Effect of Neonatal Hypothyroidism and Delayed L-Triiodothyronine Treatment on Behavioural Activity and Norepinephrine and Dopamine Biosynthetic Systems in Discrete Regions of Rat Brain

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Abstract. The influence of neonatal hypothyroidism on norepinephrine and dopamine metabolism in certain discrete regions of rat brain was studied. Intraperitoneal administration of 131I in a dose of 200 µCi to 1-day-old rats significantly impaired the ontogenesis of spontaneous locomotor activity and reduced tyrosine hydroxylase activity (by 35%) in the striatal region. A parallel decrease in norepinephrine levels was observed in hypothalamus, pons-medulla and striatum. Thyroid deficiency in neonatal life also decreased dopamine levels as well as its metabolite, 3,4-dihydroxyphenylacetic acid, in striatal region. A comparable, but statistically non-significant, change was observed in homovanillic acid as well. Whereas neonatal hypothyroidism decreased monoamine oxidase activity (by 14%) in the hypothalamus, a rise was noted in the mid-brain region. Hypothyroidism in young rats significantly increased catechol-O-methyl transferase activity in brain stem, striatum and mid-brain; however, a 40% decline in O-methylating enzyme was observed in hypothalamus. Data suggest that low levels of norepinephrine, dopamine and their metabolites can be attributed to the decreased synthesis and utilization of these catecholamines in brain. Replacement therapy with L-triiodothyronine (10 µg/100 g, s.c.) for 25 days, beginning 5 days after radiothyroidectomy, enhanced tyrosine hydroxylase activity and raised norepinephrine, dopamine and 3,4-dihydroxyphenylacetic acid levels to virtually normal values. Furthermore, the locomotor activity was significantly increased in hypothyroid rats given L-triiodothyronine treatment in infancy. However, when the initiation of L-triiodothyronine treatment was postponed until adulthood, no significant change was observed in tyrosine hydroxylase or in norepinephrine and dopamine levels of rat brain. These findings are discussed in relation to the role of catecholamines in depressed behaviour seen during 'cretinoid' syndrome and the importance of thyroid hormone during the critical period of maturation of brain monoaminergic neurons.

Key words: Hypothyroidism — L-Triiodothyronine — Norepinephrine — Dopamine — Homovanillic acid — Tyrosine hydroxylase — Monoamine oxidase — Catechol-O-methyl transferase — Spontaneous locomotor activity

The role of brain biogenic amines in major psychiatric disorders and the incorporation of endocrine knowledge into related clinical and biological research continue to be problematic issues for contemporary psychiatric thought. In recent years, studies have been carried out to present evidence that biochemical and structural ontogeny of the central nervous system, which are determinants of adult neurophysiological and behavioural processes, are susceptible to insults inflicted by various external and internal factors, especially during sensitive periods of growth. Among internal factors, alterations in physiological levels of hormones during critical periods of development are believed to exert profound effects on psychophysiological processes and brain chemistry (Eayrs and Taylor, 1951). Excessive thyroid secretion is known to be associated with hyperexcitability, restlessness and emotional instability; if left untreated these symptoms clinically may lead to psychosis (Eayrs, 1960; Whybrow and Ferrell, 1974). Animal studies have recently shown that repeated exposure of rats to L-triiodothyronine (T3) since birth markedly increased the behavioural activity as well as brain catecholamines, indoleamine (Rastogi and Singhal, 1976) and acetylcholine metabolism (Rastogi et al., 1977). These neurochemical
Changes appear to be similar to those generally seen in manic patients (Goodwin and Sack, 1973). Further, we demonstrated that administration of T₃ in adulthood produced no significant effect on catecholaminergic and serotoninergic neuronal activities (Rastogi and Singhal, 1976). On the contrary, low hormone levels in early life have been demonstrated to decrease protein, lipids and nucleic acid metabolism only in maturing brain (Sokoloff, 1970; Gelber et al., 1964; Tata et al., 1963). Clinical studies have shown that frank hypothyroidism is usually, if not always, accompanied by depression and that depressed patients tend to have rather low thyroid hormone levels (Prange et al., 1969; Whybrow and Ferrell, 1974). Indeed, Hatotoni et al. (1977) found that a number of persistently depressed patients showed a latent hypothyroidism, possibly due to hypothalamo-pituitary dysfunction, and responded well to thyroid medication in addition to tricyclic antidepressant drugs. Recently, neonatal hypothyroidism induced either by ¹³¹I or methimazole was chosen to interfere with the developmental pattern of norepinephrine (NE) in whole brain (Rastogi and Singhal, 1974; Rastogi et al., 1976). The present study was undertaken to gain deeper insight into the regional changes in NE and dopamine (DA) of developing brain in response to altered thyroid secretion.

Materials and Methods
Pregnant Sprague-Dawley rats obtained from Biotrexing Labs, Ottawa were maintained in individual cages under constant environmental conditions (24°C, 60% relative humidity and a 12-h light-dark cycle) with free access to Master Laboratory Chow and water. The litter size was reduced to eight by discarding the extra number of pups. The runts were not included. The litters were weaned at 22 days of age. The following experimental procedures were undertaken.

Experimental Hypothyroidism. Neonatal hypothyroidism in rats was induced by a single injection of ¹³¹I in a dose of 200 μCi in a volume of 0.05 ml by the i.p. route as described previously (Rastogi and Singhal, 1974). Histological studies have shown that ¹³¹I at this dose produces complete destruction of thyroid gland without affecting parathyroid (Rastogi, 1975). The littermate controls were injected with equal volume of physiological saline. The mortality induced by radioiodine was about 25% mostly seen after 15 days of age. Prior to their death, data were not obtained from animals that displayed signs of toxicity (e.g., spontaneous locomotor activity). Post-mortem examination of dead animals revealed sloughing of intestinal mucosa.

Replacement Therapy With L-Triiodothyronine. In order to examine whether changes in various neurochemical parameters related to catecholamine metabolism seen in radiothyroidectomized rats were specific to thyroid hormone, groups of ¹³¹I-treated rats were injected s.c. with T₃ at the dose of 10 µg/100 g (Schwarz et al., 1972) for 5 days beginning 5 days after ¹³¹I injection. In another series of experiments, injection of T₃ in hypothyroid rats was postponed until 120 days after ¹³¹I injection to study the effect of delayed T₃ treatment on NE and DA metabolism in various brain regions. The corresponding control rats received an equal volume of the vehicle (0.02 N NaOH).

Measurement of Spontaneous Locomotor Activity. The spontaneous locomotor activity was estimated with the use of a Selective Activity Meter, model SE (Columbus Instruments, Columbus, Ohio). The quantitation of spontaneous locomotor activity was carried out under identical conditions at the same time of the day in a temperature-controlled, sound-proof room. Radio-iodine-, saline- or T₃-treated rats of the corresponding age group were placed individually in a cage, resting on the Selective Activity Meter, for 5 min for exploration before the actual recording of the activity was made over a 30-min session.

Sample Preparation and Assay Methods. The 'near-freezing' technique of Takahashi and Aprison (1964) was employed to kill the rats. Following decapitation, brains were rapidly excised, placed on a petri dish resting on crushed ice and stripped of adherent meningeal tissue and grossly visible blood vessels. The brain was dissected into seven specific regions as described in our previous report (Rastogi and Singhal, 1976). The striatum was homogenized in 20 vol. 0.28 M ice cold sucrose and the activity of tyrosine hydroxylase (TH) was determined under linear kinetic conditions according to the procedure of McGeer et al. (1967) with modifications as described previously (Rastogi and Singhal, 1974). Endogenous levels of tyrosine (TR) in striatum were determined as described by McGeer et al. (1967) modified from Waukes and Udenfriend (1957). The activity of catechol-O-methyl transferase (COMT) and monoamine oxidase (MAO) was determined in several brain regions homogenized in 20 vol. ice cold 0.28 M sucrose according to the method of McCaman (1965) and Wurtman and Axelrod (1963), respectively, as has been described previously (Rastogi and Singhal, 1976). The levels of NE and DA were determined according to the methods of Maickel et al. (1968) and Spano and Neff (1971), respectively. For measuring the concentrations of homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), the striata were pooled from two rats and assays were carried out according to the method of Murphy et al. (1969) as described previously (Rastogi and Singhal, 1976).

Data were statistically evaluated by a two-way analysis of variance using logarithmically transformed raw scores. For those main effects found to be significant beyond the 0.05 level of probability, a Duncan's Multiple Range test was applied.

Chemicals. All reagents were of the purest grade available and dissolved in glass distilled water. D-Arterenol, L-tyrosine, and 3,4-dihydroxyphenylethylamine were purchased from Calbiochem (La Jolla, Calif.). L-Cysteine, 4-hydroxy-3-methoxyphenylacetic acid and dihydroxyphenylethylamine were purchased from Calbiochem (La Jolla, Calif.).

Results
Influence of Radiothyroidectomy on the Ontogenesis of Spontaneous Locomotor Activity. Results illustrated in Fig. 1 demonstrate the development pattern of spontaneous locomotor activity in both the normal and neonatally hypothyroid rats. The locomotor activity rose progressively during the first 30 days of age in normal rats, thereafter it tended to drop slightly. Data in Fig. 1 also show that neonatal hypothyroidism led to marked interference in the normal ontogeny of spontaneous locomotor activity. Neonatally hypothyroid