Prenatal stress induces intrauterine growth restriction and programmes glucose intolerance and feeding behaviour disturbances in the aged rat

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Abstract

There is growing evidence that prenatal adversities could be implicated in foetal programming of adult chronic diseases. Since maternal stress is known to disturb the foetal glucocorticoid environment, we examined the consequences of prenatal stress on foetal growth, on glucose–insulin metabolism and on feeding behaviour in the aged male rat. In foetuses at term, maternal stress reduced body, adrenal and pancreas weight as well as plasma corticosterone and glucose levels. In aged male rats (24 months of age), prenatal stress induced hyperglycaemia and glucose intolerance and decreased basal leptin levels. Moreover, after a fasting period, they showed an increased food intake. These data suggest that maternal stress induces a long-lasting disturbance in feeding behaviour and dysfunctions related to type 2 diabetes mellitus. This programming could be linked to the early restricted foetal growth and to the adverse glucocorticoid environment *in utero*.

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Introduction

In humans, an inverse relationship between birth weight and adult ischaemic heart disease has been described, suggesting the role of prenatal 'programming' as a determinant of adult diseases (Barker & Osmond 1986). This suggests that environmental influences acting on foetal life are reflected in altered birth size/phenotype and permanently affect structure and metabolism, thereby leading to a greater risk of developing coronary heart disease, hypertension, insulin-resistance syndromes and osteoporosis (Barker 1998, Nathanielsz & Thornburg 2003). However, while increasing epidemiological evidence supports the role of early developmental growth patterns in the development of specific adult diseases, the determinant of the foetal growth restriction remains unclear. Maternal hormonal and nutritional status may be particularly important (Holness *et al.* 2000, Lesage *et al.* 2002). In this view, in humans and in rodents prenatal glucocorticoid overexposure induced by hormonal treatment or by maternal stress during gestation has been proposed to programme an adverse adult cardiovascular, metabolic, neuroendocrine and behavioural phenotype (Seckl 2001).

In rats, prenatal stress (PS) is known to disturb the foetal environment and to programme permanently neuroendocrine and behavioural responses in adult offspring (Maccari *et al.* 2003). For example, PS increased stress-induced adrenocorticotrophin (ACTH) and corticosterone (CORT) secretion and decreased binding capacity of hippocampal glucocorticoid receptors (GRs) (Koehl *et al.* 1999). These hypothalamo–pituitary–adrenal (HPA) axis dysfunctions have been suggested to be mediated by maternal glucocorticoids during pregnancy. Indeed, adrenalectomy of pregnant dams suppressed the effects of maternal stress on the HPA axis of the offspring (Barbazanges *et al.* 1996). Even if PS constitutes a model of glucocorticoid overexposure *in utero*, few studies have explored its long-lasting consequences on metabolic parameters. It was reported that in young adult animals PS increased basal glycaemia and reduced both body weight and food intake (Vallée *et al.* 1996). However, although the vulnerability to develop certain metabolic disorders such as type 2 diabetes mellitus strongly increases with ageing (Holness *et al.* 2000), metabolic alterations in aged PS rats have never been investigated. The aim of the present study was to evaluate if PS induces an *in utero* growth restriction and increases the vulnerability to develop metabolic disorders with ageing such as altered glucose–insulin metabolism and disturbed feeding behaviour.

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Materials and Methods

Subjects

Female Sprague–Dawley rats (250 g) were mated with a male for one night. The next day was considered as day 0 of pregnancy if spermatozoa were found in the vaginal smears. Pregnant females were then transferred to individual cages. During the last week of pregnancy (from embryonic day E14 to E21), pregnant females in the stress group ($n=10$) were placed for 45 min in a transparent plastic cylinder in a lighted environment three times per day (0900, 1200 and 1700 h). Control females ($n=10$) were left undisturbed. For studies in foetuses at term (E21), the maternal stress procedure was continued until E20. This stress procedure was previously described by Maccari *et al.* (1995). After weaning, offspring were housed in groups of four animals and left undisturbed for 23 months. Then, aged male rats were individually housed for 1 month before the beginning of experiments. All rats were maintained on a 12 h light:12 h darkness cycle (lights on 0800–2000 h), with free access to food and water. Manipulation of the animals was performed following the principles of laboratory animal care published by the French Ethical Committee and the rules of the European Union Normative (86/609/EEC).

Plasma and tissue collections

On day 21 of gestation, pregnant females at term ($n=6$ females/group) were killed rapidly by decapitation between 1000 and 1200 h. Each litter usually contained 8–12 foetuses, which were collected by caesarean section, rapidly weighed and killed by decapitation.

Trunk blood samples of foetuses were collected after decapitation in tubes pre-rinsed with EDTA. Blood glucose was measured using a glucometer (One Touch II; Lifescan, Roissy, France). Body length was measured in foetuses and the adrenals and the pancreas were removed and weighed.

For aged male rats (24 months old), a maximum of two males from the same litter were used. After food intake measurements and oral glucose tolerance tests (OGTTs), rats were killed at rest by decapitation between 1000 and 1200 h. Trunk blood was collected and blood glucose was measured.

Adrenals, pancreas and the perirenal and perigonadal fat pads were removed and weighed. All blood samples were centrifuged at 3200 ***g*** for 10 min at 4 °C and plasma samples were kept at − 30 °C prior to CORT and leptin assays.

OGTTs

OGTTs were performed on eight or nine animals from both groups at 24 months of age after a 16 h fasting. Animals were given 2 g glucose/kg body weight with an oral cannula. Blood samples were collected from the tail vein 5 min before (time 0) and 60 and 120 min after glucose load. Blood glucose was measured and plasma aliquots were kept at − 30 °C until assayed for insulin.

Food intake measurement

Basal feeding behaviour was evaluated by measuring consumption of food in the home cages of the animals for a 24 h period. Cumulative food intake was also determined for 1, 2, 3 and 24 h periods after 24 h of fasting. Food consumption was determined by placing 150 g of chow in the home cage and weighing the residual food at indicated intervals.

RIAs

Plasma CORT levels were measured with an ^{125}I RIA kit (ICN Biomedicals, Irvine, CA, USA) using a highly specific CORT antibody and a detection threshold of 0·1 μg/100 ml.

Plasma insulin levels were measured using monoiodinated ^{125}I-labelled porcine insulin (Sorin Biomedica, Sallugia, Italy) as a tracer, guinea pig anti-insulin antibody kindly provided by Dr Van Schravendijk (Brussels, Belgium) and purified rat insulin (Novo Nordisk, Boulogne, France) as standard. Charcoal was used to separate free from bound hormone. The sensitivity of the assay was 0·25 ng/ml.

Plasma leptin concentration was measured using a rat/mouse leptin RIA kit (LEP-R61; Mediagnost, Tuebingen, Germany). Standards and ^{125}I-labelled tracer were prepared from recombinant mouse leptin. No cross-reactivity was found with insulin and insulin-like growth factor-I. Sensitivity was 6 pg/ml in undiluted plasma samples.

Statistical analysis

All data are presented as means ± S.E.M. Morphometric and biological parameter comparisons between control and PS rats were performed using independent Student's *t*-tests. One-way ANOVA followed by a Newman–Keuls (NK) post-hoc test was used to compare groups for parameters with repeated measures (OGTT test and food intake measurement). $P<0·05$ was considered significant.

Results

Effect of maternal stress on physiological parameters of foetuses at term

Foetuses from stressed mothers showed reduced body weight both in males ($P<0·001$) and in females ($P<0·01$),

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Table 1 Effects of maternal stress during the last week of gestation on morphometric and biological parameters of foetuses at term (day 21 of gestation). Data are means ± S.E.M. (n=29 male foetuses/group and n=31–39 female foetuses/group for body weight values; n=13–20 male foetuses/group for others parameters)

	Control	PS
Body weight (g)		
Males foetuses	6·09 ± 0·09	5·34 ± 0·10***
Female foetuses	5·85 ± 0·09	5·46 ± 0·08**
Body length (cm)	4·89 ± 0·04	4·84 ± 0·05
Adrenals (mg)	3·33 ± 0·28	2·20 ± 0·25**
Pancreas (mg)	30·78 ± 0·88	25·49 ± 0·86***
Plasma glucose (mg/dl)	77·10 ± 4·44	60·76 ± 4·24*
Plasma CORT (µg/dl)	7·07 ± 0·76	2·40 ± 0·35***
Plasma leptin (ng/ml)	2·71 ± 0·29	3·35 ± 0·28

*P<0·05, **P<0·01, ***P<0·001, PS vs control.

as well as reduced adrenal (P<0·01) and pancreas (P<0·001) weight in males (Table 1). Plasma glucose and CORT levels were also significantly reduced (P<0·05 and P<0·001 respectively) in these foetuses (Table 1). However, plasma leptin levels were not affected by maternal stress (Table 1).

Effect of PS on physiological parameters of aged male rats

PS had no effect on body, adrenal, pancreas and fat depots weights (Table 2). Basal plasma CORT levels tended to increase in PS rats (Table 2) but statistical analysis was not significant (P=0·08). In contrast, plasma leptin levels, at rest, were reduced (P<0·05) in PS rats (Table 2).

As shown in Fig. 1, the OGTT induced an increase of plasma glucose and insulin levels (time effect, glucose, F(2,28)=58·33, P<0·001; insulin, F(2,28)=14·78, P<0·001). Aged PS rats had higher glucose levels than control rats at all investigated times (group effect, F(1,14)=4·68, P<0·05) (Fig. 1A). In contrast, insulin secretion after OGTT was similar between experimental groups (Fig. 1B).

Table 2 Effects of PS on body and organ weights and basal plasma CORT and leptin levels in 24-month-old male rats. Data are means ± S.E.M. (n=7–8 animals/group)

	Control	PS
Body weight (g)	676·14 ± 17·53	647·25 ± 17·02
Adrenals (mg)	77·93 ± 6·92	67·21 ± 4·91
Pancreas (g)	1·07 ± 0·07	1·02 ± 0·06
Perirenal fat (g)	6·52 ± 0·72	5·44 ± 0·23
Gonadal fat (g)	4·76 ± 0·56	4·38 ± 0·23
Plasma CORT (µg/dl)	9·29 ± 2·15	16·72 ± 4·34
Plasma leptin (ng/ml)	13·55 ± 0·99	10·28 ± 0·57*

*P<0·05, PS vs control.

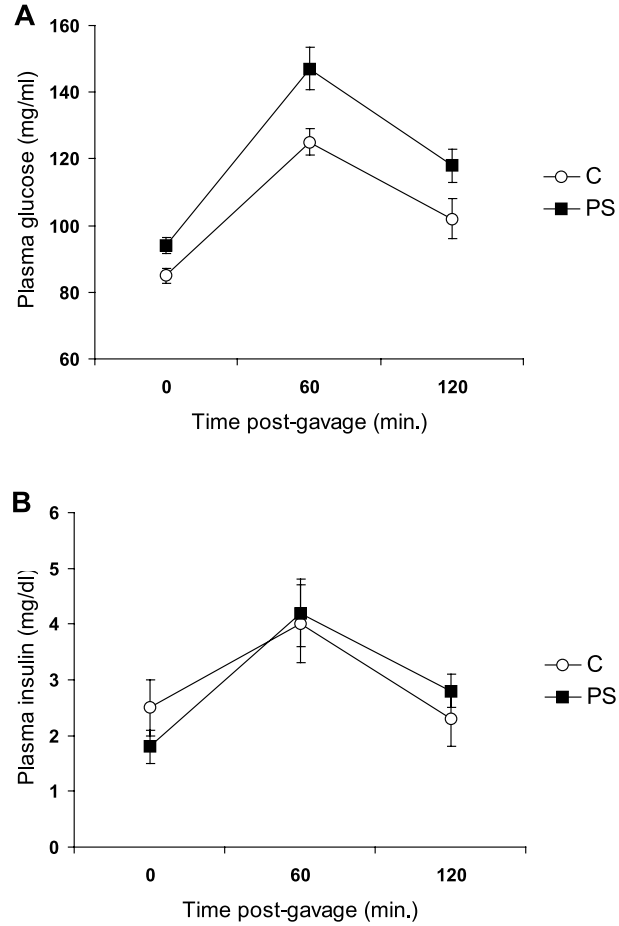

Figure 1 Effect of PS on plasma glucose (A) and insulin levels (B) during an OGTT in 24-month-old male rats. Values are means ± S.E.M. from eight or nine animals/group.

Effect of PS on feeding behaviour of aged male rats

PS had no effect on the basal food intake in aged animals (control, 25·7 ± 1·8 g; PS, 25·1 ± 0·6 g). In contrast, after 24 h of fasting, the cumulative food intake over a period of 3 h was higher in PS rats than in controls (group effect, F(1,12)=14·59, P<0·01; Fig. 2). The time course of food consumption differed between control and PS rats. Indeed, PS rats continued to increase their food intake 2 h (NK post-hoc, P<0·001 compared with the 1 h period) and 3 h (NK post-hoc, P<0·05 compared with the 2 h period) after food was placed in their home cage, whereas in controls there was no significant increase of food intake after the first hour.

Discussion

In the present study, we report that prenatal restraint stress induces a restriction of intrauterine growth in both male

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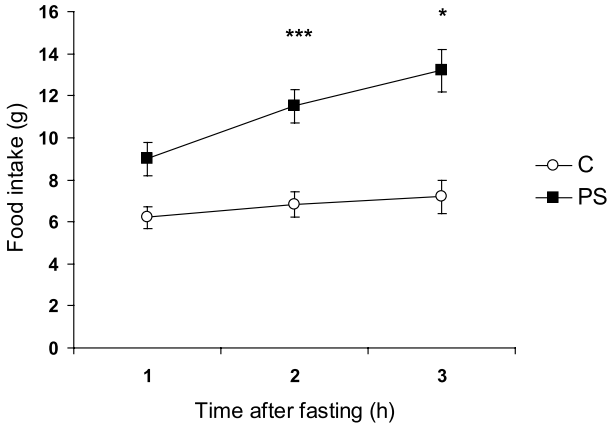

Figure 2 Effect of PS on cumulative food intake in 24-month-old male rats after 24 h of fasting. Values are means ± S.E.M. from eight or nine animals/group. ***$P<0{\cdot}001$, 2 h vs 1 h values; *$P<0{\cdot}05$, 3 h vs 2 h values.

and female foetuses. Interestingly, we demonstrate for the first time in aged PS animals an increase of plasma glucose levels without a change in plasma insulin concentration after an OGTT procedure. Moreover, aged rats had reduced plasma leptin levels and showed an increase of food intake after a fasting period. These results suggest that PS could increase later vulnerability to metabolic diseases with ageing.

Our study indicates that maternal stress decreases foetal body weight at term, indicating a foetal growth restriction. Previous reports on the effects of PS on body weight are conflicting. Indeed, a reduced body weight of pups has been reported in some cases (Drago *et al.* 1999, Patin *et al.* 2002), whereas other studies have not revealed any differences (Power & Moore 1986, Von Hoersten *et al.* 1993). These discrepancies could be a result of postnatal factors such as maternal milk yield. In our case, pups were removed by caesarean procedure, excluding the possibility of differences in milk secretion between control and stressed mothers (Lau 1992) or in milk intake in newborns.

Processes by which maternal stress affects pups development are unknown. However, numerous studies have shown that excessive glucocorticoids exposure *in utero* reduces birth weight and alters organ maturation in a variety of mammalian species, including primates and man (Reinisch *et al.* 1978, Novy & Walsh 1983). Maternal hypersecretion of glucocorticoids as well as exogenous administration of CORT or dexamethasone has been reported to reduce both foetal adrenal growth and activity in parallel with a drastic reduction of both hypothalamic corticotrophin-releasing hormone content and plasma ACTH concentration (Dupouy *et al.* 1987, Lesage *et al.* 2001). Chronic restraint stress is known to increase CORT levels in pregnant dams (Barbazanges *et al.* 1996). We report here a reduction of CORT secretion and an atrophy of adrenal glands in the foetus at term. These modifications confirm the foetal glucocorticoid overexposure, since adrenal atrophy is a physiological adaptation in the foetus to attenuate high glucocorticoids levels. Our present results also indicate that maternal stress reduces both plasma glucose levels and pancreas weight in foetuses. In a previous study, we showed that the development of pancreatic islets and β-cells is extremely sensitive to glucocorticoids from both maternal or foetal adrenal glands (Blondeau *et al.* 2001). Indeed, inhibition of foetal steroid production drastically increases both islet number and β-cell mass, whereas overexposure to maternal glucocorticoids has opposite effects (Blondeau *et al.* 2001). The reduction of plasma glucose levels in foetuses from stressed mothers could result from numerous disturbances such as a reduced efficiency in placental (maternal-to-foetal) glucose transfer, or an increased foetal glucose metabolism.

When they were 24 months old, PS rats had no alterations in their body or organs weights. However, PS rats exhibited hyperglycaemia under basal conditions and after a glucose load, whereas insulinaemia was not affected. This is the first experimental proof suggesting that maternal stress could programme type 2 diabetes mellitus in aged offspring. These results extend other reports in the literature. Indeed, it has been shown that PS rats have high plasma glucose levels at the age of 5 months (Vallée *et al.* 1996). Present data suggest a persistent effect of PS on glycaemia. Administration of synthetic glucocorticoids during late gestation causes hyperglycaemia and glucose intolerance in adult rat offspring (Nyirenda *et al.* 1998). Moreover, inhibition of placental 11β-hydroxysteroid dehydrogenase type 2, which protects the foetus from an excess of glucocorticoids of maternal origin, reduces birth weight and leads to impaired glucose tolerance in adult rats (Saegusa *et al.* 1999). So, our results reinforce the hypothesis that prenatal programming of glucose metabolism may be mediated by the glucocorticoid environment during foetal life. It was reported that early stress paradigms reduce the food intake in young adult rats (Vallée *et al.* 1996, Penke *et al.* 2001). We show that basal food intake was not altered in aged PS rats. We also report an increase of food intake after a fasting period in aged PS rats, suggesting an alteration of the feeding behaviour during stressful situations in these animals. PS reduces leptin secretion in aged offspring. Leptin is well documented to activate hypothalamic proopiomelanocortin/cocaine-amphetamine-regulated transcript anorexigenic neurons and to inhibit NPY/AgRP orexigenic ones, resulting in a decreased food intake (Schwartz *et al.* 2000). So, it could be hypothesised that low leptin levels in PS rats could be involved in the increase of food intake. However, in PS rats this increase of food intake is only triggered after a fasting period. PS has been well described to provoke HPA axis hyperactivity in response to stress through life (Vallée *et al.* 1999). As CORT is implicated in the feeding behaviour after fasting (Castonguay 1991, Hamelink *et al.* 1994), it

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could be hypothesised that a differential corticosteroid response to the stress of fasting in PS rats could be implicated in the differences in food intake. The reduction of plasma leptin levels in aged PS rats, in spite of an unchanged weight of several adipose tissues, is quite surprising. However, an altered adipocyte metabolism may be involved since recent data demonstrate that prenatal dexamethasone exposure is associated with increased GR expression and attenuated fatty acid uptake in adult visceral adipose tissue (Cleasby *et al.* 2003).

Several data in humans suggest an early programming of adult diseases including an increased risk for developing a type 2 diabetes mellitus with ageing in subjects with a low body weight at birth. We demonstrate here that maternal stress induced an intrauterine growth restriction in rat foetuses and programmes a type 2 diabetes mellitus and eating disorders in aged offspring. These data support the concept of a prenatal programming of chronic adult diseases and demonstrate that stress during perinatal life may have a profound impact on health throughout life.

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