Neonatal hyperthyroidism: Maturation acceleration and learning deficit in triiodothyronine-stimulated rats

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Rat pups injected on Postnatal Days 2-4 with subcutaneous doses of triiodothyronine (T_3) up to 4 micrograms/g showed substantial accelerations in the maturation of swimming behavior, righting reflexes, and eye opening which were greater than the accelerations produced by neonatal thyroxine (T_4) in a previous study. They also showed significantly higher activity in stabilimeter cages on Postnatal Day 13 but not an expected earlier peak than normal rats in the ontogeny-of-arousal functions obtained in stabilimeter testing. As adults, the T_3 -treated rats displayed large maze learning deficits which were comparable in size to those produced by fairly severe thyroid deficiency in the perinatal period.

A previous study (Davenport & Gonzalez, 1973), which extended initial findings by Eayrs (1964) and Schapiro (1968), has shown that when hyperthyroidism is induced neonatally in rats by injections of thyroxine (T_4 , 2 micrograms/g body weight, SC, Postnatal Days 2-5), the rat pups show accelerated behavioral maturation in the second and third postnatal weeks but in later life they are deficient in maze learning. The accelerations appeared between the 7th and 20th postnatal days in swimming behavior, righting reflexes, and age of eye opening, and learning deficits appeared in passive avoidance testing at Days 35 and 36 as well as in symmetrical maze problems at the ages of 70-85 and 180-195 days. The early T_4 treatment also induced a 13% decrement in physical growth in terms of body weight at 230 days of age.

While the behavioral effects found by Davenport and Gonzalez were consistent and statistically significant, both the early accelerations and the subsequent maze learning deficits were small in magnitude, perhaps leaving the impression that behavioral abnormalities resulting from an excess of thyroid hormone around the time of birth constitute a much less serious developmental problem than those produced by perinatal thyroid deficiency, as indicated by intellectual deficits in human cretinism (e.g., Wilkins, 1965) or experimental studies of early hypothyroidism in rats (e.g., Davenport & Dorsey, 1972; Eayrs, 1961). The T_4 dose used by Davenport and Gonzalez (1973), however, may be regarded as near the low end of a dose-response continuum, as was that (1 microgram/g, IP) used by Schapiro (1968). The more potent and rapid-acting thyroid hormone, triiodothyronine (T_3), was used by Eayrs (1964, 1968), in doses up to 5 micrograms/g, and these manipulations yielded an important indication of

more severe maze learning deficit than in the T_4 studies.

In the present research, we have examined the effects of T_3 , given on Postnatal Days 2-4, in doses of 1, 2, and 4 micrograms/g; the highest of these proved to be a lethal dose for over 50% of the pups. To the measures of behavioral acceleration employed by Davenport and Gonzalez (1973), we have added stabilimeter activity in order to determine the effect of neonatal hyperthyroidism on what Campbell and his associates (Campbell, Lytle, & Fibiger, 1969; Campbell & Mabry, 1972) call the ontogeny of behavioral arousal. Despite its obviously gross nature, this type of dependent variable is useful in studying behavioral maturation because of the characteristic ontogenetic pattern in stabilimeter activity displayed in normal rat pups—a steep rise from near-zero activity at Postnatal Day 7 to a peak around Day 15, followed by a sharp decline to low activity levels in the 4th week. The work by Campbell's group has indicated that this pattern reflects, among other maturational processes, the sequential development of adrenergic, serotonergic, and cholinergic mechanisms in the central nervous system (CNS). In view of the maturational accelerations in swimming and reflexes already demonstrated in neonatally hyperthyroid rats, and because we have obtained preliminary data in 47 rats showing delays in the stabilimeter activity pattern in early hypothyroidism, we were particularly interested in whether neonatal T_3 would produce an earlier-than-normal peak in arousal and perhaps otherwise alter this ontogenetic function in such a way as to shed some light on the role of thyroid hormone in the development of important neurotransmitter systems.

METHOD

Subjects and Rearing Conditions

Pregnant rats purchased from the Holtzman Company, Madison, Wisconsin, were housed in 46 x 25 x 15 cm plastic nesting cages. After birth, litter size was reduced to eight, and,

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within each of six litters, the pups were randomly assigned to the control and three T_3 dose conditions. The pups received T_3 (3,3,5-triiodo-L-thyronine, ICN Nutritional Biochemicals Corp., doses of 1, 2, or 4 micrograms/g body weight) or the saline vehicle in daily subcutaneous injections on Postnatal Days 2-4. The original Ns in the four conditions were 12/group (six of each sex), but during the week following injections, one, one, four, and nine pups died in control and 1-4 micrograms/g T_3 groups, respectively.

Thereafter, procedures closely followed those used by Davenport and Gonzalez (1973) except for the added stabilimeter testing, the omission of avoidance conditioning tasks, and the age at which maze testing was conducted. After the behavioral testing in the first 3 weeks, the rats were weaned on Day 30 and housed individually under ad lib feeding conditions until they were 178 days old, when feeding was restricted so as to bring weights down to 85% of normal free-feeding values in preparation for the maze testing.

Behavioral Testing

The pups were tested for stabilimeter activity daily on Days 7-20, in the swimming task daily on Days 7-15, for the grasp-hold reflex on Alternate Days 7-19, and for the righting reflex on Alternate Days 8-18. On each day in which three tests were given, the order was stabilimeter activity (10-min session length), followed by one of the reflex tests (three trials), and then the swimming task (one trial), a regimen which took no longer than 20 min for a given squad of four pups. Since the pups were poikilothermic in the early days of this testing, they were returned to their nesting cages between the activity and reflex testing for a few minutes on all days. Procedures for the swimming and reflex testing are described in detail by Davenport and Gonzalez (1973). The pups were observed for eye opening during daily weighing in this early testing phase.

For the activity testing, four identical stabilimeter cages, constructed of aluminum (floors and ceilings) and wire mesh (side walls), were used. In size (17.2 x 9.8 x 9.2 cm), they were larger than those used by Campbell and Mabry (1972) for 5-15-day old rats and smaller than those which they used for rats over 15 days of age. Each cage was mounted on a central fulcrum that allowed the cage to tilt with shifts in weight. Electronic contact relays (BRS/LVE DR 901/221-05), connected to electromechanical counters in an adjacent room, provided for monitoring of the activity. The cages were located in separate visually isolated compartments of a BRS/LVE squirrel monkey test chamber which had an exhaust fan for effective masking of sounds from the rest of the laboratory. Each compartment was illuminated by a bare type 1819 bulb powered by 20 V ac approximately 25 cm above the stabilimeter cage ceiling. The bases of the cages rested on heating pads which maintained the air temperature in the compartments within the range of 29°-31°C.

Tests in the symmetrical maze, described elsewhere (Davenport, Hagquist, & Rankin, 1970), were started when the rats were 186 days old. After magazine training (two 40-pellet sessions), the four standard practice problems were given (12 trials each), followed by seven test problems (T 6, T 9, T 10, T 8, T 7, T 11, and T 12), each of which was run in a single session to the usual criterion of five consecutive trials containing at least four errorless trials or (if criterion was not attained by the rat) a maximum of 48 trials. One additional death occurred, during the practice problems, in the male 2 micrograms/g T_3 subgroup.

RESULTS AND DISCUSSION

Overall Development

Striking abnormalities in skeletal maturation and physical growth occurred in the T_3 -injected rats which

were easily visible within the first 2 postnatal weeks and in adulthood. These effects, which did not appear in our T_4 -stimulated rats (Davenport & Gonzalez, 1973), conformed closely to some verbal and photographic descriptions of T_3 -treated rats by Eayrs (1966, 1968). Among the most prominent changes at preweaning ages were accelerated development of the pinnae, elongation of the snout, and lengthening of the spine and tail, combined with reduced body weight and an overall thin appearance (best described as emaciation in the cases of the pups which died in the week after injections). As adults, most of the survivors showed a somewhat deformed facial appearance from faulty growth of the nasal bones, overall abnormalities in bodily proportions, and permanently arrested growth. One of the three survivors in the highest dose group and the rat which died during maze testing displayed obvious motor defects in adulthood involving postural asymmetry and whirling behavior which resembled symptoms of middle ear disease.

The dose-related deficits in terms of body weight are shown in Figure 1. Group differences within each sex were highly significant at all ages in F tests, and the deficits in growth were clearly greater than in our T_4 studies. Best and Duncan (1969), however, have shown adult body-weight deficits from eight IP injections of 27 micrograms T_4 over the first 2 postnatal weeks which are comparable to those resulting from 2 micrograms/g T_3 in the present study.

Precocious eye opening occurred in the T_3 pups (Figure 2), in agreement with several previous studies of neonatal hyperthyroidism, and like the body-weight differences, this effect was dose related. In Fisher least significance difference (LSD) tests following analysis of variance of the eye-opening data ($F = 69.56$, $df = 3/29$, $p < .001$), the highest-dose group (for which N was 3 in the analysis, whereas Figure 1 includes a fourth pup which died on Day 12) showed significantly ($p < .01$) earlier eye opening than both the 1 microgram/g T_3 group and the controls. Considering the lethality of our highest dose, the 4-day acceleration of eye opening found here probably represents the maximum precocity that can result from neonatal hyperthyroidism.

During the week following injections, most of the T_3 pups displayed tremor which was particularly marked in their appendages. This subsided gradually over Days 9-12.

Reflexes

The groups did not differ in the grasp-hold reflex, perhaps because the T_3 pups were handicapped in this task by the just-noted muscular tremor in their forelimbs. Acceleration was found, however, in the righting reflex (Figure 3). The overall group differences in righting were significant ($F = 8.05$, $df = 3/29$, $p < .001$, for total righting responses over Days 8-18); subsequent LSD tests showed reliable T_3 -control differences in all three comparisons ($p < .01$) but no

Figure 1. Mean body weights of the female (left) and male (right) groups from weaning to Day 178.

Figure 2. Histograms showing ages of eye opening in the four groups.

significant differences among the T₃ groups. Again, the T₃ effect was greater than the righting-reflex acceleration found with T₄ (Davenport & Gonzalez, 1973).

Swimming

We also found much greater acceleration of swimming behavior in these T₃ pups than in our T₄-stimulated rats. In fact, as Figure 4 shows, the T₃ animals were relatively

proficient at swimming on the first test at 7 days of age. Analysis of the sums of swimming scores over Days 7-15 reflected the large overall group differences ($F = 19.75$, $df = 3/29$, $p < .001$); again, by LSD tests, only T₃-control differences were significant ($p < .01$).

Stabilimeter Activity

Ontogeny-of-arousal functions which assumed the same general trends over age levels as in Campbell's rat studies of stabilimeter activity are shown in Figure 5. Analysis of these data revealed reliable groups ($F = 2.99$, $df = 3/29$, $p < .05$) and days ($F = 21.34$, $df = 13/377$, $p < .001$) effects with no significant interaction ($F < 1.0$). Subsequent one-way analyses of each day separately (variability changed over days) showed significant ($p < .05$) group differences only on Day 13 ($F = 3.17$), and on this day, the control group displayed reliably ($p < .01$) lower activity than the 1- and 2-micrograms/g T₃ groups; the highest dose pups more closely resembled the other T₃ groups than the controls in their performance.

These results showed generally higher activity in the T₃ rats but clearly did not conform to our original expectation of an earlier peak of stabilimeter activity in such rats. If anything, Figure 5 suggests that the controls peaked a day or two earlier than the T₃ groups, and that acceleration only occurred with respect to activity levels prior to the peaks. The figure also suggests some working hypotheses related to the maturation of neurotransmitter mechanisms in thyroid-altered rats. While the generally higher activity scores on Days 8-15 may have been a manifestation of accelerated

Figure 3. Acceleration of the righting reflex by neonatal T₃.

Figure 4. Mean swimming scores of the four groups over Days 7 through 15.

Figure 5. Mean stabilimeter activity scores of the four groups over Days 7 through 20.

noradrenergic mechanisms, much of the T_3 -control difference prior to Day 13 can be accounted for by the T_3 -induced tremor (which probably triggered some extra counts when a pup was near the fulcrum of the stabilimeter cage), and the remaining portion of the difference is perhaps better interpreted in terms of accelerated motor capacity, in accordance with Nicholson and Altman's (1972) finding of accelerated synaptogenesis in (presumably nonnoradrenergic pathways of) the cerebellum in neonatally hyperthyroid rats. The interesting possibility remains, however, that our failure to find an earlier peak of arousal in the T_3 rats stemmed from a deficiency in the hippocampal serotonergic system which Mabry and Campbell (1974) hypothesize as the first major inhibitory mechanism to emerge, around 15 days of age in the normal rat, in counteraction to the noradrenergically mediated activation of behavior which develops earlier. Such a deficiency could result from the same processes, apparently involving premature termination of neuronal proliferation, by which synaptogenesis is eventually reduced by early hyperthyroidism in the cerebellum at later ages (Nicholson & Altman, 1972) and seemingly at earlier ages in the cerebral cortex (Balazs, Cocks, Eayrs, & Kovacs, 1971). Drug challenges, such as Mabry and Campbell's administrations of parachlorophenylalanine

and amphetamine to rats in stabilimeter cages at varying preweaning ages, might shed considerable light on this possible serotonergic defect in T_3 -treated rats.

Maze Learning

Figure 6 shows deficits in symmetrical maze performance which appeared in the T_3 rats in adulthood. Since sex differences in favor of better learning by male rats nearly always show up in this apparatus, error scores (totaled over four problems) for males and females are distinguished in the figure. The extreme error scores of the 4 micrograms/g T_3 rats were not included in the statistical analysis.

Among the other three conditions, significant differences in error scores appeared in both males ($F = 4.14$, $df = 2/12$, $p < .05$) and females ($F = 9.70$, $df = 2/11$, $p < .005$). LSD tests showed reliably better performance by the control subgroups than by the 1 microgram/g ($p < .02$) and 2 micrograms/g ($p < .05$) T_3 subgroups in both sexes. Failures to achieve criterion within 48 trials were found on at least one of the seven problems in two female rats in the 1 microgram/g group, one female rat in the 2 micrograms/mg group, and all three 4 micrograms/g rats. Significant sex differences within the T_3 conditions are readily apparent from the dot distributions in Figure 6.

Figure 6. Total errors over seven symmetrical maze problems for individual rats. The solid and dashed lines connect mean values for the males and females, respectively.

The maze learning deficits found at all three doses of T_3 were considerably larger than those in the Davenport and Gonzalez (1973) T_4 study, particularly if comparison is made with respect to the errors on problems common to the two studies (T 6, T 9, T 10, and T 12). The deficits in the present work seem comparable in size to those in Eayrs' (1964) T_3 studies, to the extent that it is possible to judge by his T_3 -control difference scores obtained in a different (Hebb-Williams) maze.

More importantly, the present deficits were also comparable to those which we have found on the same problems of the symmetrical maze series in thyroid-deficient rats exposed to thiouracil throughout the pre- and postnatal critical period for the induction of learning deficit in rats (Davenport & Dorsey, 1972; Davenport, Gonzalez, Hennies, & Hagquist, in press). The clinical literature (e.g., Selenkew, Robbin, & Refetoff, 1969) clearly indicates that in Graves' disease during pregnancy, transplacental passage of the long-acting thyroid stimulator (LATS) readily occurs, rendering the offspring hyperthyroid prior to birth and for several weeks after birth. Considering the close correspondence of critical periods of hypo- and hyperthyroidism in rats (Eayrs, 1968) and the

probability that the critical period of thyroid deficiency begins at a relatively earlier fetal age in humans than in rats, this timing seems maximally unfortunate from the standpoint of the offspring's later intellectual development. The clinical literature is also sprinkled with case reports of accelerated maturation and (occasionally) mental retardation in such offspring (e.g., Riggs, Wilroy, & Etteldorf, 1972), but apparently no systematic survey of intelligence levels in perinatal-hyperthyroid children has been undertaken. Since there seems to be no reason for believing that the results of animal studies of early hyperthyroidism are any less applicable to thyroid disorders in human infancy than those from studies in early hypothyroid animals, further examination of the intelligence question seems mandatory. The size of the learning deficits found in the present study suggests that the intellectual consequences of perinatal hyperthyroidism in humans may be even more serious than heretofore suspected.

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