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Reduced Life Expectancy in Rats After Neonatal Dexamethasone Treatment

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ABSTRACT: The glucocorticoid dexamethasone (Dex) is widely used in preterm infants for the prevention of chronic lung disease. However, major concern has arisen about the long-term sequelae of this therapy. Here we report that neonatal treatment with dexamethasone significantly shortens the lifespan by 25% of male rats (28.6 ± 1.1 to 21.3 ± 0.8 mo) and by 18% of female rats (26.9 ± 1.8 to 22.0 ± 0.7 mo). Histopathological examination indicated end-stage cardiac and renal failure as the cause of premature death. Furthermore, Dex-treated rats showed symptoms of hypertension at young adult age, which worsened with increasing age. Thus, a brief period of glucocorticoid treatment during early life results in untimely death presumably due to cardiovascular and renal disease later in life. These serious, adverse long-term consequences call for prudence with glucocorticoid treatment of human preterm infants and careful follow-up of young adults with a history of neonatal glucocorticoid treatment. (***Pediatr Res*** **61: 72–76, 2007**)

Severe respiratory distress syndrome with concomitant chronic lung disease carries a high incidence of morbidity and mortality (1,2). Inflammation is thought to be an important factor in its pathogenesis. Therefore, treatment with glucocorticoids (GCs), particularly dexamethasone (Dex), was introduced in the eighties resulting in a reduction in chronic lung disease (3–5).

Because of these potential benefits in relation to short-term effects on lung function and mechanics (3–5), neonatal Dex became common practice in many centers around the world despite the fact that there had been previous animal studies that showed potential adverse CNS effects of this treatment (6,7). More recently, animal studies indicated that neonatal treatment with GCs affects neurodevelopment and has long lasting adverse effects on cognition and behavior (8,9). In 2000, two papers were published that cautioned the use of Dex during the neonatal period in humans (10,11). Since then, more adverse effects of neonatal GC have been shown in rodents on the immune (12) and neuroendocrine systems (13), on the NMDA receptor (14) and in human follow-up studies of prematurely born children treated with Dex during the neonatal period (15,16).

Although the available data leave no doubt that neonatal GC treatment can have long-term adverse effects on a wide variety of brain and bodily functions in animals, it remains unclear whether or not this treatment results in higher rates of mortality and cardiovascular morbidity in the long run. In rat pups we have found that neonatal Dex suppresses proliferation of cardiomyocytes leading to a lower number of cardiomyocytes (17). This reduced cardiomyocyte number was associated with a substantial cardiomyocyte hypertrophy and increased fibrosis at adult age (18). Furthermore, Le Cras *et al.* (19) described that GC treatment of neonatal rats causes lung hypoplasia and increased pulmonary arterial pressures. We hypothesized that the myocardial changes previously found in rats induced by neonatal Dex treatment, whether or not in combination with hemodynamic instability, may have implications for the life expectancy. To test this hypothesis, we compared the survival of rats neonatally treated with Dex with that of saline (Sal) controls. Subsequently, we investigated if the observed effects on mortality were associated with hypertension and/or histopathological alterations in organ tissues.

MATERIALS AND METHODS

Animals. Ten days pregnant Wistar rats (250–280 g, Central Animal Laboratory, University Medical Center Utrecht, The Netherlands) were housed individually. For the experiments described in this manuscript animals from 22 litters were used. Pups were born on day 22–23 of gestation. On the day of birth (day 0) the pups were removed from their nest and 8 pups (4 females and 4 males) were randomly placed back with each dam. Pups were weaned at day 21 of age and then group-housed with same-sex littermates until experimentation. At weaning animals were randomly assigned to the various experimental conditions. Rats had *ad libitum* access to food and water. Light/dark cycle (dark phase 1900–0700 h), temperature (21°C) and humidity (60%) were kept constant. All experimental procedures were approved by the Committee for Animal Experimentation of the University Medical Center Utrecht.

Glucocorticoid treatment design. Rat pups were injected intraperitoneally with dex 21-phosphate (Dex; Organon International B.V., Oss, The Netherlands) on neonatal day 1 (0.5 mg/kg body weight), day 2 (0.3 mg/kg) and day 3 (0.1 mg/kg) or with equal volumes (10 mL/kg) of sterile pyrogen free saline (Sal) for a total of 3 d. All pups in a nest received the same treatment, *i.e.*, either Dex or Sal. The dose and duration of the treatment was based on a 21-d tapering course of Dex (starting dose, 0.5 mg/kg) to prevent or reduce chronic

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Abbreviations: Dex, dexamethasone; **GCs,** glucocorticoids; **Sal,** saline

lung disease in preterm infants. The separate experiments were performed using different groups of male and female offspring.

Lifespan determination. Groups of male rats (Dex $n = 17$; Sal $n = 18$) were allowed to reach senescence. The age of natural death was recorded for each individual. To reveal if long-term consequences of neonatal Dex treatment on mortality was gender-specific, the same experiment was performed with female rats (Dex $n = 13$; Sal $n = 12$).

Histopathology. To obtain an indication of the possible cause of death, groups of Dex ($n = 5$) and Sal ($n = 6$) male rats were kept under similar conditions and treated following the same protocol as in the lifespan experiment. Rats were killed at 15 mo of age for histopathological examination using 5-μm sections of formalin perfused, formalin postfixed and paraffin embedded parts of brain, lung, liver, spleen, and adrenal routinely stained with hematoxylin and eosin for conventional histopathological analysis. Histopathological sections (5-μm) from the hearts, in a plane parallel to the equator, were routinely stained with hematoxylin and eosin for conventional histopathological analysis. Staining with a modification of the AZAN technique was used to highlight cardiomyocyte profiles including intercalated discs. The kidneys were sliced in 5-μm sections and stained with hematoxylin and eosin.

Telemetry. Two cohorts of Dex and Sal rats were kept under similar conditions and treated following the same protocol as in the life-span experiment. Groups of male rats were equipped with implanted telemetric transmitters at 3 mo of age (Dex $n = 16$; Sal $n = 14$) or at 11 mo of age (Dex $n = 4$; Sal $n = 5$) to enable continuous recording of heart rate, blood pressure and somatomotor activity under freely moving conditions in their home cages. Surgery was performed in a sterile laminar flow cabinet to minimize the risk of infection. Telemetric transmitters were implanted in the abdominal cavity according to the procedure described previously (20). Briefly, a small longitudinal incision was made on the linea alba at the anterior of the abdomen. Two electrodes originate from the top of the transmitter, one of which was fixed to the dorsal surface of the xiphoid and the other guided under the sternohyoideus muscle and then along the trachea into the anterior mediastinum. Postoperatively, the animals received the long-acting opiate analgesic Buprenorfine hydrochloride (Temgesic[a], Reckitt and Colman, Kingston-upon-Hull, UK; 0.1 mL sc). After surgery, animals were housed individually in Plexiglas cages (25 × 25 × 35 cm) and allowed to recover for 14 d. The telemetry system consisted of small wireless transmitters model TA11CTA-F40 and receivers model RLA1020 (Data Sciences, St. Paul, MN, USA). As described previously (20) for every rat, digital data and electrocardiograms were transmitted every 10 min for 3 successive days, collected by the receiver, and led to a DataQuest 4 system (Data Sciences), after which the mean heart rate, blood pressure and somatomotor activity of those 3 d was computed.

Statistical analysis. Data are presented as mean ± SEM. Survival data were analyzed using a Kaplan-Meier test. Telemetric data were analysed by Analysis of Variance for repeated measurements, with one between-subjects factor (treatment) and one repeated within-subjects factor (time). All other group differences were analyzed by t test. $P < 0.05$ considered statistically significant.

RESULTS

Neonatal dex treatment reduces lifespan. Fig. 1 shows the survival curve of male rats neonatally treated with Dex ($n = 17$) or Sal ($n = 18$). Dex-treated rats died at considerably younger age than Sal-treated rats ($p < 0.0001$). While Sal-treated rats on average reached an age of 28.6 ± 1.1 mo, Dex-treated rats reached an age of 21.3 ± 0.8 mo. Thus, neonatal treatment with Dex shortened the average lifespan by approximately 25% ($p < 0.0001$). The last Dex-treated male rat died at 27 mo of age. At that age, 50% of the Sal (control) male rats were still alive. The last Sal-treated rat died at 36 mo.

Figure 2 shows the survival of female rats neonatally treated with Dex ($n = 13$) or Sal ($n = 12$). As was the case with Dex-treated males, female Dex-treated rats too died at considerably younger age than their Sal-treated controls ($p < 0.005$). Dex-treated female rats reached an average age of 22.0 ± 0.7 mo, whereas Sal-treated female rats reached an average age of 26.9 ± 1.8 mo. Thus, neonatal treatment with Dex shortened the average lifespan of female rats with approximately 18% ($p < 0.005$). The last Dex- and Sal-treated female rats died at 25 mo and 35 mo of age respectively.

Dex leads to hypertrophic cardiomyopathy and early severe nephropathy. The lifespan experiment showed that the first Dex-treated male rat died at 15 mo of age. To reveal if the observed effect on mortality was associated with changes in vital organs, groups of male Dex- and Sal-treated rats were killed at 15 mo of age for histopathological examination (Dex $n = 5$; Sal $n = 6$). The heart and kidneys of Dex-treated rats showed gross abnormalities compared with those of Sal-treated controls (Figs. 3 and 4). The heart of Dex-treated rats was hypertrophic, with thickening of the left ventricular wall. Compared with Sal-treated rats (Fig. 3*A, C*) cardiomyocytes were elongated and increased in diameter (Fig. 3*B, D*) with pronounced interstitial fibrosis, comparable with histopathological hallmarks of end-stage cardiac failure. The kidneys of Dex-treated rats showed signs of chronic progressive glomerulonephritis with extensive scarring and glomerulosclerosis, and dilatation, atrophy and some regeneration of the tubular system (Fig. 4*B*). A high proportion of the tubuli contained large quantities of protein. Interstitial fibrosis with irregular accumulation of lymphocytes and macrophages indicated chronic inflammation. These findings are compatible with

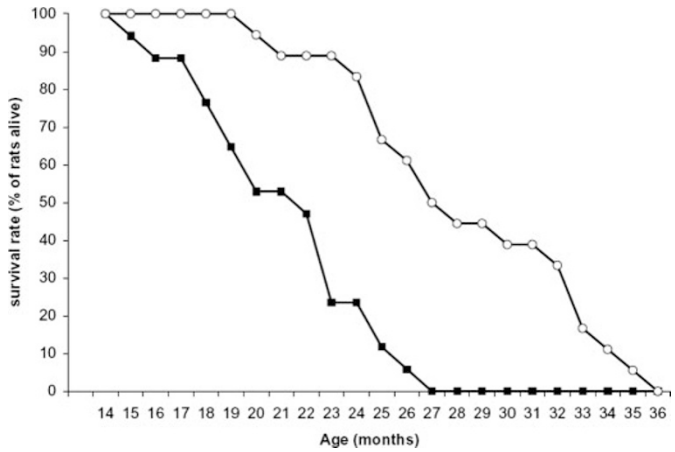

Figure 1. Survival rate of male rats neonatally treated with dexamethasone (■ $n = 17$) or saline (○ $n = 18$). Dex rats show reduced lifespan compared with Sal rats ($p < 0.0001$; Kaplan-Meier Survival analysis).

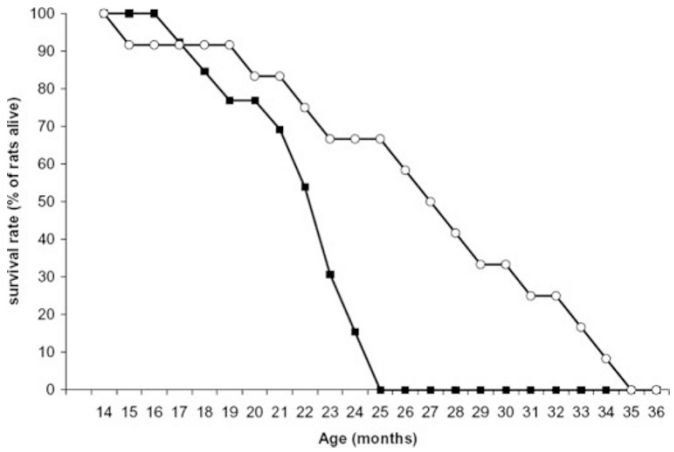

Figure 2. Survival rate of female rats neonatally treated with dexamethasone (■ $n = 13$) or saline (○ $n = 12$). Dex rats show reduced lifespan compared with Sal rats ($p < 0.005$; Kaplan-Meier Survival analysis).

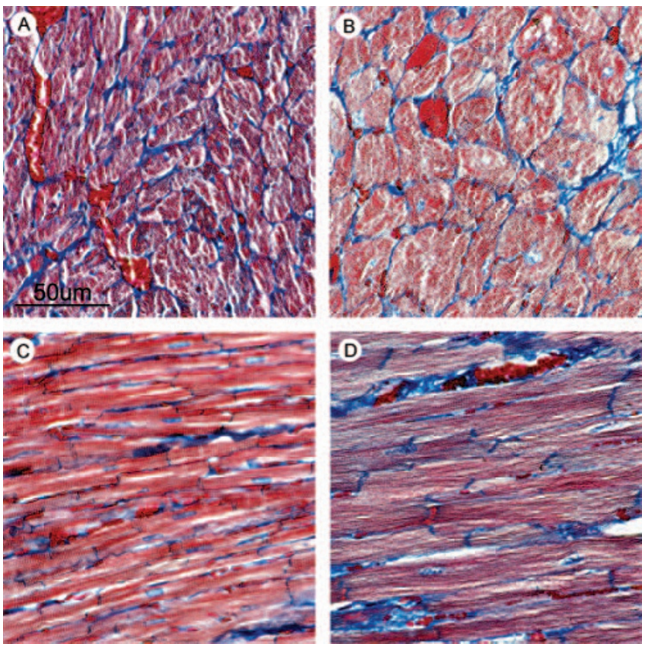

Figure 3. Stained sections (×200) of the heart of 15-mo-old rats neonatally treated with saline (Sal; *A, C*) or dexamethasone (Dex; *B, D*). Paraffin-embedded cross (*A, B*) and longitudinal sections of hearts (*C, D*) were stained with modified Azan. Blue staining indicates collagen.

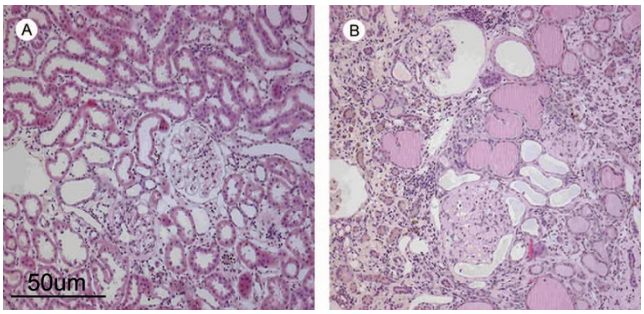

Figure 4. Stained sections (×200) of the kidney of 15-mo-old rats neonatally treated with saline (Sal; *A*) or dexamethasone (Dex; *B*). Paraffin-embedded sections of kidneys were stained with hematoxylin-eosin.

early and very severe old rat nephropathy. No histopathological abnormalities were observed in the other organs examined (lungs, liver, brain, adrenals and spleen). No data are presented here of female rats.

As shown in Table 1, the 15-mo-old Dex-treated male rats had a lower body weight than their Sal-treated male controls. When corrected for body weight, the weight of the heart, kidney and lungs was increased ($p < 0.05$) in Dex compared with Sal-treated rats, which is often observed in end-stage cardiomyopathy (21,22) and nephropathy (23,24). Together, these data point to heart and end-stage kidney failure as being the most likely cause of the premature death of Dex-treated rats.

Neonatal dex treatment leads to hypertension. Since hypertension is one of the most frequent sequelae of kidney failure and may cause hypertrophic cardiomyopathy with heart failure, blood pressure and heart rate were continuously telemetrically monitored over 3 complete diurnal cycles in freely moving, young adult male Dex- and Sal-treated rats of

Table 1. *Effect of neonatal dexamethasone (Dex; n = 5) or saline (Sal; n = 6) treatment on body- and organ weight at 15 months of age*

	Weight in grams		Weight as % body weight	
	Sal (n = 6)	Dex (n = 5)	Sal %	Dex %
Body	633 ± 70	489 ± 51*	100	100
Heart	1.9 ± 0.2	2.4 ± 0.9	0.30 ± 0.01	0.50 ± 0.08*
Brain	2.8 ± 0.1	2.4 ± 0.1*	0.45 ± 0.02	0.50 ± 0.02
Kidney§	4.1 ± 0.3	4.1 ± 0.3	0.65 ± 0.04	0.84 ± 0.08*
Liver	26.6 ± 1.7	18.4 ± 1.1*	4.21 ± 0.23	3.77 ± 0.16
Lung§	1.9 ± 0.1	2.8 ± 0.4	0.31 ± 0.02	0.58 ± 0.09*

§ Total weight of both the left and right organ/muscle (* $p < 0.05$).

3 mo of age (Dex $n = 16$; Sal $n = 14$). The systolic blood pressure of Dex-treated rats appeared to be consistently increased compared with that of Sal rats throughout the dark phase of the diurnal cycle when rats are active (Dex: 127.1 ± 2.1; Sal: 120.0 ± 2.2 mm Hg; $p < 0.05$), as well as during the light phase when rats are inactive (Dex: 123.9 ± 2.2; Sal: 116.5 ± 2.3 mm Hg; $p < 0.05$)(Fig. 5*B*). No significant differences in heart rate, diastolic blood pressure or mean arterial blood pressure were observed (Fig. 5*A, C, D*).

To reveal if this early symptom of hypertension in adult rats induced by neonatal Dex treatment amplifies with age, hemodynamics were determined in 11-mo-old male Dex- and Sal-treated rats (Dex $n = 4$; Sal $n = 5$) (see Fig. 6). In Dex-treated rats, systolic blood pressure ($p < 0.01$), diastolic blood pressure ($p < 0.01$) and mean arterial blood pressure ($p < 0.01$) appeared to be consistently increased compared with those of Sal-treated rats. The increase in heart rate was not significant, but only reached a tendency ($p = 0.08$).

Thus, the hemodynamic changes in 11-mo-old Dex-treated male rats were considerably more pronounced than those found in 3-mo-old Dex-treated rats. The systolic blood pressure during the dark phase of the diurnal cycle was 144.8 ± 1.2 mm Hg in Dex-treated rats and 130.1 ± 1.1 mm Hg in Sal-treated rats ($p < 0.05$). During the light phase, when rats are inactive, systolic blood pressure amounted to 141.2 ± 1.2 mm Hg in Dex-treated rats and 128.2 ± 1.0 mm Hg in Sal-treated rats ($p < 0.05$)(Fig. 6*B*).

DISCUSSION

The present study shows that neonatal treatment with clinically relevant dosages of Dex substantially reduces lifespan of rats. This appeared to be true for male as well as for female rats. The reduced lifespan likely is the functional implication of the hypertension and the histopathological findings, as reported in the present study, which showed gross abnormalities fitting with end-stage nephropathy and a progressive hypertrophic cardiomyopathy as reported earlier by our group (18).

Early, short-term effects of Dex on the neonatal cardiovascular system such as myocardial hypertrophy and hypertension have been previously reported in experimental animal and human studies. These effects were mostly considered to be transient (25–27). Although long-term effects of antenatal glucocorticoid exposure on the cardiovascular system have been observed in animals and humans (28,29), less is known

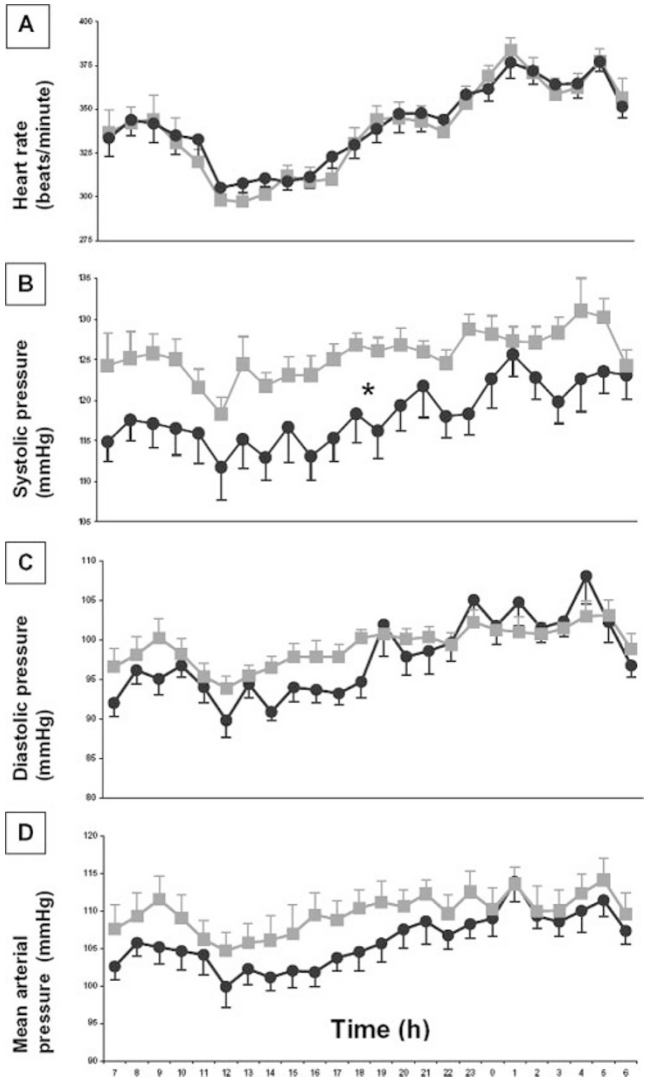

Figure 5. Effect of neonatal dexamethasone (▒ $n = 16$) or saline (● $n = 14$) treatment on heart rate (*A*), systolic blood pressure (*B*), diastolic blood pressure (*C*) and mean arterial pressure (*D*) assessed over the complete diurnal cycle in freely moving 3-mo-old rats (* $p < 0.05$). The light phase was from 700–1900 h. The horizontal axis values are the same for all four parameters.

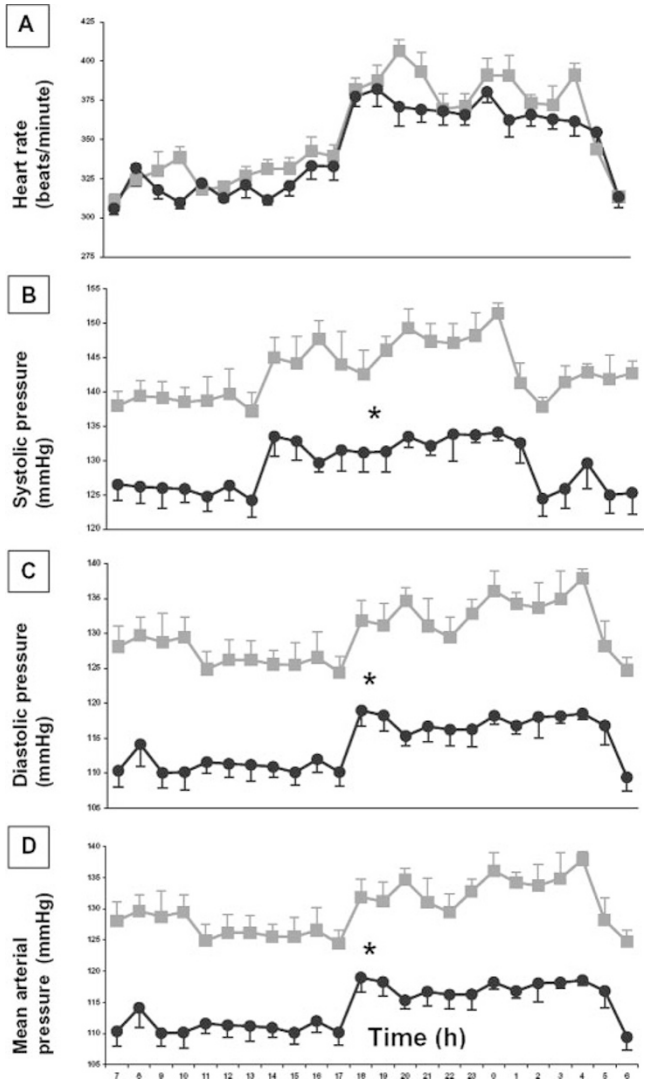

Figure 6. Effect of neonatal dexamethasone (▒ $n = 4$) or saline (● $n = 5$) treatment on heart rate (*A*), systolic blood pressure (*B*), diastolic blood pressure (*C*) and mean arterial pressure (**D**) assessed over the complete diurnal cycle in freely moving 11-mo-old rats (* $p < 0.05$). The light phase was from 700–1900 h. The horizontal axis values are the same for all four parameters.

about the long-term effects of neonatal Dex treatment on the heart and kidneys. Rudolph *et al.* reported earlier that perinatal Dex treatment inhibits or even halts physiologic myocardial hyperplasia, leaving hypertrophy as the only possibility for the still undeveloped heart to grow (30). We indeed found myocardial hypertrophy and myocardial fibrosis in the neonatally Dex-treated adult rat at 11 mo of age together with a decreased number of cardiomyocytes (18), which originated from a direct effect of neonatal Dex administration (17). In a recent study of our group we studied the hearts of rats at 4, 8 and 50 wk of age after neonatal Dex treatment. We found a significant increase in cardiomyocyte length of the Dex-treated rats compared with controls in all three age groups. This was accompanied by a significant increase in cardiomyocyte width in the 50-wk-old rats. This all resulted in significantly increased cardiomyocyte volume at 8 and 50 wk indicating cellular hypertrophy (article submitted).These above mentioned changes in neonatally Dex-treated rats may indicate early myocardial degeneration, leading to untimely ageing of the myocardium and ultimately to premature death. This process may be further complicated and deteriorated by the hypertension that started to develop already at an age of 3 mo. This corroborates observations of Le Cras *et al.*, who found an increased risk of developing pulmonary hypertension in rats treated with Dex in neonatal life (19). To our knowledge, renal abnormalities, such as signs of chronic glomerulonephritis and atrophy of the tubular system have not been reported earlier in adult rats, neonatally treated with Dex. These abnormalities may be the result of ongoing and increasing hypertension. On the other hand, however, they may have resulted from a direct effect of Dex on the developing kidney and contributed to the hypertension. Indeed, several investigators have reported that antenatal glucocorticoid treatment causes permanent changes in the kidney morphology and renal function with hypertension in the offspring (31–33).

The present study was not designed to unravel the exact mechanism(s) underlying the premature death, but merely to

test the hypothesis that neonatally Dex-induced alterations in vital organ systems such as the heart and kidneys are associated with the premature death of these treated rats. The results clearly confirm our hypothesis for the male rat. In previous studies, we found no indications for the existence of gross differences between male and female rats regarding the effects of neonatal Dex treatment, for example on the HPA-axis (13). It is therefore likely, that the cause of premature death in females is the same as in males.

Evidently, it is hazardous to directly extrapolate the present findings in the rat to the human situation. There are, however, human follow-up studies covering follow-up data up to 12 y of age, and these show some similarities with our present and previous results in the rat both with respect to short-term and long-term adverse effects due to GC treatment. In particular, these similarities concern transient effects such as myocardial hypertrophy (25,27,34) and cessation of body and skull growth during neonatal Dex treatment, and long-term adverse effects on neurologic and motor development (16,35,36), cognitive performance and social behavior (14,15,37). On the other hand, the developing myocardium in the neonatal rat differs from that of the human fetus and premature neonate. In rat neonates there is still a considerable cardiomyocyte proliferation (38) while this is less obvious in the human neonatal heart, at least in term neonates as investigated by Huttenbach *et al.* and by Mayhew *et al.* (39,40). The same investigators, however, reported still cardiomyocyte proliferative activity in myocardial tissue during the preterm period in humans. Thus, although the development of the heart of neonatal rats may not be fully comparable to that of human infants, the fact remains that cardiomyocyte proliferation may exist in the preterm infant. Therefore the present results should be taken seriously. Moreover, this study in the rat will hopefully urge researchers to investigate possible long-term adverse effects on the heart (and kidneys) of prematurely born babies treated with GCs in this vulnerable period of life.

In conclusion, the present study showed that neonatal Dex treatment in the rat leads to long-term cardiovascular and renal morbidity and to a reduced lifespan, presumably because of the longstanding cardiomyocyte hypertrophy, hypertension and end-stage nephropathy.

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