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Development of Polyamine and Biogenic Amine Systems in Brains and Hearts of Neonatal Rats Given Dexamethasone: Role of Biochemical Alterations in Cellular Maturation for Producing Deficits in Ontogeny of Neurotransmitter Levels, Uptake, Storage and Turnover[1]

T. A. SLOTKIN, G. BARNES, C. LAU, F. J. SEIDLER, P. TREPANIER, S. J. WEIGEL and W. L. WHITMORE
Department of Pharmacology, Duke University Medical Center, Durham, North Carolina
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ABSTRACT

Slotkin, T. A., G. Barnes, C. Lau, F. J. Seidler, P. Trepanier, S. J. Weigel and W. L. Whitmore: *Development of polyamine and biogenic amine systems in brains and hearts of neonatal rats given dexamethasone: Role of biochemical alterations in cellular maturation for producing deficits in ontogeny of neurotransmitter levels, uptake, storage and turnover.* J. Pharmacol. Exp. Ther. **221:** 686–693, 1982.

Excessive levels of glucocorticoids are thought to interfere with synaptic development in the central nervous system. In the present study, dexamethasone given to newborn rats produced deficits in brain growth associated with shifts in the developmental pattern of the ornithine decarboxylase/polyamine system consistent with delays in cellular maturation. The effects on the brain were of smaller magnitude and shorter duration than those on the heart or on general growth and were indicative of "brain sparing." Although some biochemical indices of synaptic development of central noradrenergic systems ([^{3}H] norepinephrine uptake into synaptosomes) were quantitatively deficient on a whole-brain basis, the reductions never exceeded the magnitude of effect on brain weight; for other indices (transmitter levels, [^{3}H]norepinephrine into synaptic vesicles), there were little or no apparent developmental deficits. Thus, the effects of dexamethasone on synaptic development in the brain may not reflect a specific action of glucocorticoids over and above their more general effects on cellular maturation. In contrast to the lack of specific action on biochemical indices of synaptic outgrowth, neonatal dexamethasone did cause alterations in norepinephrine synthesis and turnover in both central and peripheral sympathetic neurons which could contribute to the physiological and behavioral abnormalities associated with glucocorticoid treatment during development.

Coordination of neuronal ontogeny and development of synaptic function are both thought to be subject to endocrine control. It is well established that thyroid hormones play an essential role in regulation of nerve cell proliferation and differentiation as well as in nerve terminal outgrowth; consequently, abnormalities of thyroid status are invariably accompanied by perturbations of development of nervous system biochemistry and morphology, followed by alterations of synaptic transmission and behavior (Hamburgh *et al.*, 1971; Nicholson and Altman, 1972; Lau and Slotkin, 1979, 1980a, 1982). Although adrenal corticosteroids also appear to participate in endocrine regulation of nervous system maturation, their role is less clearly defined. Neonatal administration of glucocorticoids produces a delay in appearance of several physiological and behavioral patterns, such as swimming capability (Anderson and Schanberg, 1975), startle reflex and ability to exhibit seizures (Salas and Schapiro, 1970; Schapiro, 1971) and maturation of sensory evoked potentials (Vernadakis, 1961); these effects are accompanied by reductions in brain weight and DNA content and by delays in myelination (Field, 1955; Howard, 1968; Cotterell *et al.*, 1972, Bohn and Lauder, 1980).

Because of these and related findings, glucocorticoids are generally considered to exert actions opposite to those of thyroid hormones, with the former prolonging and the latter accelerating the time course of neuronal development. However, examination of ontogenesis of biogenic amine neurotransmitter systems in the central nervous system and peripheral sympathetic nervous system have indicated a much greater degree of complexity for glucocorticoid actions. Effects of neurotransmit-

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ABBREVIATIONS: ODC, ornithine decarboxylase; AMPT, α-methyl-*p*-tyrosine.

ter levels in some brain regions may be biphasic, with early reductions in neonatal life followed by subsequent elevations (Yuwiler and Geller, 1973; Lengvari *et al.*, 1980). In the periphery, catecholamine histofluorescence in developing sympathetic ganglia is markedly increased by glucocorticoids, despite deficits in the key enzymes in catecholamine biosynthesis (Eranko and Eranko, 1972; Ciaranello *et al.*, 1973; Ciaranello and Axelrod, 1975; Sorimachi, 1977); although the findings for histofluorescence and enzyme levels seem to be contradictory, the enzyme deficits may actually be secondary to glucocorticoid-induced suppression of adrenomedullary development (Lau and Slotkin, 1981), as it has been shown recently that epinephrine from the adrenal medulla regulates neonatal development of ganglionic tyrosine hydroxylase (Markey and Sze, 1981). In any case, the alterations in neuronal ontogeny may not even be primary in the effects of glucocorticoids on the expression of neurotransmission in any given pathway; for example, treatment of neonatal rats with dexamethasone delays the onset of cardiac sympathetic neurotransmission, but the major deficit does not result from effects on nerve terminal outgrowth or development of the number or affinity of synaptic receptors and instead reflects uncoupling of receptors from target tissue responses (Lau and Slotkin, 1981).

Accordingly, the possibility thus remains that the actions of glucocorticoids on development of neurotransmission reflect general retardation of organ development, rather than a specific effect directly upon synaptic maturation. Indeed, glucocorticoids are known to interrupt or retard cell division and growth in a variety of tissues (Howard, 1964; Henderson and Loeb, 1970; Loeb and Sternschein, 1973; Carson *et al.*, 1973; Baxter, 1978; Lau and Slotkin, 1981). In the current study, detailed analysis has been made of the relationships among organ growth, development of neural tissue and development of neurotransmission in central and peripheral nervous systems of neonatal rats treated with dexamethasone. An important component of this comparison is the determination of the effects of dexamethasone on the ODC/polyamine system; ODC and its polyamine end-products are major regulators of protein and RNA synthesis during tissue growth and each organ has specific developmental patterns of ODC and polyamines (Bachrach, 1973; Anderson and Schanberg, 1975; Slotkin, 1979; Russell, 1980). Numerous studies have demonstrated the utility of this system in rapid and sensitive detection of perturbation of cellular development by exposure to drugs or hormones in the perinatal period (reviews, Russell and Durie, 1978; Slotkin, 1979; Slotkin and Thadani, 1980; Russell, 1980), and a previous study with cortisol (Anderson and Schanberg, 1975) has shown that steroids do affect the developmental pattern of brain ODC. To determine the degree of specificity of glucocorticoid effects for neural tissues, the current study also makes comparisons with the pattern of development of ODC and polyamines in the heart, a non-neural tissue which, like the brain, ceases cellular proliferation and differentiation early in postnatal development; DNA replication and cell division in the myocardium of the rat are essentially completed by 2 weeks postpartum (Claycomb, 1975).

In the work described below, the time course and magnitude of effects of dexamethasone on organ growth and on ODC and polyamines have been compared with a variety of biochemical markers for nerve terminal development and synaptic activity; these include synaptosomal and synaptic vesicular uptakes of [^{3}H]norepinephrine which provide indices of outgrowth of noradrenergic terminals and development of transmitter storage capacity (Coyle and Axelrod, 1971; Kirksey *et al.*, 1978) as well as levels and turnover rates of catecholamine neurotransmitters in brain and cardiac sympathetic neurons.

Methods

Treatment of rats. Timed pregnant Sprague-Dawley rats (Zivic-Miller, Allison Park, PA) were housed individually in breeding cages and allowed food and water *ad libitum*. Pups from all litters were randomized at birth and redistributed to the nursing mothers, with litter sizes kept at 9 to 11 pups to maintain a standard nutritive status. Randomization within each treatment group was carried out at intervals of several days and, in addition, pups of both sexes were selected from several different cages in each experiment. Neonates were given dexamethasone sodium phosphate (1 mg/kg s.c.) daily beginning the day after birth and continuing for a total of four injections, whereas control animals received equivalent volumes of saline (1 ml/kg). This dose was chosen because it has been shown previously to affect development of peripheral catecholamine neuronal systems (Wurtman *et al.*, 1967; Lau and Slotkin, 1981). Pups were weighed and killed by decapitation at various ages; during the course of dexamethasone administration, animals were killed 24 hr after the previous injection. Body, heart and brain weights were recorded for each experiment.

ODC and polyamine analyses. To determine ODC activity, brains and hearts were homogenized in 19 volumes of 10 mM Tris (pH 7.2) and ODC activity was determined in duplicate in the 26,000 × *g* supernatant by a modification (Lau and Slotkin, 1979) of the method of Russell and Snyder (1968). The incubation medium contained final concentrations of 50 μM pyridoxal-5'phosphate, 1.5 mM dithiothreitol and a subsaturating concentration (4.5 μM) of *l*-ornithine so as to detect changes in enzyme activity due to shifts either in affinity for substrate or in maximal velocity (Lau and Slotkin, 1979, 1980b).

For determination of polyamine levels, tissues were homogenized in 10 volumes of 0.56 N perchloric acid containing 2 mM EDTA and were centrifuged at 26,000 × *g* for 10 min. The supernatant was adjusted to pH 5 to 6 with 2 N KOH and centrifuged. Separation and analyses of the polyamines were then carried out essentially as described by Endo (1978). A 3-ml aliquot was passed through a CM-cellulose column (6 × 100 mm) and washed sequentially with 15 ml of 0.01 and 0.03 M sodium-phosphate buffer (pH 6.2) and 30 ml of a buffer (pH 6.2) and 30 ml of a buffer (pH 9.0) containing 0.02 M H_3BO_3, 0.02 M KCl and 0.02 M Na_2CO_3. Putrescine was eluted with 20 ml of borate buffer (pH 9.0) containing 0.03 M NaCl, spermidine with 25 ml of borate with 0.075 M NaCl and spermine with 20 ml of borate with 0.15 M NaCl. Before analysis, the eluates were adjusted to pH 8.5 (putrescine) or pH 10 (spermidine and spermine) by the addition of 50 μl of 0.268 M H_3BO_3 or Na_2CO_3, respectively. Columns with standard amounts of polyamines were run concurrently with each set of analyses to determine recoveries, which were typically 100% for putrescine, 90% for spermidine and 80% for spermine; tissue values were corrected for recovery. Column eluates from tissue samples and standards were analyzed in triplicate for polyamines by addition of fluorescamine in dioxane (Endo, 1978).

Synaptosomal and vesicular uptakes of [^{3}H]norepinephrine. Brains were homogenized (Teflon-glass, 4 up-down strokes) in 4 volumes of ice-cold 0.3 M sucrose containing 25 mM Tris-HCl (pH 7.4) and 10 μM iproniazid. For determinations of synaptosomal uptake, the homogenate was diluted with an equal volume of sucrose-Tris-iproniazid and centrifuged at 1000 × *g* for 10 min. Aliquots of the supernatant representing 10 mg wet weight of tissue were added to Krebs-Henseleit medium containing final concentrations of 1.25 μM iproniazid, 2 μM ascorbic acid and 0.1 μM [^{3}H]norepinephrine in a final volume of 1 ml. Samples were incubated for 5 min at 37°C; blanks consisted of identical incubations containing an addition of 0.1 mM cocaine HCl, a specific inhibitor of synaptosomal uptake. Uptake was stopped by placing the tubes on ice and adding 3 ml of ice-cold Krebs-Henseleit medium. The labeled synaptosomes were trapped by rapid vacuum filtration on Gelman cellulose acetate filters (0.2 μm pore size), washed three times

with 3 ml of medium and counted by liquid scintillation spectrometry. This synaptosomal preparation has been characterized previously in both adult and neonatal rats and has been shown to provide an index of nerve terminal proliferation in the developing brain (Coyle and Snyder, 1969; Coyle and Axelrod, 1971; Kirksey *et al.*, 1978).

For determinations of vesicular uptake, the original tissue suspension was rehomogenized in an all-glass apparatus and then centrifuged at 1,000 × *g* for 15 min and the supernatant was recentrifuged at 20,000 × *g* for 30 min. The supernatant of the latter centrifugation was sedimented at 100,000 × *g* for 30 min and the crude vesicle-containing pellet was resuspended (Teflon-glass, 2 up-down strokes) gently in a volume of 130 mM potassium phosphate (pH 7.4) equal to that of the glass-to-glass homogenate. Incubations contained final concentrations of 0.1 μM [^{3}H]norepinephrine, 1 mM ATP-Mg^{++}, 2 μM ascorbic acid, 1.25 μM iproniazid and vesicles derived from 133 mg of original brain tissue in a final incubation volume of 1.67 ml of phosphate buffer. Samples were incubated for 4 min at 30°C, whereas duplicate tubes used as blanks contained 1 μM reserpine, a specific inhibitor of vesicular uptake. Uptake was stopped by the addition of 1.7 ml of ice-cold phosphate buffer and the labeled vesicles were trapped, washed and counted as described above. Although this microsomal fraction contains many particles and organelles, uptake of amines *in vitro* occurs primarily into the catecholamine synaptic vesicles (Seidler *et al.*, 1977; Slotkin *et al.*, 1978).

Catecholamine content and turnover rate. To determine levels of brain norepinephrine and dopamine and of cardiac norepinephrine, tissues were removed and homogenized within 15 sec of decapitation in 2 ml of 0.1 N perchloric acid containing 3,4-dihydroxybenzylamine as an internal standard. After centrifugation at 26,000 × *g* for 15 min, the supernatant solution was added to 50 mg of acid-activated alumina (ICN Nutritional Biochemicals, Cleveland, OH) and the pH was adjusted to 8.6 by the addition of 2 ml of 3 M Tris containing 50 mM EDTA. The samples were shaken vigorously for 10 min and the supernatant solution was aspirated and the alumina was washed once with Tris-EDTA and twice with water. Norepinephrine, dopamine and dihydroxybenzylamine were then eluted with 0.1 N perchloric acid and frozen for later assay. A 20-μl aliquot of the sample was analyzed by high-pressure liquid chromatography using electrochemical detection (Bioanalytical Systems, W. Lafayette, IN) essentially as described by Felice *et al.* (1978). Samples were chromatographed on a reverse-phase ion-paired column containing microparticulate-bound C_{18} as the stationary phase and 0.1 M sodium-phosphate buffer (pH 3) containing 100 mg/l of sodium octyl sulfate as the mobile phase. Retention times were 8 min for norepinephrine, 22 min for dihydroxybenzylamine and 45 min for dopamine; recoveries averaged 80 to 90%. Values reported are corrected for recovery of the internal standard.

Catecholamine turnover was assessed in both controls and dexamethasone-treated animals of various ages by administering saline or AMPT methyl ester HCl (300 mg/kg i.p.). Five hours later, the animals were killed by decapitation and hearts and brains were removed and prepared for norepinephrine and dopamine analyses as described above. AMPT inhibits tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis (Spector *et al.*, 1965) and the disappearance of endogenous catecholamines after AMPT estimates the turnover rate (Brodie *et al.*, 1966; Javoy and Glowinski, 1971; Widerlov and Lewander, 1978; Seidler and Slotkin, 1981). The 5-hr point was chosen because significant depletion of catecholamines occurs within this span in neonatal rats, thus permitting estimation of turnover (Seidler and Slotkin, 1981).

Statistics. Values are reported as means and S.E.s, with significance determined by the Student's *t* test (two-tailed). Activities and contents were calculated per gram of wet tissue weight as well as per organ.

Materials. *l*-[7-^{3}H]Norepinephrine (2.8 Ci/mmol) was obtained from New England Nuclear (Boston, MA) and *l*-[1-^{14}C]ornithine (55 mCi/mmol) was obtained from Amersham Corp. (Arlington Heights, IL), Dexamethasone sodium phosphate was obtained from Merck, Sharp & Dohme (Rahway, NJ), dithiothreitol from Bachem Feinchemikalien AG (Liestal, Switzerland) and AMPT methyl ester HCl, iproniazid phosphate, pyridoxal-5'-phosphate, dihydroxybenzylamine HBr, norepinephrine HCl, dopamine HCl, putrescine dihydrochloride, spermidine phosphate, spermine diphosphate and CM-cellulose from Sigma Chemical Co. (St. Louis, MO).

Results

Body and tissue weights. Over the first 3 weeks of postnatal development, control rats increased in body weight from 9 g at 2 days of age to 56 g (table 1). Rats given dexamethasone displayed an immediate and persistent deficit in body weight gains throughout this time period (fig. 1); after discontinuing dexamethasone, body weights remained approximately 20 to 40% subnormal. Although brain weights showed an initial deficit equivalent to that seen for body weight, substantial recovery occurred by 21 days of age, when brain weights in the treated animals were ≥ 90% of control values. The time course and magnitude of heart weight deficiencies caused by neonatal dexamethasone administration differed from those of the brain; significant differences in heart weight first appeared at 4 days of age and greater deficits were seen subsequently. As with body weights, heart weights eventually became 20 to 30% subnormal.

ODC and polyamines. In agreement with previous reports (Anderson and Schanberg, 1975; Slotkin *et al.*, 1976; Bartolome *et al.*, 1977), the developmental pattern of brain ODC activity in control rats consisted of initially high values followed by a rapid decline over the first 2 weeks postnatally (fig. 2, top). In rats treated neonatally with dexamethasone, activity was extremely low at 2 days of age; a period of high activity still occurred, but was displaced to 4 days of age. Brain ODC activity in both control and dexamethasone-treated animals then de-

TABLE 1

Development of tissue weights, [^{3}H]norepinephrine uptake and catecholamine levels in control rats

Data represent means and S.E. of determinations in 14 to 62 animals for weights, 7 or 8 for [^{3}H]norepinephrine uptakes and 8 to 10 for catecholamine levels.

Age	Weight			[^{3}H]Norepinephrine Uptake		Catecholamine Levels		
	Body	Brain	Heart	Synaptosomes	Vesicles	Brain		Heart
						Norepinephrine	Dopamine	Norepinephrine
days	*g*			*pmol/g*		*ng/g*		*ng/g*
2	9.1 ± 0.2	0.356 ± 0.004	0.049 ± 0.001	41 ± 4	3.4 ± 0.3	112 ± 2	201 ± 4	53 ± 3
4	12.3 ± 0.2	0.497 ± 0.004	0.062 ± 0.001	69 ± 9	7.2 ± 0.8	146 ± 6	234 ± 8	94 ± 6
8	21.4 ± 0.4	0.862 ± 0.006	0.092 ± 0.002	59 ± 4	7.7 ± 0.5	147 ± 4	251 ± 11	244 ± 11
11	27.9 ± 0.3	1.11 ± 0.01	0.110 ± 0.001	82 ± 4	7.1 ± 0.4	161 ± 5	267 ± 8	306 ± 12
15	36.0 ± 0.7	1.35 ± 0.01	0.146 ± 0.002	103 ± 3	10.1 ± 0.5	186 ± 6	359 ± 9	515 ± 22
18	41.9 ± 0.6	1.45 ± 0.01	0.184 ± 0.003	89 ± 4	11.1 ± 0.4	240 ± 11	393 ± 19	836 ± 51
21	56.3 ± 1.8	1.51 ± 0.01	0.261 ± 0.006	93 ± 5	12.9 ± 0.6	231 ± 8	423 ± 14	701 ± 44

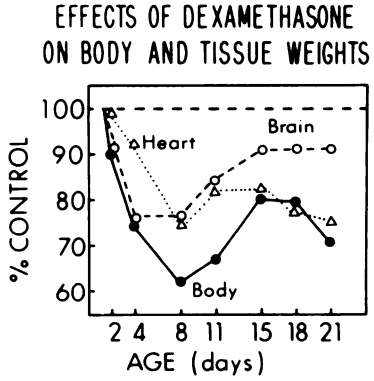

Fig. 1. Effects of dexamethasone (1 mg/kg s.c. begun at 1 day postnatally and given daily for 4 days) on body and organ weights. Control values appear in table 1. Values represent means of up to 62 animals at each age; S.E.s were <3%. All values are significantly different from controls ($P < .05$ or less) except for hearts at 2 days of age.

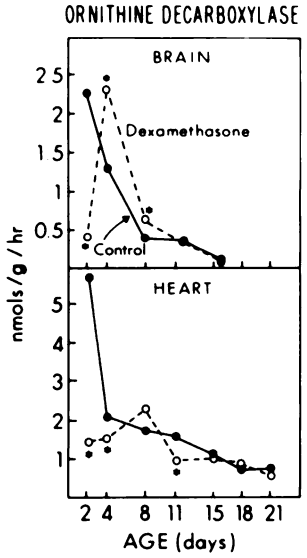

Fig. 2. ODC activities in brains and hearts of developing control rats and rats treated neonatally with dexamethasone on days 1 to 4. Values represent means of 8 to 10 animals in each group at each age; S.E.s were <12% for brain ODC and <16% for heart ODC. Asterisks denote significant differences ($P < .05$ or less).

clined toward zero by the middle of the third week postnatally. The effects of dexamethasone on cardiac ODC were more profound than those seen in the brain (fig. 2, bottom), with total suppression of the period of high activity.

In keeping with its effects on ODC activity, dexamethasone treatment resulted in marked initial deficits in brain putrescine levels which abated by 8 days of age (fig. 3, top). However, spermidine levels were essentially normal in the treated pups (fig. 3, middle) and spermine may even have been slightly elevated by dexamethasone (fig. 3., bottom). In contrast, in the heart, the levels of all three polyamines were decreased substantially by dexamethasone (fig. 4); the deficits in putrescine were the most pronounced but shortest in duration, those for spermidine were intermediate in intensity and duration and those for spermine were of the smallest magnitude but lasted longest.

Brain catecholamine systems. Synaptosomal uptake of [^{3}H]norepinephrine per g of brain tissue was essentially normal in dexamethasone-treated pups throughout development (fig. 5, top); uptake per brain was below normal solely because of the brain weight deficits. Uptake into synaptic vesicles showed even less of a deficit after dexamethasone (fig. 5, bottom); with these organelles, the only significant decrease in uptake per brain was seen at 4 days of age and uptake per gram was actually increased at 8 and 11 days. Similarly, dexamethasone tended to cause an initial increase in levels of brain norepinephrine and dopamine per gram of tissue (fig. 6). Deficits were seen on a per brain basis only at 4 days for norepinephrine and at 15 and 18 days for dopamine; in the latter instances, the deficits in amine levels per brain never exceeded the percentage shortfall of brain weight. However, dexamethasone did cause a significant increase in the turnover rate of norepinephrine expressed either per gram of tissue or per brain, as measured after acute

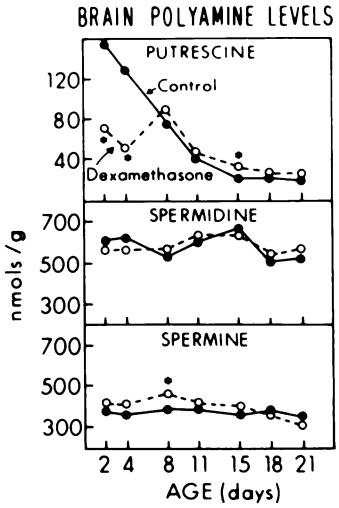

Fig. 3. Brain polyamine levels in developing control rats and rats treated neonatally with dexamethasone. Values represent means of six to nine animals; S.E.s were <10%. Asterisks denote significant differences ($P < .05$ or less).

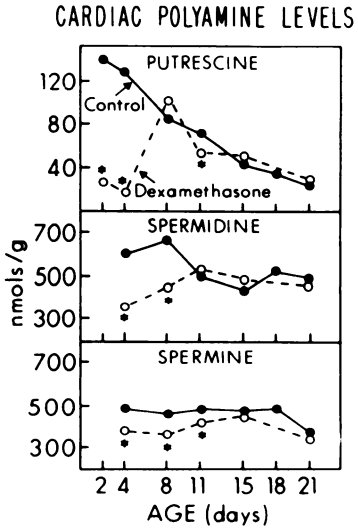

Fig. 4. Heart polyamine levels in developing control rats and rats treated neonatally with dexamethasone. Values represent means of six to nine animals; S.E.s were <10%. Asterisks denote significant differences ($P < .05$ or less).

inhibition of catecholamine biosynthesis by AMPT (fig. 7, left); the effect disappeared as weaning was approached. Although dopamine turnover was elevated per gram, this again reflected primarily an effect on brain weight, as no consistent difference was seen on a whole-brain basis (fig. 7, right).

Cardiac sympathetic system. In contrast to the relatively small changes seen for brain catecholamine levels, dexamethasone produced a profound deficit in cardiac norepinephrine levels expressed either per gram or per heart beginning at 8 days of age (fig. 8). Values remained subnormal throughout the preweanling period. Despite the transmitter deficiency, the turnover rate for cardiac norepinephrine (fig. 9) became markedly elevated during the 2nd week of postnatal life, but still displayed the sharp increase normally characteristic of the late preweanling phase of development (Seidler and Slotkin, 1981).

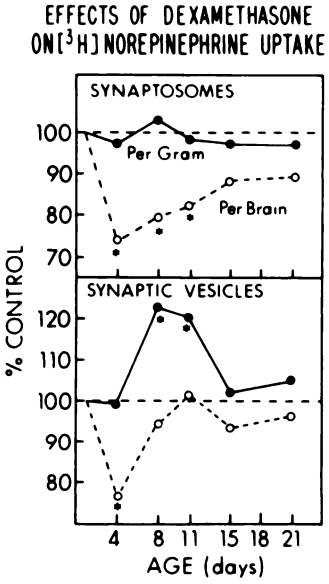

Fig. 5. Effects of neonatal dexamethasone treatment on synaptosomal and synaptic vesicular uptakes of [^{3}H]norepinephrine expressed per gram or per brain. Values represent means of seven to eight animals; S.E.s were <10%. Asterisks denote significant differences ($P < .05$ or less) from control values given in table 1.

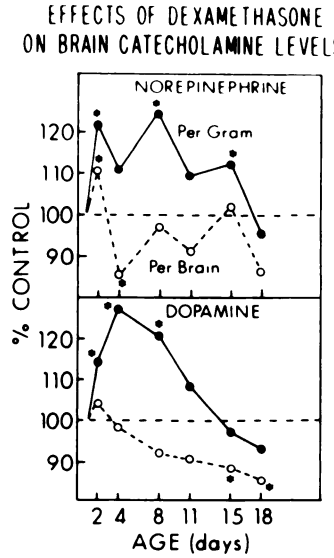

Fig. 6. Effects of neonatal dexamethasone treatment on brain catecholamine levels expressed per gram or per brain. Values represent means of 6 to 10 animals; S.E.s were <5%. Asterisks denote significant differences ($P < .05$ or less) from control values given in table 1.

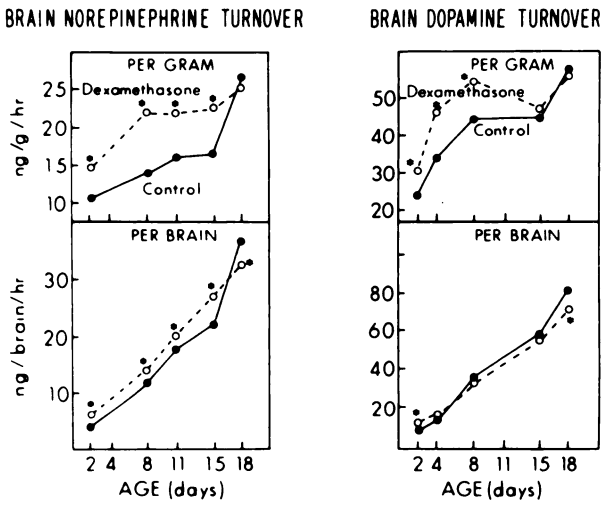

Fig. 7. Turnover rates of brain norepinephrine (left) and dopamine (right) in developing control rats and rats treated neonatally with dexamethasone. Values represent means of 6 to 10 animals; S.E.s were <7%. Asterisks denote significant differences ($P < .05$ or less).

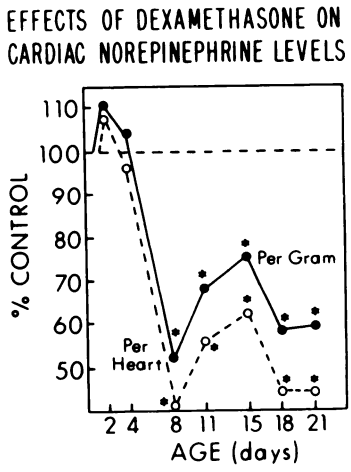

Fig. 8. Effects of neonatal dexamethasone treatment on cardiac norepinephrine levels expressed per gram or per heart. Values represent means of 8 to 10 animals; S.E.s were <8%. Asterisks denote significant differences ($P < .05$ or less) from control values given in table 1.

Discussion

Results obtained in this study are consistent with the view that neonatal administration of glucocorticoids produces a general delay in cellular maturation of various tissues, as exemplified by perturbations in the developmental patterns of ODC activity and polyamine levels. ODC catalyzes the first reaction in the biosynthesis of the polyamines putrescine, spermidine and spermine (Pegg and Williams-Ashman, 1968; Russell and Snyder, 1968), which have been shown to play regulatory roles in both nucleic acid and protein synthesis during growth and development (Bachrach, 1973; Russell and Durie, 1978; Abraham and Pihl, 1981); additionally, the ODC molecule itself may be an initiation factor for RNA polymerase I activity (Russell and Durie, 1978). ODC activity and polyamine concentrations

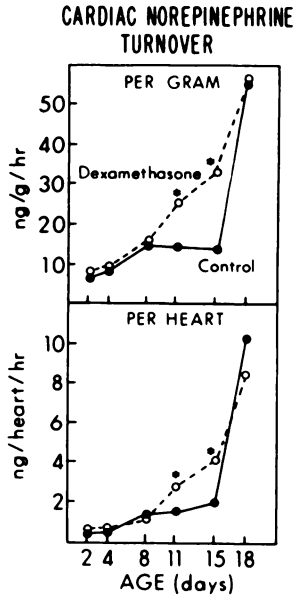

Fig. 9. Turnover rate of cardiac norepinephrine in developing control rats and rats treated neonatally with dexamethasone. Values represent means of 7 to 10 animals; S.E.s were <12%. Asterisks denote significant differences ($P < .05$ or less).

(particularly putrescine) are highest during rapid growth and cellular multiplication or differentiation and decrease as these processes cease or as the number of growing or dividing cells decline (Bachrach, 1973; Russell and Durie, 1978; Slotkin, 1979). Consequently, each organ has specific ODC and polyamine developmental patterns and alterations in these patterns caused by changes in perinatal environment have been shown to provide extremely sensitive measures of perturbation of the subcellular processes occurring in maturation (Slotkin, 1979; Russell, 1980).

In the current study, the ODC/polyamine developmental patterns for both brain and heart were abnormal in dexamethasone-treated neonates and for each organ, the type of alteration was consistent with the subsequent magnitude and persistence of growth deficits. Normally, in the brain, ODC activity is high at birth and declines rapidly during the first 1 to 2 weeks of postnatal life (Anderson and Schanberg, 1975; Slotkin *et al.*, 1976; Bartolome *et al.*, 1977; Slotkin, 1979). Dexamethasone did not obliterate this developmental pattern of brain ODC, but rather shifted the period of high activity such that it occurred 2 days later. Whereas putrescine levels were abnormally low initially, the effects were not persistent and no deficits occurred in levels of spermidine or spermine. Consistent with the transient nature of the effects on the ODC/polyamine system, brain weights in the dexamethasone-treated pups were decreased initially, but did show a substantial recovery toward normal by weaning.

The effects of dexamethasone on the ODC/polyamine system of the heart, although also indicative of delayed cellular maturation, were quantitatively and qualitatively different from those in the brain. Dexamethasone totally eliminated the period of high ODC activity in the heart and the consequent deficits in polyamine levels involved spermidine and spermine as well as putrescine. These profound effects were associated with more persistent deficits in cardiac growth which, unlike brain growth, showed no signs of restitution toward normal by weaning. Thus, relative to the effects of neonatal dexamethasone administration on body weight and heart development, the brain is "spared" at the levels of both biochemical maturation and overall organ growth.

Although this study also confirms that neonatal dexamethasone does indeed slow the development of central catecholaminergic synapses, this effect appears to be secondary to the more general alterations in cellular development and overall growth. Uptake of [^{3}H]norepinephrine into synaptosomes, a biochemical index of outgrowth of nerve terminals (Coyle and Axelrod, 1971; Kirksey *et al.*, 1978), was reduced per brain in the treated pups, but only to the same extent as for brain weight; there was no effect when results were expressed per gram. Even more striking was the absence, with only one exception, of effects on [^{3}H]norepinephrine uptake into synaptic vesicles, an index of nerve terminal transmitter storage capabilities (reviews, Philippu, 1976; Slotkin and Bareis, 1980); for this parameter of nerve terminal development, uptake per gram was actually elevated at two age points. These data suggest that the slowing of synaptic development by neonatal dexamethasone treatment is not related to a unique effect of the steroid on synaptogenesis, but rather is a reflection of the actions of the hormone on cellular development in general, displaying the same extent (or even a greater extent) of sparing as seen for brain growth or for the ODC-polyamine developmental pattern.

The same sparing and lack of specific effect was apparent for central catecholamine transmitter levels; for both norepinephrine and dopamine, content per brain never displayed deficits of greater magnitude than those of brain weight and, in fact, were often unchanged per brain and elevated per gram of tissue. The indication that the extent of suppression of development of neurotransmitter levels by dexamethasone is less than the degree of general brain growth retardation again suggests that actions on this aspect of neuronal maturation are not unique events over and above more general effects of the steroid.

In contrast to the lack of specific or unique effects of dexamethasone on biochemical indices of central synaptogenesis or transmitter levels, evidence for a transmitter-specific developmental alteration by dexamethasone was obtained for effects on turnover rate. Brain norepinephrine turnover was consistently elevated both per organ and per gram of tissue, whereas dopamine turnover per brain was normal (the elevation seen for dopamine turnover per gram merely reflected the lowered brain weight in dexamethasone-treated animals). This action was not restricted to central noradrenergic systems, as a similar elevation of turnover was seen in cardiac peripheral sympathetic neurons. It is particularly interesting that increased turnover in the latter occurred despite a marked deficit of transmitter levels; previous studies have shown that sympathetic neuron outgrowth *per se* may be largely unaffected (Lau and Slotkin, 1981) or even enhanced (Eranko and Eranko, 1972) by steroids, yet the transmitter levels are clearly subnormal. One likely explanation is the specific reduction in tyrosine hydroxylase activity, the rate-limiting factor in catecholamine biosynthesis, reported to occur in steroid-treated neonates (Ciarenello *et al.*, 1973; Ciaranello and Axelrod, 1975; Lau and Slotkin, 1981); administration of exogenous steroids suppresses adrenocorticotropin release, leading to adrenal atrophy and deficient adrenomedullary development (Ganong, 1963; Sorimachi, 1977; Lau and Slotkin, 1981). Because circulating epinephrine from the adrenal may control development of tyrosine hydroxylase in peripheral sympathetic neurons (Markey and Sze, 1981), dexamethasone treatment could thus produce a reduction in tyrosine

hydroxylase leading to deficits in transmitter levels, followed by a compensatory centrally mediated increase in sympathetic activity with a consequent elevation of turnover rate of the remaining transmitter. In support of this hypothesis, cardiac norepinephrine turnover did not become elevated until after the 1st week of postnatal age, the point at which central control of sympathetic tone first develops (Seidler and Slotkin, 1979, 1981; Bartolome *et al.*, 1980; Smith *et al.*, 1982).

In conclusion, dexamethasone causes organ-specific alterations of developmental patterns of ODC/polyamine systems which are correlated with the subsequent magnitude and duration of deficits in organ growth. The effects of the steroid on biochemical indices of central synaptogenesis reflect these more general actions on cellular development and display "sparing" relative to other tissues and to general growth. In contrast, neonatal dexamethasone causes more specific alterations in transmitter synthesis and turnover which may contribute to physiological or behavioral abnormalities caused by glucocorticoid treatment during development.

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Send reprint requests to: Dr. T. A. Slotkin, Box 3813, Duke University Medical Center, Durham, NC 27710.