could not yet be verified in normal kidneys *in vivo*, mainly because of its severe systemic effects. Therefore, the present study was designed to investigate the effect of PTHrP on both renal blood flow (RBF) and GFR in anaesthetized rats by use of a recently described method for infusing drugs continuously into the renal artery (Parekh, 1995). This method promotes an even distribution of drugs within the renal blood stream. By infusing the agonists directly into the renal artery, the cardiac, peripheral and central effects of the test agent can be largely avoided.

Methods

Experimental procedures

Experiments were performed on female Wistar rats weighing 180–230 g. The animals had free access to water, were fasted overnight before the experiments and were anaesthetized with 100 mg kg^{-1} body weight thiobarbitone (Inactin, Byk Gulden, Konstanz, Germany). Cannulae were placed into the left femoral vein for infusion of saline (0.9% NaCl or 0.6% NaCl + 0.5% creatinine) at 3 ml h^{-1} and into the left femoral artery for recording MAP and HR and collecting blood samples for determining haematocrit and creatinine concentrations. An electromagnetic flow probe (0.6 mm diameter, Carolina Medical Electronics, King, NC) was placed around the left renal artery for measuring RBF. In experiments in which clearance of exogenous creatinine (GFR) was determined, both ureters were catheterised via an abdominal incision. After surgery, an equilibration time of 30 to 45 min was allowed before the experiments were started.

For infusion of test agents into the renal artery, a previously described catheter system was used (Parekh, 1995). The catheter system consisted of a teflon cannula connected to multiple polyethylene cannulae, which in turn were connected to a magnetic membrane pump and to a desired number of infusion pumps. The teflon cannula was inserted through the right femoral artery into the abdominal aorta above the renal artery. The left renal artery was then dissected free through a subcostal flank incision, and infusion of lissamine green solution (1%) at 2–5 $\mu l\ min^{-1}$ was started and the tip of the teflon cannula (o.d. 150 μm) inserted under visual control into the left renal artery. The magnetic membrane pump connected to the multiple catheter system was operated periodically for 60 ms (ejection period) and switched off for 600 ms (suction period) to oscillate about 4 μl blood between the renal artery and the teflon cannula. Blood oscillation causes turbulence in the renal artery around the cannula tip during the ejection period and ensures thereby a thorough mixing of the infusate with the arterial blood stream, which otherwise does not occur in laminar arterial flow. Concentrations of the infused agents (C) and infusion rate ($\dot{Q}$, range from 5 to 20 $\mu l\ min^{-1}$) were adjusted to obtain the required final concentration in renal artery plasma (C_{pl}), whereby $C_{pl} = C \times \dot{Q}/RBF(1 - Hct)$.

Experimental protocols

Experiments were done on 5 groups of rats ($n = 4–6$ each). The effects of hPTHrP(1–34) and rPTH(1–34) on renal circulation were measured in the first and second groups, respectively (see Table 1). The effects of PTHrP after PTH/PTHrP receptor blockade with two different agents were studied in a third and fourth group. The protocol started with control measurements of mean arterial pressure (MAP), HR and RBF for 10 min to ascertain a steady-state of the measured parameters. In the first and second experimental groups, the last control measurement was followed by six sequential infusions of either PTH or PTHrP into the left renal artery to produce increasing concentrations in renal plasma ($C_{pl} = 2 \times 10^{-11}$, 6×10^{-11}, 2×10^{-10}, 6×10^{-10}, 2×10^{-9} and 6×10^{-9} M) for 5 to 10 min. In the third and fourth groups, the control measurement was followed by an infusion of either [$Nle^{8,18}$, Tyr^{34}]-bPTH(3–34)amide or [Leu^{11},D-Trp^{12}]-hPTHrP(7–34)amide, to maintain a C_{pl} of 2×10^{-8} M throughout the experiment. Ten min after the beginning of the antagonist infusion, kidneys were exposed to increasing concentrations of PTHrP. In the fifth group effects of PTHrP on GFR and $\dot{V}$ were determined. After a priming dose of 0.6 mg creatinine, an i.v. infusion was started to maintain a plasma creatinine concentration of circa 0.15 mg ml^{-1}. Clearance of exogenous creatinine has been shown to be an accurate measure of GFR (Zager, 1987). After two control urine collections, each of 10 min duration, from both kidneys, left renal PTHrP concentration was adjusted to about 10^{-10} M to increase left RBF by about 10%. Ten min after attaining a stable increase in RBF, urine samples were collected for a 10 min period. Creatinine concentrations were measured in urine samples and plasma samples taken at the beginning and the end of the experiment with an analyser (Creatinin Analyzer2, Beckman, Munich, Germany). The right kidney served as a time control of GFR and $\dot{V}$.

Table 1 Control values for mean arterial pressure (MAP), heart rate (HR) and renal blood flow (RBF) in 5 experimental groups of rats

Peptides		*Mean arterial pressure* (mmHg)	*Heart rate* (beats min^{-1})	*Renal blood flow* (ml min^{-1})
hPTHrP(1–34)	(6)	115 ± 2	365 ± 17	8.7 ± 0.6
rPTH(1–34)	(4)	108 ± 3	370 ± 15	8.3 ± 1.0
hPTHrP(1–34) + (3–34)analogue	(4)	121 ± 3	350 ± 15	7.2 ± 1.2
hPTHrP(1–34) + (7–34)analogue	(5)	114 ± 1	375 ± 12	7.8 ± 0.7
hPTHrP(1–34)	(4)	116 ± 10	382 ± 13	6.3 ± 0.8

The results are expressed as mean ± s.e.mean. Number of animals are given in parentheses. The values for MAP, HR and RBF were measured before intrarenal infusions of the peptides were started. Glomerular filtration rate was determined only in the last group.

Peptides

The following synthetic peptides were obtained from Bachem Feinchemikalien AG (Bubendorf, Switzerland) or Cambridge Research Biochemical (Northwich, Cheshire, U.K.): rat(r)PTH(1–34), human(h)PTHrP(1–34) and [Nle^{8,18},Tyr^{34}]-bovine(b)PTH(3–34)amide. [Leu^{11},D-Trp^{12}]-human(h)PTHrP (7–34) amide was purchased from Peninsula Laboratories (Belmont, CA, U.S.A.). All peptides were dissolved in 10^{-3} M HCl containing 0.1% bovine serum albumin at a final concentration of 2.5×10^{-4} M and stored at −70°C in 25 µl aliquots. Before use, peptides were further diluted to the desired concentration in 0.9% NaCl containing 0.1% bovine serum albumin.

Statistical analysis

All values were presented as means ± s.e.mean. Effects of increasing concentrations of peptides on different parameters were tested statistically by two-way analysis of variance followed by the Student-Newman-Keul's test for multiple comparisons. For a single intervention, paired Student's t test was used. Differences with P values less than 0.05 were considered statistically significant.

Results

Table 1 shows control values for MAP, HR and RBF values in the five groups. A typical recording of renal vasodilatation induced by increasing renal plasma concentrations of PTHrP is depicted in Figure 1. Similar patterns were observed in experiments with PTH instead of PTHrP. RBF increased in a concentration- and time-dependent manner. Up to a renal peptide concentration of 2×10^{-9} M, RBF increased at each step within 3 to 5 min and reached a stable plateau within 5 to 10 min, whereas MAP did not change significantly. At a renal concentration of 6×10^{-9} M, the peptide caused a transient rise in RBF followed by a steep drop in both RBF and MAP, indicating a spillover of the peptide into the systemic circulation. At this point, the infusion was stopped. MAP recovered rapidly after the infusion had stopped, but RBF remained more than 10% above the control value for at least 30 min. In additional experiments, PTHrP renal concentration was raised to 2×10^{-9} M in a single step, and the corresponding rise in RBF was found to be comparable to that obtained in sequential step experiments at the same concentration. This rules out the possibility that the renal vasodilator response is desensitised by continuous exposure of the kidney to the peptide.

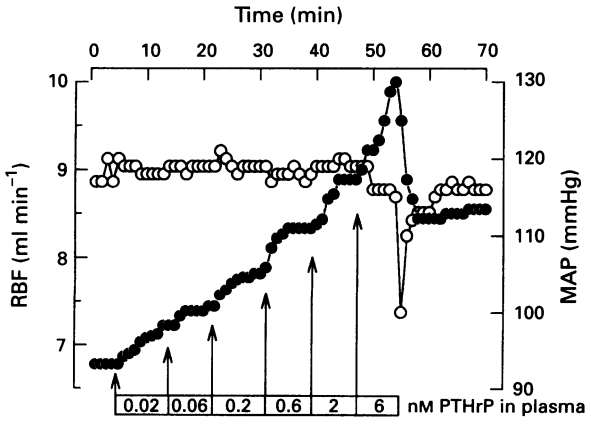

Figure 1 Recording of mean arterial pressure (MAP, ○) and renal blood flow (RBF, ●) illustrating the effects of increasing concentration of hPTHrP(1–34) in renal arterial plasma.

Mean values of MAP and relative change in RBF at different concentrations of PTHrP and PTH are shown in Figure 2. RBF increased significantly by 3.5 ± 1.7% at a threshold concentration of PTHrP (2×10^{-11} M), and by 34.3 ± 3.6% at the highest concentration (6×10^{-9} M). Since the maximal RBF response could not be obtained due to systemic hypotension at the highest concentration, an EC_{50} value could not be calculated. The relative changes in measured parameters due to PTH were practically identical to those at corresponding molar concentrations of PTHrP.

At a renal concentration of 2×10^{-8} M, the antagonists [Nle^{8,18},Tyr^{34}] - bPTH(3 – 34) amide and [Leu^{11},D - Trp^{12}]-hPTHrP(7–34)amide infused before hPTHrP(1–34) had no measurable effect on basal MAP, HR and RBF over a period of 10 min. Analogues were equipotent in antagonizing PTHrP-induced increase in RBF. At a molar ratio of (3–34) or (7–34) to PTHrP of 10:1, the RBF increase produced by 2×10^{-8} M PTHrP was inhibited by 63% (Figure 2).

Table 2 shows values of RBF, GFR, filtration fraction (FF) and $\dot{V}$ in the left PTHrP-infused and right time-control kidneys before and after an infusion of 10^{-10} M PTHrP. Relative changes induced by PTHrP on those parameters are shown in Figure 3. In these experiments MAP decreased slightly but significantly by 4.5 ± 0.9%, most likely due to prolonged duration of PTHrP infusion required for these measurements. At this low concentration PTHrP increased RBF in the left kidney by 10.0 ± 2.1% and GFR by 20.6 ± 4.2% ($P < 0.05$). FF was increased by 9.7 ± 3.4% but the rise was not statistically

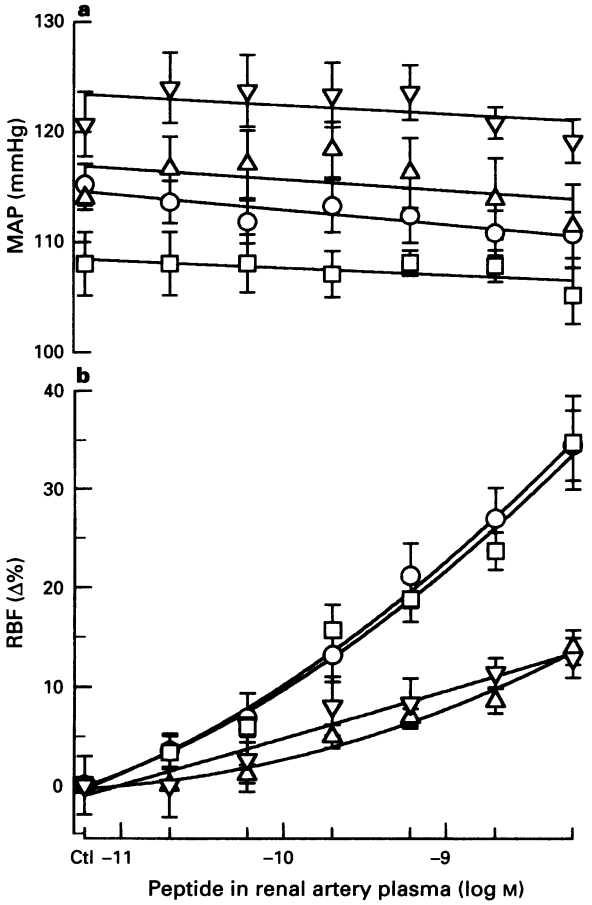

Figure 2 Effects of increasing renal concentrations of hPTHrP(1–34) (○, $n=6$) and rPTH(1–34) (□, $n=4$), on mean arterial pressure (a) and renal blood flow (b) under control conditions and in presence of 2×10^{-8} M of the PTH/PTHrP receptor antagonist [Nle^{8,18}, Tyr^{34}]-bPTH(3–34)amide (▽, $n=4$) or [Leu^{11}, D-Trp^{12}]-hPTHrP(7–34)amide (△, $n=5$).

Table 2 Effects of hPTHrP(1–34) on renal plasma flow (RPF), glomerular filtration rate (GFR), filtration fraction (FF) and diuresis ($\dot{V}$)

	Left kidney		*Right kidney*	
Parameters	*Baseline*	*hPTHrP(1–34)*	*Baseline*	*hPTHrP(1–34)*
RPF (ml min^{-1})	3.3±0.4	3.7±0.5*	ND	ND
GFR (ml min^{-1})	0.69±0.07	0.84±0.12*	0.78±0.05	0.79±0.08
FF (GFR/RPF, %)	21.3±2.0	23.5±2.5	ND	ND
$\dot{V}$ (μl min^{-1})	14.9±3.9	21.9±4.2*	15.0±4.18	11.4±3.4

PTHrP was infused only into the left renal artery (10^{-10} M); the contralateral right kidney served as a time-control ($n=4$). *$P<0.05$, ND = not determined.

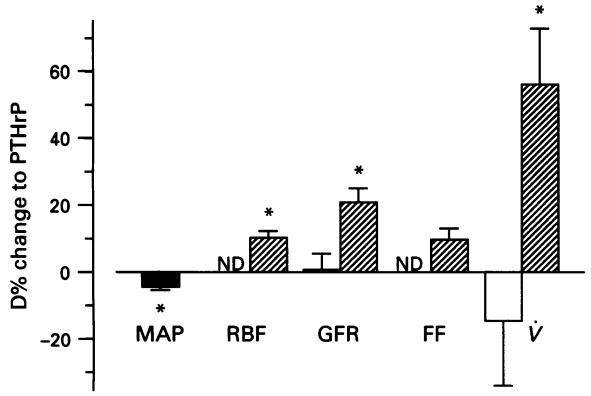

Figure 3 Relative changes in mean arterial pressure (solid column), and right (open columns) and left kidney (hatched columns) renal blood flow (RBF), glomerular filtration rate (GFR), filtration fraction (FF) and diuresis ($\dot{V}$) in response to 10^{-10} M hPTHrP(1–34) into the left experimental kidney. Values represent means ±s.e.mean ($n=4$). *$P<0.05$ vs baseline. ND = not determined.

significant ($0.1<P<0.05$). The peptide increased $\dot{V}$ by 56.3+16.4% in the left kidney. GFR and $\dot{V}$ in the right contralateral kidney were not affected by PTHrP infusion.

Discussion

Renal effects of PTH or its recently discovered related protein PTHrP on RBF and GFR *in vivo* are still unclear. The main reason for this is the large cardiovascular and central effects of the peptides, which affect renal functions. Renal vasodilatation due to PTHrP has been shown in studies with rat or rabbit kidney preparations *in vitro* (Musso *et al.*, 1989a,b; Trizna & Edwards, 1991). The renal vasodilator effect in response to PTH has been shown in former studies *in vivo* in dogs (Charbon & Hulstaert, 1974; Wang *et al.*, 1984; Crass *et al.*, 1987). However, in the rat kidney infusion of either PTH (Ichikawa *et al.*, 1978; Schor *et al.*, 1981; Brenner *et al.*, 1982) or PTHrP (Roca-Cusachs *et al.*, 1991) did not increase RBF. The discrepancy between these results might reflect a species-dependent difference in sensitivity of RBF to the peptides. Yet RBF changes depend not only upon direct renal effects but also upon accompanying systemic effects and the experimental procedure. Roca-Cusachs *et al.* (1991) also compared the effect of intravenous infusions of PTHrP and PTH on vascular resistance in different organs in rat by use of a microsphere technique. The peptides were most effective in reducing flow resistance in the heart and skin, had a moderate effect on the kidney and were least effective in splanchnic organs. Endlich *et al.* (1995) demonstrated for the first time PTHrP- and PTH-dependent renal vasorelaxation *in vivo* in the absence of systemic effects. Using videomicroscopic techniques they measured the rise in glomerular blood flow in the split hydronephrotic rat kidney suspended in a tissue bath and exposed to a desired bath concentration of the peptide without perturbing systemic effects. In the present study we infused the peptides into the artery of a normal kidney, thus avoiding or minimising systemic effects of the peptides and being able to adjust their concentration in renal plasma. Uneven distribution of infused test agents in arterial blood, inherent to laminar flow in arteries, was overcome by use of a recently developed device (Parekh, 1995).

We found a dose-dependent rise in RBF up to 35% above control value, and the curves for PTHrP and PTH were practically superimposable. We could not determine the maximal increase in RBF needed for calculating EC_{50} values, as at renal concentrations of 6×10^{-9} M and above there was a spillover of the peptides into the systemic circulation. However, on the assumption that the highest measured rise in RBF in our experiments is close to the maximal response, the mean EC_{50} value of 5×10^{-10} M determined in the hydronephrotic kidney (Endlich *et al.*, 1995) appears to be applicable also to the intact rat kidney. In addition, in the hydronephrotic rat kidney the effect of PTH and PTHrP on glomerular blood flow was similar in molar terms to those observed in RBF in the present study. In rat isolated, perfused kidney (Musso *et al.*, 1989b) and in isolated perfused afferent and efferent arterioles of the rabbit kidney, the corresponding EC_{50} values for PTHrP have been found to be 3×10^{-9} M and 10^{-8} M, respectively. It is also interesting to note that in our experiments a 25% rise in RBF was attained at a peptide concentration of 2×10^{-9} M, whereas in a previous study using the same methodology it was obtained with 2×10^{-7} M acetylcholine (Parekh, 1995). Thus, on a molar basis the vasodilator potency of PTHrP and PTH appears about 100 times higher than acetylcholine in the kidney *in vivo*.

In our experiments PTHrP and PTH increased RBF gradually (5 to 10 min) after the start of the infusion and decreased slowly (>30 min) after the infusion had stopped whereas in the isolated perfused kidney of the rat the corresponding changes have been found to be rapid and short-lasting (Musso *et al.*, 1989a,b). This difference might reflect an interaction of the peptides with blood-derived components or a difference in binding- and degradation-kinetics under *in vivo* and *in vitro* conditions.

Amino-terminally truncated PTH and PTHrP peptides are potent receptor antagonists. A single receptor has been shown to be responsible for the expression of the biological activity of both PTH and PTHrP in bone and renal tubules (Abou-Samra *et al.*, 1992; Orloff *et al.*, 1992). This is apparently true for the renal glomerulo-vascular system as well, in which hPTHrP(1–34) has been shown to bind to a single class of receptors in isolated, renal cortical microvessels (Nickols *et al.*, 1990). Moreover, [Nle^{8,18}, Tyr^{34}]-bPTH(3–34)amide, the first PTH antagonist described *in vitro* (Rosenblatt *et al.*, 1977), and its further truncated [Tyr^{34}]-bPTH(7–34)amide (Horiuchi *et al.*, 1983), strongly antagonized PTHrP-induced adenylyl cyclase stimulation in isolated renal microvessels (Musso *et al.*, 1989b), in glomeruli (Massfelder *et al.*, 1993) and PTHrP-induced vasodilatation in the isolated perfused kidney of the rat (Musso *et al.*, 1989b). In the present study, the PTH analogue [Nle^{8,18}, Tyr^{34}]-bPTH(3–34)amide, and the PTHrP analogue [Leu^{11}, D-

Trp[12]]-hPTHrP(7–34)amide (Nutt *et al.*, 1990), were equipotent in inhibiting the *in vivo* renal vascular effects of PTHrP. The rise in RBF was inhibited by 63% at a molar agonist-antagonist ratio of 1:10 suggesting a PTH/PTHrP receptor-mediated mechanism. Furthermore, infusion of peptide antagonists for 10 min had no effect on RBF. This indicates either a low level of endogenous PTHrP or a slow replacement from receptors by antagonists. Since it is presently not known whether PTHrP is constitutively secreted, extracellular concentrations of PTHrP could be low despite the presence of the immunoreactive peptide in renal vascular smooth muscle and endothelial cells. On the other hand, a slow clearance of PTHrP from receptors was indicated from the rise in RBF which persisted more than 30 min after the PTHrP infusion had stopped. Our experimental design is not overly suitable for quantifying the role of the endogenous peptide if the above factors are operative. A small change in RBF due to prolonged infusion of antagonists is difficult to distinguish from basal fluctuations with time.

In the present study, subnanomolar concentration of PTHrP produced a substantial increase in GFR and $\dot{V}$, and a close to significant ($0.1 > P > 0.5$) increase in FF. Earlier studies in which an i.v. infusion of PTH was administered failed to detect the rise in GFR or single nephron GFR, but found a significant reduction in the glomerular ultrafiltration coefficient (Ichikawa *et al.*, 1978; Schor *et al.*, 1981; Brenner *et al.*, 1982). In a previous study on the hydronephrotic kidney (Endlich *et al.*, 1995), PTHrP and PTH dilated all preglomerular but no postglomerular vessels. Applied to the normal filtering kidney, this should increase glomerular filtration pressure and hence FF. On the other hand, the reduction of the glomerular ultrafiltration coefficient described above should reduce FF. Which mechanisms were operative in our experiments, and to what degree, is not known.

In conclusion, the present study provides the first direct evidence that exogenous PTHrP, in absence of its systemic effects, increases RBF, GFR and urine flow *in vivo*. PTHrP and PTH were equipotent in increasing RBF, and their renovascular effects could be substantially inhibited by two different structural analogues suggesting a common PTH/PTHrP receptor-mediated mechanism. The role of this putative paracrine hormone in regulating the renal microcirculation and excretory functions under different physiological and pathophysiological conditions requires further investigation.

Abbreviations

FF: filtration fraction; GFR: glomerular filtration rate; HR: heart rate; MAP: mean arterial pressure; rPTH(1–34) or hPTHrP(1–34): 1–34 fragment of rat parathyroid hormone or human PTH-related protein; RBF: renal blood flow; RPF: renal plasma flow; $\dot{V}$: diuresis.

This work was supported by the French National Institute of Health and Medical Research (INSERM), grant CJF 9409, the French Ministry of Higher Education (MENESR), EA 1314, the French Medical Foundation, the European Community Program PROCOPE and the German Research Foundation (DFG) Ste 107/12-2.

References

ABOU-SAMRA, A.B., JÜPPNER, H., FORCE, T., FREEMAN, M.W., KONG, X.F., SCHIPANI, E., URENA, P., RICHARDS, J., BONVENTRE, J.V., POTTS JR, J.T., KRONENBERG, H.M. & SEGRE, G.V. (1992). Expression cloning of a common receptor for parathyroid hormone and parathyroid hormone-related peptide from rat osteoblast-like cells – A single receptor stimulates intracellular accumulation of both cAMP and inositol trisphosphates and increases intracellular free calcium. *Proc. Natl. Acad. Sci. U.S.A.*, **89,** 2732–2736.

BURTIS, W.J., WU, T., BUNCH, C., WYSOLMERSKI, J.J., INSOGNA, K.L., WEIR, E.C., BROADUS, A.E. & STEWART, A.F. (1987). Identification of a novel 17,000-dalton parathyroid hormone-like adenylate cyclase-stimulating protein from a tumor associated with humoral hypercalcemia of malignancy. *J. Biol. Chem.*, **262,** 7151–7156.

BRENNER, B.M., SCHOR, N. & ICHIKAWA, I. (1982). Role of angiotensin II in the physiologic regulation of glomerular filtration. *Am. J. Cardiol.*, **49,** 1430–1433.

BURTON, D.W., BRANDT, D.W. & DEFTOS, L.J. (1994). Parathyroid hormone-related protein in the cardiovascular system. *Endocrinology*, **135,** 253–261.

CHARBON, G.A. & HULSTAERT, P.F. (1974). Augmentation of arterial hepatic and renal flow by extracted and synthetic parathyroid hormone. *Endocrinology*, **95,** 621–626.

CRASS III, M.F., JAYASEELAN, C.L. & DARTER, T.C. (1987). Effects of parathyroid hormone on blood flow in different regional circulations. *Am. J. Physiol.*, **253,** R634–R639.

ENDLICH, K., MASSFELDER, T., HELWIG, J.J. & STEINHAUSEN, M. (1995). Vascular effects of parathyroid hormone (PTH) and PTH-related protein in the split hydronephrotic rat kidney. *J. Physiol.*, **483,** 481–490.

HALLORAN, B.P. & NISSENSON, R.A. (1992). '*Parathyroid Hormone-Related Protein: Normal Physiology And Its Role In Cancer*', Boca Raton, FL, U.S.A.: CRC Press.

HELWIG, J.J., MUSSO, M.J., JUDES, C. & NICKOLS, G.A. (1991). PTH and calcium: Interactions in the control of renin secretion in the isolated non-filtering rat kidney. *Endocrinology*, **129,** 1233–1242.

HONGO, T., KUPFER, J., ENOMOTO, H., SHARIFI, B., GIANNELLA-NETO, D., FORRESTER, J.S., SINGER, F.R., GOLTMAN, D., HENDY, G.N., PIROLA, C., FAGIN, J.A. & CLEMENS, T.L. (1991). Abundant expression of parathyroid hormone-related protein in primary rat aortic smooth muscle cells accompanies serum-induced proliferation. *J. Clin. Invest.*, **88,** 1841–1847.

HORIUCHI, N., HOLICK, M.F., POTTS JR, J.T. & ROSENBLATT, M. (1983). A parathyroid hormone inhibitor in vivo: design and biological evaluation of a hormone analog. *Science*, **220,** 1053–1055.

ICHIKAWA, I., HUMES, H.D. & DOUSA, T.P. (1978). Influence of parathyroid hormone on glomerular filtration in the rat. *Am. J. Physiol.*, **3,** F393–F401.

ISHIKAWA, M., OUCHI, Y., AKISHITA, M., KOZAKI, K., TOBA, K., NAMIKI, A., YAMAGUCHI, T. & ORIMO, H. (1994). Immunocytochemical detection of parathyroid hormone-related protein in vascular endothelial cells. *Biochem. Biophys. Res. Commun.*, **199,** 547–551.

KRAMER, S., REYNOLDS, F.H., CASTILLO, M., VALENZUELA, D.M., THORIKAY, M. & SORVILLO, J.M. (1991). Immunological identification and distribution of parathyroid hormone-like protein polypeptides in normal and malignant tissues. *Endocrinology*, **128,** 1927–1937.

MASSFELDER, T., SAUSSINE, C., SIMEONI, U., ENANGA, B., JUDES, C. & HELWIG, J.J. (1993). Evidence for adenylyl cyclase-dependent receptors for parathyroid hormone (PTH)-related protein in rabbit kidney glomeruli. *Life Sci.*, **53,** 875–881.

MOSELEY, J.M., KUBOTA, M., DIEFFENBACH-JAGGER, H., WETTENHALL, R.E.H., KEMP, B.E., SUVA, L.J., RODDA, C.P., EBELING, P.R., HUDSON, P.J., ZAJAC, J.D. & MARTIN, T.J. (1987). Parathyroid hormone-related protein purified from a human lung cancer line. *Proc. Natl. Acad. Sci. U.S.A.*, **84,** 5048–5052.

MUSSO, M.J., BARTHELMEBS, M., IMBS, J.L., PLANTE, M., BOLLACK, C. & HELWIG, J.J. (1989a). The vasodilator action of parathyroid hormone fragments on isolated perfused rat kidney. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **340,** 246–251.

MUSSO, M.J., PLANTE, M., JUDES, C., BARTHELMEBS, M. & HELWIG, J.J. (1989b). Renal vasodilation and microvessel adenylate cyclase stimulation by synthetic parathyroid hormone-like protein fragments. *Eur. J. Pharmacol.*, **174,** 139–151.

NICKOLS, G.A., NICKOLS, M.A. & HELWIG, J.J. (1990). Binding of parathyroid hormone and parathyroid hormone-related protein to vascular smooth muscle of rabbit renal microvessels. *Endocrinology*, **126,** 721–727.

NICKOLS, G.A., NANA, A.D., NICKOLS, M.A., DIPETTE, D.J. & ASIMAKIS, G.K. (1989). Hypotension and cardiac stimulation due to the parathyroid hormone-related protein, humoral hypercalcemia of malignancy factor. *Endocrinology*, **125,** 834–841.

NUTT, R.F., CAULFIELD, M.P., LEVY, J.J., GIBBONS, S.W., ROSENBLATT, M. & MCKEE, R.L. (1990). Removal of partial agonism from parathyroid hormone (PTH)-related protein-(7–34)NH2 by substitution of PTH amino acids at position-10 and position-11. *Endocrinology*, **127,** 491–493.

ORLOFF, J.J., RIBAUDO, A.E., MCKEE, R.L., ROSENBLATT, M. & STEWART, A.F. (1992). A pharmacological comparison of parathyroid hormone receptors in human bone and kidney. *Endocrinology*, **131,** 1603–1611.

PAREKH, N. (1995). A novel method for infusing drugs continuously into the renal artery of rats. *Am. J. Physiol.*, **37,** F967–F971.

PHILBRICK, W.M., WYSOLMERSKI, J.J., GALBRAITH, S., HOLT, E., ORLOFF, J.J., YANG, K.H., VASAVADA, R.C., WEIR, E.C., BROADUS, A.E. & STEWART, A.F. (1996). Defining the roles of parathyroid hormone-related protein in normal physiology. *Physiol. Rev.*, **76,** 127–173.

PIROLA, C.J., WANG, H.M., KAMYAR, WU, S., ENOMOTO, H., SHARIFI, B., FORRESTER, J.S., CLEMENS, T.L. & FAGIN, J.A. (1993). Angiotensin II regulates parathyroid hormone-related protein expression in cultured rat aortic smooth muscle cells through transcriptional and post-transcriptional mechanisms. *J. Biol. Chem.*, **268,** 1987–1994.

PIROLA, C.J., WANG, H.M., STRGACICH, M.I., KAMYAR, A., CERCEK, B., FORRESTER, J.S., CLEMENS, T.L. & FAGIN, J.A. (1994). Mechanical stimuli induce vascular parathyroid hormone-related protein gene expression *in vivo* and *in vitro*. *Endocrinology*, **134,** 2230–2236.

ROCA-CUSACHS, A., DIPETTE, D.J. & NICKOLS, G.A. (1991). Regional and systemic hemodynamic effects of parathyroid hormone-related protein – Preservation of cardiac function and coronary and renal flow with reduced blood pressure. *J. Pharmacol. Exp. Ther.*, **256,** 110–118.

ROSENBLATT, M., CALLAHAN, E.N., MAHAFFEY, J.E., PONT, A. & POTTS JR, J.T. (1977). Parathyroid hormone inhibitors. Design, synthesis, and biologic evaluation of hormone analogues. *J. Biol. Chem.*, **252,** 5847–5851.

SAUSSINE, C., MASSFELDER, T., PARNIN, F., JUDES, C., SIMEONI, U. & HELWIG, J.J. (1993). Renin stimulating properties of parathyroid hormone-related peptide in the isolated perfused rat kidney. *Kidney Int.*, **44,** 764–773.

SCHOR, N., ICHIKAWA, I. & BRENNER, B.M. (1981). Mechanisms of action of various hormones and vasoactive substances on glomerular ultrafiltration in the rat. *Kidney Int.*, **20,** 442–451.

SIMEONI, U., MASSFELDER, T., SAUSSINE, C., JUDES, C., GEISERT, J. & HELWIG, J.J. (1994). Involvement of nitric oxide in the vasodilatory response to parathyroid hormone-related peptide in the isolated rabbit kidney. *Clin. Sci.*, **86,** 245–249.

SOIFER, N.E., VANWHY, S.K., GANZ, M.B., KASHGARIAN, M., SIEGEL, N.J. & STEWART, A.F. (1993). Expression of parathyroid hormone-related protein in the rat glomerulus and tubule during recovery from renal ischemia. *J. Clin. Invest.*, **92,** 2850–2857.

STREWLER, G.J., STERN, P.H., JACOBS, J.W., EVELOFF, J., KLEIN, R.F., LEUNG, S.C., ROSENBLATT, M. & NISSENSON, R.A. (1987). Parathyroid hormone-like protein from human renal carcinoma cells. Structural and functional homology with parathyroid hormone. *J. Clin. Invest.*, **80,** 1803–1807.

TRIZNA, W. & EDWARDS, R.M. (1991). Relaxation of renal arterioles by parathyroid hormone and parathyroid hormone-related protein. *Pharmacology*, **42,** 91–96.

WANG, H.H., DRUGGE, E.D., YEN, Y.C., BLUMENTHAL, M.R. & PANG, P.K.T. (1984). Effects of synthetic parathyroid hormone on hemodynamics and regional blood flows. *Eur. J. Pharmacol.*, **97,** 209–215.

WINQUIST, R.J., BASKIN, E.P. & VLASUK, G.P. (1987). Synthetic tumor derived human hypercalcemic factor exhibits parathyroid hormone-like vasorelaxation in renal arteries. *Biochem. Biophys. Res. Commun.*, **149,** 227–232.

ZAGER, R.A. (1987). Exogenous creatinine clearance accurately assesses filtration failure in rat experimental nephropathies. *Am. J. Kidney Diseases*, **6,** 427–430.

(Received January 31, 1996
Revised April 15, 1996
Accepted May 3, 1996)