Effect of neonatal dexamethasone exposure on growth and neurological development in the adult rat

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Neal, Charles R., Jr., Gabrielle Weidemann, Mohamed Kabbaj, and Delia M. Vázquez. Effect of neonatal dexamethasone exposure on growth and neurological development in the adult rat. Am J Physiol Regul Integr Comp Physiol 287: R375–R385, 2004. First published April 29, 2004; 10.1152/ajpregu.00012.2004.—Until recently, the synthetic glucocorticoid dexamethasone was commonly used to lessen the morbidity of chronic lung disease in premature infants. This practice diminished as dexamethasone use was linked to an increased incidence of cerebral palsy and short-term neurodevelopmental delay. Of more concern is the fact that we know little regarding dexamethasone effects on long-term neurodevelopment. To study the effects of neonatal dexamethasone exposure on long-term neurodevelopment, we have developed a rat model where newborn pups are exposed to tapering doses of dexamethasone at time points corresponding to the neurodevelopmental age when human infants are traditionally exposed to this drug in the neonatal intensive care unit. Using a within-litter design, pups were assigned to one of three groups on postnatal day 2 (P2): handled controls, saline-injected controls, and animals receiving intramuscular dexamethasone between P3 and P6. Somatic growth was decreased in dexamethasone-treated animals. Dexamethasone-treated animals demonstrated slight delays in indexes of neurodevelopment and physical maturation at P7 and P14, but not P20. In adolescence (P45), there was no difference between groups in an open field test. However, as adult dexamethasone-treated animals were less active in the open field and spent more time in closed arms of the elevated plus maze. The serum corticosterone response to crowding stress in dexamethasone-treated animals was no different from controls, but they demonstrate a delay in return of corticosterone levels to baseline. These differences in behavior and hormonal stress responsiveness suggest that neonatal dexamethasone exposure may permanently alter function of the neuroendocrine stress axis.

SEVERE RESPIRATORY DISTRESS syndrome is commonly experienced in extremely low birth weight (ELBW) infants, resulting in various degrees of ventilator and/or oxygen dependency and subsequent onset of chronic lung disease (CLD). Until recently, administration of a prolonged course of postnatal dexamethasone to ELBW infants was frequently practiced in an attempt to lessen the progression of CLD. Although clinical trials using dexamethasone in this fashion failed to consistently demonstrate improvement in mortality or length of hospitalization, exposures of up to 42 days were commonly used in many neonatal units (4, 8, 17, 55, 56). A recent study examining the outcome of ELBW infants (birth weight between 500 and 750 g) found that 43% of those infants born from 1990 to 1992 received dexamethasone compared with 84% of ELBW infants born between 1993 and 1995 (40). Routine use of dexamethasone continued until Yeh and colleagues (102) published the results of a followup study that demonstrated a significant increase in neurodevelopmental dysfunction in those neonates treated with dexamethasone to limit progression of CLD.

In clinical practice, this window of dexamethasone exposure in critically ill ELBW infants spans an extensive period of perinatal viability, ranging from 24 to 40 wk postconception. During this time, the human brain is undergoing significant structural and functional transformations, making it particularly vulnerable to external influences.

Acute side effects of prolonged dexamethasone use in ELBW infants are well documented and include systemic hypertension, bowel perforation, infection, ventricular hypertrophy, metabolic derangements, and alteration of limbic-hypothalamic-pituitary-renal (LHPA) axis function (2, 9, 14, 31, 62, 76, 77). Although dexamethasone readily crosses the blood-brain barrier and binds glucocorticoid receptors (19, 66, 92), little animal data exist regarding possible glucocorticoid effects on long-term axis or central nervous system (CNS) in general.

Clinical studies examining acute dexamethasone effects on physiology and CNS function in premature infants have been limited. It has been shown that premature infants receiving prolonged dexamethasone therapy experience reduced linear growth, decreased weight gain, and smaller head circumferences (10, 38, 90). During the acute phase of dexamethasone exposure, changes in gross neuromotor function have also been noted (11, 102). Not surprisingly, clinical studies are increasingly linking dexamethasone therapy to long-term neurological effects, including cerebral palsy and decreased cerebral volumes (27, 46–48, 70, 80, 89). As a result, use of dexamethasone to improve pulmonary function in ventilator-dependent ELBW infants is undergoing significant modification toward more judicious treatment: dexamethasone therapy is given less often, and shorter courses are now used.

In addition to evidence supporting early dexamethasone effects on LHPA function and early infant neurodevelopment, there is an emerging literature of human followup data investigating dexamethasone effects on subtle behavioral outcomes...
related to LHPA function and mental health in general, in older survivors of prematurity (6, 21, 27, 28, 39, 41, 50, 53, 54, 57, 61, 63, 70, 79, 80, 84, 85, 89, 100, 102, 103). A recent series of Cochrane reviews has concluded that risks of adverse neurodevelopmental outcome in premature infants receiving postnatal dexamethasone outweigh the benefits of their use, and it has been recommended that only cautious use of this drug should be practiced in select cases of CLD (46–48).

Although dexamethasone use in premature infants has been curtailed significantly, few alternative treatment options exist for severe CLD in the extremely premature infant. Betamethasone, given exclusively to mother in preterm labor to limit severity of respiratory distress syndrome in the premature neonate, has not been studied or used in postnatal trials. Although use of hydrocortisone to treat CLD has recently been reported, its clinical benefit is questionable (94). Given these limitations, dexamethasone will continue to be used, albeit sparingly, in this vulnerable population, and uncovering the mechanisms of its deleterious CNS effects remains important.

In an attempt to better understand consequences of early glucocorticoid exposure, animal models have been developed to investigate long-term neurodevelopmental effects of perinatal steroids (12, 29, 30). Most recently, studies in this laboratory have reported neonatal dexamethasone effects on adolescent rat behavior, and adult LHPA neurochemistry, using one such model (32, 74). Behavioral and physiological effects of neonatal dexamethasone in any developmental model are interpreted on the assumption that, although timing differs significantly between species, the general sequence of brain growth is similar (25, 26, 32). Therefore, while much caution is necessary when extrapolating from animal models to the human condition, one can still take advantage of similarities in sequence and timing of brain development between species (25, 26). For example, in humans, neuronal proliferation is completed before 24 wk postconception, after which glia continue to proliferate and oligodendroglia maintain myelination. The peak in brain growth in the human occurs near term (38–40 wk gestation). In contrast to humans, rodents experience their peak brain growth postnatally. In terms of brain growth velocity, germinal matrix composition, neurochemical expression, electroencephalographic patterns, and synapse formation, the postnatal day 9 (P9) rat brain is estimated to be roughly equivalent in neurodevelopment to that of a full-term human infant (25, 26, 42). Extrapolating on this model of cross-species neurodevelopmental approximation, the brain of a rat pup at birth (P1) corresponds to that of a human fetal brain at or near 20–22 wk postconception (25, 69, 100). Using brain growth velocity estimates of Dobbing (25, 26) and Morgane et al. (69) to extrapolate further, the P2 rat brain approximates that of a 24- to 26-wk human in terms of gross neurodevelopment, the P3 brain corresponds to that of a 26- to 27-wk human brain, and the P6 rat brain to that of a 30–32 wk human.

Using these approximate relationships, we have developed a neonatal rat model to investigate long-term effects of neonatal dexamethasone exposure on the developing CNS. This model was recently used to demonstrate an association between dexamethasone exposure in the neonatal rat pup and changes in LHPA function in the adolescent, including increased anxiousness in the light-dark test of anxiety and, in response to a mild novelty stress, a blunted corticosterone response (32). Although behavioral and physiological alterations were observed well after glucocorticoid exposure (dexamethasone exposure between P3 and P6 with testing on P3), it remains unclear whether these alterations are permanent. The specific aim of the present study was to extend this model into adulthood and test whether effects of neonatal dexamethasone exposure on LHPA axis function persist to adulthood. We have recently demonstrated that dexamethasone exposure in the neonatal rat leads to alteration in orphanin receptor expression in the adult hippocampus and periventricular hypothalamic nucleus (74). This receptor system likely plays a key role in LHPA circuitry (22–24, 67, 71, 72, 82), raising concerns that stress-related behaviors may be permanently effected by early dexamethasone treatment.

We hypothesize that a 4-day tapering course of dexamethasone treatment in the neonatal rat pup, which correlates to several weeks of brain development, leads to permanent changes in the adult animal in terms of stress-related behaviors and their physiological response to a stressful stimulus. The present study is based on a previously published neonatal rat model (32, 74). It is not designed to evaluate the effects of a 2-day, four-dose dexamethasone protocol used in many neonatal units for treatment of refractory hypotension but rather to provide a tapering dose of dexamethasone during a postnatal age in the rat that corresponds to the neurodevelopmental time point at which human premature infants have historically received prolonged glucocorticoid therapy for CLD (46–48). This is in contrast to animal models that use short courses or single doses of dexamethasone (5, 7, 20, 28, 53, 54).

METHODS

Animals

Litter management, animal handling, and drug treatment in this animal model of neonatal dexamethasone (Dex) exposure have been previously reported (32).

Adult Sprague-Dawley rats (Charles Rivers, Wilmington, MA) were housed in our animal unit and maintained in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. All animals were kept under constant temperature (25 ± 2°C) and photoperiodicity (14:10-h light-dark cycle) and provided with food and water ad libitum. Female rats were mated using a trio mating system (2 females:1 male). Assuming a 21-day gestation, females were housed in pairs until estimated gestational day 18, at which point they were housed individually. They were then checked twice daily until pups were born, the day of birth designated P1.

Twenty pregnant females were initially used for this study. A within-litter design was used in the present analysis as an approach to studying neonatal glucocorticoid exposure (32). This design provides the advantage of genetic contribution from a given family being represented in all treatment groups. In addition the early life environment contributed by maternal behavior is also uniform. This is important because of the major role maternal responses play in litter manipulations (for review see Refs. 16, 34, 64). To control for the effect of maternal behavior toward an entire litter of experimentally manipulated pups, we have chosen to represent all three experimental groups within each litter. On P2, each litter was sexed and culled to 12 pups (6 males:6 females) to ensure equality in nutrition and maternal care within litters. Pups were separated into three treatment groups on P3, with each group represented within a litter to control for variations in maternal behavior. On P8, a male and a female pup from each treatment group were killed, culling the litter to six animals (3 males:3 females). On P21, animals were weaned from their mother, and males and females were separated on P30. On P45, all male animals used in

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this study were separated and singly housed into adulthood. This was done following the “Guidelines for the Care and Use of Laboratory Animals” at the University of Michigan, which bases the number of animals in a cage on the size of the animals and expected growth without food restriction. Females were group-housed until adulthood. Behavioral testing was initiated in males on P60. Females did not undergo behavior testing. All animals were killed on P120.

Although the goal of this investigation was to study Dex effects on male LHPA activity, to minimize confounding maternal-pup and pup-pup interaction effects it was imperative to maintain sex balance in the litter until animals were weaned and separated. Accepted practice generally includes culling of litters to four to six per sex (1, 81). It is clear that litter imbalance leading to sexual asymmetry will affect outcomes more consistently than culling (1, 59, 60, 81). Mothers interact differently with male and female offspring in rats, including increased anogenital licking of male pups (68). Given the important role of maternal licking on adult behavior (87), allowing sexual imbalance in a litter could markedly confound observations of LHPA activity in the adult. For these reasons, all litters remained balanced by sex until separated on P30. Females were maintained until P120 to collect somatic growth and brain weight data.

Drug Treatment

Three treatment groups were assigned for each litter: handled controls (Han), saline-injected animals [vehicle (Veh)], and Dex-treated animals (Dex). All pups within each litter were removed from their home cage and separated from their mother for injection and handling, and somatic measurement, between the hours of 1100 and 1300 for a period of 5 min. Pups in the Dex group received a daily intramuscular injection of Dex (American Pharmaceutical Partners, Los Angeles, CA) between P3 and P6. Dex was given in tapering doses of 0.5 mg/kg on P3, 0.25 mg/kg on P4, 0.125 mg/kg on P5, and 0.05 mg/kg on P6. Animals in the Veh group received equivalent volumes of intramuscular sterile saline as the pups in the Dex group. Animals in the Han group were removed from the home cage, handled, and measured during the same time period on P5 through P6.

Animal Measurements

Weights were recorded during handling and before injections on P3-P8, P14, P20, P45, P60, and P120 for each treatment group. Length was measured from the nose to the base of the tail (rump length) before handling or injection on P3, P7, P14, P20, and P60 for each treatment group. Procedures for testing neurodevelopmental responses and physical maturation were derived and adapted from those reported by Altman and McCrady (3), Fox (33), and Wahlsiten (97). Results using this model of neurodevelopmental and maturation assessment on rat pups have been previously reported by this study (32, 98). Twenty days after adult open field testing was completed, animals underwent testing in the elevated plus maze. This maze consists of four arms elevated 2 ft above soft landing material. Two opposing arms are surrounded by Plexiglas walls (closed arms) while the other arms are devoid of walls (open arms). External clues were minimized and lighting was indirect and minimal. Rats were placed in the center of the apparatus, facing an open arm, to begin the test period. Number of entries into closed and open arms was scored as well as time spent in the open arms, closed arms, and the center. Entry is defined as having four paws in an arm of the plus maze. Animals were tested once in this paradigm (83). Total testing time was 5 min.

Twenty days after elevated plus maze testing was completed, animals were placed in the light-dark box. The testing apparatus consisted of a covered 30 × 60 × 30 cm Plexiglas shuttle-box with a stainless steel grid floor suspended above corncob bedding. Boxes were divided into two equal-sized compartments with a 12-cm-wide opening. The light compartment was constructed of white Plexiglas and was brightly illuminated; the dark compartment was constructed of black Plexiglas and was minimally lit. Each animal was placed in the dark compartment and scored for number of transitions, latency to enter the light side, reentry to the light side, and activity in the light and dark. Locomotor activity as well as time spent in each compartment were scored via photocells located on the wall of each box, with the number of photocell beams interrupted per unit time recorded with a microprocessor. Total testing time was 5 min (32).

Adrenocortical Response to Crowding Stress

Twenty days after light-dark testing was completed, animals were subjected to 30 min of crowding, a reliable means to elicit a moderate stress response (36, 37, 51). Representative adult males (Dex, Veh, Han) from two random litters were placed into a small cage for 30 min (6 animals total). Blood sampling was performed via tail nick at 30, 60, 90, and 120 min after exposure to crowding. A basal time point was obtained 24 h before the procedure. Blood samples after the 120-min time point were not collected for analysis.

For both corticosterone and ACTH measurements, blood was collected in prechilled tubes containing EDTA and spun at 2,000 rpm for 7 min. Serum was promptly removed and stored at −20°C. Using 200-μl serum aliquots, plasma ACTH concentrations were quantified using the Allegro HS ACTH IRMA from Nichols Institute (San Juan Capistrano, CA). This assay uses unextracted plasma with EDTA used as the anticoagulant. With this kit, the intra-assay coefficient of variation is 3.5%, and the inter-assay coefficient of variation is 7%. Corticosterone was measured using a radioimmunoassay as previously described (32, 96). Stored plasma samples were thawed to room temperature and diluted (1:100) in 50 mM sodium phosphate buffer containing 2.5% BSA, pH 7.5. Samples were heated to 80°C to separate corticosterone from corticosterone binding protein. The corticosterone antibody used (a gift from Huda Akil, University of Michigan) cross-reacts 2.2% with cortisol and <1% with other endogenous steroids. Trinitiated corticosterone (Amersham, Arlington Heights, IL) was used as a radiolabeled trace. Bound [3H]corticosterone was separated from free ligand using a suspension of 2% charcoal and 0.2% dextran. The detection limit of the assay is 1 pg/ml with intra-assay coefficient of variation at 2% and inter-assay coefficient of variation 3%.
Brain Weights

When litters were culled on P8, male and female wet brain weights were obtained. All remaining animals (male and female) were killed 1 wk after crowding studies were completed in the male animals. Wet brain weights were also obtained for males and females during the P120 necropsy. After weighing, all brains were quick-frozen in liquid isopentane at −40°C and stored at −80°C for later processing and analysis.

Statistical Analysis

The goal for this study was to produce a minimum of six animals per sex per treatment group in the adult population. A total of 20 females was bred with 16 females delivering acceptable litters (size and sex) for culling. From these 16 litters, 10 were culled to exactly six male and six female pups. From P1 through P8, there were 120 total pups, 60 male and 60 female, with 20 males and 20 females per experimental group. After litter reduction on P8, 30 male and 30 female pups remained, with 10 males and 10 females per group. Before weaning, one Han male died, and the remainder of that litter was excluded, leaving nine males and nine females for the P20 analysis. After weaning, one Dex male and another Han male died unexpectedly. To maintain continuity, these two litters were also excluded, leaving seven males and seven females per treatment group for the remainder of this study.

Brain weight, hormonal values, and behavioral data were averaged across treatment groups and ages. Results were subject to ANOVA considering age and treatment simultaneously. Body weight and length were subject to repeated-measures ANOVA using the General Linear Models Procedure considering age and treatment simultaneously. Total and individual maturation and neurodevelopment scores were averaged across treatment groups and ages, and these results were subject to analysis using the Kruskal-Wallis test of nonparametric values. For all tests and measures performed before P30, when sex differences were determined to be nonsignificant, the data were combined across the respective variable. The level of significance $P$ value was set at $P \leq 0.05$. Post hoc comparisons were done using the Tukey Kramer test.

RESULTS

Growth Measurements

Female and male weights were similar in each group through P20, but females weighed significantly less than males in each treatment group on P45, P60, and P120 ($P < 0.0001$). Repeated-measures ANOVA revealed a group ($P < 0.0002; F = 13.13$) and time ($P < 0.0001; F = 1662.53$) effect across all time points studied in males. In females there was a time effect ($P < 0.0001; F = 1201.82$) but no group effect ($P = 0.64; F = 3.105$). Post hoc analysis revealed that P3 females had similar rump lengths, but Dex-treated females had smaller rump lengths compared with Veh and Han controls on P7, P14, and P20 ($P < 0.05$). By P60, there was no length difference between groups in females. Similar to females, males had similar rump lengths on P3, whereas Dex-treated males had significantly smaller rump lengths compared with controls on P7, P14, and P20 ($P < 0.05$). There were no differences in length between groups in adult male rats.

Brain Weights

On P8 there was no difference in brain weights between male and female pups. However, on P120 gross brain weights were significantly greater in males compared with females ($P < 0.0001$). Brain weight in Dex-treated male and female rats was decreased on P8 and P120 compared with both vehicle and handled controls (P8 males $P > 0.03$; P8 females $P < 0.01$; P120 males $P < 0.003$; P120 females $P < 0.01$). However, when corrected for body weight in P120 animals, difference in brain weight only reached significance between Dex and Han females ($P < 0.04$; Fig. 2).

Neurodevelopment and Physical Maturation

Comprehensive test scores for each treatment group were assigned by adding individual components of the total neurodevelopmental examination. In the present study, no sex differences in the neurological exam were noted within treatment groups on P7 ($P = 0.122$), P14 ($P = 0.782$), or P20 ($P = 0.868$), so male and female data were combined for analysis. Animals exposed to Dex in the neonatal period exhibited lower total neurodevelopmental scores compared with Veh and Han groups only on P20 (mean total neurodevelopmental score ± SE: Dex 86.0 ± 0.5; Veh 88.0 ± 0.3; Han 88.0 ± 0.2; $P < 0.004$). There was no significant difference in total maturation and neurodevelopmental score on P7 (mean total score ± SE: Dex 37.0 ± 0.6; Veh 38.0 ± 0.6; Han 38.0 ± 0.5) or P14 (mean total score ± SE: Dex 68.0 ± 0.6; Veh 69.0 ± 0.6; Han 69.0 ± 0.5).

Differences in total maturation and neurodevelopmental indexes between groups on P20 were largely due to the minimal variability on neurological exam components within groups. As neurological measures became more mature, small differences within groups reflected more significantly on total neurological score. For example, on P7, there were no differences between groups in total score, but differences were observed on specific indexes in P7 Dex-treated animals (Fig. 3A). These differences included mild delays in neurodevelopment (immature posture, $P < 0.003$; hindlimb grasp, $P < 0.03$; postural righting reflex, $P < 0.05$) and acceleration in physical maturation (tooth eruption, $P < 0.03$). Similarly, no difference in total score was observed between groups on P14, but Dex-
treated animals still exhibited differences on individual indexes of physical maturation and neurodevelopment (Fig. 3B), including ear opening, ear unfolding, eye opening and fur development ($P < 0.0001$), forelimb placing ($P < 0.03$), and hindlimb grasp ($P < 0.01$). In contrast to mild delays typically observed in maturational indexes of Dex-exposed animals, a marked acceleration in eye opening was observed in the Dex animals at this postnatal age ($P < 0.0001$). By P20 (Fig. 3C), delays in Dex animals included ear opening ($P < 0.04$), tooth eruption ($P < 0.03$), fur development ($P < 0.0001$), and postural extension ($P < 0.02$).

**Behavioral Testing**

**Open field.** In the P45 open field session (Fig. 4), there were no significant differences observed between groups in number of squares crossed, time spent rearing, or number of defecation and urination events. Animals in the Dex group did spend less time grooming than did Veh controls ($P < 0.03$), but not less than the Han controls ($P = 0.37$). After 15 days of single housing, all P60 animals demonstrated decreased locomotor activity on repeated measure compared with P45. However, this decrease in activity only reached significance in the Dex group (mean number of squared crossed $\pm$ SE: P45 Dex 31.0 $\pm$ 6.0; P60 Dex 6.0 $\pm$ 2.1, $P < 0.04$; P45 Veh 29.0 $\pm$ 4.9; P60 Veh 17.0 $\pm$ 5.8, $P = 0.31$; P45 Han 39.0 $\pm$ 6.1; P60 Han 24.0 $\pm$ 6.1, $P = 0.19$). On P60 there was again no difference in time spent grooming and rearing or in amount of defecation and urination between groups. However, Dex-treated adult males crossed significantly fewer squares than Han controls ($P < 0.04$) and trended toward fewer squares crossed compared with the Veh groups ($P = 0.18$).

**Elevated plus maze.** In this study (Fig. 5), there was no difference observed between groups in the amount of time spent investigating the open arm of the maze (mean time in open arm $\pm$ SE: Dex 31.0 $\pm$ 8.4 s, Veh 37.0 $\pm$ 5.4 s, Han 45.0 $\pm$ 13.4 s). However, animals in the Dex group spent significantly more time in the closed arm of the maze compared with Han ($P < 0.003$) and Veh ($P < 0.04$) controls (mean time in closed arm $\pm$ SE: Dex 212.00 $\pm$ 22.1 s, Veh 159.0 $\pm$ 8.8 s, Han 127.0 $\pm$ 18.8 s). Subsequently, animals in the Dex group spent less time in the center portion of the maze compared with Han ($P < 0.03$) and Veh ($P < 0.05$) controls (mean time in
closed arm ± SE: Dex 57.0 ± 14.1 s, Veh 115.0 ± 17.6 s, Han 128.0 ± 21.2 s).

Light-dark box. There was no difference between groups in the elapsed time to enter the lighted side of the box (mean time to enter the light ± SE: Dex 97.0 ± 23.5 s, Veh 79.0 ± 22.9 s, Han 72.0 ± 25.9 s), total time spent in the dark side of the box (mean time in dark ± SE: Dex 231.0 ± 16.8 s, Veh 228.0 ± 9.7 s, Han 213.0 ± 12.0 s) or total time spent in the lighted side of the box (mean time in light ± SE: Dex 62.0 ± 16.6 s, Veh 67.0 ± 8.1 s, Han 80.0 ± 11.1 s). In addition, there were no differences in dark activity or light activity between treatment groups.

Adrenocortical Response to Crowding Stress

No difference in basal corticosterone or ACTH levels was noted between female groups (mean serum corticosterone concentration in μg/dl ± SEM: Dex 1.45 ± 0.26; Veh 1.84 ± 0.33; Han 1.36 ± 0.15: mean serum ACTH concentration in pg/ml ± SE: Dex 32.66 ± 8.79; Veh 32.73 ± 7.13; Han 28.10 ± 5.24). It should be noted that stage of female estrous cycle was not determined before corticosterone sampling. In males, no difference was noted in basal corticosterone levels between groups (mean serum corticosterone concentration in μg/dl ± SE: Dex 1.87 ± 0.49; Veh 2.23 ± 0.58; Han 3.24 ± 0.90), although basal ACTH levels were decreased in adult male Dex and Veh groups compared with Han controls (Dex vs. Han, P = 0.071; Veh vs. Han, P < 0.04), signifying a treatment effect on basal ACTH release (mean serum ACTH concentration in pg/ml ± SE: Dex 12.9 ± 1.75; Veh 12.43 ± 1.53; Han 17.71 ± 1.92).

In response to crowding stress, Dex-treated animals demonstrated an adequate corticosterone peak in response to crowding stress. No difference was noted between groups at 30 min (mean serum corticosterone concentration in μg/dl ± SE: Dex 12.23 ± 1.15; Veh 12.68 ± 2.03; Han 10.79 ± 1.07) or 60 min (mean serum corticosterone concentration in μg/dl ± SE: Dex 13.45 ± 1.11; Veh 12.13 ± 0.94 Han 14.24 ± 1.92) after crowding was initiated (Fig. 6). However, at 120 min, corticosterone concentration approached baseline levels in both Han and Veh controls but remained elevated in Dex-exposed animals (mean serum corticosterone concentration in μg/dl ± SE: Dex 9.88 ± 1.61; Veh 4.20 ± 0.76; Han 5.66 ± 1.51). As a result, corticosterone levels in Dex-exposed animals were significantly different from those of Han (P < 0.04) and Veh (P < 0.006) controls at the 120-min time point.

DISCUSSION

Clinical reports linking postnatal dexamethasone therapy to long-term neurological sequelae have led to a marked decrease in the use of this drug for prolonged periods in ELBW infants (27, 46–48, 70, 80, 89). Although dexamethasone is still offered to select infants with severe CLD, it is used uncommonly and with significantly shorter courses than in the past (39, 46–48, 52, 86). Despite this change in current clinical practice, generations of past survivors of prematurity that were exposed to dexamethasone postnatally are now entering adolescence and early adulthood.

Previous studies have reported neonatal glucocorticoid effects in rats using various regimens of dexamethasone administration (5, 7, 20, 28, 53, 54). In the present protocol, dexamethasone was administered over a longer period (P3 through P6) in tapering doses in an attempt to mimic the longer treatment regimen cited by many neonatal intensive care units (17). The timing of drug administration is critical in this model, corresponding to third-trimester human gestation, a period when the neonatal brain appears highly vulnerable to insults (25, 26). It is important to note that this model is not intended to mimic shorter 48-h dexamethasone bursts used for refractory hypotension, 7- to 10-day glucocorticoid courses, or prolonged postnatal treatment with other glucocorticoids, which is uncommon. Given this, our findings suggest that prolonged exposure to dexamethasone during the neonatal period may have long-lasting consequences.

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Animal Housing

All males were housed alone for the majority of the study period, and there are a number of ways to interpret our study findings. One is that transitory behavioral effects of neonatal steroid exposure are evident in the adolescent animal, but adaptations that emerge during the neonatal and adolescent period are still evolving. The permanent effects that we observed, such as slow termination in the LHPA stress response and anxious behavior in open field testing, are perhaps only evident in the adult animal. However, it is possible that our animals may have been affected by their single-housing environment after P45. This raises the possibility of dexamethasone exposure creating a vulnerable state in the animal, leading to alterations in LHPA function only after experiencing what could be construed as prolonged social isolation. However,
studies of long-term social isolation and LHPA function do not support the notion of housing animals alone acting as a chronic social stress (13, 35, 43–45, 49, 58). While it is unlikely that housing animals alone from P45 to P120 constitutes a significant social isolation stress, it is important to consider that dexamethasone alone may not be responsible for all observations in this study and, without group-housed animals for comparison, one cannot exclude the possibility that observations are a result of the interaction between dexamethasone exposure and social isolation.

Somatic and Brain Growth

A regimen of decreasing dexamethasone exposure early in life has a lasting impact on somatic growth. We presume that observed differences in somatic and brain weight are due to direct dexamethasone effects on catabolism and tissue accretion. One possible explanation for the decreased somatic growth observed in the dexamethasone-treated pups is inadequate nutritional intake during the postnatal period due to inability to attach to the mother’s nipples or poor suckling. Although we did not quantify these behaviors, it has been shown that the dam spends more time providing nutrition, stimulation, and warmth to a litter that is perceived to have poor health (15, 65, 91, 101). On the other hand, dexamethasone prevents adequate growth, rapidly inducing protein catabolism beyond the capacity for an anabolic state, resulting in reduced growth and lean body mass (10, 62, 90, 99). Our findings are consistent with this reasoning in that dexamethasone was administered during the stress hyporesponsive period, inducing a catabolic state during a time when lower circulating corticosteroid levels promote an anabolic state (18, 95).

Dexamethasone-treated animals also had decreased gross brain weights. When corrected for variations in somatic weight, brain weights did not differ between groups in males but were still lower in females. Similar findings have been reported in human clinical studies where a 30% reduction in cerebral tissue volume is observed in premature infants treated with dexamethasone compared with untreated age-matched controls (70). Interestingly, in studies of premature infants treated with dexamethasone, increased incidence of cerebral palsy has been reported (6, 46–48, 80, 89), an outcome that has been attributed primarily to white matter damage (75). Decreased gross brain weights observed in this study are not specific to behavioral or hormonal effects observed in dexamethasone-exposed animals. One cannot rule out dexamethasone effects on neurogenesis, gliogenesis, or myelination within specific vulnerable brain structures. Studies on changes in hippocampal neurogenesis in animals exposed to neonatal dexamethasone are on-going in our laboratory.

Neurodevelopment and Physical Maturation

Dexamethasone-treated animals experienced transient variations in neurodevelopment and physical maturation compared with controls. However, no gross neurological deficits were observed in our dexamethasone-treated pups by P20, suggesting that the pathways relevant to organization of reflexes and behavior assessed on our exam were developing normally by the time of weaning. Primitive reflexes appear and disappear in defined sequences during development, with an absence or persistence of any reflex beyond expected time of extinction typically indicating significant brain dysfunction (75). The fact that most of these reflexes were either completely mature by...
P20 or only slightly delayed suggests a transient effect of dexamethasone on their development. Clinical evidence and limited animal studies suggest that neurodevelopmental delay resulting from a prolonged course of dexamethasone during early neonatal life may be due primarily to an inhibition of the normal myelination processes (30, 70, 78, 88, 93). Neuroanatomic evaluations to ascertain changes in morphology or myelination were not performed in the present study, and it remains unclear at present whether permanent CNS changes have occurred that are too subtle to detect by our neurodevelopmental assessment.

Measures of Stress Reactivity

In the present study, increased anxiety-like behaviors were observed in single-housed dexamethasone animals in the elevated plus maze and open field, but we found no differences between groups in the light-dark test. Although these animals exhibit increased anxiety-like behavior in threatening environments, they demonstrate no such behavior when placed in a less threatening novel environment. Basal corticosterone levels were different between groups in adult males and females. In contrast, basal ACTH levels were decreased in adult male dexamethasone and Veh groups compared with Han controls, signifying a likely treatment effect on basal ACTH release. In response to crowding stress, animals in the dexamethasone groups demonstrated an adequate corticosterone response, but with slow termination to baseline. The animals could mount a response but were unable to turn it off appropriately, even at up to 120 min after crowding stress began.

The delayed return to baseline in serum corticosterone after crowding stress may be due to increased corticosterone secretion or decreased clearance. Preliminary findings in this laboratory demonstrate that neonatal dexamethasone exposure leads to decreased glucocorticoid receptor (GR) mRNA expression but no change in mineralocorticoid receptor (MR) mRNA expression in adult rat hippocampus (73). Unchanged MR mRNA expression is consistent with the basal corticosterone findings in dexamethasone-exposed rats in this study. In addition, decreased hippocampal GR would lead to diminished feedback inhibition of corticosterone, which is what was observed in this study. Given these observations, delayed return to baseline in serum corticosterone after crowding stress is likely due to loss of regulatory feedback inhibition of corticosterone secretion.

In conclusion, early exposure to a tapering dose of dexamethasone has long-lasting effects on neurodevelopment and neuroendocrine function in the male rat. These findings raise concerns about maladaptive behavioral strategies that may be subtle and not recognizable until later in development. Such effects may have important implications on learning, mood and ultimately quality of life in survivors of prematurity.

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