Maternal Protein Restriction Influences the Programming of the Rat Hypothalamic-Pituitary-Adrenal Axis[1,2]

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ABSTRACT The role of glucocorticoids in the intrauterine programming of hypertension was assessed in the progeny of rats fed either 18 g casein/100 g diet (control diet) or 9 g casein/100 g diet (low protein diet), before conception and throughout pregnancy. Rats exposed to the low protein diet had significantly ($P < 0.05$) higher systolic blood pressures than control animals, when weaned. These rats had elevated brain and liver activities of specific glucocorticoid-inducible marker enzymes, relative to controls. Glycerol 3-phosphate dehydrogenase activity was also higher (377%) in whole brains of newborn rats exposed to low protein diet in utero, but no similar effect of corticosteroids was noted in brains of d 20 fetuses. Weanling rats of the low protein group exhibited a blunted diurnal pattern of adrenocorticotrophin (ACTH) concentrations in plasma. Plasma corticosterone concentrations were unaltered by prenatal dietary experience and exhibited a normal pattern of diurnal variation. Brain regional 11β-hydroxysteroid dehydrogenase activities were unaltered by prenatal dietary experience, as was binding of ^{3}H-corticosterone to type I glucocorticoid receptors in hippocampus, hypothalamus and liver. Type II glucocorticoid receptor binding capacity and receptor numbers in male rats were apparently elevated in hippocampus of low protein–exposed rats and were significantly lower in liver ($P < 0.05$), relative to control rats. Programming of the hypothalamic-pituitary-adrenal axis is inferred, and the observation that binding of steroid to type II receptor sites in vascular tissue is increased in low protein exposed rats may provide a direct mechanism for modulation of blood pressure by glucocorticoids in this model. J. Nutr. 126: 1578–1585, 1996.

INDEXING KEY WORDS:

• *rats* • *glucocorticoids* • *glucocorticoid receptors* • *pregnancy and nutrition* • *hypertension*

An extensive series of epidemiological investigations of populations in both developed (Barker 1995) and developing nations, has indicated that the intrauterine environment is a major determinant of disease in adult life. It is proposed that maternal undernutrition may "program" permanent physiological and biochemical changes in the fetus, initiating cardiovascular pathologies that will appear in later life (Barker 1995, Barker et al. 1993).

Animal studies have provided strong support for this hypothesis. Genetic models of hypertension such as the Spontaneously Hypertensive Rat appear to be associated with intrauterine growth retardation (Iwase et al. 1995) and may be ameliorated by nutritional manipulation (McCarty and Fields-Okotcha 1994). Recent studies in rats have demonstrated that fetal exposure to mild maternal protein restriction results in increased blood pressure in later life (Langley and Jackson 1994, Langley-Evans and Jackson 1995, Langley-Evans et al. 1994). This phenomenon, also seen in humans that have undergone intrauterine growth retardation (Law et al. 1993), is detectable early in life and apparently lifelong (Langley-Evans et al. 1994). The low protein diet model in the rat may therefore provide a useful approach to the elucidation of molecular mechanisms to explain the association of maternal nutrition with later cardiovascular disease.

Edwards et al. (1993) proposed that glucocorticoids may program cardiovascular disease in utero and that the placental enzyme 11β-hydroxysteroid dehydrogenase (11βHSD)[4] plays a key role in protecting the fetus

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[4] Abbreviations used: ACTH, adrenocorticotrophin; GPDH, glycerol 3-phosphate dehydrogenase; GS, glutamine synthetase; HPA, hypothalamic-pituitary-adrenal axis; 11βHSD, 11β-hydroxysteroid dehydrogenase; MD, malate dehydrogenase; PK, pyruvate kinase; TAT, tyrosine aminotransferase.

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TABLE 1

Composition of purified control (18 g casein/100 g) and low protein (9 g casein/100 g) diets[1]

	Dietary protein	
	Control	Low protein
	g/100 g diet	
Casein	18	9
Cornstarch	42.5	48.5
Cellulose fiber	5	5
Sucrose	21.3	24.3
Choline chloride	0.2	0.2
DL-Methionine	0.5	0.5
AIN-76 mineral mix[2]	2	2
AIN-76 vitamin mix[2]	0.5	0.5
Corn oil	10	10

[1] Diets were provided to the rats as balls (60–100 g dry weight).
[2] Supplied by SDS (Cambridge, UK).

from adverse effects of corticosteroids. In support of this hypothesis, we have demonstrated that rat placental 11βHSD activity is lowered by maternal low protein diets (Phillips et al. 1994). Furthermore, maternal diet–induced hypertension in rats is abolished by inhibition of maternal and fetal corticosterone synthesis (Langley-Evans et al. 1996b). Our working hypothesis is that maternal protein restriction in pregnancy leads to programming of lifelong changes to hypothalamic-pituitary-adrenal axis function. This in turn may be responsible, directly or indirectly, for the resetting of homeostatic controls over cardiovascular function and eventually for the development of disease. In the current paper, we present evidence that the hypothalamic-pituitary-adrenal axis is indeed programmed in utero by maternal nutrition.

MATERIALS AND METHODS

Chemicals. Standard chemicals and reagents were purchased from Sigma (Poole, UK). ^{3}H-corticosterone was obtained from Amersham (Little Chalfont, UK). RU486 was the kind gift of Roussell-UCLAF (Romainville, France).

Animals. Animal experimentation was performed under license from the British Home Office and was in compliance with the 1986 Animal Act. Rats were of the Wistar strain, bred in the University of Southampton animal facility. The animals were housed at 24°C in wire mesh cages, with a 12-h light:dark cycle. All rats had free access to food and water.

A total of fifty-six virgin female rats (200–225 g) were used to generate the offspring used in the experiments. The dams were habituated over 14 d to purified diets (**Table 1**) containing either 18 g casein/100 g diet (control diet) or 9 g casein/100 g diet (low protein diet), as previously described (Langley et al. 1994). After 14 d, the rats were mated and fed the same diets until they gave birth. Within 1–12 h of delivering, all rats were transferred to a standard, nonpurified diet (CRMX, Special Diet Services, Cambridge, UK), and litters were culled to a maximum of eight (4 male, 4 female) pups (Langley-Evans et al. 1994). The offspring differed, therefore, only in terms of prenatal dietary experience. At 4 wk of age, the pups were weaned onto the nonpurified diet.

A proportion of the pregnancies (6 of 28 in each dietary group) were terminated at 20 of gestation, and fetuses removed and killed for tissues. The offspring of a further six dams in each group were killed at birth. Of the remaining 16 litters in each group, 11 were killed at weaning and the remainder at 7 wk of age.

Tissue collection. Pups killed at d 20 of gestation or at birth were decapitated and whole brains removed. Weanling rats were either decapitated (if hormonal analysis was required) and blood collected, or killed by cervical dislocation. Blood was taken into lithium heparin tubes and plasma prepared. Brain and liver were rapidly excised. Hippocampus, hypothalamus and cerebellum were dissected from the brains as described previously (Langley and York 1990a). All tissues were frozen immediately in liquid nitrogen and later stored, with plasma, at −80°C for up to 3 mo prior to biochemical analyses.

Measurement of systolic blood pressure. Systolic blood pressure was determined, no more than 3 d prior to killing, using an indirect tail-cuff method, as described previously (Langley-Evans and Jackson 1995). Pressures of both male and female rats were recorded and no gender-related differences observed. An IITC model 29 blood pressure monitor linked to a computer software package to determine pressures using a preset algorithm was used (Linton Instrumentation, Diss, UK). To further avoid observer bias, the operator was unaware of the in utero dietary exposure of the animals.

Enzyme assays. The glucocorticoid-inducible enzymes glycerol 3-phosphate dehydrogenase (GPDH, EC 1.1.1.8), glutamine synthetase (GS, EC 6.3.1.2), tyrosine aminotransferase (TAT, EC 2.6.1.5) and the glucocorticoid-insensitive enzymes malate dehydrogenase (MD, EC 1.1.1.37) and pyruvate kinase (PK, EC 2.7.1.40) were assayed using the method of Langley and York (1990b). TAT activity differs between male and female rats and only male livers were assayed. For all other activities, tissues from both male and female rats were used because there were no gender-related differences. Assay of 11βHSD was conducted in brain regions of male rats using the method of Benediktsson et al. (1993), with either NAD or NADP as cofactors to distinguish the two isoforms.

Enzyme activities were expressed per milligram of protein. Because buffers used in some of the enzyme assays competed with copper-based protein assays, pro-

TABLE 2

Day 20 fetal and placental weights and full-term birthweights of rats exposed to different levels of maternal dietary protein[1,2]

Maternal diet	Control	Low protein
	g	
Fetal weight	2.82 ± 0.08	3.25 ± 0.08*
Placental weight	0.54 ± 0.01	0.59 ± 0.02*
Birthweight	5.56 ± 0.09	5.67 ± 0.11

[1] All data are presented as means ± SEM, $n = 55–89$; *significantly different than 18 g casein/100 g control group, $P < 0.05$.

[2] Dams fed 18 g/100 g (control) or 9 g/100 g (low protein) casein diets ($n = 6$ per group) were killed at d 20 and fetal tissue and placenta obtained. The offspring of 6 dams per group were killed at birth and tissues obtained.

tein was estimated using the methods of Lowry et al. (1951), Bradford (1976) and Smith et al. (1985).

Hormone determinations. Plasma adrenocorticotrophin (ACTH) was determined in female rats using a commercial RIA kit (RIK 8502, Penninsula Laboratories, Belmont, CA), following acid extraction. Corticosterone was assayed in ethanolic extracts of plasma from female rats, as described by Langley and York (1990a).

Receptor binding. Type I and type II glucocorticoid receptor binding was estimated in male rats, using the method of Langley and York (1990b). ^{3}H-corticosterone (20 nmol/L) was incubated with semipurified 100,000-g cytosol preparations of liver, hippocampus, hypothalamus and thoracic aorta from adrenalectomized rats. Tissue was obtained 12 h after surgery to allow clearance of endogenous steroid from receptors, but not allowing upregulation of receptor numbers (McEwen et al. 1974). Nonspecific ligand binding was determined by competition of ^{3}H-corticosterone with a 500-fold excess of RU486 [type II binder, (Moguilewsky and Philibert 1984)] or aldosterone [type I binder (Reul and DeKloet 1985)]. Bound and unbound steroid were separated by gel filtration. Scatchard analyses (Langley and York 1990b) of binding in liver (4–5 individual rats per group), hippocampus and hypothalamus (pooled brain regions from 15 rats in each group), were performed using a concentration range of 2–25 nmol/L ^{3}H-corticosterone.

Statistical analysis. Where appropriate, one- or two-way ANOVA was performed with a Tukey test used for post-hoc analysis. Other analyses used Student's *t* test. A probability of 5% or less was accepted as statistically significant. All data are presented as means ± SEM.

RESULTS

At d 20 of gestation, the weights of fetuses from dams fed the low protein diet were significantly greater than the weights of control fetuses (**Table 2**). Placental weights were also greater in the low protein group on d 20. By full term, no significant differences in birthweights of the pups were apparent.

Activities of glucocorticoid-inducible (GS, GPDH) and glucocorticoid-insensitive (MD, PK) marker enzymes were determined in whole brains from d 20 fetuses and newborn pups. Maternal protein restriction had no effect on total brain protein at either age (**Table 3**), and activities of GS and MD were similarly unaltered by prenatal dietary experience. In the d 20 fetuses, GPDH activity in whole brain was similar in the two groups of rats, whereas PK activity was significantly (14%) lower in the low protein–exposed group. At full term, PK activity was similar in the two groups, but activity of GPDH was massively (377%) elevated in the brains of the low protein–exposed pups, relative to control pups.

The same enzyme activities were assessed in specific brain regions of weanling rats. Hippocampus, hypothalamus and cerebellum were selected as known glucocorticoid target tissues. No maternal diet–related differences in total protein concentrations were noted in any of the three regions (**Table 4**). Activity of GPDH was higher in hippocampus and hypothalamus of rats exposed to low protein diets in utero, relative to control rats. Similarly, GS activity was significantly higher in all three brain regions of low protein–exposed rats. Activity of MD in hippocampus and hypothalamus was unaltered by prenatal diet, whereas in cerebellum a 21% lower activity was noted in the low protein group, relative to control rats.

Activity of the hepatic glucocorticoid-sensitive enzyme, TAT, was 100% higher in rats exposed to maternal low protein diets (18 g casein per 100 g diet, $n = 8$, 2.00 ± 0.58 nmol/(min · mg protein); 9 g casein/100 g diet, $n = 6$, 4.14 ± 0.87 nmol/(min · mg protein), $P < 0.05$). Systolic blood pressures of the weanling rats were also assessed, and pressure in both males and females was significantly higher in the low protein group (control, 109 ± 2 mm Hg, $n = 23$; low protein, 123 ± 4 mm Hg, $n = 21$, $P < 0.05$).

Hormones of the hypothalamic-pituitary-adrenal axis were determined in plasma at four different points in the light cycle to assess diurnal variations. In control animals, plasma ACTH concentrations varied between 395 and 552 ng/L, with a maximum concentration at 0300 h and a minimum at 0900 h (**Fig. 1**). In weanling rats which had undergone intrauterine protein restriction, there was no evidence of diurnal variation, and plasma ACTH concentrations varied only between 413 and 435 ng/L over the 24 h studied. At 0300 h, plasma ACTH concentrations were significantly lower in the low protein group. Two-way ANOVA indicated that plasma ACTH concentration was significantly influenced by maternal diet ($P < 0.01$) and time of day ($P < 0.02$), and that maternal diet tended to interact with time of day ($P = 0.09$). Plasma corticosterone (**Fig. 2**) concentrations were similar in the two groups of rats at

TABLE 3

Activities of glucocorticoid-inducible and glucocorticoid-insensitive enzymes in whole brains of d 20 fetuses and newborn rat pups, exposed to different levels of maternal dietary protein[1,2]

Age	Day 20 fetus		Newborn	
Maternal diet	Control	Low Protein	Control	Low Protein
Total protein, *mg/g tissue*	114.1 ± 7.0	113.3 ± 10.7	140.2 ± 15.3	110.3 ± 6.5
GPDH,[3] *units*	2.75 ± 0.43	2.54 ± 0.34	1.85 ± 0.30	8.84 ± 2.37*
GS, *units*	119.2 ± 4.5	112.7 ± 3.9	117.5 ± 5.6	112.8 ± 3.6
MD, *units*	158 ± 4	151 ± 5	231 ± 8	242 ± 5
PK, *units*	435 ± 12	375 ± 20*	338 ± 16	373 ± 37

[1] All data are presented as means ± SEM, n = 7–10 observations; *indicates a significant effect of low protein exposure ($P < 0.05$).

[2] Dams fed 18 g/100 g (control) or 9 g/100 g (low protein) casein diets (n = 6 per group) were sacrificed at d 20 and fetal tissue and placenta obtained. The male and female offspring of 6 dams per group were killed at birth and tissue obtained.

[3] GPDH, glycerol 3-phosphate dehydrogenase; GS, glutamine synthetase; MD, malate dehydrogenase; PK, pyruvate kinase. Units of enzyme activity are nmol product formed/(min · mg protein).

all times of day. In both groups, plasma corticosterone concentrations rose with the onset of the dark cycle, with maximal concentrations at 2100 h, and fell with the onset of the light cycle. Plasma corticosterone concentrations at 0900 and 1500 h were significantly lower than at 2100 h ($P < 0.05$).

Specific binding of ^{3}H-corticosterone to type I and type II glucocorticoid receptors was assessed in liver, hippocampus, hypothalamus and thoracic aorta (**Table 5**). A tissue-specific pattern of maternal diet–related differences was noted. Rats exposed to low protein diets in utero bound significantly more ^{3}H-corticosterone at type II sites in hippocampus (41% greater binding, $P < 0.05$) and thoracic aorta (188% greater binding, $P < 0.05$). Type II binding tended to be lower in the livers of low protein–exposed rats than in controls ($P < 0.1$). Assessment of type II binding by Scatchard analysis (**Table 6**) indicated that there were significantly fewer receptors in the livers of the low protein group ($P < 0.05$). Binding affinity for corticosterone of these liver receptors tended to be greater in the low protein groups ($P < 0.2$). Type II receptor number was apparently elevated in hippocampus, and affinities of the receptors for corticosterone were similar in both groups of rats. In hypothalamus, type II receptor number and affinity were unaltered by prenatal dietary experience.

Binding of ^{3}H-corticosterone to type I receptors of liver, hippocampus and hypothalamus was similar in both groups of rats (Table 5). Access to the type I receptor is regulated by tissue 11βHSD activity. The activities of both the NAD- and NADP-dependent isoforms of the enzyme were assayed in hippocampus and hypothalamus (**Table 7**). Maternal protein restriction had no significant effect on either activity in these brain regions.

DISCUSSION

Retrospective studies of human populations suggest that patterns of disproportionate fetal growth retarda-

TABLE 4

Activities of glucocorticoid-inducible and glucocorticoid-insensitive enzymes in brain regions of weanling rats, exposed to different levels of maternal dietary protein[1,2]

Brain region	HC[3]		HY		CER	
Maternal diet	Control	Low protein	Control	Low protein	Control	Low protein
Total protein, *mg/g tissue*	134 ± 9	139 ± 12	133 ± 9	150 ± 3	130 ± 11	155 ± 15
GPDH,[4] *units*	ND	ND	43.6 ± 2.5	52.9 ± 2.6*	14.8 ± 1.3	21.0 ± 0.9*
GS, *units*	152 ± 7	187 ± 13*	186 ± 4	237 ± 9*	184 ± 8	243 ± 12*
MD, *units*	22.9 ± 0.9	18.2 ± 1.1	26.9 ± 0.9	27.0 ± 1.7	32.8 ± 1.2	25.9 ± 0.8*

[1] All data are presented as means ± SEM, n = 9–10 observations; *indicates a significant effect of low protein exposure ($P < 0.05$). ND, not determined.

[2] The male and female offspring of dams fed 18 g/100 g (control) or 9 g/100 g (low protein) casein diets (n = 6 per group) were killed at weaning (4 wk of age) and whole brains removed for immediate dissection of regions.

[3] HC, hippocampus; HY, hypothalamus; CER, cerebellum.

[4] GPDH, glycerol 3-phosphate dehydrogenase; GS, glutamine synthetase; MD, malate dehydrogenase. Units of enzyme activity are nmoles product formed/(min · mg protein).

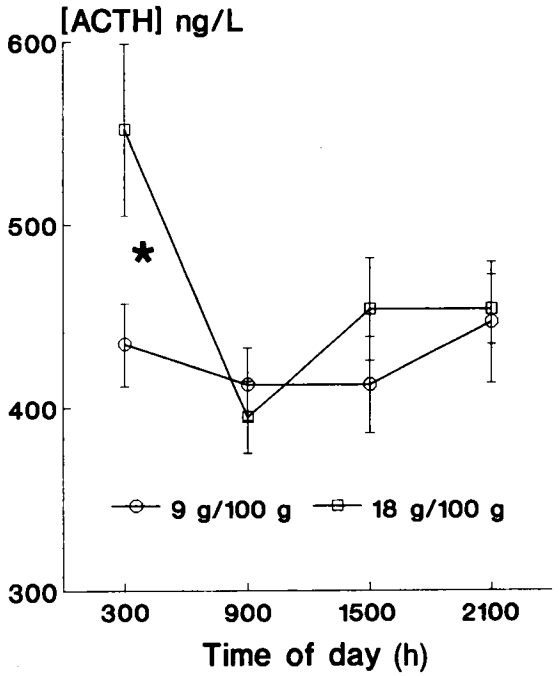

FIGURE 1 Diurnal variation of plasma adrenocorticotrophin (ACTH) concentrations in rat pups exposed to different protein diets in utero. All data are means ± SEM for 4–6 observations per group. Female rats aged 4 wk were killed at 0900, 1500, 2100 and 0300 h and plasma obtained* indicates a significant effect of maternal dietary restriction. Two-way ANOVA indicated a significant effect of maternal diet ($P < 0.01$) and time of day ($P < 0.02$) on plasma ACTH concentration and that there was a tendency for the effect of time to be modulated by maternal diet ($P = 0.09$).

tion are strongly associated with cardiovascular disease and noninsulin-dependent diabetes in later life (Barker 1995). In keeping with observations in humans, we have previously shown that undernutrition of pregnant rats induces increases in blood pressure in the resulting offspring (Langley and Jackson 1994). The hypertensive effect of protein restriction, as demonstrated in the present paper, is consistently associated with an increased placental weight at d 20 of gestation and would appear to be coupled to late gestation growth retardation. As previously described, the low protein–exposed fetuses were larger than controls at d 20, but by full term were of lower (Langley-Evans et al. 1994) or normal birthweight. During the last 2 d of gestation, the body weight of the rat fetus doubles, and growth of peripheral organs such as the liver during this period appears to be vulnerable to the adverse effects of maternal low protein diets (Langley-Evans et al. 1996a). Brain growth of low protein–exposed fetuses, however, appears to be spared.

The diets used in these experiments were balanced with respect to energy, fiber, fat, minerals and vitamins (Langley et al. 1994). The low protein diet represented only a mild restriction of maternal protein intake, because the pregnant rat dam requires 12 g protein/100 g diet (National Research Council 1978). Much of the effect of prenatal exposure to this diet should be attributable to protein restriction, because maternal protein intake is reduced by 50% and carbohydrate intake increased by only 14% in dams consuming the diet, changes which typically do not alter overall food intake (Langley and Jackson 1994).

In the current investigation, we examined a number of indices of hypothalamic-pituitary-adrenal (HPA) axis function. The experiments of Edwards et al. (1993) have suggested a role for intrauterine exposure to glucocorticoids in programming of hypertension. This hypothesis is consistent with our model of nutritional manipulation (Langley and Jackson 1994, Langley-Evans et al. 1994). Pharmacological blockade of corticosterone synthesis abolishes the effect of maternal low protein diets on the blood pressures of young adult rats (Langley-Evans et al. 1996b). This indicates that nutritional programming of cardiovascular function is an adrenal steroid–dependent phenomenon. To investigate HPA axis function, we used three approaches. Activities of marker enzymes for glucocorticoid action were determined in central and peripheral tissues. Although glucocorticoid-inducible, these activities were unlikely to have any direct role in programming of blood pressure.

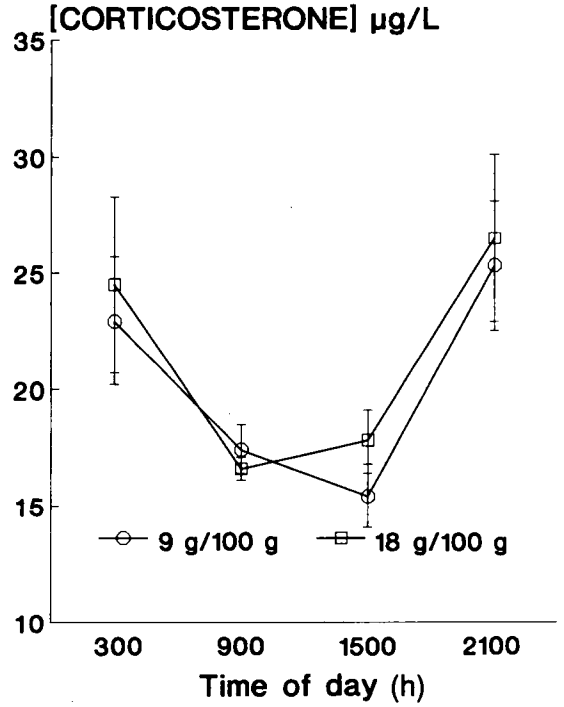

FIGURE 2 Diurnal variation of plasma corticosterone concentrations in rats exposed to different protein diets in utero. All data are means ± SEM for 5–6 observations per group. Female rats aged 4 wk were killed at 0900, 1500, 2100 and 0300 h and plasma obtained. Two-way ANOVA indicated a significant effect of time of day ($P < 0.0005$) on plasma corticosterone concentration. For both groups, plasma corticosterone was significantly higher at 2100 h than at 0900 h ($P < 0.05$) and 1500 h ($P < 0.04$). For the low protein group, a significant difference between concentrations at 0300 and 0900 h was also indicated ($P < 0.04$).

TABLE 5

Specific binding of 20 nmol/L ^{3}H-corticosterone to glucocorticoid receptors in liver, hippocampus, hypothalamus and thoracic aorta of rats exposed to maternal low protein diets in utero[1,2]

	Type I receptors		Type II receptors	
Maternal diet	Control	Low protein	Control	Low protein
	fmol bound/mg protein			
Liver	32 ± 8	42 ± 17	344 ± 95	234 ± 56
Hippocampus	87 ± 6	135 ± 20	51 ± 8	72 ± 3*
Hypothalamus	115 ± 8	137 ± 3	42 ± 7	43 ± 4
Thoracic aorta	ND	ND	40 ± 10	115 ± 25*

[1] All data are presented as means ± SEM for $n = 4$–12 observations (aorta $n = 14$–18); *indicates a significant effect of exposure to low protein diets ($P < 0.05$). ND, not determined.

[2] The male offspring of dams fed 18 g/100 g (control) or 9 g/100 g (low protein) casein diets were adrenalectomized for clearance of endogenous steroids and killed 12 h postsurgery. Whole brains were removed for immediate dissection of regions.

Second, HPA axis hormone concentrations were measured at four different points in the light cycle to assess diurnal secretion patterns. Finally, we used competitive ligand binding assays to determine type I and type II glucocorticoid receptor binding activity in central, peripheral and vascular tissue.

Glucocorticoid-inducible enzyme activities in brain regions and in the livers of low protein–exposed weanling rats were higher than in control animals. This appeared to be a specific steroid-mediated effect because all three marker enzymes had higher activity, although unrelated, and glucocorticoid-insensitive enzymes and

TABLE 6

Glucocorticoid type II receptor numbers and affinities in liver and brain regions of rats exposed to different levels of maternal dietary protein, as determined by Scatchard analyses[1,2]

	B_{max}[3]		K_d	
Maternal diet	Control	Low protein	Control	Low protein
	g/100 g			
Liver	941 ± 94	186 ± 51*	5.98 ± 1.49	5.60 ± 1.53
Hippocampus	41	81	2.49	4.42
Hypothalamus	159	190	3.32	4.90

[1] Liver data represents means ± SEM, $n = 4$ (control) or 5 (low protein). Hippocampus and hypothalamus data are single determinations; *indicates significantly different than 18 g/100 g control ($P < 0.05$).

[2] The male offspring of dams fed 18 g/100 g (control) or 9 g/100 g (low protein) casein diets were adrenalectomized for clearance of endogenous steroids and killed 12 h postsurgery. Whole brains were removed for immediate dissection of regions.

[3] B_{max}, maximum binding; K_d, dissociation constant.

TABLE 7

Effect of exposure to maternal low protein diets on activities of 11β-hydroxysteroid dehydrogenase (11βHSD) isoforms in rat pup hippocampus and hypothalamus[1,2]

	NAD		NADP	
Maternal diet	Control	Low protein	Control	Low protein
	11βHSD units[3]			
Hippocampus	4.5 ± 0.4	5.6 ± 0.5	11.8 ± 1.4	9.7 ± 1.1
Hypothalamus	4.3 ± 0.3	5.1 ± 0.5	6.6 ± 0.6	5.6 ± 0.3

[1] All data are presented as means ± SEM, $n = 6$.

[2] The male offspring of dams fed 18 g/100 g (control) or 9 g/100 g (low protein) casein diets were killed and whole brains rapidly removed for immediate dissection of regions.

[3] Units of 11βHSD activity are percentage of corticosterone converted to 11-dehydrocorticosterone/(min · mg protein).

total protein concentrations were either unaltered or lowered. Although GPDH and GS are present in fetal rat brain, no steroid induction appeared to have occurred in d 20 fetuses of the low protein group. By full term (d 22), GPDH activity was nearly four fold higher in the low protein–exposed group. These apparent temporal shifts in glucocorticoid effects may be explained in a number of ways. The use of whole fetal brains may have masked steroid-induced increases in enzyme activity in specific brain regions or nuclei. The hippocampus, hypothalamus and cerebellum (comprising at most 20% of total brain weight) are rich in receptors at this time in development (Rosenfeld et al. 1988), whereas the cortex, comprising much of the total brain mass, has a relatively lower receptor density. Alternatively, the enzyme data may indicate that brain receptor changes required to mediate increases in sensitivity to corticosteroids do not develop until postpartum. Furthermore, insufficient fetal corticosterone may be present to elicit any responses because the peak in fetal adrenal corticosterone secretion occurs later, at around the time of birth (Atkinson and Waddell 1995, Chatelain et al. 1980). A further explanation is that the Edwards hypothesis (Edwards et al. 1993) is incorrect and that rather than representing a primary intrauterine programming stimulus, glucocorticoid action in programming hypertension becomes important only in the postnatal period. In this respect, however, a resetting of the fetal HPA axis by intrauterine conditions may be critical. Stewart et al. (1995) argue that rather than providing a protective screen for the fetus against generally adverse effects of maternal corticosteroids, placental 11βHSD activity has a crucial role in the development of the fetal adrenal. A lower activity of this enzyme, as observed in rats fed low protein diets (Phillips et al. 1994), may provide a greater impact of maternal steroids on the fetal HPA axis and establish an altered pattern of function.

Consistent with the above proposal, weanling rats from the low protein group were found to have blunted diurnal variation in plasma ACTH concentrations. In control animals, peak ACTH concentrations were observed in the middle of the dark phase (0300 h), with a trough early in the light phase (0900 h), as reported by other workers (Atkinson and Waddell 1995). At 0300 h, the low protein–exposed animals had failed to increase plasma ACTH concentrations and had significantly lower levels of the hormone than controls. Corticosterone concentrations in both groups of rats followed a diurnal pattern similar to that seen for plasma ACTH concentrations in the control group. Clearly, HPA axis tone in low protein–exposed rats was altered by undernutrition in utero, and the presence of normal diurnal secretion of corticosterone with blunted ACTH secretion may indicate hyper-responsiveness to ACTH in the adrenal. Further investigations of HPA axis function should include dexamethasone suppression tests to assess regulation of ACTH secretion from the pituitary as well as synacthen (an ACTH analog) treatment to examine hormonal interactions within the axis.

Although the marker enzyme activities suggest increased corticosterone action in brain and liver of low protein diet–exposed rats, no evidence of increased plasma corticosterone concentrations was obtained. Increased activity of the corticosterone-sensitive enzymes, concomitant with low to normal hormone concentrations, is indicative of increased sensitivity to the glucocorticoids, mediated by the hormone receptors.

The finding of greater type II receptor numbers in the hippocampus of low protein–exposed rats is consistent with increased sensitivity to corticosterone being mediated at the receptor level. GPDH and GS are both regulated through the classical type II glucocorticoid receptor (DeKloet and Reul 1987). Increased hormone binding at these sites would be expected, however, to decrease receptor number, as was observed in the liver. Type II receptors are downregulated by corticosterone (Sapolsky et al. 1984). It would appear, however, that other regulatory mechanisms may operate in brain tissue. ACTH and monoamine neurotransmitters are putative regulators of central receptor numbers (DeKloet et al. 1987). A similar phenomenon of elevated central type II receptor numbers, in association with increased sensitivity to glucocorticoids, has been previously described in genetically obese *fa/fa* rats (Langley and York 1990b).

Type I receptor binding was unaffected by prenatal dietary experience. Unexpectedly, binding to type I sites in brain regions exceeded binding to type II sites. The type II site is generally the more numerous receptor in central regions (Reul and de Kloet 1985). Access to the type I sites is controlled by tissue 11βHSD activity (Funder et al. 1988). The type I receptor is identical to the aldosterone receptor (Reul and Dekloet 1985), and in mineralocorticoid target tissues, higher 11βHSD activity is required to prevent inappropriate binding of corticosterone. The lack of increased type I binding and the normal 11βHSD activity observed in low protein–exposed rats suggest that glucocorticoid-specific effects in these animals are not mediated by the type I receptor. Hypothalamic type I and type II receptors have differential effects on blood pressure in the rat. Type I agonists promote increases in pressure, whereas type II agonists have a hypotensive effect (van den Berg et al. 1990). The present data do not appear to support a role for hypothalamic receptors in the hypertension of low protein–exposed animals. However, receptor number or relative binding following clearance of endogenous steroid may not necessarily correlate with the number of free receptors in vivo in adrenal-intact animals. A large percentage (70–80%) of type I sites are normally occupied in vivo (Reul et al. 1987); thus changes in binding capacity should exert proportional changes in tonic stimulation of type I functions. The type II receptor however, is only 10–20% occupied in vivo (Reul et al. 1987), unless the animal is stressed; it is also of lower affinity than the type I site (Reul and DeKloet 1985). Large changes in receptor number would therefore be required to exert any major change in tone through this site.

A more likely mechanism by which glucocorticoids may be directly modulating blood pressure in the low protein–exposed animal is indicated by the higher level of type II receptor binding in thoracic aorta. Binding of corticosterone to receptors in resistance arteries increases blood pressure, and further studies will consider resistance vessels in other regions that may play a greater role in control of systemic blood pressure than does the aorta. The binding of glucocorticoids to type II receptors in vascular smooth muscle cells increases uptake of Na^+ and Ca^{2+} (Kornel et al. 1995). Furthermore, in spontaneously hypertensive rats, type II binding increases angiotensin II receptor numbers (Provencher et al. 1995). Angiotensin II is a potent pressor agent, and modulation of its receptor number by glucocorticoid action may represent a major mechanism by which glucocorticoids control blood pressure. Interestingly, dependence of maternal diet–induced hypertension in the rat depends upon generation of angiotensin II through activity of angiotensin-converting enzyme, although plasma angiotensin II concentrations are apparently normal (Langley-Evans and Jackson 1995).

The present study has demonstrated that many aspects of HPA axis function can be programmed in utero by maternal protein restriction. Diurnal variation of ACTH secretion was blunted, and despite normal corticosterone concentrations in circulation, activities of corticosterone-inducible enzymes were significantly elevated. It would appear that type II glucocorticoid receptors are inappropriately regulated in rats exposed to maternal low protein diets. Elevations of receptor number in vascular tissue may provide a primary mechanism through which maternal diet modulates the later blood pressure of the fetus.

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