Knockdown of parathyroid hormone related protein in smooth muscle cells alters renal hemodynamics but not blood pressure

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Raison D, Coquard C, Hochane M, Steger J, Massfelder T, Moulin B, Karaplis AC, Metzger D, Chambon P, Helwig J-J, Barthelmebs M. Knockdown of parathyroid hormone related protein in smooth muscle cells alters renal hemodynamics but not blood pressure. Am J Physiol Renal Physiol 305: F333–F342, 2013. First published May 29, 2013; doi:10.1152/ajprenal.00503.2012.—Parathyroid hormone-related protein (PTHrP) belongs to vasoactive factors that regulate blood pressure and renal hemodynamics both by reducing vascular tone and raising renin release. PTHrP is expressed in systemic and renal vasculature. Here, we wanted to assess the contribution of vascular smooth muscle cell endogenous PTHrP to the regulation of cardiovascular and renal functions. We generated a mouse strain (SMA-CreERT2/PTHrP<sup>+/-</sup> or premutant PTHrP<sup>-/-</sup>/), which allows temporally controlled, smooth muscle-targeted PTHrP knockdown in adult mice. Tamoxifen treatment induced efficient reduction of PTHrP-floxed alleles and decreased PTHrP expression in vascular and visceral smooth muscle cells of PTHrP<sup>-/-</sup>/ mice. Blood pressure remained unchanged in PTHrP<sup>-/-</sup>/ mice, but plasma renin concentration and creatinine clearance were reduced. Renal hemodynamics were further analyzed during clearance measurements in anesthetized mice. Conditional knockdown of PTHrP decreased renal plasma flow and glomerular filtration rate with concomitant reduction in filtration fraction. Similar measurements were repeated during acute saline volume expansion. Saline volume expansion induced a rise in renal plasma flow and reduced filtration fraction; both were blunted in PTHrP<sup>-/-</sup>/ mice leading to impaired diuresis. These findings show that endogenous vascular smooth muscle PTHrP controls renal hemodynamics under basal conditions, and it is an essential factor in renal vasodilation elicited by saline volume expansion.

Parathyroid hormone-related protein; blood pressure; renal hemodynamics; saline volume expansion

Parathyroid hormone-related protein (PTHrP) was discovered as the agent responsible for humoral hypercalcemia of malignancy. This protein shares amino-terminal sequence homology with PTH and acts through a common PTH/PTHrP receptor (PTH1R) to promote bone resorption and inhibit calcium excretion (37). PTHrP acts as an endocrine effector in cancer and fetal development, but its plasma levels are usually undetectable in normal adults. Indeed, PTHrP is expressed in a wide variety of normal tissues, where it acts through autocrine, paracrine, or intracrine pathways to regulate local functions (4, 16). In the cardiovascular system, PTHrP mRNA and protein are mainly localized in the vessel’s smooth muscle layer, but PTHrP expression has also been reported in endothelial cells and in the heart (32, 33). PTHrP shares with PTH hypotensive and chronotropic effects when infused to conscious rats, together with a direct relaxation on aortic strips (24). The renal vasculature is an important target for PTHrP. Intravenous administration of PTHrP to healthy subjects at a dose devoid of hypotensive effects induced a marked increase of renal blood flow (41). In the rat, when regional hemodynamics were analyzed by the radioactive microsphere technique, PTHrP preserved renal blood flow, although blood pressure decreased (29). PTHrP and PTH1R are expressed all through the intrarenal arterial tree, and exogenous application of PTHrP (1–36) increases renal blood flow and glomerular filtration rate in anesthetized rat by preferentially dilating the preglomerular vessels (6, 17, 18). Moreover, PTHrP elicits vasorelaxation and renin release on the isolated perfused kidney (21, 31). Cultured arterial vascular smooth muscle cells express both PTHrP and PTH1R (34). The strong induction of PTHrP by vasoconstrictors and mechanical stretch suggests that PTHrP may control vascular tone through a feedback loop. It is, however, not clear whether these cardiovascular and renal effects of PTHrP are elicited by endogenous PTHrP produced under physiological settings.

Knockout mice for PTHrP or PTH1R genes did not help to answer this question because they died at birth or in utero with premature calcification of chondrocytes and skeletal dysplasia (11, 12). Rescue PTHrP-null mice by transgenic expression of PTHrP targeted to chondrocytes survived less than 3 wk with multiple abnormalities (42), and PTH1R-null mice with targeted expression of a constitutively active PTH1R in the growth plate died perinatally (35). On the other hand, transgenic mice overexpressing PTHrP or PTH1R selectively in the smooth muscle developed a cardiovascular phenotype with decreased blood pressure and altered vascular reactivity (13, 28). Moreover, exaggerated renal vasodilation after volume expansion and blunted vasoconstriction in response to ANG II were reported in mice overexpressing the PTH1R (25). Finally, PTH1R overexpression in normotensive and hypertensive rats by systemic PTH1R cDNA plasmid delivery induced changes in blood pressure, renal vascular tone, and plasma renin activity (8, 19). Altogether, these findings support the contribution
of PTHrP to the regulation of cardiovascular and renal functions when PTHrP or PTH1R are overexpressed.

The aim of the present study was to evaluate the role of endogenous PTHrP in cardiovascular and renal functions by using for the first time a CreLox mouse model. This mouse strain allows smooth muscle-specific, tamoxifen (Tam)-inducible PTHrP deletion in adult mice. We report that knockdown of endogenous PTHrP in smooth muscle does not affect blood pressure but alters renal functions, particularly renal hemodynamics and renin release. Moreover, the renal response to volume expansion is blunted.

MATERIALS AND METHODS

Generation of transgenic mice. Premutant PTHrP<sup>OM-/-</sup> mice were obtained by crossing mating mice carrying PTHrP-floxed gene (PTHRP<sup>p2.5/2.5</sup>) (10) with SMA-Cre<sup>ERT2</sup> mice that express, selectively in vascular and visceral smooth muscle cells [α-smooth muscle actin (SMA) promoter], a chimeric Cre recombinase (Cre<sup>ERT2</sup>), which can be activated by Tam treatment (40). The PTHrP<sup>p2.5/2.5</sup> mice carry two loxP sites flanking exon 4 of the PTHrP gene, which encodes most of the protein (10). Premutant PTHrP<sup>OM-/-</sup> [SMA-Cre<sup>ERT2</sup>(<sup>p</sup>)/P<sup>THrP</sup>]<sup>p2.5/2.5</sup> mice and their littermates (PTHRP<sup>p2.5/2.5</sup>) were used. Mice genotypes were determined on tail DNA by PCR for Cre<sup>ERT2</sup> recombinase (primer sequences in Table 1). Mice breeding, care, and procedures, in compliance with guidelines of the European Community (2010/63/EU) and the French government concerning the use of animals, were approved by the local Animal Ethics Committee (Mouse Clinic-Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France). Only males were used to avoid hormonal effects in physiological tests.

Adult mice, 2–4 mo of age, were treated intraperitoneally for 5 consecutive days with vehicle (sunflower oil) or 1 mg tamoxifen (Tam; Sigma-Aldrich, St. Quentin Fallavier, France), which was shown to efficiently induce Cre-mediated recombination in SMA-Cre<sup>ERT2</sup> mice (40). SMA-Cre<sup>ERT2</sup>/ACZL mice were used to verify effective Cre-mediated recombination in intrarenal arterioles (40). One day after the last injection (day 6), animals were euthanized by exsanguination under intraperitoneal anesthesia with thiobutabarbital (80 mg/kg), supplemented with ketamine (100 mg/kg body wt) and xylazine (10 mg/kg body wt). Anesthesia was considered safe when no attempt to withdraw the limb after pinching could be observed. Organs were then isolated for RNA extraction.

Thoracic aorta and bladder were opened and scraped to remove endothelial and epithelial cells, respectively. Second- and third-order mesenteric arterioles were carefully dissected under a microscope (Nikon). Intact aorta, bladder, heart, and kidney were also fixed for 3 h in 4% paraformaldehyde (pH 7.4) and paraffin-embedded for histological purposes. Abdominal aorta was used to verify recombination of the PTHrP-floxed alleles by PCR (primers shown on Table 1). The presence of amplicons was verified by electrophoresis on 1.5% agarose gel.

Real-time RT-PCR analysis. Total RNAs were extracted from the organs using the TRIzol reagent (Invitrogen, Cergy Pontoise, France), according to the manufacturer’s protocol. Five micrograms of RNA were reverse-transcribed to cDNA at 37°C for 1 h, in a reaction buffer with monospecific primer p(dT)15 (Roche Diagnostics, Meylan, France). Quantitative real-time PCR reactions were performed with the LightCycler-FastStart DNA Master SYBR Green kit (Roche Diagnostics). The sequences of primer pairs are given in Table 1 for PTHrP, PTH1R, and GAPDH as a housekeeping gene. Results were analyzed with the LightCycler software (Roche Diagnostics), normalized according to GAPDH mRNA, and the ratio was set at 1 for the control group in each tissue.

Immunohistochemistry. Immunohistochemistry was performed on paraffin-embedded tissues sections (6 μm) from aorta, bladder, heart, and kidney, using the DakoCytomation EnVision+ System-HRP (AEC) kit, according to the manufacturer’s instructions (Dako-France, Trappes, France). After quenching endogenous peroxidase activity, successive slices were incubated for 1 h at room temperature with rabbit anti-PTHRP(24–35) antiserum (C13 antisera, a gift from Dr. P. Esbrit; 1/600 dilution), or with rabbit anti-α-SMA antibody (Sigma-Aldrich; 1/200), and revealed by polymer-peroxidase complex and 3-amino-9-ethylcarbazole (AEC+) substrate chromogen. Some slices were incubated without primary antibodies or with nonimmune rabbit serum (Millipore, Molsheim, France; 1/600 dilution) as negative controls. Image acquisition was performed on similar areas on the slices using a Zeiss AxiosImager Z2 microscope equipped with a Zeiss MRM B/W camera. Vascular smooth muscle cells can then be easily identified, particularly on renal and cardiac (ventricle) tissues.

Blood pressure and heart rate measurements. Systolic blood pressure and heart rate were measured in conscious mice using a computerized sphygmomanometric tail-cuff system (Panlab and Powerlab software, Bioseb, Chaville, France). Mice were trained daily for 1 wk before measurements. Each value is the average of at least 10 separate determinations.

Urine collection in conscious mice. Mice were housed individually in metabolic cages (Tecniplast, Buguggiate, Italy) and were allowed to get accustomed for 5 days before a 24-h urine collection. At the end of urine collection, blood was obtained by retro-orbital puncture under ketamine-xylazine anesthesia as described above. Sodium, potassium, chloride, calcium, and phosphorus, together with creatinine (enzymatic method) were measured on plasma and urine (3). Creatinine clearance was calculated as usual.

Plasma renin concentration measurements. In separate groups of mice, blood samples were collected on EDTA tubes, from the retro-orbital plexus, under slight ketamine-xylazine anesthesia as described above. Plasma renin concentration was determined on the first day following completion of Tam or oil treatments (day 6). The plasma renin concentration was determined by RIA of ANG I generated after incubating the plasma for 1 h at pH 8.5 in the presence of an excess of rat angiotensinase as reported by Griol-Charhbili et al. (9).

Renal hemodynamic studies. Also on day 6, mice were prepared for renal clearance studies, as described by other groups (2, 25), but we used FITC-sinistrin as a highly water-soluble marker for glomerular filtration rate (27). Mice were anesthetized by an intraperitoneal injection of thiobutabarbitral (80 mg/kg), supplemented with ketamine.

Table 1. Nucleotide sequences of the primers used for PCR or real-time RT-PCR

<table>
<thead>
<tr>
<th>Mouse gene</th>
<th>Primer Sequence 5’ to 3’</th>
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<tr>
<td>Cre-&lt;sup&gt;ERT2&lt;/sup&gt; recombinase</td>
<td>TK139: ATTTGCCCTGGAATTACGGTC TK141: ATCAACGTTTTCTTTTCGG</td>
</tr>
<tr>
<td>PTHrP floxed</td>
<td>920: TTTGGAGGGTGCCTACCTTAGACAA 923: CGACAAAAATGGTAGGGAACCA</td>
</tr>
<tr>
<td>Real-time RT-PCR</td>
<td></td>
</tr>
<tr>
<td>PTHrP</td>
<td>GAGCCGAGAATCGAGAGCTACG CTCCTGTTCCTGCTGGTTC</td>
</tr>
<tr>
<td>PTH1R</td>
<td>GGCCAAGAAGAATGGGATGATT GGCATGAAGAGAAGTGTAGT</td>
</tr>
<tr>
<td>GAPDH</td>
<td>ATGGAGAAGGAGGAGGCTTC AGAGGATCATGTTGAGGAG</td>
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(25 mg/kg) and xylazine (2.5 mg/kg), so that noxious pinching of the limbs did not evoke any motor reflexes. Body temperature was maintained at 37°C, and mice were provided with a steady stream of 100% O₂ to breathe. Catheters were placed in the left carotid artery for blood pressure and heart rate measurements, in the right jugular vein for intravenous perfusions, in the bladder for urine collection and in the femoral artery for blood sampling. After surgery, FITC-sinistrin and PAH were administered as a bolus (3 μl/g body wt, 1% FITC-sinistrin, 4% PAH in PBS), followed by a maintenance infusion (0.15 μl·min⁻¹·g⁻¹, 0.5% FITC-sinistrin, 2% PAH). After a 60-min equilibration period, two consecutive 30-min urine samples were collected, flanked by arterial blood sampling (50 μl) for measurements of hematocrit, FITC-sinistrin, and PAH. At the end of the study, blood was directly withdrawn in some mice from the renal vein to estimate renal PAH extraction. The kidneys were then removed, drained, and weighed. In a separate group of mice, isotonic saline volume expansion (SVE) was performed as described by Noonan et al. (25). Infusion of isotonic saline (0.75 μl·min⁻¹·g body wt⁻¹) was initiated at the midpoint of the equilibration period and was then continued all over the study, corresponding to a volume overload of 6.7%. FITC fluorescence was determined in plasma and urine samples at 535 nm (excitation 485 nm) using a SpectraFluor Tecan spectrometer (Tecan, Lyon, France). PAH concentration was measured using a colorimetric test (15). Renal plasma flow was calculated from PAH clearance corrected for renal PAH extraction (0.80 ± 0.08%, means ± SE; n = 5). Renal vascular resistance and filtration fraction were calculated as usual. The mean values over the two clearance periods are given.

Statistical analysis. Results are expressed as means ± SE. Statistical analysis was performed when appropriate using Student’s t-test, one-way or two-way ANOVA followed by Tukey’s multiple-comparison test. The test of Grubb’s (α = 5%) was used to detect for the presence of outliers. Statistics were run with SigmaStat (Jandel Scientific, San Rafael, CA) or Prism (GraphPad, San Diego, CA). Differences with P < 0.05 were considered statistically significant.

RESULTS

Tam treatment of premutant PTHrP<sup>ΔSM−/−</sup> mice induces selective and efficient knockdown of PTHrP in vascular and visceral smooth muscle cells. Tam treatment for 5 days (1 mg/day ip) of premutant PTHrP<sup>ΔSM−/−</sup> mice resulted in conditional activation of CreER<sup>2</sup>. Efficient excision of exon 4 of PTHrP gene was confirmed by PCR analysis on aortic DNA performed on day 6, following the end of Tam treatment in PTHrP<sup>ΔSM−/−</sup> mice (Fig. 1, A and B). Moreover, at day 6, the level of PTHrP mRNA was decreased in various organs from the PTHrP<sup>ΔSM−/−</sup> mice compared with control mice (oil-treated premutant PTHrP<sup>ΔSM−/−</sup> or Tam-treated PTHrP<sup>L2/L2</sup>) (Fig. 2A).

This effect was particularly strong in the bladder and in capacitance or resistance arteries (aorta, mesenteric arterioles; 80% decrease, or more), but it did not reach significance in colon and kidney. However, intrarenal arterioles expressed Tam-activated CreER<sup>2</sup>, as documented by X-Gal staining on kidneys from SMA-CreER<sup>2</sup>/ACZL mice (Metzger D, unpublished).
lished results). PTHrP protein immunostaining was found in the kidney not only on vessels but also on the tubules, mainly the proximal ones (Fig. 3). A marked decrease in PTHrP immunostaining was observed on sections from aorta, bladder, coronary arteries, and intrarenal arterioles of PTHrP<sup>SM</sup>/−/− mice compared with control Tam-treated PTHrP<sup>L2/L2</sup> mice (Fig. 3). Decreased PTHrP expression had no effect on the expression of PTH1R in the aorta or the kidney (Fig. 2B). Because PTHrP knockdown was efficient on PTHrP expression of PTH1R in the aorta or the kidney (Fig. 2B) (Fig. 3). Decreased PTHrP expression had no effect on the time point and under basal conditions, before Tam treatment. and arterioles, functional experiments were performed at this time point and under basal conditions, before Tam treatment.

Comparison of basal parameters of PTHrP<sup>L2/L2</sup> and premutant PTHrP<sup>SM</sup>/−/− mice. Before any treatment (Tam or oil), there was no difference between PTHrP<sup>L2/L2</sup> and premutant PTHrP<sup>SM</sup>/−/− mice for the parameters considered in this study, including blood pressure, heart rate, and renal functions (Table 2).

Blood pressure, heart rate, and renal functions in awake PTHrP<sup>SM</sup>/−/− mice. As shown in Fig. 4A, systolic blood pressure measured by tail cuff sphygmomanometry in awake animals was similar on day 6 in PTHrP<sup>SM</sup>/−/− mice and control mice (oil-treated premutant PTHrP<sup>SM</sup>/−/− or Tam-treated PTHrP<sup>L2/L2</sup>), but a decrease in heart rate was observed in PTHrP<sup>SM</sup>/−/− mice. On animals placed in metabolic cages, food and water intake, diuresis, sodium, calcium, and phosphate excretions did not differ in PTHrP<sup>SM</sup>/−/− mice from values in control mice (Table 3). Creatinine clearance was significantly lower on day 6 in PTHrP<sup>SM</sup>/−/− mice (Fig. 4B) without, however, any impact on plasma creatinine (Table 3). Functional parameters were similar in Tam-treated PTHrP<sup>L2/L2</sup> mice and oil-treated premutant PTHrP<sup>SM</sup>/−/− mice (Table 3, Fig. 4).

Plasma renin concentration in PTHrP<sup>SM</sup>/−/− mice. We previously reported that PTHrP increases renin release from the kidney (31). Therefore, we measured plasma renin concentration in PTHrP<sup>SM</sup>/−/− mice on day 6. Under ketamine/xylazine anesthesia, we found a decrease in plasma renin concentration in PTHrP<sup>SM</sup>/−/− mice (Fig. 4C).

Renal hemodynamics in anesthetized PTHrP<sup>SM</sup>/−/− mice. To further confirm that the vascular loss of PTHrP modifies glomerular filtration rate, we performed clearance studies in anesthetized mice on day 6 (Fig. 5). Here, again mean blood pressure was similar between groups, but heart rate was no longer reduced in the PTHrP<sup>SM</sup>/−/− mice. Overall, heart rate was lower in anesthetized mice compared with awake ones,
indicating decreased sympathetic tone. We observed a 30% reduction in renal plasma flow in PTHrP<sup>SM</sup>−/− mice compared with controls. Because this effect occurred without changes in mean blood pressure, it reflected an increase in renal vascular resistance. The decrease in glomerular filtration rate was even higher (50%), resulting in a concomitant decrease in filtration fraction. No difference was observed between Tam-treated PTHrP<sup>L2/L2</sup> mice and oil-treated premutant PTHrP<sup>PSM</sup>−/− mice.

**DISCUSSION**

In this paper, we present a new mouse model, allowing inducible and smooth muscle-specific PTHrP knockdown to analyze the contribution of endogenous PTHrP to the regulation of cardiovascular and renal functions in adult mice. Tam-inducible PTHrP knockdown results from Cre/loxP recombination in a mouse strain obtained by cross-mating mice bearing PTHrP-floxed alleles and SMA-CreER<sup>T2</sup> mice, which express the Tam-dependent CreER<sup>T2</sup> recombinase selectively in smooth muscle cells (10, 40). In our study, smooth muscle-targeted expression of CreER<sup>T2</sup> recombinase led to recombination of floxed PTHrP, which was strictly Tam-dependent, in agreement with X-gal staining described previously in reporter mice (40). Recombination led to a decrease in PTHrP mRNA in α-SMA-rich tissues, and in protein immunostaining in aorta, bladder, coronary arteries, and intrarenal arterioles on day 6, after the 5-day Tam treatment of premutant PTHrP<sup>PSM</sup>−/− mice. Thus, all functional studies were performed on day 6. PTH1R expression was not modified in the aorta after PTHrP knockdown, in line with the absence of feedback of PTHrP on PTH1R expression in vascular smooth muscle cells (26).

When we analyzed cardiovascular and renal functional parameters in PTHrP<sup>SM</sup>−/− mice, we found no change in systolic blood pressure, but heart rate, creatinine clearance, and plasma renin concentration were decreased. However, since measurements on day 6 were performed just after the end of Tam treatment, functional changes could have been due to Tam treatment per se. Tam is a first-generation estrogen receptor modulator and acts as an estrogen receptor agonist on arteries. Tam has been found to relax coronary arteries in vitro (7), to increase responses to various vasoconstrictors in mesenteric vascular bed after a 3-wk treatment (14) but did not affect blood pressure or plasma renin activity (36). We observed no difference, whatever the functional parameter considered, when we compare 5-day oil-treated premutant PTHrP<sup>PSM</sup>−/− mice with 5-day Tam-treated PTHrP<sup>L2/L2</sup> mice, these mice strains being similar before any treatment. Hence, we concluded that changes observed on day 6 in the PTHrP<sup>SM</sup>−/− group were linked to Tam-induced knockdown of PTHrP in smooth muscle cells.

Hypotension has extensively been reported after systemic administration of PTHrP (5, 24, 29) and in transgenic mice, which overexpress either PTHrP or PTH1R in smooth muscle (13, 28), as well as in rats after systemic delivery of naked PTH1R cDNA (8, 19). In all of these studies, hypotension was the result of enforced overexpression of the ligand or the receptor. In the present study, efficient knockdown of PTHrP in arteries and arterioles with normal PTH1R expression did not affect blood pressure, when measured either indirectly in awake mice or intra-arterially in anesthetized animals. We cannot exclude that compensatory mechanisms are triggered to maintain cardiovascular homeostasis. The concomitant decrease in heart rate observed in conscious mice may reflect...
buffering through baroreflex. However, when the effects on heart rate were blunted under anesthesia, blood pressure again remained stable. Another possibility is that PTHrP secreted by endothelial cells acts in a paracrine way on the PTH1R present on smooth muscle cells, or in an autocrine way on endothelial cells themselves. Although PTHrP-induced vasodilation is clearly independent of the endothelium in rats (18, 23), endothelium-dependent aortic relaxation has been reported in mice (38). It is likely that compensatory mechanisms, mainly endothelium-dependent, help in maintaining blood pressure stability in mice with specific knockdown of PTHrP in vascular smooth muscle layer.

Cardiac cells are targets of PTHrP, which was found to exert both positive chronotropic and inotropic effects on isolated rat hearts (24). PTHrP is locally expressed at high levels in cardiomyocytes in the atria (1). In the rat ventricle, PTHrP was claimed to derive mainly from coronary endothelial cells (32). However, PTHrP immunostaining was repeatedly reported in ventricular cardiomyocytes in other species, including human (1, 20). We confirm this result in mice ventricular cardiomyocytes, where the intense PTHrP immunostaining persisted after knockdown of PTHrP in the coronary arteries. Accordingly, no change in PTHrP transcript expression was observed in the heart from PTHrP \( ^{\text{SM}^{-/-}} \) mice. The decrease in heart rate observed in our study in conscious mice most probably may not be directly linked to a PTHrP defect in the heart.

PTHrP belongs to the vasoactive factors that regulate renal hemodynamics through changes in vascular tone and renin secretion (4). The renal vasculature is an important target for PTHrP. As a matter of fact, at a dose that does not affect blood pressure, PTHrP increases renal blood flow in humans (41) and, at a hypotensive dose, renal blood flow is well preserved in rats (29). PTHrP induced vasodilation in the isolated perfused rat kidney by a cAMP-dependent pathway linked to the activation of PTH1R (22). Although relaxation was similar in vitro on isolated afferent and efferent arterioles (39), PTHrP increased in vivo renal blood flow and glomerular filtration rate by dilating preglomerular vessels, while vasodilation was blunted on the efferent arteriole by concomitant ANG II-induced vasoconstriction (6, 17). PTHrP itself stimulated renin release by acting directly on juxtaglomerular cells (30). PTHrP is expressed throughout the renal arterial tree as revealed by

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Fig. 4. Cardiovascular and renal effects of Tam-induced conditional PTHrP knockdown. 
A: systolic blood pressure and heart rate were measured (tail cuff method in awake mice) before (basal) Tam or oil injection, and at day 6, the day following the last injection. 
B: creatinine clearance on 24-h urine collected in metabolic cages from awake mice, at basal and at day 6. 
C: plasma renin concentration in blood samples collected under ketamine/xylazine anesthesia on day 6. Values are expressed as means ± SE, and analyzed by one-way ANOVA followed by Tukey’s test, * \( P < 0.05 \) PTHrP \( ^{\text{SM}^{-/-}} \) vs. control groups. In the third group (black box), basal values were obtained in premutant PTHrP \( ^{\text{SM}^{-/-}} \) before this group was Tam-treated (PTHrP \( ^{\text{SM}^{-/-}} \) group).
immunostaining in a previous study (18) and confirmed in the present one. We now report that conditional inactivation of PTHrP in vascular smooth muscle cells elicits renal responses quite the opposite of those mentioned above. Actually, plasma renin concentration decreases and renal vascular resistance increases. Glomerular filtration rate falls when measurements are made in conscious or anesthetized mice. The decrease in filtration rate suggests that the increase in preglomerular vascular tone was greater than in postglomerular arterioles. Because plasma levels of PTHrP are normally very low in adults, altogether, present results provide evidence that endogenous renal vascular PTHrP, with normal PTH1R expression, contributes to the regulation of renal hemodynamics. In this respect, the renal vascular bed differs from other vascular beds. Indeed, blood pressure remained unchanged probably because of PTHrP production by endothelial cells or PTHrP-induced endothelium-dependent vasodilation, but such responses have not yet been reported for PTHrP on the mouse kidney.

Finally, we used acute isotonic SVE as an experimental condition to examine whether endogenous vascular PTHrP plays a role in the renal hemodynamic response elicited by SVE. This response contributes to restore fluid and sodium...
homeostasis. An increase in PAH clearance has previously been reported after SVE, and this response was enhanced in mice overexpressing the PTH1R in the smooth muscle (25). In the present study, under identical volume overload, we found that SVE-induced renal vasodilation is impaired, as well as the decrease in filtration fraction in PTHrP<sup>SM<sup>-/-</sup></sup> mice. In control mice, volume overload elicited an increase in renal plasma flow with no change in glomerular filtration rate, suggesting that vasodilation occurred on both preglomerular and postglomerular arterioles with no change in glomerular capillary hydrostatic pressure. The blunting of this response after conditional smooth muscle knockdown of PTHrP provides evidence that locally produced PTHrP is essential for the renal hemodynamic response to volume overload. SVE may enhance vascular PTHrP synthesis through the increase in blood pressure, as reported previously in renal arterioles from hypertensive rats (19), or by shear stress, as found on vascular smooth muscle cells in culture (34). Interestingly, PTHrP is able to induce vasodilation on both afferent and efferent arterioles after inhibition of the renin-angiotensin system (6). Several mechanisms inhibit renin release during SVE (increase in blood pressure, sodium overload, atrial release of natriuretic peptide) and oppose the stimulatory effect of PTHrP per se. SVE-induced decrease in filtration fraction is part of the renal response that contributes to enhance sodium and water excretion. Accordingly, when filtration fraction was no longer changed during SVE in PTHrP<sup>SM<sup>-/-</sup></sup> mice, diuresis was impaired. In the present study, we did not measure sodium excretion during SVE. It will be of great interest in future investigations to establish whether the natriuresis was impaired as well in PTHrP<sup>SM<sup>-/-</sup></sup> mice subjected to saline overload. Altogether, the present results demonstrate that vascular PTHrP is essential for the renal hemodynamic response to manage volume overload and restore fluid and sodium balance.

Table 4. Two-way ANOVA output concerning the analysis of acute SVE effects on parameters evaluated on day 6 by the clearance study in anesthetized mice

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>MBP</th>
<th>Heart Rate</th>
<th>RPF</th>
<th>RVR</th>
<th>GFR</th>
<th>Filtration Fraction</th>
<th>Haematocrit</th>
<th>Diuresis</th>
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<tr>
<td>Group (control vs. PTHrP&lt;sup&gt;SM&lt;sup&gt;-/-&lt;/sup&gt;&lt;/sup&gt; )</td>
<td>0.958</td>
<td>0.989</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.063</td>
<td>0.252</td>
<td>0.050</td>
</tr>
<tr>
<td>SVE (−SVE vs. +SVE)</td>
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<td>0.017</td>
<td>&lt; 0.001</td>
<td>0.020</td>
<td>0.694</td>
<td>0.002</td>
<td>&lt; 0.001</td>
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<td>Interaction</td>
<td>0.507</td>
<td>0.876</td>
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<td>0.035</td>
<td>0.115</td>
<td>0.004</td>
<td>0.791</td>
<td>0.022</td>
</tr>
</tbody>
</table>

The evolution of most of the parameters is presented on Fig. 6, with post hoc localization of individual differences by the Tukey’s test. Data are from control mice (premutant PTHrP<sup>SM<sup>-/-</sup></sup> + oil and PTHrP<sup>L2562L</sup>+Tam) and PTHrP<sup>SM<sup>-/-</sup></sup> mice, treated for 5 days (Tam or oil) before measurements on day 6. MBP, mean blood pressure; RPF, renal plasma flow; RVR, renal vascular resistance; GFR, glomerular filtration rate.
In summary, the present study describes a novel, inducible and smooth muscle-specific PTHrP knockout model to analyze the contribution of endogenous PTHrP to the regulation of hemodynamics, in mice with normal PTH1R expression. Tam-induced PTHrP vascular knockdown did not modify blood pressure, but it was associated with a renal phenotype characterized by renal vasoconstriction and decrease in renin release, resulting in a decrease in glomerular filtration rate and filtration fraction. Moreover, after acute isometric SVE, an experimental condition in which renal hemodynamics adapt to restore fluid and sodium homeostasis, our results show that renal vasodilation induced by endogenous PTHrP is essential for this response.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES

24. Okano K, Wu S, Huang X, Pirola CJ, Juppner H, Abou-Samra AB, Segre GV, Iwasaki K, Fagin JA, Clemens TL. Parathyroid hormone (PTH)/PTH-related protein (PTHrP) receptor and its messenger ribo-


