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# EFFECT OF THYROXINE AND CORTISOL ON BRAIN ORNITHINE DECARBOXYLASE ACTIVITY AND SWIMMING BEHAVIOR IN DEVELOPING RAT*

THOMAS R. ANDERSON and SAUL M. SCHANBERG†

Laboratory of Neuropharmacology, Center for Cerebrovascular Research and Department of Pharmacology, Duke University Medical Center and Veterans Administration Hospital, Durham, N.C. U.S.A.

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**Abstract**—Thyroxine and cortisol were tested to determine their effects on the ontogenetic pattern of ornithine decarboxylase (ODC) (EC 4.1.1.17) activity in rat brain. The effects of both hormones were significant, but varied with the brain region studied and with the age of the rat. Thyroxine (1 μg/g body wt/day injected i.p. × 3) altered ODC activity in all brain regions studied, if given during the first 10 postnatal days The overall effect was to compress the time course in which ODC activity passed through its ontogenetic pattern; both the peak in activity and the drop to the characteristic low adult activity level occurred earlier and were accelerated. The magnitude of the response to thyroxine was greater in the cerebellum, followed by the cerebrum and brainstem respectively. Cortisol, when given on the first postnatal day (0·5 mg i.p. × 1), initially depressed ODC activity and subsequently prolonged the time required to reach the low level of activity found normally in adult brain. The magnitude of the response to cortisol generally was greater than that of thyroxine, except in the cerebellum. The effects of these hormones on the ontogenetic pattern of ODC activity are consistent with previously observed effects in rats both on biochemical parameters, e.g. polyamine content and nucleic acid synthesis, and behavioral parameters, e.g. development of swimming ability. Our data are consistent with the hypothesis that thyroxine and cortisol, when administered to the neonate, may exert some of their effects on nucleic acid metabolism and ultimately growth, development and behaviour via their effects on polyamine metabolism.

Ornithine decarboxylase (ODC) (L-ornithine carboxylyase; EC 4.1.1.17) catalyzes the first step in polyamine biosynthesis, which is the conversion of L-ornithine to putrescine [1]. Our recent demonstration that the activity of this enzyme changes markedly during perinatal development of the rat brain [2] and that these changes correlate with those of the polyamine spermidine [3] is in agreement with considerable evidence showing a dramatic increase in the activity of the enzyme very early in the transition of tissues from a non-growing to a growing state [4]. Much evidence in the literature suggests that polyamines influence nucleic acid metabolism [1], and that they accumulate in parallel with RNA in many mammalian systems [5]. In normally developing rat brain, changes in spermidine concentration with time are related directly to net RNA and DNA synthesis [4].

To a great degree, hormones affecting growth and development act in the cell nucleus by inducing the synthesis of additional RNA [6]. Growth hormone administration induce a simultaneous increase in ODC and RNA polymerase activity in rat liver [7], and three other hormones have been shown to induce ODC in the appropriate target tissue: testosterone in the prostate [8], estradiol in the uterus [9], and LH in the ovary [10]. It has recently been shown that thyroxine and cortisol alter patterns of cell growth in developing rat brain [11, 12], and that these hormones also affect behavioral and neurophysiological patterns of development [13, 14].

It is the purpose of this study to examine the effects of thyroxine and cortisol on ODC activity and to see how closely these effects correlate with those on cell growth and on behavior.

## METHODS

Each litter of Sprague–Dawley rats (Zivic–Miller) was divided into an experimental and a control group, and kept with their respective mothers. The experimental rats received subcutaneous injections (0·1 ml) of either thyroxine sodium dissolved in 0·01 N NaOH or cortisol acetate suspended with Tween 80 in 0·9% NaCl solution. In all experiments involving thyroxine, a dose of 1 μg/g body wt was administered. Cortisol

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was administered either as a single injection of 0·5 mg the day after birth, or as a series of daily injections of 10 μg/g body wt (doses of 0·5 mg were not used in animals 1- to 2-weeks-old because of their high mortality due to a decreasing $LD_{50}$). The postnatal age of the rats was assigned by taking conceptual day 22 as day 0.

The development of swimming behavior in rats was determined in a rectangular 15-gal fish tank (1ft × 2ft × 1ft high) according to the criteria used by Schapiro *et al.* [13].

At various times, rats were killed by decapitation and the brains quickly removed and placed on ice-cold glass plates. Brain regions were obtained by dissecting the brain into cerebral hemispheres, cerebellum and brainstem. Tissues were extracted for ODC according to the method previously described [2], except 40—rather than 20—parts (w/v) of cold (5°) 10 mM *N*-Tris (hydroxymethyl) methyl-2-aminoethane sulphonic acid (TES) buffer was used for the homogenation.

The activity of ODC was determined by assaying for $^{14}CO_2$. The incubation medium for the cerebral hemispheres and brainstem contained 20 μmoles TES buffer (pH 7·2), 0·05 μmole pyridoxal phosphate/ml, about 2–3 mg undialyzed supernatant protein, and 0·5 μCi DL-[1-$^{14}$C]ornithine (11·9 mCi/nmole) in a total volume of 2 ml. For the cerebellum, the same concentration of substances was used, except that the total volume of the incubation mixture was reduced to 1 ml. The $^{14}CO_2$ released was collected and counted as described previously [2]. Incubation was for 40 min at 37° in a shaking water bath. The kinetics of the reaction was linear over the incubation period. The Student's *t*-test was used for the statistical analysis.

The assay for ODC previously has been verified with column chromatography by isolating the putrescine product of the reaction [2].

DL-[1-$^{14}$C]ornithine (sp. act. 11·9 mCi/nmole) was obtained from New England Nuclear Corp. (Boston, Mass.). Thyroxine sodium and hydrocortisone acetate were obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio); pyridoxal 5′-phosphate was from Sigma Chemical Co. (St. Louis, Mo.); and *N*–Tris (hydroxymethyl) methyl-2-aminoethane sulphonic acid (TES) was from CalBiochem (Los Angeles, Calif.).

## RESULTS

*Effect of early postnatal administration of thyroxine and cortisol on tissue wet weight in developing brain.* The effects of thyroxine and cortisol treatment on the wet weights of the major brain regions during postnatal development are summarized in Fig. 1. In general, the rate of increase in wet weight from 3 to 12 postnatal days was highest in the cerebral hemispheres, followed in decreasing order by brainstem and cerebellum, regardless of treatment. However, the absolute increase in cerebellar weight during this period was more than six-fold compared to a three-fold or less increase for brainstem and cerebral hemispheres. The effect of thyroxine on brain weight did not become apparent until

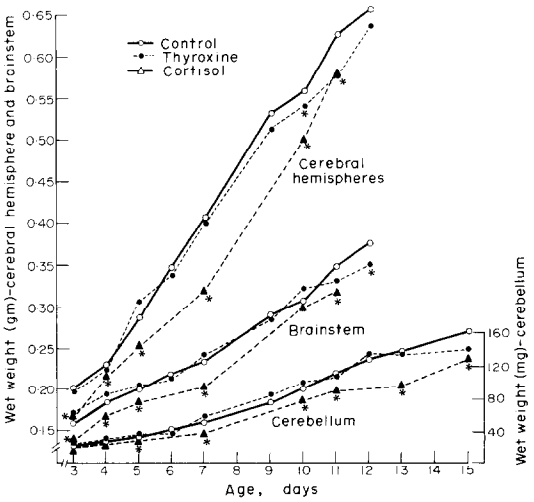


Fig. 1. Effect of thyroxine or cortisol on the wet weight of the major regions of developing rat brain plotted as a function of postnatal age. Either a single injection of 1 μg/g body wt s.c. of thyroxine sodium was given daily 2–4 postnatal days, or a single injection of 0·5 mg cortisol acetate was given 1 day postnatally. The asterisks indicate points where experimental animals differed significantly from controls with $P < 0.001$ to $< 0.05$. For control and experimental rats, each point represents the mean of 10–25 and 6–8 determinations respectively. The S.E.M. was generally ± 5 per cent or less of the mean value

the end of the 2nd postnatal week. A significant loss in weight was not seen in cerebral hemispheres, brainstem and cerebellum until 10, 12 and 15 postnatal days respectively. Cortisol treatment caused a significant reduction in brain weight throughout the development period studied. The weight of the cerebral hemispheres and brainstem was reduced several days earlier than that of the cerebellum.

*Effect of early postnatal administration of thyroxine and cortisol on the activity of ornithine decarboxylase in developing brain.* The effects of thyroxine and cortisol on ODC activity are shown in Fig. 2. Graph a represents the cerebral hemispheres. In control rats, ODC activity was high initially and declined rapidly to low adult values by day 11. Thyroxine treatment elevated ODC activity relative to controls from 3 to 6 postnatal days. Activity then declined significantly, and remained below control levels until adult values were reached at 11 postnatal days. Conversely, cortisol treatment significantly reduced the level of ODC activity relative to controls from days 2 to 4 postnatally. Activity then increased to a magnitude significantly greater than that of controls at postnatal day 5 and remained significantly above control levels, but declined in parallel with them from postnatal day 5 until the final measurement at day 11.

The effects of thyroxine and cortisol on brainstem are shown in graph b. Thyroxine treatment did not

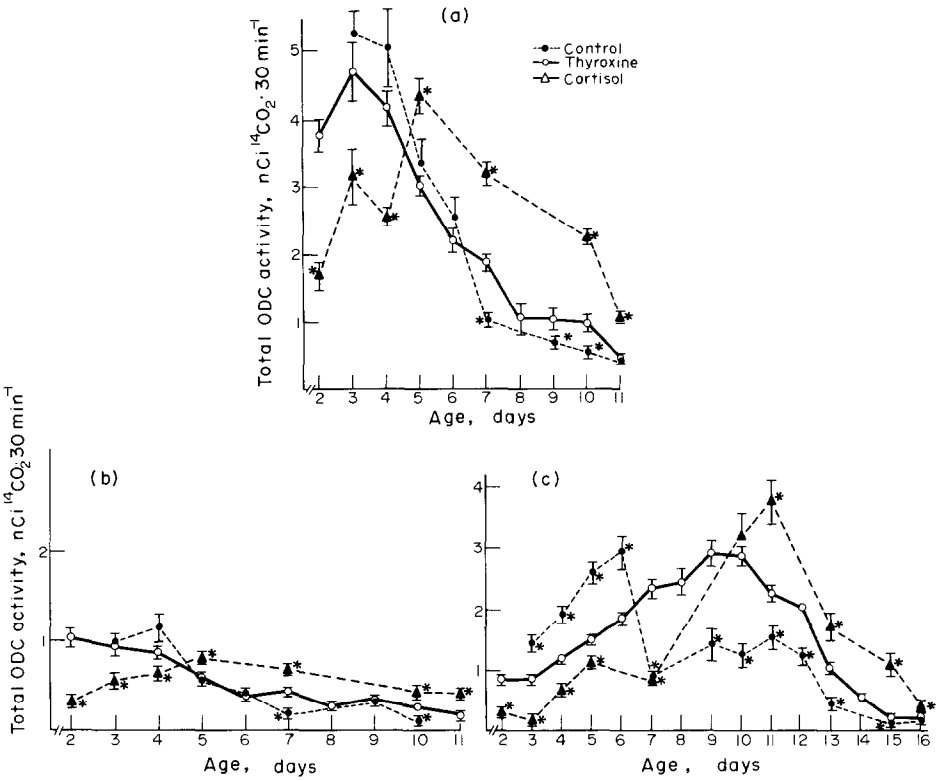


Fig. 2. Effect of hormones on ODC activity in cerebral hemispheres (graph a), brainstem (graph b), and cerebellum (graph c) of developing rats. Hormone dosage and time of administration were the same as for Fig. 1. Each point represents the mean of 10–25 and 6–8 determinations for control and experimental rats respectively. Vertical bars represent the S.E.M. The statistical significance varied from $P < 0.001$ to $< 0.05$ for points indicated by an asterisk.

affect consistently the ontogenetic pattern of ODC activity relative to controls. However, the effect of cortisol was similar to that seen in the cerebral hemispheres. ODC activity was reduced significantly from 2 to 4 days postnatally, surpassed control level of activity at day 5, and remained elevated significantly through day 11. The effects of thyroxine and cortisol on the cerebellum are shown in graph c. Cerebellar tissue was analyzed for activity through day 16 because of the later development of this brain region. In contrast to cerebral hemispheres and brainstem, the control level of ODC activity increased rather than decreased from 2 to 10 postnatal days. The effect of thyroxine treatment on ODC activity in the cerebellum was similar to that in the cerebral hemispheres, but the response was much more marked in the former. Activity remained significantly above control levels from 3 to 6 postnatal days, then rapidly declined to levels significantly less than that of controls at day 7 and remained suppressed until adult values were reached by day 15. The over-all effect of thyroxine treatment was to compress the time-course in which ODC activity passed through its ontogenetic pattern; both the peak in activity and the drop to the characteristic low adult activity level occurred earlier and were accelerated. The effect of cortisol on the pattern of ODC activity in cerebellum was similar to that of cerebral hemispheres and brainstem. Activity was reduced significantly by cortisol from 2 to 7 postnatal days but significantly surpassed that of controls at postnatal day 11, and was still elevated at the final measurement on day 16. The over-all effect of cortisol treatment was to delay and spread out the normal ontogenetic pattern of ODC activity.

The magnitude of the response to cortisol was significant at most points in all three brain regions, and was greater than that of thyroxine, except in cerebellum.

*Critical periods of sensitivity of developing brain to the effects of thyroxine and cortisol on ornithine decarboxylase activity.* Rats were treated with thyroxine or cortisol at various postnatal times to determine the development period in which ODC activity was influenced most by drugs. In general, thyroxine was effective in all brain regions studied if given during the first 10 postnatal days (Fig. 3). The magnitude of the

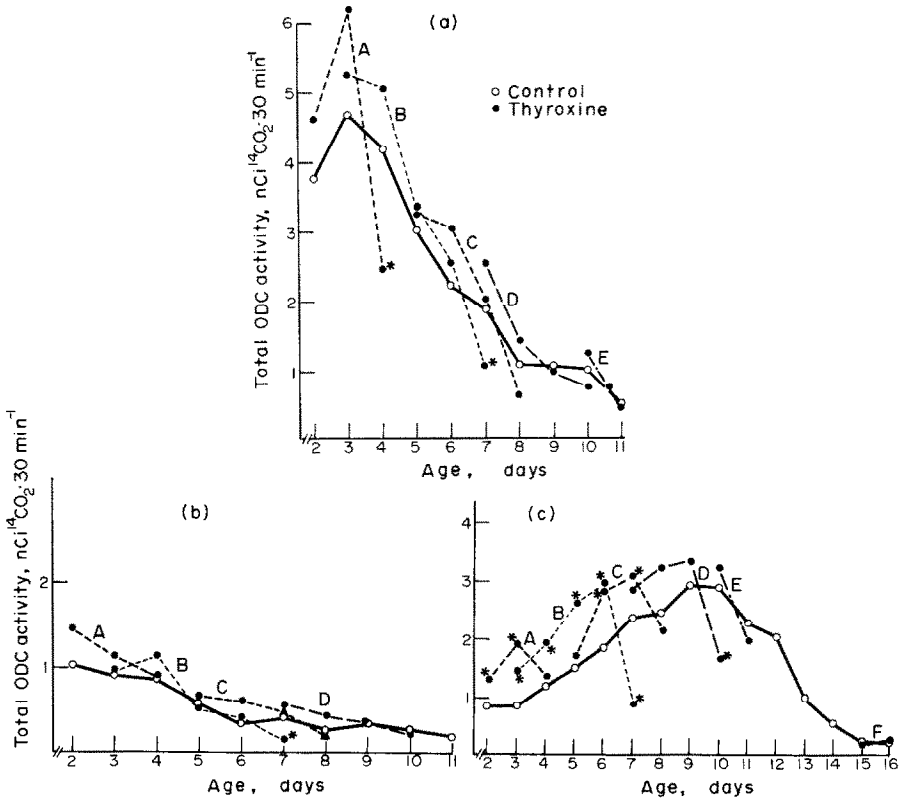


Fig. 3. Period of sensitivity of developing rat brain to the effects of thyroxine on ODC activity. Cerebral hemispheres, (graph a); brain stem, (graph b); and cerebellum, (graph c). In each group, the rats were injected daily for 3 consecutive days with 1 μg/g body wt of thyroxine sodium. The injections were 0–2 days for group A, 2–4 days for B, 4–6 days for C, 6–8 days for D, 9–11 days for E and days 14–16 for F. The rats of each point were injected at least 24 hr before kill. The control curves are the same as in Fig. 2. For experimental rats, each point represents the mean of 6–8 determinations. The S.E.M. was generally ± 15 per cent or less of the mean value, except for the thyroxine value at day 3 where the S.E.M. was ± 20 per cent of the mean value. The statistical significance varied from $P < 0.001$ to $< 0.05$ for points indicated by an asterisk.

response throughout this period, however, was in the cerebellum (graph c), followed by the cerebral hemispheres (graph a), and brainstem (graph b). The earlier the administration of thyroxine the greater and more sustained was the response. Cortisol, in contrast, still was quite effective in altering the ODC activity pattern when administered 2 weeks postnatally (Fig. 4).

*Effect of thyroxine and cortisol on swimming behavior of infant rats.* The effect of thyroxine and cortisol administration on the swimming behavior of infant rats is summarized in Fig. 5. Over-all, thyroxine advanced by about 2 days the age at which infant rats were able to keep their heads completely above water. Conversely, cortisol retarded the attainment of the adult swimming posture.

## DISCUSSION

*Effect of thyroxine on ODC activity.* Considerable clinical and histologic evidence demonstrates that thyroxine is necessary for normal growth and differentiation of the CNS [15]. Biochemical investigations indicate that one of the early effects of excess thyroxine on rat brain maturation is an increase in RNA, protein and DNA synthesis [12, 16], followed in the second and third postnatal weeks by a reduction in the synthesis of these substances [12]. Recently, these alterations in nucleic acid metabolism have been shown to result in a permanent decrease in the number of cells in the cerebellum [11]. It is clear from the present study that the influence of thyroxine on ODC activity follows the same pattern as that observed for nucleic acid and protein synthesis associated with microneurogenesis [11, 12]. However, the onset of the periods of enhanced and suppressed ODC activity precede the periods described for the alteration of nucleic acid and protein synthesis.

The period during postnatal development when ODC activity is responsive to the thyroxine treatment

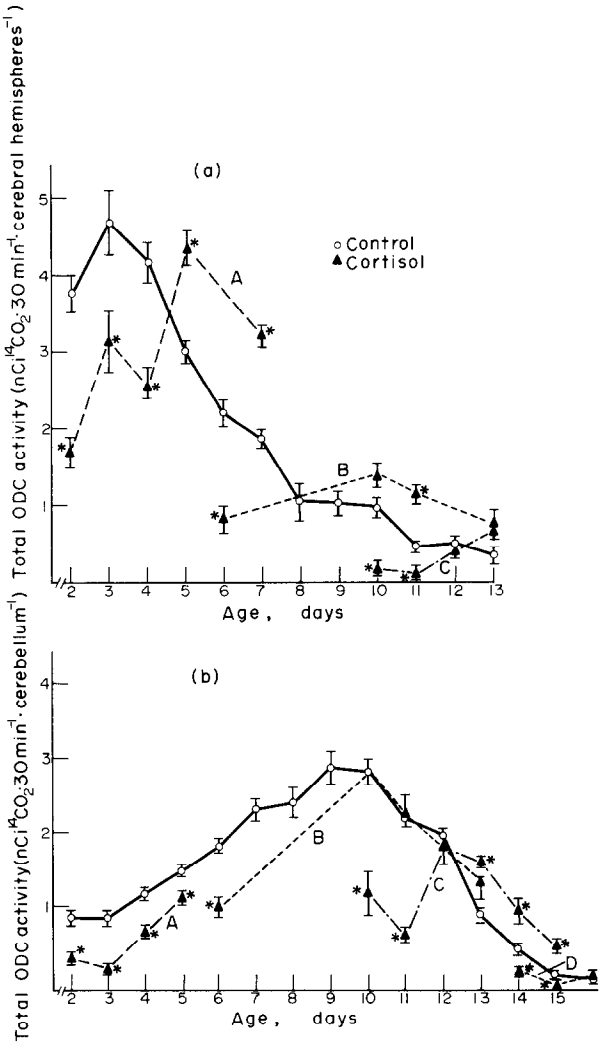


Fig. 4. Period of sensitivity of developing rat brain to the effects of cortisol on ODC activity. Cerebral hemispheres, graph a; and cerebellum, graph b. In group A, the rats were injected with 0·5 mg cortisol acetate 1 day postnatally; in groups B–D, rats received daily injections of 10 μg/g body wt for 3–4 consecutive days; in group B, injections were from 3 to 6 days, in C, from 8 to 10 days and in D from 13 to 15 days. The rats of each point were injected at least 24 hr before kill. The control curve is the same as in Fig. 2. For experimental rats, each point represents the mean of 6–8 determinations. Vertical bars represent the S.E.M. The statistical significance varied from $P < 0{\cdot}001$ to $< 0{\cdot}05$ for points indicated by an asterisk.

(Fig. 3) corresponds to the period when ODC is most active and when thyroxine is most critical for the normal development of the CNS [17].

*Effect of cortisol.* The effect of cortisol on levels of ODC activity is the inverse of that of thyroxine. The suppression in ODC activity correlates with the suppression in both the deposition of DNA and the rate of DNA synthesis which occurs during the period of cortisol administration to infant rats [18]. The enhancement in ODC activity correlates with but precedes the enhancement in DNA deposition and rate of DNA synthesis [18]. This enhancement may reflect an attempt by the system to compensate for the earlier suppressive effect of cortisol on cell proliferation. The magnitude of the response of ODC activity to cortisol relative to controls generally was greater than that of thyroxine, except in the cerebellum. As cell proliferation is high in the forebrain of the cerebral hemispheres at birth and low in the cerebellum [19], it is reasonable that early treatment with cortisol would have a more pronounced suppressive effect on the forebrain. Whereas the initial action of thyroxine to increase ODC is somewhat delayed (24–36 hr) it is not surprising that the cerebellum, which proliferates and

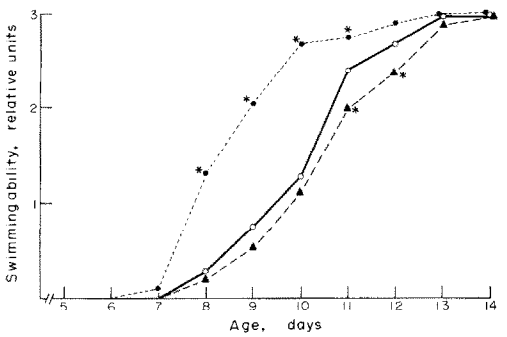


Fig. 5. Effect of thyroxine (●···●) or cortisol (▲--▲) on the development of swimming ability in infant rats. Each point represents the mean of 16–25 determinations. The S.E.M. was generally ± 20 per cent or less of the mean value. The statistical significance varied from $P < 0.001$ to $< 0.05$ for points indicated by an asterisk.

differentiates primarily after birth, is more sensitive to the action of thyroxine than the cerebral hemispheres which already have undergone a major portion of their development before birth. Since cell proliferation in the brainstem at this time is low, these hormones have a correspondingly less significant effect on this brain region.

Cortisol has no clear-cut "critical" period relative to ODC activity which agrees with the critical period attributed to the action of the hormones on brain excitability [20]. The effect of cortisol follows the same pattern and is of similar magnitude during the entire developmental period (Fig. 4).

The rapid increase in the wet weight of the brain during the first 2 postnatal weeks is due mostly to new cell formation and cell enlargement [21]. The initially slow rate of increase in the wet weight of the cerebellum is consistent with morphological observations showing the late development of this brain region; the higher rate of growth during the second postnatal week reflects an accelerated rate of cell proliferation in the cerebellar cortex [22]. In both thyroxine- and cortisol-treated rats, the reduced weight of the brain regions studied appears to be due to a reduced cell number rather than reduced cell size, as the hormones do not alter the total cellular content of protein and RNA but significantly reduce the content of brain DNA [12, 17].

The consequent acceleration and retardation in the ontogenetic pattern of ODC activity by thyroxine and cortisol, respectively, correlate well with the corresponding effect these two hormones have on the maturation of swimming behavior in infant rats as first demonstrated by Schapiro *et al.* [13] and repeated in part in this study (Fig. 5). A similar pattern of effects resulting from hormone treatment has been shown for other indices of brain function. While thyroxine treatment seems to advance the appearance of the startle reflex and the cortical EEG as well as enhance spontaneous locomotor activity, cortisol retards the appearance of these functions [13, 23]. As both thyroxine and cortisol treatments reduce the final cell number in rat brain, an explanation for their opposing effects on behavior probably lies either in the stage of development affected or in the specificity of their action with regard to cell types.

These present data show that the effect of administered thyroxine and cortisol on ODC activity precedes and correlates well with the pattern of their effects on the rate of synthesis and accumulation of DNA, and on the development of swimming behavior in rats. Recently, we have demonstrated that these alterations in ODC were accompanied by immediate changes in the content of putrescene and subsequent changes in the contents of spermidine and spermine [24]. As alterations in the content of polyamines have been shown to modify nucleic acid metabolism in various tissues [4, 5], the data are consistent with the hypothesis that thyroxine and cortisol, when administered early in the postnatal period, may exert their effects on nucleic acid metabolism and ultimately growth, development and behavior via their effects on polyamine metabolism.

The actions of the hormones are not necessarily specific to ODC. Berlin and Schimke [25] have shown that, in a situation where an inducing agent stimulates general protein synthesis, enzymes with a fast turnover increase much more rapidly than those with a slow turnover. Hence, since ODC has an extremely short half-life, a general stimulation of all protein synthesis could lead to a rapid increase in ODC. However, as indicated by Tabor and Tabor [26], although such an increase in an enzyme may be non-specific, it still could be physiologically important. The fast turnover of ODC would enable it to increase quickly preparatory to rapid growth without the need of specific induction.

Since the brain is very sensitive to imbalances in the hormonal environment and to external factors such as nutrition [27], handling of neonates [28], and radiation [29] during periods of rapid neurogenesis, an assay of ODC activity after an insult to the brain or a change in the internal or external environment may prove a quick and sensitive means of determining when and in which brain regions neurogenesis has been affected.

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