

PTH/PTHrP Receptor and Mid-molecule PTHrP Regulation of Intrauterine PTHrP: PTH/PTHrP Receptor Antagonism Increases SHR Fetal Weight

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Parathyroid hormone-related protein (PTHrP) has important roles in fetal growth and development through stimulation of placental calcium transport, vasodilatation of the uteroplacental vasculature and regulation of cellular growth and differentiation. The growth restricted spontaneously hypertensive rat (SHR) has reduced fetal plasma, placental and amniotic fluid PTHrP concentrations compared to its progenitor, the Wistar Kyoto (WKY) rat. The aim of this study was to determine whether intrauterine PTHrP infusions can restore PTHrP levels and promote SHR fetal growth. PTHrP(1–34), mid-molecule PTHrP(67–94), the PTH/PTHrP receptor antagonist [Asn¹⁰, Leu¹¹]-PTHrP(7–34) or vehicle were infused via a mini-osmotic pump between 10 and 20 days of gestation into the uterine lumen of SHR and WKY rats. Uterine, placental, amniotic fluid and plasma (fetal and maternal) PTHrP were measured via N-terminal radioimmunoassay. PTH/PTHrP receptor antagonism and mid-molecule PTHrP(67–94) induced endogenous intrauterine PTHrP production with receptor antagonism eliciting a greater and more wide spread effect. The PTH/PTHrP receptor antagonist [Asn¹⁰, Leu¹¹]-PTHrP(7–34) acting through a receptor other than the PTH/PTHrP receptor increased SHR fetal and placental weights above vehicle ($P < 0.05$) to that of the WKY and restored SHR amniotic fluid volume ($P < 0.05$). This was associated with a highly significant up regulation of placental, uterine and plasma (fetal and maternal) PTHrP ($P < 0.05$). Modest increases in placental and uterine PTHrP ($P < 0.05$) following intrauterine infusions of PTHrP(1–34) and PTHrP(67–94) had no effect on WKY and SHR fetal weight. Effective growth promoting actions of increased endogenous PTHrP were observed following PTH/PTHrP receptor antagonism rather than exogenous PTHrP administration. A novel finding was that mid-molecule PTHrP also up regulates endogenous intrauterine N-terminal PTHrP production supporting the existence of a mid-molecule receptor. This study highlights that an increase in endogenous uterine, placental and fetal plasma PTHrP following PTH/PTHrP receptor antagonism was associated with increased SHR fetal growth presumably by improving placental growth and function.

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INTRODUCTION

Both PTHrP and the PTH/PTHrP receptor are essential for fetal development, as disruption of these genes is lethal. PTHrP-deficient mice die at birth due to severe skeletal dysplasia [1,2] whilst mice with homologous deletion of the PTH/PTHrP receptor gene are growth restricted and die mid-gestation [3,4]. PTHrP has central roles in placental function and fetal growth. During fetal life PTHrP may act to regulate epithelial cell growth and differentiation [5], normal bone development [1], placental calcium transport [2,4,6,7], uterine smooth muscle tone [8] and fetal-placental vessel tone [9,10].

Our recent work demonstrates that fetal plasma, placental and amniotic fluid PTHrP concentrations are significantly reduced in association with growth restriction in the spontaneously hypertensive rat (SHR) of the Okamoto strain compared to the Wistar Kyoto (WKY) rat [11]. Other hormonal and metabolic abnormalities are evident in the SHR and these include lower fetal blood glucose and haematocrit, higher blood lactate and altered levels of insulin-like growth factors and their binding proteins [12]. We have shown that the deficiency in placental, uterine and fetal PTHrP may contribute to the compromised fetal growth and development observed in the SHR. Our embryo transfer studies have demonstrated that SHR fetal weight, as well as amniotic fluid PTHrP concentrations, are largely determined by the fetus or gestational tissues and are independent of the maternal environment or hypertension [13]. The SHR is an inbred

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strain exhibiting fetal growth restriction and spontaneous, genetically determined hypertension, the etiology of which exhibits many similarities to human essential hypertension. The SHR is a model of intrauterine growth restriction that arises spontaneously without nutritional, surgical, or endocrine intervention. Intrauterine growth restriction contributes significantly to perinatal morbidity and mortality as well as a predisposition to diseases including hypertension and diabetes later in life [14]. Studies have also implicated a role for PTHrP in the development and/or maintenance of hypertension in the SHR [15,16]. Pregnancies complicated by intrauterine growth restriction are characterized by poor placentation, impaired placental blood flow, increased vascular resistance and placental dysfunction that PTHrP is known to influence.

In the present study it was hypothesized that intrauterine PTHrP infusion during the period of rapid fetal growth in the late gestation SHR could restore uterine, placental and fetal PTHrP and by improving placental function thereby increase fetal weight in this model of growth restriction. We administered mid-molecule PTHrP peptides as they have been shown to stimulate ovine [6] placental calcium transport. Infusion of the PTH/PTHrP receptor antagonist was to establish whether the actions of PTHrP are mediated by the PTH/PTHrP receptor and whether the up regulation of gestational tissue and fetal PTHrP improves fetal growth by other mechanisms.

MATERIAL AND METHODS

Experimental protocol

This study had the ethical approval of The University of Melbourne's Animal Experimentation Ethics Sub-committee. Nine-week-old female WKY and SHR were obtained from and housed (2 to 4 rats per cage) in the Biological Research Facility, Department of Physiology and Pharmacology, University of Melbourne (Parkville, Victoria, Australia). Animals were subjected to a 12 h light/dark cycle and were fed food pellets and standard tap water ad libitum. They were mated with a breeder of the same strain after vaginal smears in the morning indicated that they were in pro-oestrus and presumably in oestrus on that night. The presence of sperm in the vaginal smear the following morning was taken as day 1 of pregnancy. Systolic blood pressure was measured on day 1 of pregnancy [11] by an indirect, tail-cuff method using a programmed electro-sphygmomanometer with a pneumatic pulse transducer (PE-300, Narco Bio-System Inc, Houston, Texas, USA).

The Alzet mini-osmotic pump (Model-2002, Alza Corporation, Palo Alto, California, USA) at 37°C has a pumping rate of 0.5 µl/h (±0.1 µl/h), reservoir volume of 240 µl and is thus able to deliver solutions continuously for approximately 14 days. A polyethylene cannula (length: 5 cm, inner diameter: 0.80 mm, outer diameter: 1.20 mm) was attached to the flow moderator. The filled mini-osmotic pump was incubated in sterile saline at 37°C for at least 4 h prior to

surgery to ensure that the pump started infusing immediately upon insertion. The mini-osmotic pump infused either PTHrP(1–34) (1 and 10 pmol/h), PTHrP(67–94) (1 and 10 pmol/h), PTH/PTHrP receptor antagonist ([Asn¹⁰, Leu¹¹]-PTHrP(7–34)amide, 1 pmol/h) or vehicle (2 per cent BSA in isotonic saline) (*n*=6–8 animals per treatment). On day ten of gestation, rats were anaesthetized via intraperitoneal injection (Ketamine; Parnell Laboratories Pty Ltd, Alexandria, NSW, Australia; 50 mg/kg and xylazine Ilium Xylazil-20; Troy Laboratories, Pty Ltd, Smithfield, NSW, Australia; 10 mg/kg). An incision was made in the lateral flank and the left uterine horn exposed. The cannula attached to the mini-osmotic pump was inserted into an incision made at the ovarian end of the left uterine horn and stitched into place with 4-0 silk suture. Both the uterus and mini-osmotic pump were returned to the abdominal cavity. The layers of muscle and fat were each stitched closed with 4-0 chromic gut and the skin incision was closed with 4-0 suture silk.

Tissue and fluid collection

At 20 days' gestation, rats were euthanased via tail vein injection with pentobarbitone sodium (Nembutal, Boehringer Ingelheim, Sydney, Australia; 120 mg/kg body weight). Following measurement of maternal body weight, maternal blood was collected by cardiac puncture. The uterus was dissected from the rat, and the number of fetal sacs within each horn of the uterus were noted. The fetal sacs were weighed individually. Amniotic fluid was aspirated from the fetal sacs. Fetal blood was collected via cardiac puncture with ammonium heparinized capillary tubes and pooled within left and right uterine horns. Placentae and fetal membranes (amnion and chorion) were separated from their respective fetuses, weighed, snap frozen in liquid nitrogen and tissues from left and right uterine sides were stored separately at –80°C. Amniotic fluid volume was calculated by subtracting the fetal, placental and membrane weights from the fetal sac weight. The left and right horns of the uterus were weighed and frozen in liquid nitrogen and stored separately at –80°C. Aprotinin (5.2 TIU/mg solid, Sigma Chemical Co., St Louis, MO, USA) was added to the tubes used for PTHrP analysis. Maternal and fetal blood was centrifuged at 2500 rpm at 4°C for 15 min and the plasma fraction was removed. Amniotic fluid and plasma samples were frozen in liquid nitrogen and stored at –20°C until analysed.

Tissue and fluid analysis

Samples of frozen placental and uterine tissues (1.0 g) were homogenized for 20 sec at 24 000 rpm in 5 ml acetic acid (1 M) using previously established techniques [11]. Duplicate 500 µl aliquots of the homogenate were removed for DNA assay. The remaining homogenate was incubated for 2–3 h at 4°C and centrifuged at 20 000 g for 15 min at 4°C. The supernatant

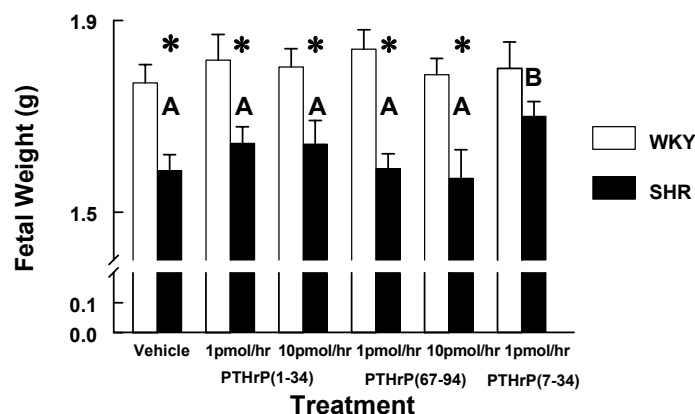


Figure 1. Fetal weight for WKY and SHR for the various treatments (mean \pm SEM, $n=6-8$). Infusion of the PTH/PTHrP receptor antagonist [Asn¹⁰, Leu¹¹]-PTHrP(7-34) significantly increased SHR fetal weight by 9 per cent above vehicle treatment to values that were not different to WKY fetal ($P<0.03$). Significant differences between strains for a given treatment are indicated by an * ($P<0.05$). Significant differences across the treatments for a given strain (SHR in upper case letters) are indicated by letters with different letters indicating significant differences such that data with an *A* is different to data with a *B* ($P<0.05$).

was then dialysed against 5 l deionized water for 22–26 h at 4°C using Spectra-Por 3 dialysis tubing (MW 6000–8000, Cole Palmer, Niles, IL, USA). The extract was stored at –20°C for PTHrP radioimmunoassay. Plasma, amniotic fluid and tissue concentrations of N-terminal PTHrP were quantified by a sensitive N-terminal radioimmunoassay that measures all forms of PTHrP that contain the N-terminal but does not recognize PTH nor mid-molecule PTHrP peptides [11,17]. Ionic calcium concentrations in amniotic fluid and maternal plasma were measured using ion selective electrodes (Ciba-Corning model 644, Medfield, MA, USA).

Statistical analyses

Homogeneity of variance was analysed using Bartlett's test. Data were analysed by three way analysis of variance with strain (WKY and SHR), treatment and horn (infused and non-infused) as factors (SPSS-X, SPSS Inc., USA). There were no significant differences in all parameters between infused and non-infused uterine horns. Therefore, data were analysed and presented as combined data from both infused and non-infused uterine horns. When a significant interaction was found between strain and treatment, differences across the treatments within a strain following one way analysis of variance were determined by post-hoc Student Newman Keuls test and differences between strains for each treatment determined with a *t*-test for independent samples. Data are presented as mean \pm SEM and $P<0.05$ was taken as statistically significant.

RESULTS

SHR and WKY differences in the vehicle group

SHR fetuses in the vehicle treated group weighed 10 per cent less than WKY, had a lower fetal : placental weight ratio and

had a greater amniotic fluid volume with no difference in litter size ($P<0.005$; Figure 1 and Table 1). In the vehicle treated group, fetal plasma and amniotic fluid N-terminal PTHrP concentrations were 51 per cent and 40 per cent lower in the SHR compared to the WKY, respectively ($P<0.03$; Figure 2). In the vehicle group, there were no differences between strains in placental and uterine PTHrP content at 20 days of gestation (Figure 3). This data is consistent with our previous studies [11].

Maternal parameters

Maternal blood pressure in the PTHrP(67–94) group on day 1 of gestation in both WKY and SHR groups was significantly higher than vehicle ($P<0.05$; Table 1). Maternal heart weight was significantly higher in SHR compared to WKY for all treatment groups ($P<0.004$; Table 1). Maternal body and uterine weight and age were not different between the different treatments (data not shown). Paternal ($P<0.0001$; data not shown) and maternal ($P<0.0001$; Table 1) blood pressure at day 1 of pregnancy were significantly higher in SHR than WKY for all treatment groups. There were no significant differences in maternal plasma ionic calcium concentrations for either strain with no effect of treatment compared to vehicle (Table 2).

Fetal parameters

There was no change in fetal weight across treatment groups in the WKY (Figure 1). SHR fetal weight was significantly lower than WKY for all treatments ($P<0.03$; Figure 1) except following PTH/PTHrP receptor antagonist [Asn¹⁰, Leu¹¹]-PTHrP(7–34) treatment. Infusion of the PTH/PTHrP receptor antagonist [Asn¹⁰, Leu¹¹]-PTHrP(7–34) increased SHR fetal weight by 9 per cent relative to vehicle to values that were

Table 1. Fetal and maternal parameters

Strain	Treatment	Litter size	Membrane weight (g)	Placental weight (g)	Fetal : Placental weight ratio	Amniotic fluid volume (ml)	Maternal blood pressure (mmHg)	Maternal heart weight (% body weight)	Uterine weight (% body weight)
WKY	Vehicle	11.2 ± 0.6	0.12 ± 0.005	0.319 ± 0.009	5.62 ± 0.14	0.76 ± 0.017	119.1 ± 2.6 ^a	0.32 ± 0.004	1.10 ± 0.03
	PTHrP(1-34) (1 pmol/h)	10.0 ± 0.4	0.13 ± 0.003	0.353 ± 0.023	5.31 ± 0.33	0.78 ± 0.006	112.4 ± 4.2 ^a	0.32 ± 0.008	0.87 ± 0.10
	PTHrP(1-34) (10 pmol/h)	10.5 ± 0.4	0.13 ± 0.003	0.331 ± 0.007	5.49 ± 0.11	0.73 ± 0.026	113.4 ± 1.6 ^a	0.32 ± 0.008	1.08 ± 0.03
	PTHrP(67-94) (1 pmol/h)	9.9 ± 0.7	0.12 ± 0.004	0.340 ± 0.004	5.41 ± 0.12	0.75 ± 0.008	135.4 ± 5.7 ^b	0.29 ± 0.006	1.01 ± 0.06
	PTHrP(67-94) (10 pmol/h)	10.9 ± 0.6	0.14 ± 0.003	0.327 ± 0.004	5.49 ± 0.10	0.83 ± 0.022	135.2 ± 3.3 ^b	0.29 ± 0.006	0.99 ± 0.04
	PTHrP(7-34) (1 pmol/h)	9.4 ± 0.5	0.12 ± 0.003	0.311 ± 0.013	5.79 ± 0.20	0.83 ± 0.037	118.1 ± 3.5 ^a	0.32 ± 0.007	1.04 ± 0.04
SHR	Vehicle	9.3 ± 1.2	0.10 ± 0.004*	0.342 ± 0.011	4.70 ± 0.13*	0.90 ± 0.020*	161.9 ± 4.1 ^{A*}	0.41 ± 0.044*	0.99 ± 0.07
	PTHrP(1-34) (1 pmol/h)	9.7 ± 0.5	0.11 ± 0.004*	0.340 ± 0.004	4.87 ± 0.09	0.87 ± 0.006*	166.0 ± 4.2 ^{AB*}	0.42 ± 0.013*	1.03 ± 0.05
	PTHrP(1-34) (10 pmol/h)	8.7 ± 0.5	0.11 ± 0.005*	0.361 ± 0.013	4.59 ± 0.05*	0.90 ± 0.012*	165.0 ± 4.2 ^{AB*}	0.38 ± 0.014*	0.99 ± 0.08
	PTHrP(67-94) (1 pmol/h)	8.1 ± 0.8	0.10 ± 0.004*	0.348 ± 0.011	4.65 ± 0.13*	0.93 ± 0.009*	177.4 ± 4.1 ^{AB*}	0.36 ± 0.005*	1.08 ± 0.07
	PTHrP(67-94) (10 pmol/h)	9.5 ± 0.6	0.10 ± 0.005	0.347 ± 0.008*	4.57 ± 0.10*	0.91 ± 0.026*	182.9 ± 4.5 ^{B*}	0.38 ± 0.017*	1.04 ± 0.04
	PTHrP(7-34) (1 pmol/h)	10.0 ± 0.7	0.11 ± 0.003*	0.362 ± 0.006*	4.75 ± 0.10*	0.88 ± 0.018	172.8 ± 4.9 ^{AB*}	0.37 ± 0.014*	1.12 ± 0.03

Litter size, fetal membrane weight, placental weight, fetal : placental weight ratio, amniotic fluid volume, maternal blood pressure on day 1 of pregnancy and maternal heart and uterine weight (corrected for maternal body weight) for WKY and SHR for the various treatments (mean ± SEM, $n=6-8$). Significant differences between strains for a given treatment are indicated by an * ($P<0.05$). Significant differences across the treatments for a given strain are indicated by letters with different letters indicating significant differences such that data with an *a* or *A* is different to data with a *b* or *B* but the same as data with an *ab* or *AB* (WKY in lower case and SHR in upper case letters; $P<0.05$).

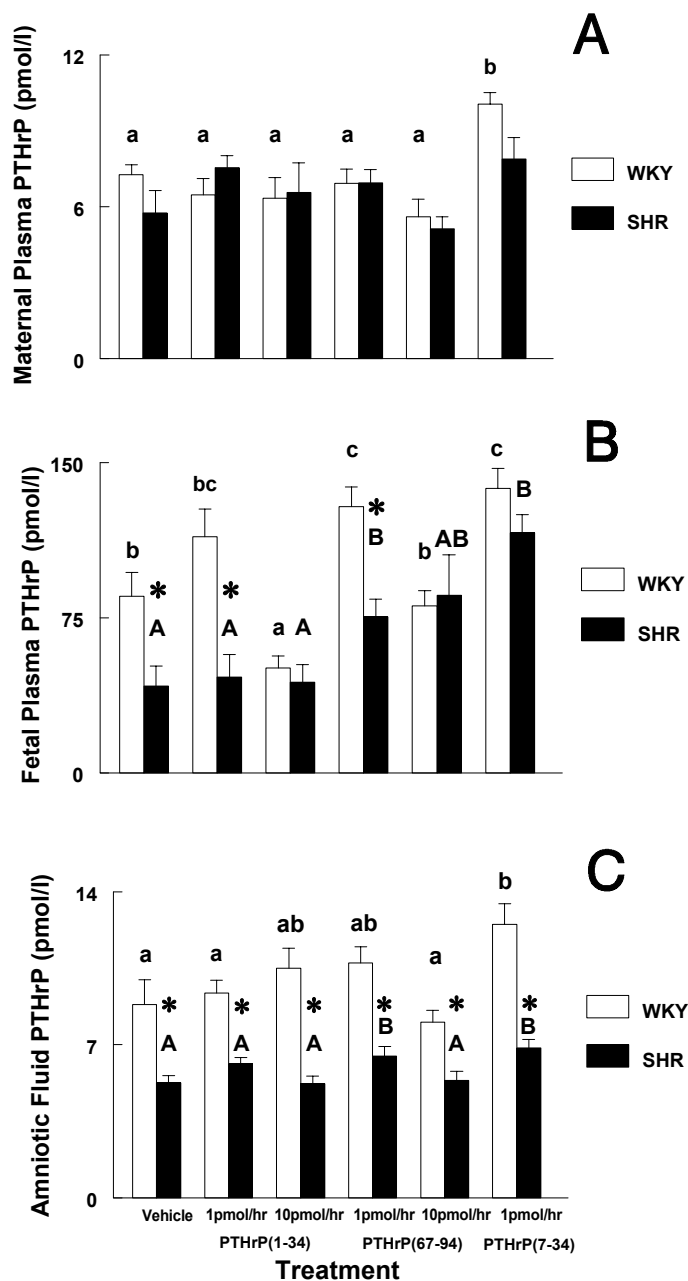


Figure 2. Maternal plasma (A), fetal plasma (B) and amniotic fluid (C) N-terminal PTHrP concentrations for WKY and SHR for the various treatments (mean \pm SEM, $n=6-8$). PTH/PTHrP receptor antagonism increased maternal plasma PTHrP concentrations in the WKY ($P<0.001$) and SHR ($P<0.08$). In the WKY, PTHrP(1-34) lowered while PTHrP(67-94) and [Asn¹⁰, Leu¹¹]-PTHrP(7-34) elevated fetal plasma N-terminal PTHrP concentrations ($P<0.05$). Amniotic fluid PTHrP concentrations increased above vehicle in response to [Asn¹⁰, Leu¹¹]-PTHrP(7-34) for WKY and SHR ($P<0.001$). Significant differences between strains for a given treatment are indicated by an * ($P<0.05$). Significant differences across the treatments for a given strain (WKY in lower case letters and SHR in upper case letters) are indicated by letters with different letters indicating significant differences such that data with an *a* or *A* is different to data with a *b* or *B* but the same as data with an *ab* or *AB* ($P<0.05$).

not different to WKY ($P<0.03$; Figure 1). Total litter size was unchanged between treatment groups, supporting the conclusion that any increases in fetal weight cannot be attributed to alterations in litter size (Table 1). Placental weights were significantly higher in SHR compared to WKY for PTHrP(67-94) (at 10 pmol/l) and [Asn¹⁰, Leu¹¹]-PTHrP(7-

34) but not the other treatment groups ($P<0.05$; Table 1). Fetal : placental weight ratio was significantly lower in SHR than WKY for PTHrP(67-94) and [Asn¹⁰, Leu¹¹]-PTHrP(7-34) groups but not PTHrP(1-34) (at 1 pmol/l) ($P<0.001$; Table 1). WKY fetal membrane weights were larger than SHR in PTHrP(1-34) and [Asn¹⁰, Leu¹¹]-PTHrP(7-34) groups but

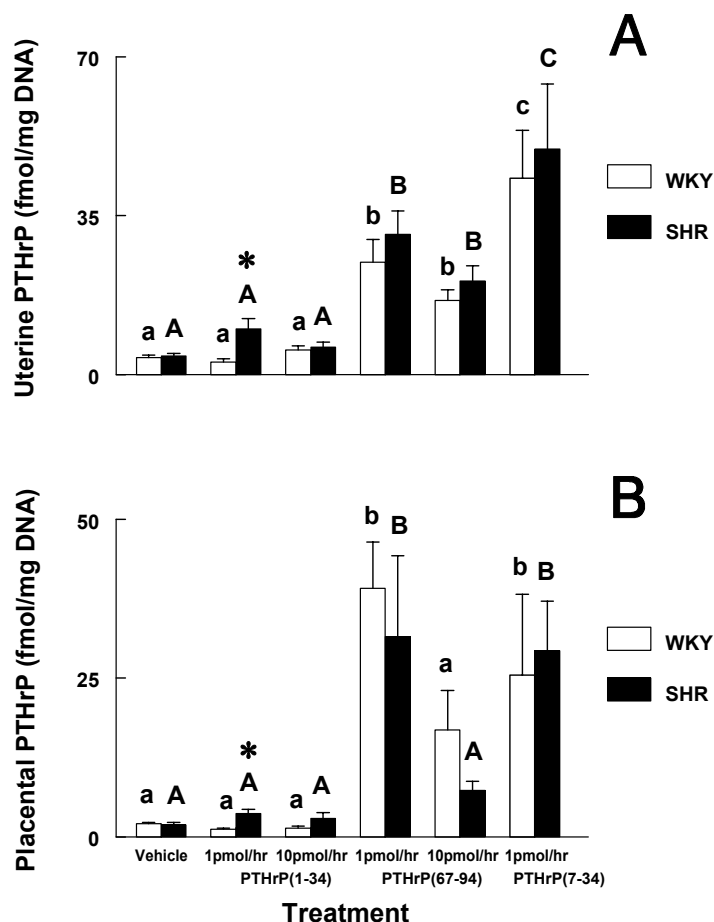


Figure 3. Uterine (A) and placental (B) N-terminal PTHrP tissue content for WKY and SHR for the various treatments (mean \pm SEM, $n=6-8$). PTHrP(1-34) increased SHR placental and uterine N-terminal PTHrP 1.5-fold above WKY ($P<0.05$). Placental and uterine N-terminal PTHrP content in both WKY and SHR increased 7-to-12-fold following infusion of PTHrP(67-94) and [Asn¹⁰, Leu¹¹]-PTHrP(7-34) ($P<0.05$). Significant differences between strains for a given treatment are indicated by an * ($P<0.05$). Significant differences across the treatments for a given strain (WKY in lower case letters and SHR in upper case letters) are indicated by letters with different letters indicating significant differences such that data with an *a* or *A* is different to data with a *b* or *B* but the same as data with an *ab* or *AB* ($P<0.05$).

not in response to PTHrP(67-94) (at 10 pmol/l) ($P<0.05$; Table 1). Amniotic fluid volume was significantly greater in the SHR compared to WKY for all treatment groups except [Asn¹⁰, Leu¹¹]-PTHrP(7-34) ($P<0.05$; Table 1).

Fetal plasma and amniotic fluid PTHrP and calcium concentrations

Fetal plasma PTHrP concentrations are ten fold higher than in amniotic fluid and twenty fold higher than in maternal plasma, suggesting that PTHrP is an important fetal endocrine factor possibly derived from the placenta, fetal parathyroids or other fetal tissues [15,18]. Amniotic fluid ionic calcium concentrations were significantly different between the strains for PTHrP(1-34) (at 10 pmol/h) and PTHrP(67-94) (1 pmol/h) infusion but not the other treatments ($P<0.05$; Table 2). Amniotic fluid PTHrP concentrations (and amniotic fluid PTHrP content; data not shown) in the SHR were significantly

lower for all treatment groups compared to WKY ($P<0.03$; Figure 2). Amniotic fluid PTHrP concentrations increased by 42 per cent and 30 per cent above vehicle in response to [Asn¹⁰, Leu¹¹]-PTHrP(7-34) for WKY and SHR, respectively ($P<0.001$; Table 2).

Endogenous PTHrP concentrations in maternal plasma were significantly elevated (by 38 per cent) following PTH/PTHrP receptor antagonism in the WKY ($P<0.001$; Figure 2) but the 37 per cent increase in the SHR failed to reach statistical significance ($P=0.08$; Figure 2). PTH/PTHrP receptor antagonist [Asn¹⁰, Leu¹¹]-PTHrP(7-34) increased 2.7-fold and PTHrP(67-94) (at 1 pmol/h) increased SHR fetal plasma PTHrP concentrations above vehicle ($P<0.0001$; Figure 2). In the WKY, PTHrP(1-34) (at 10 pmol/h) significantly suppressed (by 41 per cent) (to SHR levels) while PTHrP(67-94) (at 1 pmol/h) and [Asn¹⁰, Leu¹¹]-PTHrP(7-34) significantly elevated fetal plasma PTHrP concentrations compared to vehicle ($P<0.05$; Figure 2).

Table 2. Maternal plasma and amniotic fluid composition

Strain	Treatment	Maternal plasma ionic calcium (mmol/l)	Amniotic fluid ionic calcium (mmol/l)
WKY	Vehicle	1.00 ± 0.04	1.29 ± 0.12
	PTHrP(1–34) (1 pmol/h)	0.91 ± 0.02	1.52 ± 0.11
	PTHrP(1–34) (10 pmol/h)	0.96 ± 0.02	1.51 ± 0.10
	PTHrP(67–94) (1 pmol/h)	0.90 ± 0.07	1.28 ± 0.11
	PTHrP(67–94) (10 pmol/h)	1.04 ± 0.05	1.32 ± 0.10
	PTHrP(7–34) (1 pmol/h)	1.06 ± 0.03	1.31 ± 0.11
SHR	Vehicle	1.02 ± 0.07	1.61 ± 0.10
	PTHrP(1–34) (1 pmol/h)	0.89 ± 0.05	1.57 ± 0.10
	PTHrP(1–34) (10 pmol/h)	0.95 ± 0.04	1.77 ± 0.05*
	PTHrP(67–94) (1 pmol/h)	1.00 ± 0.07	1.66 ± 0.07*
	PTHrP(67–94) (10 pmol/h)	1.10 ± 0.08	1.51 ± 0.08
	PTHrP(7–34) (1 pmol/h)	0.87 ± 0.07	1.57 ± 0.05

Maternal plasma and amniotic fluid ionic calcium concentrations for WKY and SHR for the various treatments (mean ± SEM, $n=6-8$). Significant differences between strains for a given treatment are indicated by an * ($P<0.05$).

Tissue PTHrP content

In response to PTHrP(1–34) (at 1 pmol/h), SHR placental and uterine PTHrP content were 1.5-fold higher than WKY ($P<0.05$; Figure 3). Placental and uterine PTHrP content in both WKY and SHR were 7-to-12-fold higher following infusion of PTHrP(67–94) and [Asn¹⁰, Leu¹¹]-PTHrP(7–34) ($P<0.01$; Figure 3). The increase in gestational tissue N-terminal PTHrP in response to PTHrP(1–34) was small and was not associated with elevated fetal plasma and amniotic fluid PTHrP concentrations and thus was not effective at influencing fetal growth.

DISCUSSION

The data presented here are consistent with our recent findings showing that at 20 days of gestation, fetal plasma and amniotic fluid PTHrP concentrations are significantly reduced in the SHR compared to the WKY in association with growth restriction [11,13]. This supports the hypothesis that PTHrP homeostasis is altered in this strain and may contribute to the compromised fetal growth observed in the SHR. The PTH/PTHrP receptor antagonist [Asn¹⁰, Leu¹¹]-PTHrP(7–34) substantially up regulated endogenous PTHrP (or reduced its breakdown), as measured using N-terminal PTHrP radioimmunoassay, in placenta, uterus and fetal plasma in both strains which acted by non PTH/PTHrP receptor mechanisms [2]. However, the increase in fetal and placental growth was only observed in the growth restricted SHR. In contrast, PTHrP(1–34) infusion had a small effect to increase uterine and placental PTHrP with no effect on fetal weight. A novel observation is that PTHrP(67–94) also increased placental and uterine PTHrP in both strains but not to the same extent as that observed with the receptor antagonist. This was not associated with an increase in fetal weight in SHR.

There were no differences in any variables measured between infused (left) and non-infused (right) horns reflecting an overall uterine effect. It was presumed that after 10 days of infusion the entire uterine environment had established equilibrium between the horns. Interestingly, PTH/PTHrP receptor antagonism increased maternal plasma PTHrP significantly (by 38 per cent) in the WKY while there was a non significant rise in the SHR (by 37 per cent, $P=0.08$). Whether this reflects a direct effect of the receptor antagonism on maternal uterine PTHrP production or whether the antagonist reaches other maternal tissues as well is unclear. The lack of significant effect in SHR maternal plasma PTHrP may be indicative of altered PTH/PTHrP receptor-mediated actions which is in keeping with the suggested role for PTHrP in the development of hypertension in the SHR [15,16]. Our results suggest that alteration of PTHrP levels in the intrauterine environment exerts local and possibly systemic maternal effects.

Critical to our study was the finding that PTH/PTHrP receptor antagonism increased endogenous intrauterine N-terminal PTHrP either by increasing synthesis or reducing breakdown. The elevated PTHrP, acting through a receptor other than the PTH/PTHrP receptor, was associated with an increase in SHR fetal weight to values that were not different to WKY. Thus, PTH/PTHrP receptor antagonism rescued the suppressed PTHrP levels, corrected the SHR growth restriction, and restored amniotic fluid volume, presumably due to improved placental weight and function. The PTH/PTHrP receptor antagonist was effective in up regulating PTHrP in all intrauterine compartments including uterine and placental tissues (12-fold higher than vehicle), amniotic fluid and fetal (and maternal) plasma in both WKY and SHR. This is consistent with the PTH/PTHrP receptor being localized and expressed in placenta, fetal membranes and umbilical cord and the fetus itself [19–22]. Most importantly, the SHR fetal plasma PTHrP levels, which were basally suppressed relative to the WKY, were raised to WKY levels indicating that the

suppression could be overcome to some degree by PTH/PTHrP receptor antagonism. This is suggestive of a direct fetal effect but placental mediated actions cannot be excluded nor can impaired clearance of PTHrP within the fetus.

PTHrP is expressed predominantly in the surface epithelium of developing organs, and the PTH/PTHrP receptor resides on the adjacent mesenchymal cells [5,15]. We suggest that the cytokine growth factor-like effects of elevated intrauterine PTHrP, following PTH/PTHrP receptor antagonism, occurred by promoting placental growth [5,15,23] and function including placental calcium transport [2,6,7] and vasodilatation [9,10]. This action would need to be mediated by non PTH/PTHrP receptor mechanisms. It is possible that PTHrP expression is altered in the SHR early in development, and this subtly contributes to fetal growth restriction by impairing optimal development of vital fetal organs. Furthermore, we propose that the elevated intrauterine PTHrP may have directly promoted growth and/or differentiation of placental tissue, blood vessels, fetal epithelial cells and bone [1,15,23–26].

In this study PTH/PTHrP receptor antagonism had widespread effects resulting in up regulation of endogenous PTHrP which can exert its effects locally. On the other hand, exogenous infusion of mid-molecule PTHrP(67–94), which also up regulated endogenous uterine and placental PTHrP in the WKY and SHR and fetal plasma and amniotic fluid in the WKY, was not effective in increasing fetal or placental weight in the SHR possibly due to limited distribution of receptors for this mid-molecule peptide and/or that it has no role in the mechanisms leading to a growth response. This mid-molecule region of PTHrP has been shown to stimulate placental calcium transport [2,6,7] although no receptor has yet been identified. The novel observation that exogenous mid-molecule PTHrP can regulate endogenous PTHrP production in both WKY and SHR is exciting and deserves further study. Our observations provide independent evidence for the existence of a mid-molecule receptor. Overall, these results are in keeping with the recent report that endogenous (as was generated by PTH/PTHrP receptor antagonism), and not exogenous PTHrP, may be having intracrine effects by translocation into the nucleus to stimulate cell proliferation and differentiation in the SHR [26,27]. We have also shown that high levels of PTHrP(1–34) (10 pmol/h) can down regulate endogenous fetal plasma PTHrP in the WKY with normal PTHrP regulation. This is not seen in the SHR in whom PTHrP levels are suppressed. That there was no feedback effect in WKY gestational tissues while there was one in the fetus, is of interest and warrants further investigation. It was not possible to establish the source of the increased fetal plasma PTHrP but is likely to reflect enhanced endogenous fetal production or impaired breakdown.

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Although human data suggest that amniotic fluid PTHrP is derived from the fetal membrane, the amnion [28] and not derived from fetal urine [29] our recent studies in the rat suggest that uterine, placental, fetal membrane and fetal PTHrP all may contribute to rat amniotic fluid PTHrP [11]. Fetal membrane weight is lower in the SHR and reduced fetal membrane PTHrP may contribute to the lower fetal plasma and amniotic fluid PTHrP levels. This is supported by our findings that amniotic fluid PTHrP concentrations were increased in both SHR and WKY in response to PTH/PTHrP receptor antagonism in parallel with the increases in placental, uterine and fetal plasma PTHrP. In the SHR, amniotic fluid calcium concentrations increased with PTHrP(67–94) infusion which may be suggestive of an increase in placental calcium transport thereby increasing fetal plasma and amniotic fluid calcium concentrations. PTHrP(1–34) also increased SHR amniotic fluid calcium concentrations but the mechanism is unclear given that there were no changes in tissue PTHrP levels, however, it may be the result of direct local effects in placenta [7].

In summary, the consequences of reduced intrauterine (fetal plasma, placental and amniotic fluid) PTHrP in the SHR near term is likely to be reflected in reduced placental and fetal cellular growth and differentiation [5], placental calcium transport [2,6] and vasodilatation [9,10] thus contributing to the reduced fetal growth during pregnancy at a time when growth should be maximal. Fetal weight is tightly regulated and small changes in fetal weight can be crucial in terms of fetal survival. Given that the intrauterine environment is believed to be important to the programming of diseases later in life, our results suggest that PTHrP may be a significant in utero modulator of placental function, subsequent fetal growth and therefore also contribute to the development of subsequent hypertension and diabetes at maturity. Whether the increased intrauterine PTHrP also occurs in the vasculature with subsequent effects on the perinatal programming of hypertension in this strain remains to be established. This work highlights the role of the PTH/PTHrP receptor in placental function and fetal growth and also indicates that in a situation where this receptor is blocked the induced increase in endogenous PTHrP can mediate increased fetal growth and other responses in utero, possibly via its intracrine actions. In contrast, exogenous PTHrP administration was without effect on fetal growth. The novel observation that mid-molecule PTHrP regulates local PTHrP production may be linked to its demonstrated role in stimulating placental calcium transport but may also be indicative of other yet to be determined local roles. This study highlights the profound role of endogenous PTHrP in establishing and maintaining placental function and subsequent growth-promoting actions in utero.

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