Role of maternal glucocorticoid inducible kinase SGK1 in fetal programming of blood pressure in response to prenatal diet

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Departments of 1Physiology and 3Pharmacology, University of Tübingen, Tübingen; and 3Department of Pathology, University of Erlangen, Erlangen, Germany; 4Institute of Reproductive and Developmental Biology, Imperial College London, Hammersmith Campus, London, United Kingdom; and 5Department of Biology, Chemistry, and Pharmacy, Free University, Berlin, Berlin, Germany

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Rexhepaj R, Boini KM, Huang DY, Amann K, Artunc F, Wang K, Brosens JJ, Kuhl D, Lang F. Role of maternal glucocorticoid inducible kinase SGK1 in fetal programming of blood pressure in response to prenatal diet. Am J Physiol Regul Integr Comp Physiol 294: R2008–R2013, 2008. First published March 26, 2008; doi:10.1152/ajpregu.00737.2007.—Maternal stress and malnutrition modify intrauterine fetal development with impact on postnatal blood pressure, nutrient, water, and electrolyte metabolism. The present study explored the possible involvement of maternal serum- and glucocorticoid-inducible kinase (SGK)-1 in fetal programming of blood pressure. To this end, wild-type (sgk1+/+) males were mated with SGK1 knockout (sgk1−/−) female mice, and sgk1−/− males with sgk1+/− females, resulting in both cases in heterozygotic (sgk1−/+ ) offspring. Following prenatal protein restriction, the offspring of sgk1−/+ mothers gained weight significantly slower and had significantly higher blood pressure after birth. Moreover, a sexual dimorphism was apparent in fasting glucose and plasma corticosterone concentrations, with higher levels in female offspring. In contrast, prenatal protein restriction of sgk1−/− mothers had no significant effect on postnatal weight gain, blood pressure, plasma glucose concentration, or corticosterone levels, irrespective of offspring sex. Plasma aldosterone concentration, urinary flow rates, and urinary excretions of Na+ and K+ were not significantly modified by either maternal genotype or nutritional manipulation. In conclusion, maternal signals mediated by SGK1 may play a decisive role in fetal programming of hypertension induced by prenatal protein restriction.

METHODS

All animal experiments were conducted according to the guidelines of the American Physiological Society, as well as the German law for the welfare of animals, and were approved by local authorities. Mice deficient in SGK1 (sgk1−/−) on 129Sv background were generated and bred as previously described (19, 42). Before the experiments, all mice were fed a standard diet (1314, Altromin, Heidenau, Germany) ad libitum with free access to tap water. To yield offspring heterozygotic mice (sgk1−/+), wild-type (sgk1+/+) male

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mice were mated with SGK1 knockout (sgk1−/−) female mice, and sgk1−/+ males with sgk1+/+ females (all at the age of 4 mo). During mating and pregnancy, the diet consisted of either the control diet (C1000, 18% protein, 0.25% Na+, 0.36% Cl−, 0.71% K+; Altromin) or a low-protein diet (C1003, 8% protein, 0.25% Na+, 0.36% Cl−, 0.71% K+; Altromin) with free access to tap drinking water. After birth, the diet was changed to standard diet, and the pups were weaned at 3 wk. The compositions of the diets are provided in Table 1. To minimize handling stress, animals were not weighed at birth. The same sets of animals were used for the plasma hormone levels, electrolytes, metabolic studies, and blood pressure measurements. In theory, differences in litter size could have influenced body weight and blood pressure in offspring. The average litter size was 4.1 ± 0.9 in protein-restricted and 2.8 ± 0.3 in protein-replete sgk1−/− mothers, and 5.7 ± 0.2 in protein-restricted and 5.0 ± 0.2 in protein-replete sgk1+/+ mothers (n = 8–9 for each group). Litter size was not statistically significantly different between protein-replete and protein-restricted mothers of either genotype. Moreover, under protein-restricted diet, there was no significant difference of litter size between sgk1−/− and sgk1+/+ mothers. However, in protein-replete mothers, the litter size was significantly smaller in sgk1−/− than in sgk1+/+ animals. Body weight and blood pressure of offspring from protein-replete mothers were not significantly different between the two genotypes.

One to three male and one to three female offspring were randomly selected from each of the eight to nine mothers per group and used for blood pressure and body weight measurements. For plasma glucose, hormone levels, electrolytes, and metabolic studies, two male and two female offspring were randomly selected from three mothers in each group.

For evaluation of renal excretion, at the age of 13 wk, offspring sgk1−/+ mice were placed individually in metabolic cages (Tecniplast Hohenpeissenberg) for 24-h urine collection (37). They were allowed a 3-day habituation period, when food intake, water intake, urinary flow rate, urinary salt excretion, and body weight were recorded every day to ascertain that the mice were adapted to the new environment. Subsequently, 24-h collection of urine was performed for 3 consecutive days to obtain the basal urinary parameters. To ensure quantitative urine collection, the inner wall of the metabolic cages was siliconized, and urine was collected under water-saturated oil.

Data are provided as means ± SE; n represents the number of independent experiments. All data were tested for significance using paired or unpaired Student t-test or two-paired ANOVA, where
required, using GraphPad InStat or Prism version 5.00 for Windows (GraphPad Software, San Diego CA; http://www.graphpad.com). A P value <0.05 was considered statistically significant.

RESULTS

The body weight of heterozygotic (sgk1+/−) offspring was comparable 6 wk after birth, irrespective of sex, prenatal diet, or maternal genotype. However, the subsequent increase in body weight between 7 and 12 wk postnatally was significantly lower in offspring from wild-type (sgk1+/+) mothers fed a protein-restricted diet during pregnancy than in offspring from sgk1+/− mothers fed a control diet (Fig. 1). In contrast, prenatal protein restriction had no significant effect on body weight gain in offspring from SGK1-deficient (sgk1−/−) mothers. In addition, food intake per gram body weight was significantly enhanced in offspring upon prenatal protein restriction of sgk1+/+ mothers, whereas such dietary interventions did not alter food intake of offspring from sgk1−/− mothers (Table 2). Thus the effects of protein deficiency during pregnancy on postnatal weight gain and food intake of the offspring was dependent on maternal SGK1 expression.

Fasting plasma glucose concentrations in female but not male offspring from sgk1+/+ mothers were significantly enhanced upon prenatal protein restriction. In contrast, plasma glucose concentrations in male or female offspring from sgk1−/− mothers were not significantly affected by the maternal diet (Table 2). Moreover, plasma glucose concentrations in female offspring were significantly lower upon protein restriction of pregnant sgk1−/− mice compared with sgk1+/+ mothers. Plasma insulin concentrations, however, were comparable between offspring of sgk1+/+ and sgk1−/− mothers, irrespective of dietary manipulation (Table 2).

Plasma Na+ and K+ concentrations in offspring were not significantly modified by prenatal diet or maternal genotype (Table 2). Furthermore, urinary flow rates, urinary excretions of Na+ and K+, or plasma aldosterone concentrations in offspring were also not significantly modified by either prenatal diet or maternal genotype (Table 2).

However, maternal protein restriction significantly enhanced the plasma corticosterone concentration in female offspring, and there was a trend (P = 0.17) toward increased levels in male offspring from sgk1+/+ mothers (Table 2). On the other hand, there was no evidence of increased circulating corticosterone levels or sexual dimorphism in offspring from sgk1−/− mothers fed a low-protein diet during pregnancy.

The blood pressure of the offspring, determined with the tail-cuff method, was significantly higher on feeding pregnant wild-type sgk1+/+ mothers a protein-restrictive diet, irrespective of sex (male: 107.7 ± 1.6 vs. 102.8 ± 1.3 mmHg; female: 104.0 ± 1.0 vs. 97.1 ± 2.1 mmHg). In contrast, prenatal protein restriction had no significant effect on the postnatal blood pressure of male or female offspring of sgk1−/− mothers (male: 103.3 ± 1.4 vs. 102.8 ± 1.4 mmHg; female: 102.2 ± 1.0 vs. 101.0 ± 2.7 mmHg) (Fig. 2). Similar observations were made, when blood pressure was determined utilizing a Statham transducer connected to a catheter placed into the femoral artery of anesthetized animals. Utilizing this method, femoral blood pressure was again significantly higher in male offspring from protein-restricted sgk1+/+ mothers than in offspring from protein-replete sgk1+/+ mothers (Fig. 2C). Again, prenatal protein restriction had no significant effect on the postnatal femoral blood pressure of male offspring from sgk1−/− mothers (Fig. 2C). Moreover, the blood pressure was significantly lower in offspring from protein-restricted sgk1−/− mothers than in offspring from protein-restricted sgk1+/+ mothers (Fig. 2C). Accordingly, prenatal protein restriction leads to enhanced blood pressure, an effect again influenced by maternal SGK1.

DISCUSSION

The present observations confirm that low-protein diet during pregnancy predisposes for hypertension in adulthood (9, 21, 23, 26–28, 32, 33, 38, 40, 43, 44). As expected, prenatal protein restriction was associated with impaired weight gain and increased blood pressure of offspring from wild-type (sgk1+/+) mothers. In contrast, weight gain and blood pressure of offspring from SGK1 knockout (sgk1−/−) mothers was unaffected by maternal low-protein diet. Those observations suggest a critical role of maternal SGK1 in some aspects of fetal programming. Notably, the genotype of the offspring was heterozygotic (sgk1−/+ ) in both groups. Thus the offspring from sgk1−/− and sgk1+/+ mothers should be similarly sensitive to changes in amino acids or glucocorticoid exposure during pregnancy, and the differences in fetal programming...

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<tr>
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<th>sgtk+/+ Offspring</th>
<th>sgtk+/− Offspring</th>
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<tr>
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<td>Body Weight, g</td>
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<td>Food intake, g/24 h</td>
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<td>Food intake, mg/g BW−1·24 h−1</td>
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<td>Urinary K+ excretion, μmol·24 h−1·g BW−1</td>
<td>14.8±3.4</td>
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Values are means ± SE; n = 6 animals per group, 13–16 wk old. Results show food intake of offspring from female serum and glucocorticoid-inducible kinase (sgk1+) and sgtk−/− mice under control diet and low-protein diet (LPD). BW, body weight; [Na+] and [K+], Na+ and K+ concentration, respectively. *Significant difference (P < 0.05) from standard diet. †Significant difference (P < 0.05) from sgtk+/+.}

cannot be due to genetic differences within the offspring. Rather, the phenotypic differences in offspring result from genetic differences between the mothers. Thus fetal programming of blood pressure in response to prenatal protein restriction may be dependent on maternal signals that involve SGK1.

Male mice were similarly exposed to low-protein diet, and, at least in theory, alterations in sperm or semen could impact on subsequent embryonic or fetal development. However, the effect of low-protein diet was virtually absent in the offspring of sgtk−/− females mated with sgtk+/+ male mice. If dietary restriction alters sperm, then the known effect in wild-type parental animals would depend on the genotype of the male and would be lacking in offspring of sgtk−/− male, not in offspring of sgtk−/− female, mice.

Our experiments suggest that maternal SGK1 expression is a prerequisite for the programming of blood pressure in the offspring. The present study did not attempt to define SGK1-sensitive maternal mechanisms. Nutrition of the fetus is accomplished by the interaction of maternal decidualized endometrium (sgk1−/− or sgtk+/+) and fetal placenta (sgk1−/− in each group). Thus it is tempting to speculate that endometrial SGK1 expression is involved. Indeed, SGK1 is abundantly expressed in endometrial glandular cells, which provide nutrients, growth factors, and cytokines for the early fetal-placental unit (13).

Presently, it is not known if and how SGK1 expression in maternal endometrium (10) is influenced by diet or stress. Notably, amino acids inhibit SGK1 expression (39), which, in turn, suggests that prenatal protein restriction may enhance maternal SGK1 expression. On the other hand, fetal programming of blood pressure can also be triggered by a maternal protein-rich diet (7, 14, 15, 34, 36). This latter effect has been attributed to a stimulating effect of protein-rich diet on glucocorticoid release (1, 12, 35), which, again, should stimulate SGK1 expression (11). Clearly, future experiments are required to elucidate the sensitivity of endometrial SGK1 expression to dietary manipulations.

Fig. 2. Influence of maternal low-protein diet on offspring blood pressure. Values are means ± SE of blood pressure measured via tail-cuff (A and B) in male (A) and female (B) offspring from maternal sgtk+/+ mice and sgtk−/− mice exposed to control diet (open bars, n = 12 male and 12 female offspring from sgtk+/+ mice, n = 9 male and 9 female offspring from sgtk−/− mice) and low-protein diet (solid bars, n = 12 male and 12 female offspring from sgtk+/+ mice, n = 12 male and 12 female offspring from sgtk−/− mice) during pregnancy. C: mean blood pressure values (±SE) measured utilizing a Statham transducer connected to the femoral artery of offspring from maternal sgtk+/+ mice (n = 6 male offspring and sgtk−/− mice (n = 6 male offspring) exposed to control diet (open bars) and low-protein diet (solid bars) during pregnancy. *Significant difference (P < 0.05) from standard diet. †Significant difference (P < 0.05) from sgtk+/+.
The present study did not address the mechanisms that couple altered maternal SGK1 expression to impaired blood pressure regulation in the offspring. Increased blood pressure may be due to altered renal development in utero on nutritional restriction of the mother. As shown previously, maternal protein restriction causes offspring hypertension by suppressing the intrarenal renin-angiotensin system during development, leading to impaired nephrogenesis and a reduced number of nephrons at birth, which persists into adulthood (16, 38, 40, 41). The altered renal development could compromise renal electrolyte excretion and thus require a compensatory increase in blood pressure to eliminate the daily load. Low-protein diet has further been suggested to enhance sensitivity to angiotensin II (28, 33, 43) and to increase expression of the renal bumetanide-sensitive Na-K-2Cl cotransporter and the renal thiazide-sensitive Na-Cl cotransporter (26).

Maternal low-protein diet was followed by significantly enhanced blood corticosterone concentrations in female offspring of sglk1+/− mothers and a trend toward increased levels in male offspring. Furthermore, plasma corticosterone concentrations following prenatal protein restriction tended to be higher in female and were significantly higher in male offspring of sglk1+/− mothers compared with offspring of sglk1−/− mothers. It is tempting to speculate that, depending on maternal SGK1, protein restriction imposes stress on the offspring, which leads to enhanced stimulation of the adrenal gland. However, under a control diet, the plasma corticosterone levels were significantly higher in female offspring of sglk1−/− mothers than in female offspring of sglk1+/− mothers. We have no ready explanation for this difference. In any case, the plasma corticosterone levels alone cannot explain the enhanced blood pressure in offspring of sglk1+/− mothers subjected to low-protein diet. Plasma aldosterone levels in the offspring were unchanged, irrespective of prenatal diet or maternal genotype. Enhanced plasma aldosterone levels are found in sglk1−/− mice (42). However, the offspring consisted only of heterozygotic (sglk1+/−) animals, albeit from sglk1−/− and sglk1+/− mothers, and thus we would not a priori expect differences in plasma aldosterone concentration.

Besides its influence on blood pressure, prenatal protein restriction of wild-type mothers was also associated with a slight, but statistically significant, decrease in body weight gain in the offspring. Again, this effect of maternal diet was absent in offspring from sglk1−/− mothers. Decreased body weight of offspring in response to prenatal protein restriction has been reported for rats (2, 38) and mice (16, 17) and is paralleled by decreased weight of kidney, liver, spleen, heart, and brain (16). In theory, impaired body weight gain in offspring of wild-type mothers fed a protein-deficient diet could have been due to decreased food intake. However, within the short period of metabolic cage experiments, food intake was rather enhanced. We cannot rule out the possibility that food intake was actually decreased in offspring from sglk1−/− mothers early after birth. Arguably, increased food intake in older offspring could be a compensatory response for limited nutrient availability in the early phases of development.

Maternal low-protein diet was followed by enhanced blood glucose concentration in female but not male offspring of wild-type mothers. Plasma insulin levels were slightly higher, a difference, however, not reaching statistical significance. Again, plasma glucose concentration was not significantly affected by maternal diet in offspring from sglk1−/− mothers. Sexual dimorphism in fetal programming has also been reported for the liver (16). Maternal protein restriction has been shown to result in decreased normalized (to body weight) liver weight in male offspring, but increased normalized liver weight in female offspring. It is tempting to speculate that the relatively enhanced hepatic glycogen stores in female offspring from wild-type mothers account for the maintenance of blood glucose levels following overnight fasting.

**Perspectives and Significance**

This study suggests that maternal SGK1 plays an integral role in fetal programming of blood pressure in response to prenatal protein restriction. The data reveal a novel function of SGK1 and shed new light on the mechanisms that account for the increased risk in cardiovascular disease in adult offspring of nutrient-restricted mothers. The definition of a maternal gene participating in fetal programming is an important step toward understanding the maternal side of fetal programming. The present observations are expected to direct future experiments defining the influence of dietary manipulations on endometrial SGK1 expression and hopefully disclose the SGK1-dependent mechanisms in the maternal-fetal interface.

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