Altered cardiovascular haemodynamics and baroreceptor–heart rate reflex in adult sheep after prenatal exposure to dexamethasone

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ABSTRACT

Numerous epidemiological studies, together with mounting evidence from studies in animals, point to a correlation between an adverse intrauterine environment and the early onset of cardiovascular and metabolic diseases later in life. We were the first to show that sheep exposed to dexamethasone (0.28 mg kg\(^{-1}\) day\(^{-1}\) for only 2 days) at the end of the first month of pregnancy (PTG1), but not those exposed at the end of the second month of pregnancy (PTG2), had a higher basal mean arterial pressure (MAP) 19 months after birth. In the present study we report the MAP, cardiovascular haemodynamics and baroreflex sensitivity in these animals at 40 months of age. MAP in the PTG1 group was significantly higher than in the control group (91 ± 1 mmHg and 81 ± 1 mmHg respectively; \(P < 0.001\)) and also when compared with the PTG2 group (82 ± 1 mmHg; \(P < 0.001\)). There was a significant increase in cardiac output in the PTG1 group compared with the control group (108 ± 2 and 96 ± 4 ml min\(^{-1}\) kg\(^{-1}\) respectively; \(P < 0.05\)). The increase in cardiac output in the PTG1 group was due to an increase in stroke volume (1.82 ± 0.08 ml kg\(^{-1}\) beat\(^{-1}\), compared with 1.46 ± 0.06 ml kg\(^{-1}\) beat\(^{-1}\) in the control group; \(P < 0.05\)), but not in heart rate. In the hypertensive group of animals (PTG1), there was a rightward shift of the baroreflex curve. In group PTG2 (the normotensive group of animals), a lower gain was found before and during propranolol treatment. The decrease in gain of the baroreflex was not associated with changes in heart rate range, suggesting an impairment in the central processing of the baroreceptor signals. Thus sheep fetuses exposed to dexamethasone for only 2 days at the end of the first month of gestation have high blood pressure (dependent upon the increase in cardiac output) and a reset of the baroreflex at 40 months of age. Animals that have received prenatal dexamethasone closer to mid-gestation, although normotensive with normal cardiac output, showed an altered baroreceptor–heart rate response.

INTRODUCTION

Epidemiological studies have pointed to a strong link between adult hypertension and inappropriately low birth weight for gestational age [1]. It was found that if adults weighed less than 2.95 kg (6.5 lb) at birth, they had a 10-fold increased risk of developing syndrome X (hypertension, insulin resistance, central obesity and abnormal blood lipids) than those who weighed more than 4.3 kg (9.4 lb), whereas lifestyle factors, such as smoking and overeating, increased the risk up to 3-fold [2], giving a very clear message of the importance of intrauterine environment for health in later life. The recent observations that people of birth weights < 2.5 kg

Key words: dexamethasone, hypertension, sheep.
Abbreviations: CO, cardiac output; HR, heart rate; MAP, mean arterial pressure.
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had, at age 50 years, higher resting pulse rates than those who had weighed 3.3 kg or more were interpreted as evidence of sympathetic nerve activity being programmed in utero [3]. The same group found that, within the normal range of resting plasma cortisol concentrations, higher cortisol levels were found in the group with lowest birth weight. In addition, higher plasma cortisol concentrations were positively correlated with systolic blood pressure, plasma triacylglycerol levels and the degree of insulin resistance [4].

Studies in experimental animals have reproduced intrauterine growth retardation, and have confirmed the link between intrauterine growth retardation and adult hypertension or metabolic disease. The offspring of rats fed on low-protein diets (50% reduction) during pregnancy and lactation showed substantial changes in liver morphology and greatly reduced glucokinase activity in the perivenous region [5]. In addition, there was an inverse relationship between maternal protein intake during pregnancy and the systolic blood pressure in offspring of rats at 9 weeks of age [6]. In growth-restricted guinea pigs, the young male adults developed hypertension, had reduced skeletal muscle mass and increased adipose tissue, were glucose intolerant, and developed impaired cholesterol homeostasis [7,8]. Most interestingly, undernutrition of pregnant ewes in the first half of pregnancy (15% decrease in total food intake until 70 days of gestation) led to hypertension in lambs at 3 months of age [9].

There is now increasing evidence suggesting that the primary mediation of the effect of undernutrition is excessive exposure of the fetus to adrenocortical steroid hormones [10]. Carbamazepine treatment, used to inhibit the placental inactivation of glucocorticoids, resulted in both growth retardation and increased blood pressure plus insulin resistance in the adult offspring; however, the same treatment was ineffective if the dams were adrenalectomized [11,12]. In addition, metyrapone given during days 0–14 of pregnancy to rats on a low-protein diet (a diet that invariably produces offspring with high blood pressure) resulted in offspring, of both sexes, with growth retardation but normal blood pressure at 7 weeks. Corticosterone replacement in metyrapone-treated dams resulted in hypertensive female, but not male, offspring at 6 weeks [13].

In many studies, the synthetic steroid dexamethasone has been used to treat pregnant animals, as it is not metabolized to the same extent as the naturally occurring glucocorticoids (cortisol and corticosterone) by placental 11β-hydroxysteroid dehydrogenase type 2. Treatment of pregnant rats with dexamethasone throughout gestation or for only the final third of gestation increased blood pressure in offspring 16 weeks after birth. In these rats, there was altered expression of the steroid (glucocorticoid and mineralocorticoid) receptors in the hippocampus [14,15]. We have shown recently [16] that exposure of the sheep fetus to high levels of dexamethasone for a very short period (2 days only) during the last week of the first month of pregnancy (birth occurs after 5 months) resulted in offspring which had persistently higher blood pressure than lambs exposed either to no steroid or to the same treatment later in development (mid-pregnancy). This most remarkable finding has shown, for the first time in a long-gestation species, that hypertension can be programmed by stress hormones in utero. The exposure to dexamethasone was so brief, and occurred so early in pregnancy, that it had no effect on birth weight, suggesting that it is not only very-low-birth-weight babies who might be at increased risk of cardiovascular and metabolic disease in adult life, but any baby whose mother experiences periods of severe emotional or physical stress early in pregnancy. The mechanisms by which the treated sheep maintain higher than normal blood pressure remain largely unknown.

In the present study, our aims were three-fold: (i) to see if the previously demonstrated differences in mean arterial pressure (MAP) between ‘hypertensive’ and control animals increased with age; (ii) to find out whether the increase in blood pressure was due to an increase in cardiac output (CO) or a decrease in total peripheral conductance; and (iii) to determine if baroreflex sensitivity was altered in the ‘hypertensive’ animals.

METHODS

Animals

In the present study, we used the animals reported to become hypertensive after prenatal treatment with dexamethasone early in gestation [16]. Briefly, pregnant ewes were treated with dexamethasone (Decadron; Merck, Sharp and Dohme) given intravenously (0.28 mg·kg⁻¹·day⁻¹) for 48 h at 27 days (PTG1) or 64 days (PTG2) of gestation. Ewes (n = 8) in the PTG1 group gave birth to six female and three male lambs, including one set of twins. Ewes (n = 12) in the PTG2 group gave birth to seven female and four male lambs. Only female lambs were included in further studies, for practical purposes, there being fewer male offspring. We also studied seven female lambs (one set of twins) that were exposed to minimum stress during development as the control group of animals. Surgery (oophorectomy and carotid artery loops) was performed on all lambs at 50 days of age. Oophorectomy was performed to eliminate potential effects of oestrus cycles. Sheep are seasonal breeders, and normally spend a prolonged period in anoestrus.

Measurement of MAP

In each group of sheep, at approx. 40 months of age, MAP and heart rate (HR) were measured every 10 min
This produces a fall in temperature of 0.30–0.45°C. A loading dose of propranolol (20 mg, intravenous) was given to the ewe in the absence of sympathetic input to the heart. A loading dose of propranolol was used in order to study the relationship between blood pressure and HR in the absence of vagal input to the heart. The ewe was given an injection of 4 mg of atropine 10 min before the start of either phényléphrine or sodium nitroprusside infusion, followed by continuous infusion at 24 mg/h and repeated injections of 1.2 mg of atropine before each recording.

**Cardiovascular haemodynamics**

A standard thermodilution technique was used to measure CO in sheep [17]. In brief, a catheter tip thermistor was introduced approx. 20 cm into the carotid artery, so that the thermistor bead was located in the proximal brachiocephalic trunk or aortic arch. In addition, a polythene cannula (1.57 mm internal diam.; 2.08 mm outer diam.; total length 50 cm) was introduced via the jugular vein and inserted 20 cm towards the heart, to end in the cranial vena cava. CO determination was based on the temperature change induced by injection of 10.4 ml of ice-cold saline in < 2 s into the jugular cannula. This produces a fall in temperature of 0.30–0.45°C at the thermistor tip. The area under the curve was measured by a thermodilution computer built at the Howard Florey Institute. Injection of ice-cold saline was timed so that the temperature change sensed at the thermistor tip coincided with the measurement of MAP and HR as described above. MAP and HR measurements were taken at intervals of 1 min. Approx. 15–30 CO readings were taken over 30–60 min on two consecutive days in non-ruminating animals with a natural upright posture.

**Baroreflex sensitivity**

For this series of experiments, MAP and HR were measured via a Tygon cannula (1.0 mm internal diam.; 1.5 mm outer diam.) inserted into a carotid artery and connected to a pressure transducer (TD XIII; Cobe) and recorded continuously during infusions on a Gould 3000 series chart recorder (Gould Inc., Cleveland, OH, U.S.A.).

After a 30 min control period, arterial pressure was raised by intravenous injection of phényléphrine (0.4–10 mg/h) or lowered by intravenous injection of sodium nitroprusside (0.5–5 mg/h). For each ewe, data were collected at 6–8 different levels of arterial pressure. Arterial pressure was maintained for 3–5 min at each level, and the HR reading was taken when blood pressure had plateaued.

Animals were allowed a 2 h recovery period before phényléphrine and sodium nitroprusside infusions were repeated during sympathetic blockade with propranolol. Treatment with propranolol was used in order to study the relationship between blood pressure and HR in the absence of sympathetic input to the heart. A loading dose of propranolol (20 mg, intravenous) was given to the ewe 10 min before phényléphrine and sodium nitroprusside experiments began, followed by a continuous infusion of 30 mg/h. After a 24 h recovery period, phényléphrine and sodium nitroprusside infusions were repeated during parasympathetic blockade with atropine. Treatment with atropine was used to study the relationship between blood pressure and HR in the absence of vagal input to the heart.

**Statistical analysis**

The data for haemodynamic variables (basal MAP, CO, HR, stroke volume and total peripheral conductance) were analysed by one-way analysis of variance, followed by all pairwise contrasts between the groups. Statistical analysis was performed using the Sigma Stat statistical program.

The relationship between MAP and HR was analysed by a logistical sigmoid function (GraphPad Inplot; GraphPad Software, San Diego, CA, U.S.A.) using the following equation [18]:

\[
    \text{HR} = \frac{A(1)}{1 + e^{A(2) \cdot (\text{MAP} - A(3))}} + A(4)
\]

where A(1) is the HR range (beats/min), A(2) is the slope or sensitivity of the reflex response (1/mmHg), A(3) is midpoint (in mmHg) for equal pressor and depressor responses, and A(4) is the minimum HR. The gain of the baroreflex was derived from the first derivative of this equation [18].

The MAP and HR under basal conditions, as well as the MAP and HR before and during propranolol treatment, were analysed by split-unit analysis of variance, from which the following effects were evaluated:

(a) between-sheep effect of groups (Control, PTG1 and PTG2), which tested for differences in levels across all treatments; (b) within-sheep effect of treatments (Control, Propranolol and, in the case of values under basal conditions, Atropine), which tested for differences in levels across all groups; and (c) within-sheep interaction group versus treatment, which tested for the differences in profile of levels across treatments and according to group. The analyses were performed using Systat 7.01 (SPSS Inc., Chicago, IL, U.S.A.).

**RESULTS**

**MAP: follow-up study**

MAP was monitored continuously by computer at 10 min intervals for 3 days when animals were 44 ± 1 (control; n = 7), 40 ± 2 (PTG1; n = 5) and 40 ± 2 (PTG2; n = 6) months of age. MAP in the PTG1 group was...
Figure 1  Effects of prenatal glucocorticoid exposure on cardiovascular haemodynamics in female offspring in the control ($n = 6$), PTG1 ($n = 5$) and PTG2 ($n = 5$) groups when 40 months old
(A) MAP and HR measured, only for the duration of CO readings, by computer at 1 min intervals; results are means ± S.E.M. (B) Changes in CO, stroke volume (SV) and total peripheral conductance (TPC). Results are means ± S.E.M. Significance of differences: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Basal MAP and HR in control and dexamethasone-treated (PTG1 and PTG2) animals under basal conditions</th>
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<td>Values are means ± S.E.M. $P$ values resulting from split-unit analysis of variance are as follows. Between groups: MAP, 0.011; HR, 0.25; between treatments: MAP, &lt; 0.001; HR, &lt; 0.001; group $\times$ treatment: MAP, 0.15; HR, 0.23. Groups = control, PTG1 and PTG2; treatments = none, propranolol and atropine.</td>
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<tr>
<td>Treatment</td>
<td>MAP (mmHg)</td>
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<tr>
<td>None</td>
<td>70 ± 3</td>
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<td></td>
<td>82 ± 3</td>
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<td></td>
<td>77 ± 1</td>
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<td>Propranolol</td>
<td>69 ± 3</td>
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<td></td>
<td>83 ± 3</td>
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<td>Atropine</td>
<td>73 ± 4</td>
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<td>85 ± 3</td>
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<td>84 ± 2</td>
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significantly elevated compared with the control group ($91 ± 1$ mmHg and $81 ± 1$ mmHg respectively; $P < 0.001$) and also when compared with the PTG2 group ($82 ± 1$ mmHg; $P < 0.001$).

**Cardiovascular haemodynamics**
At the time of CO measurements, animal body weights were $48 ± 3$ kg (control; $n = 6$), $48 ± 2$ kg (PTG1; $n = 5$) and $47 ± 2$ kg (PTG2; $n = 5$).

MAP was monitored by computer at 1 min intervals only for the duration of CO measurements (1.5–2 h) on two consecutive days. MAP in the PTG1 group was significantly elevated compared with the control group ($84 ± 2$ mmHg and $76 ± 1$ mmHg respectively; $P < 0.01$) and also when compared with the PTG2 group ($78 ± 3$ mmHg; $P < 0.05$) (Figure 1A). There was an increase in CO in the PTG1 group compared with controls ($108 ± 2$ ml min$^{-1}.kg^{-1}$ and $96 ± 4$ ml min$^{-1}.kg^{-1}$ respectively; $P < 0.05$), and also when compared with the PTG2 group ($90 ± 2$ ml min$^{-1}.kg^{-1}$; $P < 0.001$). Total peripheral conductance was similar in all three group of animals. There was an increase in stroke volume...
Programming of hypertension in sheep

Table 2 Characteristics of the cardiac baroreflex in control and dexamethasone-treated (PTG1 and PTG2) animals before and during β-adrenoreceptor blockade with propranolol

Gain, mid-point MAP and HR range were measured using an equation described by Kent et al. [18] (see the Methods section). Values are mean ± S.E.M. P values resulting from split-unit analysis of variance are as follows. Between groups: gain, 0.03; mid-point, 0.007; HR range, 0.62; between treatments: gain, < 0.001; mid-point, 0.08; HR range, 0.008; group × treatment: gain, 0.76; mid-point, 0.62; HR range, 0.88. Groups = control, PTG1 and PTG2; treatments = before or during propranolol.

<table>
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<tr>
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<th>Before propranolol</th>
<th>During propranolol</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PTG1</td>
</tr>
<tr>
<td>Gain (beats·min⁻¹·mmHg⁻¹)</td>
<td>−4.8 ± 0.3</td>
<td>−4.8 ± 0.7</td>
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<tr>
<td>Mid-point MAP (mmHg)</td>
<td>65 ± 4</td>
<td>74 ± 2</td>
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<tr>
<td>HR range (beats/min)</td>
<td>71 ± 11</td>
<td>63 ± 5</td>
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Baroreceptor sensitivity

Baroreceptor sensitivity was tested in six control animals, five animals from the PTG1 group and seven animals from the PTG2 group. Basal MAP and HR before and after either β-adrenergic blockade with propranolol or parasympathetic blockade with atropine in control and dexamethasone-treated (PTG1 and PTG2) animals are shown in Table 1. There was a significant difference between the values for basal MAP, with a rank order of: PTG1 > PTG2 > control (between-groups effect; Table 1). Also, there was a highly significant effect of treatment on basal MAP (between-treatments effect; Table 1), due largely to the effect of atropine in the PTG2 group of animals.

Data for gain, MAP at mid-point and HR range in control and dexamethasone-treated (PTG1 and PTG2) animals before and during β-adrenoreceptor blockade with propranolol are shown in Table 2. The values for gain were significantly different (between-groups effect; Table 2), due mainly to the significantly lower gain in the PTG2 group of animals. Gain was also lower after propranolol treatment, consistently in all groups (between-treatments effect; Table 2). Values for MAP at the mid-point were significantly different (between-groups effect; Table 2), in the rank order: PTG1 > PTG2 > control (baroreceptor HR curve is shifted to the right in PTG1 and PTG2 animals compared with controls; Figure 2). There was no difference in HR range between the groups (between-groups effect; Table 2), but the HR range was consistently lower after propranolol treatment across all groups (between-treatments effect; Table 2).
The relationship between HR and MAP was abolished by atropine treatment (Figure 2).

**DISCUSSION**

This follow-up study reports on the basal MAP, baroreflex sensitivity and cardiovascular haemodynamics in 40-month-old sheep who were exposed to dexamethasone for 48 h at the end of the first month or at the end of the second month of gestation. Our initial study demonstrated that dexamethasone treatment of pregnant ewes at the end of the first month, but not at the end of the second month, of gestation led to elevated blood pressure in adult offspring at 19 months after birth [16]. In the present study we found that the differences between ‘hypertensive’ and control animals continued to increase with age, suggesting that prenatal dexamethasone treatment at this particular stage of gestation had a permanent effect on adult blood pressure.

One of the major findings of the present study was that, in the ‘hypertensive’ animals, the increase in blood pressure was dependent on an increased CO, with no change in peripheral resistance. In addition, the increase in CO was due to an increase in stroke volume, not in HR. The increase in stroke volume could result from an increase in ventricular contractility due to cardiac hypertrophy, or from an increased preload (increase in plasma volume). This type of hypertension, which is dependent on increased CO, is similar to that found with cortisol- and corticotropin (‘ACTH’)–induced hypertension in humans and sheep [17,19–23]. This raises the possibility that prenatal dexamethasone could have altered permanently the level of one or more steroids, or of their receptors, which would ultimately lead to hypertension. Our previous study [16] demonstrated that neither basal nor corticotropin-stimulated plasma cortisol concentrations were altered, indicating that the hypertension is probably not due to excess production of these hormones. However, the effect of prenatal dexamethasone treatment on the expression of hippocampal mineralocorticoid and glucocorticoid receptors, known to be altered by prenatal dexamethasone treatment in rats [15], has not yet been studied.

The second major area of the present study was the baroreceptor–HR relationship in the three groups of animals. Under basal conditions the HR did not change after propranolol treatment, but it increased dramatically after atropine treatment. In addition, when the MAP–HR relationship was studied during atropine treatment, the normal sigmoidal relationship was abolished. This indicates that, in sheep, under basal conditions, the vagus is the main neural regulator of HR.

In the hypertensive PTG1 sheep, the baroreceptor–HR relationship was altered towards higher pressures (rightward shift). This resetting means that the baroreceptor-mediated control of arterial pressure still occurred, even though the basal or ‘resting’ MAP was elevated. The rightward shift of the baroreceptor–HR relationship in hypertensive PTG1 sheep could be due to cardiac hypertrophy. This is a distinct possibility, as PTG1 animals have increased COs and stroke volumes. Resetting of the baroreflex is found, commonly, as a consequence of developed hypertension [24–26]. However, in some strains of rats (Dahl salt-sensitive, stroke-prone SHR and Sabra hypertension-prone rats), the baroreflex was altered well before the development of hypertension, suggesting that abnormality of the baroreflex function preceded hypertension and may very well be the cause, rather than the consequence, of hypertension [27–29]. In our study, it is not known whether the resetting of the baroreflex led to the development of hypertension or was secondary to it.

The PTG2 group of animals, although normotensive, showed an altered baroreceptor–HR relationship. It is quite interesting to note that, under basal conditions, atropine treatment increased MAP in the PTG2 group of animals, but not in the control or PTG1 groups. This suggests that this former group of animals is different from both the control and PTG1 groups in the regulation of blood pressure. It is almost as though, in these animals, maximum suppression of sympathetic outflow (to vasculature, kidney, etc.) is not possible. The PTG2 group of animals do show a significantly greater increase in plasma renin concentration, in response to a standard haemorrhage, than do the animals in either the control or PTG1 groups (M. Dodic, unpublished work). In addition, the PTG2 group showed a lower gain of the baroreflex than the control group both before and during propranolol treatment. The decrease in gain, with no change in the HR range, may suggest that in this group of animals there is either an alteration inafferent signalling or a defect in central processing of the afferent information.

In conclusion, the difference in MAP between the ‘hypertensive’ and control sheep continues to increase with age. In these animals, MAP is maintained at the higher level by increased CO, with no change in peripheral resistance. Animals that received dexamethasone closer to mid-gestation, although normotensive with normal CO, showed an impaired baroreceptor–HR response.

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