The Effect of Corticosteroids on Dendritic Development in the Rat Brain

MARJORIE A. S. ODA AND PETER R. HUTTENLOCHER

Departments of Pediatrics and Neurology, Yale University School of Medicine, New Haven, Connecticut 06510

Received April 30, 1974

Sprague-Dawley rats were given 0.02 ml of methylprednisolone (0.06 g/g body wt) 6, 9, or 12 days postnatally. When compared with saline-treated controls, the following effects on dendritic development in layers 3 and 5 of the parietal cortex were observed: (1) no significant alteration in number of basal dendrites; (2) initial increase (at 3 wk of age) in branchings near the perikaryon in the group treated at 12 days; (3) decrease in number of branches at distances $\geq 270 \mu m$ from the perikaryon in layers 3 and 5 in all steroid-treated groups when sacrificed at 6 wk; (4) decrease in number of intersections in layer 5 in all steroid-treated groups when sacrificed at 6 wk; and (5) decrease in number of terminations in layers 3 and 5 in all steroid-treated groups when sacrificed at 6 wk.

INTRODUCTION

Corticosteroids are used in the treatment of a variety of diseases of infancy and childhood, as well as in pregnant women (1–4). Aside from their retarding effects on growth, little is known about the long-term side effects on development of chronic steroid administration in such patients (5, 6). This is particularly true with respect to their effect on the developing nervous system.

There have been some reports of increased neurological deficits in infants and children, as well as in the fetuses of pregnant women, after steroid treatment (7, 8). Clinical experience at Yale–New Haven Hospital has suggested, also, that neurological sequelae may result from chronic steroid therapy in infancy (9).

In animals, experimental neonatal corticosteroid treatment has been shown to cause an irreversible reduction in brain weight (10). Quantitative neurochemical measurements have shown that steroids decrease the number of cells present as well as the total amount of myelin lipids. Thus, Howard measured the amount of DNA present in the brains of control and steroid-treated mice, as well as the

---

1 Supported by USPHS Grant 5 T 01 WS-05666-05 (National Institutes of Health).
2 Associate Professor of Pediatrics and Neurology, Yale University School of Medicine, New Haven, CT.
RNA/DNA ratio and the cholesterol/DNA ratio in order to determine cell size, cell number, and degree of myelination. Mice with subcutaneously implanted corticosterone (producing doses of 0.08–0.6 mg/day between 2 and 7 days of age) showed, by 17 days of age, a decrease in total DNA, the RNA/DNA ratio, and the cholesterol/DNA ratio in both the cerebrum and cerebellum (10). Follow-up studies in adult mice (350 days) similarly treated during the neonatal period showed a persisting decrease in total DNA and in the cholesterol/DNA ratio, but no significant decrease in the RNA/DNA ratio (11). Subsequently, Cotterrell et al. (12) studied reduction in cell number by labeling techniques, and confirmed the demonstration by Howard of a decrease in cellularity; furthermore, this investigation was able to attribute the reduction to a decrease in cell division as opposed to an increase in cell destruction.

Behavioral experiments have shown that steroids can cause long-term functional deficits in animals. After a single injection of 1 mg cortisol acetate on the day of birth, for example, Sprague–Dawley rats exhibit decreased spontaneous exploratory locomotor activity (13). Similarly, Howard reported that mice treated as described above with subcutaneously implanted corticosterone, initially exhibit an increased voluntary running in activity wheels, but a decreased activity in exploration in an open field. In addition, treated mice tend to show a poorer initial adjustment to mazes. These data have been interpreted as consistent with emotional lability (14).

Electrophysiological studies have shown that neonatal steroid administration in the young rat can cause a retardation in the development of evoked cortical responses to visual, auditory, and sciatic nerve stimulation (15). Further, it has been demonstrated that neonatal cortisol treatment delays the maturation of swimming ability in rats, and that this delay corresponds to ontogenetic shifts in the characteristics of the evoked potentials (16).

Although these neurochemical, behavioral, and electrophysiological studies suggest, indirectly, that steroids may interfere with the normal myelination and dendritic development of nerve cells, they supply no direct evidence for steroid-induced morphological changes in neurons. In humans and certain other species, it is known that both myelination and dendritic formation continue well into the postnatal period (17). Because certain conditions, such as hypothyroidism (18, 19), anoxia (20), X-irradiation (21), and malnutrition (22–24) during this postnatal period can cause permanent alterations in neuronal structure in animals, it is possible that steroid administration might also affect the structure of developing neurons. In an earlier report (25), we demonstrated that neonatal corticosteroid administration in rats can cause significant alterations in the myelination of developing axons. Specifically, we showed that neonatal treatment with steroids results in a decrease in the frequency of myelinated fibers, a decrease in the mean number of lamellae per axon, and a decrease in the number of lamellae for a given axon circumference. We now report that, in addition, the proliferation of neuronal dendritic processes is significantly altered after a single injection of steroid during the neonatal period.

**MATERIALS AND METHODS**

Sprague-Dawley rats were divided into four groups. One group (control) received a single intramuscular dose of 0.02 ml saline (McGaw) 6 days postnatally. The other three groups received 0.02 ml methylprednisolone (Depo-Medrol, Upjohn, 40 mg/ml) 6, 9, or 12 days postnatally. The rats were sacrificed at 3 or
6 wk. Rats were decapitated by means of a guillotine. Brains were immediately removed and weighed. Olfactory bulbs were not included. Frontal sections, about 2 mm in width were taken from the parietal cortex, and were placed into freshly prepared Golgi–Cox fixative (K$_2$Cr$_2$O$_7$, 10 mg in 450 ml distilled water, HgCl$_2$, 10 mg in 450 ml distilled water, and KCrO$_4$, 3 mg in 100 ml distilled water). The tissues were incubated in the fixative solution for 6–8 wk at 37°C. They were then dehydrated and embedded in celloidin, and cut into 90-μm sections. These sections were next exposed to 1% aqueous NH$_4$OH for about 20 sec and were then dehydrated and mounted on glass slides.

Dendritic development was evaluated using pyramidal cells from layer 3 an layer 5 of the parietal cortex (18). Fifteen to forty cells from each layer were selected randomly according to the following criteria: (1) cells were chosen from regions in which there was relatively little superimposition of cell bodies and branchings from neighboring neurons, and (2) regions were selected in which breakage of dendrites was minimal. Pyramidal cells thus selected for analysis were drawn by tracing the projected outline of each cell and its dendrites on paper using a Leitz microscope with drawing attachment.

The image of each neuronal perikaryon and its branches was projected onto the center of a target, and all dendrites extending from the perikaryon were drawn on a grid of 15 concentrically arranged circles, or zones, each of a radius which represented a distance of 18 μm on the histological section. Using this method, the following measurements were made (18, 26): (1) the number of dendrites arising from the perikaryal surface (basal dendrites); (2) the number of dendrites intersecting the zonal boundaries at successive distances of 18 μm from the cell body; (3) the number of branching points present in each zone; and (4) the number of dendritic endings in each zone.

Evaluation was done on a blind basis. Slides from experimental and control animals were coded prior to microscopic examination.

RESULTS

The changes in dendritic morphology resulting from corticosteroid treatment were qualitatively similar in layers 3 and 5.

Steroid treatment did not alter the number of basal dendrites arising from the perikaryon in either the 3- or 6-wk-old groups (Fig. 1).

Steroid treatment did have a significant effect on the distribution of branching points surrounding each axon. Specifically, by 6 wk of age, steroids caused a decrease in the degree of branching at distances greater than 27 μm from the neuronal perikaryon. This is seen in Figs. 2 and 3. Interestingly, the peak incidence of branching was not altered as a result of steroid treatment. Thus, in both control and experimental groups, the peak of branching occurred at a distance of 36–72 μm from the center of the perikaryon. In fact, at early times after steroid treatment (3-wk-old animals), the absolute number of dendrites branching close to the perikaryon was actually enhanced in rats treated at 12 days (Figs. 4 and 5). However, by the age of 6 wk (Figs. 2 and 3), there was, as noted above, a significant decrease in the number of dendrites branching farther (≥270 μm) from the perikaryon in the treated animals when compared with their littermate controls. This decrease, which was more pronounced in layer 5, is shown more clearly in Figs. 6 and 7. These figures compare the number of intersections, branchings, and terminations at distances ≥270 μm from the perikaryon, and show that there was a significant
Fig. 1. Mean number of basal dendrites (± SD) in layer 3 (top) and layer 5 (bottom) of parietal cortex of 3-wk-old and 6-wk-old rats.

Fig. 2. Distribution of branching points, layer 3, age 6 wk. (Each zone represents a distance of 18 μm on the histological section). • = control; △ = 12 day; ○ = 9 day; □ = 6 day.

Fig. 3. Distribution of branching points, layer 5, age 6 wk. (Each zone represents a distance of 18 μm on the histological section). • = control; △ = 12 day; ○ = 9 day; □ = 6 day.
decrease in the number of dendrites branching in the steroid-treated groups. Qualitatively, this difference can be seen by comparing Figs. 8 and 9 (3 wk), and Figs. 10 and 11 (6 wk).

**Fig. 4.** Distribution of branching points, layer 3, age 3 wk. (Each zone represents a distance of 18 μm on the histological section). ● = control; △ = 12 day; ○ = 9 day; □ = 6 day.

**Fig. 5.** Distribution of branching points, layer 5, age 3 wk. (Each zone represents a distance of 18 μm on the histological section). ● = control; △ = 12 day; ○ = 9 day; □ = 6 day.

**Fig. 6.** Mean number of intersections, branchings, and terminations at distances ≥ 270 μm from perikaryon, ± SD, layer 3, age 6 wk.
Fig. 7. Mean number of intersections, branchings, and terminations at distance $\geq 270$ $\mu$m from perikaryon, $\pm$ SD, layer 5, age 6 wk.

Fig. 8. Pyramidal cell in layer 5 of parietal cortex of corticosteroid-treated rat, age 3 wk.
Fig. 9. Pyramidal cell in layer 5 of parietal cortex of control rat, age 3 wk.

Fig. 10. Pyramidal cell in layer 5 of parietal cortex of corticosteroid-treated rat, age 6 wk.
In a pattern similar to that seen above, steroids caused a decrease in the number of ending and intersecting points at distances \( \geq 270 \, \mu \text{m} \) from the neuronal perikaryon. This effect is illustrated in Figs. 6 and 7.

**DISCUSSION**

We have described an experimental model which was used to evaluate the effects of corticosteroid administration on structural changes in brain development. Earlier, we reported significant effects of steroids on myelination. In the present study, we report the observation of significant alterations in dendritic proliferation in neonatally treated rats.

Whether it is valid to extrapolate the results reported here for rats to the human brain is an important question. The implications of the changes in myelination and dendrite formation which we have reported should be viewed in terms of the rationale behind some aspects of the experimental procedure.

First, the choice of rats as experimental animals is supported by comparative studies of brain development. Such studies have indicated that increases in both brain weight and cortical thickness follow a triphasic curve in both rats and humans although the rate of growth in rats is much more rapid (27, 28).

Second, rats were not injected until 6 days postnatally rather than at birth because the cerebral cortex of rats at the time of birth is somewhat less developed than the human cortex at birth. Specifically, the human brain at birth is similar in development to that of a rat of approximately 5 days of age (29, 30).

Third, the dosage per gram of body weight of steroid chosen was comparable to that used in humans on chronic steroid therapy.

Fourth, myelination and dendritic development were chosen for evaluation because it has been suggested that interference with these processes during the "vul-
nerable period” which, in humans, includes the last trimester of gestation and the first 18–24 postnatal months (31) may lead to serious motor, intellectual and behavioral studies (10–16) strongly suggest an interference with these processes.

In most mammals, structural, biochemical, and functional maturation of the brain is incomplete at birth (32). The postnatal increase in cortical dimensions is largely due to the increase in cell size, the growth of dendrites, and myelin deposition (33, 34).

Quantitative histological studies on the postnatal development in the rat have demonstrated that prior to 6 days of age, very few dendrites appear, but by 12 days, the mean number of dendrites arising from the perikaryon has reached the adult level. Subsequent development results in an increase in the extent of the dendritic field (26). Corresponding studies in the human (35) have demonstrated that differentiation of dendritic ramifications has just commenced at birth. Between 3 and 6 mo, there is a striking growth in the dendritic plexus, and by the age of 2 yr, no further change in dendritic development can be demonstrated by present methods.

It has been postulated that insults to developing organ systems during critical stages of development may have irreversible long-term sequelae (30, 31, 36, 37). In particular, the brain is likely to be most vulnerable to permanent alterations during periods of maximum growth. It is thought that growth impairment induced by injurious conditions does not alter the timing of the growth spurt in the brain (17). Rather, brain injury is reflected by a decrease in the extent of the processes which nevertheless occur at a set, preordained time. Therefore, some feel that the brain is an organ with only one opportunity to accomplish its important developmental events, and that once this opportunity is lost, it probably cannot be recovered.

Earlier, we reported that the myelination process in steroid-treated rats is considerably retarded (25). We now present evidence that the ability of dendrites to proliferate, elongate, and branch at their terminal arborizations is also significantly decreased by such treatment. This impairment is reflected by a decrease in the number of dendrites and their branches at distances farther from the perikaryon. This effect was noticeable in both layer 3 and layer 5 of the parietal cortex. An exception to this trend was shown by rats treated at 12 days and sacrificed at 3 wk of age, which demonstrated significantly increased dendritic development in layers 3 and 5. By the age of 6 wk, this advantage had been lost. In this regard, it is interesting to note that, although the mechanism of action is different (38, 39), a similar effect has been observed in synaptogenesis in hyperthyroid rats. Such animals have been found to exhibit an initial acceleration of cerebellar synapse formation but an ultimate reduction in the total number of synapses (40). Similarly, lung development in steroid-treated animals is initially accelerated, but ultimately reduced (41, 42). It appears that the consequences of an insult are complex, and may depend not only upon the age and type of cell it affects, but also on the time of the interference.

These structural changes in the pattern of dendritic development due to treatment with steroids during the neonatal period may possibly explain the results of some of the behavioral studies. The “brain damage behavior” syndrome in humans has been characterized by decreased motor coordination and emotional lability (43). More recently, young children with profound mental deficiency have been observed to have abnormalities of cortical dendritic development as the only or the major pathologic finding (44). The structural data reported here are consistent with
known neurochemical and functional changes, and suggest that brain damage syndromes might be caused by interference with the processes of neuron maturation which have their maximal rate during the brain growth spurt.

The results of the present study leave the mechanism of action of steroids on the central nervous system open to speculation. The derangements in gross brain size, myelination, and dendritic proliferation may be a direct effect of the steroids on the brain. On the other hand, the defects in CNS development documented here could be due to a secondary effect of the steroids on other organ systems, such as functional malnutrition due to defective intestinal absorption. Still a third possibility is that the steroids exert their effect via the functional thymectomy they are known to produce and that the central nervous system changes are part of the spectrum of defects known as the “runtling syndrome” (45).

Regardless of its mechanism of action, neonatal corticosteroid treatment has been demonstrated to have an adverse effect in the rat on the development of the structural components of the central nervous system, particularly with regard to myelination and dendritic development. These changes in morphology provide an explanation for formerly observed neurochemical, functional, and behavioral alterations in the central nervous system.

REFERENCES

1. Schwartz, J. F. Drug-induced neurologic disorders in children. Curr. Prob. Pediat. 1, 3 (1971).
2. Klevit, H. D., Corticosteroid therapy in neonatal period. Pediat. Clin. N. Amer. 17, 1003 (1970).
3. Baden, M., Bauer, C. R., Colle, E., Klein, G., Teusch, H. W., and Stern, L. A controlled trial of hydrocortisone therapy in infants with respiratory distress syndrome. Pediatrics 50, 526 (1972).
4. Liggins, G. C., and Howie, R. N. A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. Pediatrics 50, 515 (1972).
5. Blodgett F. M., Burgin, L., Iezzoni, D., Gribete, D. and Talbot, N. B. Effects of prolonged cortisone therapy on the structural growth, skeletal maturation, and metabolic status of children. N. Engl. J. Med. 254, 636 (1956).
6. Morris, H. G., Jorgensen, J. R., and Jenkins, S. A. Metabolic effects of human growth hormone in corticosteroid-treated children. J. Clin. Invest. 47, 427 (1968).
7. deLemos, R. A., and Haggerty, R. J., Corticosteroids as an adjunct to treatment in bacterial meningitis: A controlled clinical trial. Pediatrics 44, 30 (1969).
8. Warrell, D. W., and Taylor, R. Outcome for the foetus of mothers receiving prednisolone during pregnancy. Lancet 2, 117 (1968).
9. Oda, M. The effect of corticosteroids on the morphology of the developing rat brain. Yale Medical School Thesis, pp. 7–10, 1974.
10. Howard, E. Effects of corticosterone and food restriction on growth and DNA, RNA, and cholesterol contents of the brain and liver in infant mice. J. Neurochem. 11, 181 (1965).
11. Howard E. Reductions in size and total DNA of cerebrum and cerebellum in adult mice after corticosterone treatment in infancy. Exp. Neurol. 22, 191 (1968).
12. Cotterrell, M., Balazs, R., and Johnson, A. L. Effects of corticosteroids on the biochemical maturation of rat brain: Postnatal cell formation. J. Neurochem. 19, 2151 (1972).
13. Schapiro, S. Some physiological, biochemical, and behavioral consequences of neonatal hormone administration: Cortisone and thyroxine. Gen. Comp. Endocrinol. 10, 214 (1968).
14. Howard, E., and Granoff, D. M. Increased voluntary running and decreased motor coordination in mice after neonatal corticosterone implantation. Exp. Neurol. 22, 66 (1968).
15. Salas, M., and Schapiro, S. Hormonal influences upon the maturation of the rat brain's responsiveness to sensory stimuli. Phys. Behav. 5, 7 (1970).
16. Schapiro, S., Salas, M., and Vukovich, K. Hormonal effects on ontogeny of swimming
ability in the rat: Assessment of central nervous system development. *Science* **168**, 147 (1970).

17. Dobbing, J. Undernutrition and the developing brain. In "Developmental Neurobiology" (W. A. Himwich, Ed.), Thomas, Springfield, Ill., (1970).

18. Eayrs, J. T. The cerebral cortex of normal and hypothyroid rats. *Acta Anat.* (Basel) **25**, 160 (1955).

19. Rosman, N. P. Effect of thyroid deficiency on myelination of brain. A morphological and biochemical study. *Neurology* **22**, 99 (1972).

20. Hicks, S. P., Cavanaugh, M. C., and O’Brien, E. D., Effects of anoxia on the developing cerebral cortex of the rat. *Amer. J. Pathol.* **40**, 615 (1962).

21. Hicks, S. P., and D’Amato, D. J., Low-level radiation and changes in glia and neuron populations in the developing brain. *Fed. Proc.* **23**, 128 (1964).

22. Dobbing, J. The effect of undernutrition on myelinization in the rat central nervous system. *Biol. Neonat.* **9**, 132 (1965).

23. Cragg, B. G. Development of cortical synapses during starvation in the rat. *Brain* **95**, 143 (1972).

24. Patel, A. J., Balazs, R., and Johnson, A. L. Effect of undernutrition on cell formation in the rat brain. *J. Neurochem.* **20**, 1151 (1973).

25. Gumbinas, M., Oda, M., and Huttenlocher, P. The effects of corticosteroids on myelinization of the developing rat brain. *Biol. Neonat.* **22**, 355 (1973).

26. Eayrs, J. T., and Goodhead, R. Postnatal development of the cerebral cortex in the rat. *J. Anat.* **93**, 385 (1959).

27. Donaldson, H. H. A comparison of the albino rat with man in respect to the growth of the brain and of the spinal cord. *J. Comp. Neurol.* **18**, 345 (1908).

28. Dobbing, J. Effect of experimental undernutrition on development of the nervous system. In "Malnutrition, Learning and Behavior" (N. S. Scrimshaw and J. E. Gordon, Eds.), M.I.T. Press, Boston, 1968.

29. Smith, C. G. The volume of the neocortex of the albino rat and the changes it undergoes after birth with age. *J. Comp. Neurol.* **60**, 319 (1934).

30. Dobbing, J. Vulnerable periods of brain development. In "Lipids, Malnutrition and the Developing Brain", Ciba Foundation Symposium, Amsterdam, 1972.

31. Dobbing, J. Vulnerable periods in the developing brain. In "Applied Neurochemistry" (A. N. Davison and J. Dobbing, Eds.), Blackwell, Oxford, 1968.

32. Bass, N. H., Netsky, M. G., and Young, E., Microchemical studies of postnatal development in rat cerebrum. *Neurology* **19**, 258 (1969).

33. Davison, A. N., and Dobbing, J. The developing brain. In "Applied Neurochemistry" (A. N. Davison and J. Dobbing, Eds.), Blackwell, Oxford, 1968.

34. Caley, D. W. Differentiation of the neural elements of the cerebral cortex in the rat. *UCLA Forum Med. Sci.* **14**, 73 (1971).

35. Schadé, J. P., and van Groenigen, W. B., Structural organization of the human cerebral cortex. *Acta Anat.* **47**, 74 (1961).

36. Smart, J. L. Vulnerability of developing rat brain. *Brain Res.* **28**, 85 (1971).

37. Winick, M. Biological correlations. *Amer. J. Dis. Child.* **120**, 416 (1970).

38. Balazs, R., and Cotterrell, M. Effect of hormonal state on cell number and functional maturation of the brain. *Nature (London)* **236**, 348 (1972).

39. Richter, D. Endocrine factors affecting maturation of the brain. *Proc. Roy. Soc. Med.* **65**, 585 (1972).

40. Nicholson, J. L., and Altman, J. Synaptogenesis in rat cerebellum: Effects of early hypothyroidism. *Science* **176**, 530 (1972).

41. Kotas, R. V., and Avery, M. E. Accelerated appearance of pulmonary surfactant in the fetal rabbit. *J. Appl. Physiol.* **30**, 358 (1971).

42. Carson, S. H., Taeusch, H. W., and Avery, M. E., Inhibition of cell division associated with accelerated differentiation in hydrocortisone-treated fetal rabbits (abstract). *Fed. Proc.* **31**, 154 (1972).

43. Birch, H. G. The problem of "brain damage" in children. In "Brain Damage in Children" (H. G. Birch, Ed.), Williams & Wilkins, Baltimore, 1964.

44. Huttenlocher, P. R. Dendritic development in neocortex of children with mental defect and with infantile spasms. *Neurology* **24**, 203 (1974).

45. Winick, M., and Coscia, A. Cortisone-induced growth failure in neonatal rats. *Pediat. Res.* **2**, 451 (1968).