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Effect of Neonatal Hypothyroidism on the Serotonin System of the Rat Brain

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The effects of neonatal thyroidectomy and thyroid hormone replacement therapy on the development of serotonin-containing neurons in discrete rat brain nuclei were studied. Newborn male rats were rendered hypothyroid by the injection of 125 μCi ^{131}I, and, after 45 days, were compared with normal littermate controls and ^{131}I-injected animals subsequently maintained by daily T_4 injections. The serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) contents of discrete brain nuclei removed by punches of frozen brain slices were measured by HPLC with electrochemical detection. 5-HT and 5-HIAA contents were significantly increased in many nuclei of the hypothyroid rat brain. By blocking the biosynthesis of 5-HT with *p*-chlorophenylalanine we found that the activity of tryptophan hydroxylase is an important step in the stimulatory effect of hypothyroidism on the 5-HT and 5-HIAA contents. Furthermore, we demonstrated after blockage of monoamine oxidase activity with pargyline, a less pronounced decline of 5-HIAA in neonatal hypothyroid animals, thus causing a relative accumulation of this metabolite. These results demonstrate that there are important modifications of the 5-HT system in the brain of neonatal hypothyroid rats. This may have an important role in the development of hypothyroid-induced impairments of central nervous system function.

INTRODUCTION

The importance of thyroid gland function in the normal development of the central nervous system (CNS) in man and animals is well known[11,17]. Abnormalities of thyroid status are invariably accompanied by perturbations of development of nervous system biochemistry and morphology, followed by alteration of synaptic transmission and behavior[15,27–29,35]. In the rat, the neonatal period is characterized by low serum thyroid hormone concentrations at birth, increasing to adult values by the third postnatal week[10].

The serotonin (5-HT) neuronal system of most species is relatively immature at birth[1,21,22]. A critical period exists in the early life of rats during which thyroid hormone must be present for the optimal development of 5-HT metabolizing systems in maturing brain[42]. After birth, the 5-HT system development accelerates with the most important maturational events occurring after the first postnatal week and reaching a mature stage in the rat brain between the 3rd and the 6th postnatal week[1,5,16,30].

There is a marked increase in brain tryptophan hydroxylase activity between 7 and 14 days of age[7,40,45] and Rastogi and Singhal[40,46] demonstrated, in total brain, that these developmental increases are inhibited by neonatal thyroidectomy. Neonatal hypothyroidism had similar effects on the developmental increases in both tyrosine hydroxylase activity and norepinephrine levels[41,46]. However, Dupont et al.[9] did not find any variation of the norepinephrine content in 32 discrete brain nuclei of neonatal hypothyroid rats and they suggested that studies on whole brain or large brain regions can mask effects elicited in discrete nuclei. Interestingly, they observed an increase in substance P concentrations of 19 out of 32 dissected brain regions.

In this work, we have measured the 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) contents of many discrete brain nuclei of normal and neonatal hypothyroid rats. Evaluation of the 5-HT metabolism has been possible by using pargyline and *p*-chlorophenylalanine (PCPA) treatment. Brain nuclei studied were selected in accordance with the highest level of serotonin in the rat brain.

MATERIALS AND METHODS

We obtained about 1000 newborn Sprague–Dawley rats within a 4-day-period. Each morning the sex

of animals was determined and males were randomly distributed in litters of 9–11 pups with a lactating mother until postnatal day 21 and subsequently with unrestricted access to water and Purina rat chow. In our experimental model, we used 9 different groups, each one requiring 5 litters. Group 1: littermates maturing normally. Group 2: littermates made hypothyroid by injection of carrier-free ^{131}I (125 μCi, s.c.) on the first day after birth. Group 3: littermates made hypothyroid (as in group 2) and injected daily with thyroxine (T_4; 1 μg/100 g body wt., s.c.). Group 4: littermates (as in group 1) received two injections of *p*-chlorophenylalanine (PCPA; 300 mg/kg, body wt., i.p.) 48 and 24 h before sacrifice. Group 5: littermates made hypothyroid (as in group 2) having received two injections of PCPA (cf. group 4). Group 6: littermates made hypothyroid and injected daily with T_4 (as in group 3) received two injections of PCPA (cf. group 4). Group 7: littermates (as in group 1) received an injection of pargyline (75 mg/kg, body wt., i.p.) 2 h before sacrifice. Group 8: littermates made hypothyroid (as in group 2) received an injection of pargyline (cf. group 7). Group 9: littermates made hypothyroid and injected daily with T_4 (as in group 3) received an injection of pargyline (cf. group 7). Note that normal and PCPA-treated rats received a saline injection 2 h before sacrifice in order to control for possible actions of stress in the interpretation of the results.

On the 43th day after birth, rats were weighed in order to evaluate the efficacy of the ^{131}I-injection and the T_4 replacement therapy. We selected 25 animals in each group. Half of the animals of each group were killed by decapitation on the morning of postnatal day 45, and the others on the next day. Brains were rapidly removed and frozen on crushed dry ice. Discrete rat brain nuclei were dissected by a punch technique on serial coronal brain slices cut in the stereotaxic plane described by König and Klippel[25] according to the method of Palkovits[37].

Tissues were homogenized in 0.1 N perchloric acid for extraction of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), centrifuged and supernatants were frozen at —80 °C until measurement (less than 1 month) by liquid chromatography and electrochemical detection as reported by Di Paolo et al.[8] In brief, this involved a separation of the perchloric acid extract on a stainless steel chromegabond MC-18 column (octadecyl bonded on silica C18, 10 μM, 4.6 × 300 mm) using a Beckman liquid chromatograph. The mobile phase for chromatography was 0.1 M $CH_2ClCOOH$, pH 3.0, 13% MeOH, delivered at a flow rate of 2.0 ml/min. The eluate content in 5-HT and 5-HIAA was determined with an electrochemical detector (LC-4, Bioanalytical Systems) using a CP-0 carbon paste electrode; the potential was set at 0.65 V with respect to a $Ag^+/AgCl$ reference electrode. 0.5, 1 and 2 ng of standard 5-HT and 5-HIAA were chromatographed and coefficients of variations of 3% or less were obtained.

Protein content was measured[31] using a sample of the pellet. Statistical significance of differences between means was assessed by the multiple range-test of Kramer[26] after analysis of variance.

RESULTS

The body weight was used as a criterion of the efficacy of the treatment. We reported previously[9], by using the same experimental model, that growth reflected by body weight, body length, plasma T_4 concentration and pituitary growth hormone content was lower in neonatal hypothyroid rats. T_4 replacement therapy was shown to completely abolish effects of radiochemical thyroidectomy on body growth. Hypersensitivity to experimental manipulations manifested by complaint and kicking, difficulty to succeed the bar-test for equilibrium (unpublished observations) and suppressed mobility revealed by typical walk were characteristics inherent to hypothyroid rats.

In agreement with previous studies[18,38,44,47], serotonin was found to be distributed throughout the rat brain with the highest concentration in the n. dorsalis raphes followed by n. medianus raphes, area ventralis tegmenti, substantia nigra and n. amygdaloideus basalis pars lateralis (Fig. 1). Marked changes of the 5-HT and 5-HIAA content were present in the brain of hypothyroid animals and were completely abolished by chronic T_4 therapy. In hypothyroid animals, the serotonin concentration was significantly increased in 12 of the 20 brain nuclei dissected (Fig. 1). The 5-HIAA content, reflecting the catabolism of 5-HT, was significantly increased in almost all the dissected brain nuclei of neonatal hypothyroid rats (Fig. 2).

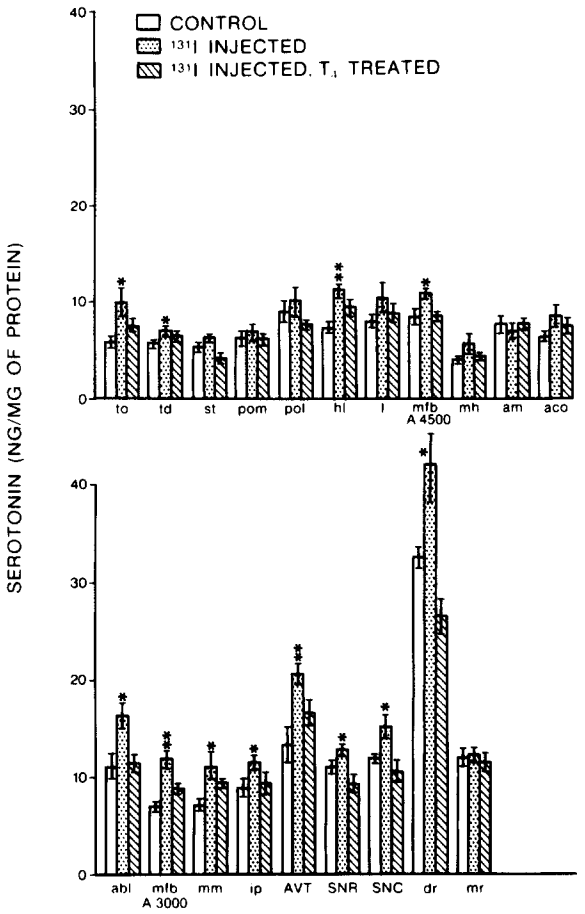


Fig. 1. Changes in the distribution of 5-HT after neonatal thyroidectomy and T_4 replacement therapy. The 5-HT content of discrete rat brain nuclei was measured as described in Materials and Methods. Results shown are the mean ± S.E.M. of 4 groups of 5 animals each. The significance of differences between means was tested by the multiple-range test of Kramer. $^*P < 0.05$; $^{**}P < 0.01$ (compared either to control group or ^{131}I-and T_4-treated group). For abbreviations, see list.

The ratio of 5-HT/5-HIAA is slightly lower in the brain nuclei of rats with neonatal thyroid deficiency, except for the infundibulum where it decreased by 50% when compared to littermate controls or to those treated with T_4 (Table I). Since both 5-HT and 5-HIAA were increased by neonatal hypothyroidism, a lower 5-HT/5-HIAA ratio could indicate a greater increase of the degradatory rather than the biosynthetic enzymes of 5-HT metabolism.

PCPA is a drug known to deplete brain 5-HT by the inhibition of tryptophan hydroxylase, the rate-limiting enzyme in 5-HT biosynthesis[13,14,19,24]. In fact, 24 h after the second injection of PCPA to euthyroid rats, we observed that the serotonin levels

TABLE I

Ratio of 5-HT/5-HIAA in discrete rat brain nuclei of ^{131}I-treated, ^{131}I- and T_4-treated and control animals

The significance of differences between means was tested by the multiple range test of Kramer. * $P < 0.05$, ** $P < 0.01$ (compared either to control group or ^{131}I- and T_4-treated group). For abbreviations, see list. No significant variations of the ratio was observed in to, pol, hl, aco A3500, abl A3500, mm, ip, SNR and dr.

Brain nucleus	*Neonatal rats*		
	Control	*^{131}I-treated*	*^{131}I + T_4-treated*
td	0.94 ± 0.01	0.81 ± 0.02**	1.08 ± 0.05
st	0.78 ± 0.03	0.67 ± 0.03*	0.85 ± 0.04
pom	0.97 ± 0.06	0.80 ± 0.04*	1.00 ± 0.04
I	2.15 ± 0.21	1.00 ± 0.10**	2.23 ± 0.14
mfb A4500	1.29 ± 0.06	0.95 ± 0.04**	1.24 ± 0.03
mh	0.91 ± 0.03	0.57 ± 0.05**	0.75 ± 0.09
am A3500	1.94 ± 0.10	1.47 ± 0.10*	2.01 ± 0.07
mfb A3000	1.35 ± 0.06	1.01 ± 0.05**	1.32 ± 0.04
AVT	1.48 ± 0.09	1.10 ± 0.05**	1.70 ± 0.08
SNC	1.27 ± 0.02	1.03 ± 0.05**	1.34 ± 0.07
mr	0.65 ± 0.02	0.51 ± 0.02*	0.70 ± 0.03

were decreased between 2- to 10-fold depending on the brain area studied (Fig. 3). In addition, following PCPA-injections, the 5-HIAA content of all the brain nuclei studied was also decreased (Fig. 4). The hypothyroid-induced accumulation of 5-HT in the n. tractus diagonalis, the medial forebrain bundle, the n. mamillaris medialis, the n. interpeduncularis, the area ventralis tegmenti and the substantia nigra zona reticulata and zona compacta was abolished by PCPA injections and the 5-HT content of the infundibulum and the n. dorsalis raphes of the neonatal hypothyroid rats were lower when compared to euthyroid animals (Fig. 3). Twenty-four hours after the second injection of PCPA, the hypothyroid-induced accumulation of 5-HIAA was abolished all over the rat brain except in the infundibulum where it persisted (Fig. 4).

In hypothyroid animals, the decline of 5-HT, as represented by the LOG of the ratio of 5-HT measured before and after PCPA injections, was slightly increased in n. interstitialis striae terminalis, n. preopticus medialis, n. preopticus lateralis and n. amygdaloideus basalis pars lateralis whilst it was accelerated 2-fold in the infundibulum, the area ventralis tegmenti and the n. dorsalis raphes (Table II).

Injection of pargyline, a monoamine oxidase inhibitor[4,12], produced a 2- to 5-fold accumulation of

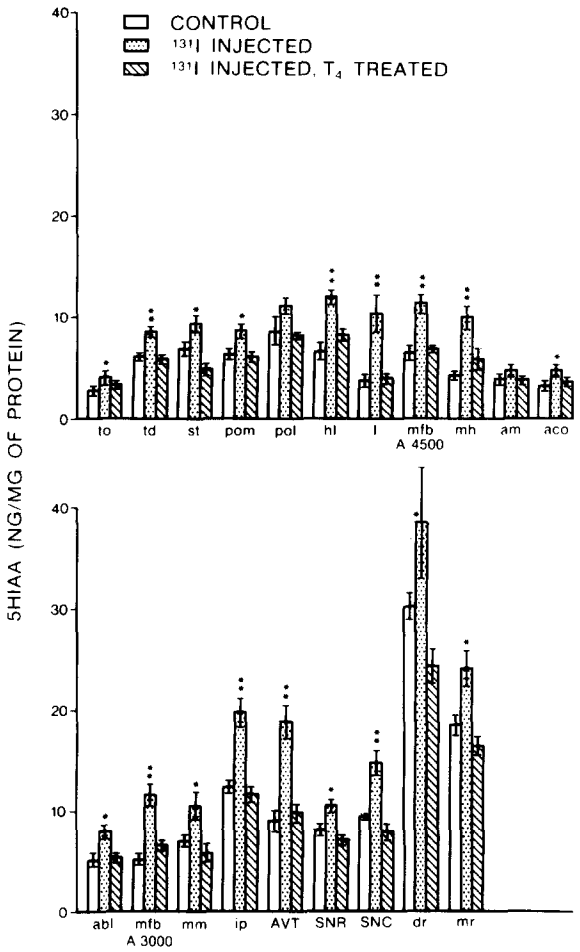


Fig. 2. Changes in the distribution of 5-HIAA after neonatal thyroidectomy and T_4 replacement therapy. The 5-HIAA content was measured as described in Materials and Methods. Results shown are the mean ± S.E.M. of 4 groups of 5 animals each. The significance of differences between means was tested by the multiple-range test of Kramer. $*P < 0.05$, $**P < 0.01$ (compared either to control group or ^{131}I- and T_4-treated group). For abbreviations, see list.

brain 5-HT (Fig. 5) and a decline of 1.5- to 2-fold in brain 5-HIAA (Fig. 6), as has already been described in euthyroid rats[51]. The hypothyroid-induced accumulation of serotonin in the rat brain was abol-

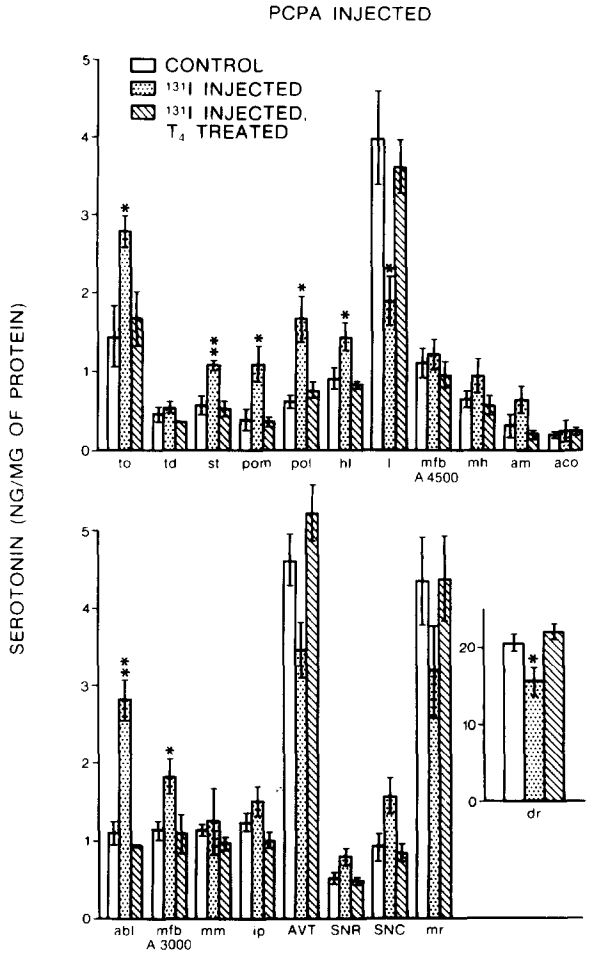
Fig. 3. Changes in the distribution of 5-HT after neonatal thyroidectomy and T_4 replacement therapy in rats having received two injections of PCPA (i.p., 300 mg/kg, body wt.) 48 and 24 h before the sacrifice. The 5-HT content was measured as described in Materials and Methods. Results shown are the mean ± S.E.M. of 4 groups of 5 animals each. The significance of differences between means was tested by the multiple-range test of Kramer. $*P < 0.05$, $**P < 0.01$ (compared either to control group or ^{131}I- and T_4-treated group). For abbreviations, see list.

TABLE II

Decline of 5-HT: the LOG (Ao/A) of the ratio of 5-HT measured before (Ao) and after PCPA injections (A) in euthyroid, neonatal hypothyroid and neonatal hypothyroid rats having received T_4-replacement therapy

The significance of differences between means was tested by the multiple range test of Kramer. * $P < 0.05$, ** $P < 0.01$ (compared either to control group or ^{131}I- and T_4-treated group). For abbreviations, see list. No significant variations of the ratio was observed in to, td, hl, mfb A4500, mh, am, aco, mfb A3000, mm, ip, SNR, SNC and mr.

Brain nucleus	*Neonatal rats*		
	Control	*^{131}I-treated*	*^{131}I + T_4-treated*
st	2.24 ± 0.19	1.77 ± 0.06*	2.08 ± 0.21
pom	2.76 ± 0.28	1.85 ± 0.20*	2.84 ± 0.09
pol	2.67 ± 0.20	1.81 ± 0.22*	2.30 ± 0.14
I	0.70 ± 0.17	1.70 ± 0.21**	0.90 ± 0.13
abl	2.31 ± 0.14	1.77 ± 0.13*	2.50 ± 0.07
AVT	1.06 ± 0.15	1.80 ± 0.12**	1.16 ± 0.11
dr	0.46 ± 0.06	1.00 ± 0.14**	0.20 ± 0.08



ished by pargyline-injection except in the n. lateralis of the hypothalamus, the medial forebrain bundle, the n. medialis habenulae and the n. mamillaris medialis (Fig. 5). In contrast the stimulatory effect of neonatal hypothyroidism on the 5-HIAA levels was not abolished by pargyline injection (Fig. 6), but was even accentuated following this treatment. The decline of 5-HIAA, as represented by the LOG of the ratio of 5-HIAA measured before and after pargyline injection, was greatly decreased in almost all nuclei of the neonatal hypothyroid rat brain (Table III).

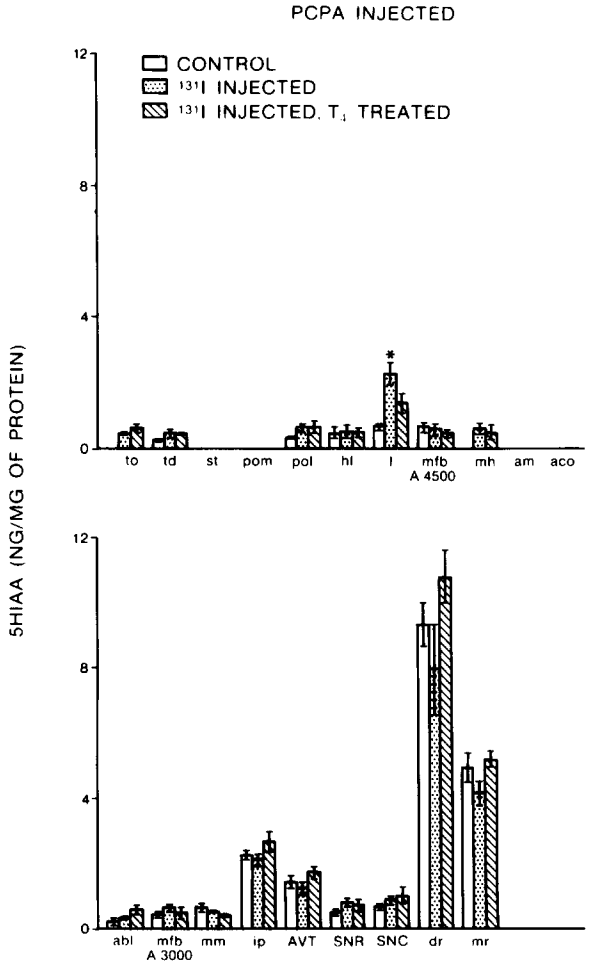


Fig. 4. Changes in the distribution of 5-HIAA after neonatal thyroidectomy and T_4 replacement therapy in rats having received two injections of PCPA (i.p., 300 mg/kg, body wt.) 48 and 24 h before the sacrifice. The 5-HIAA content was measured as described in Materials and Methods. Results shown are the mean ± S.E.M. of 4 groups of 5 animals each. The significance of differences between means was tested by the multiple-range test of Kramer. $*P < 0.05$, $**P < 0.01$ (compared either to control group or ^{131}I- and T_4-treated group). For abbreviations, see list.

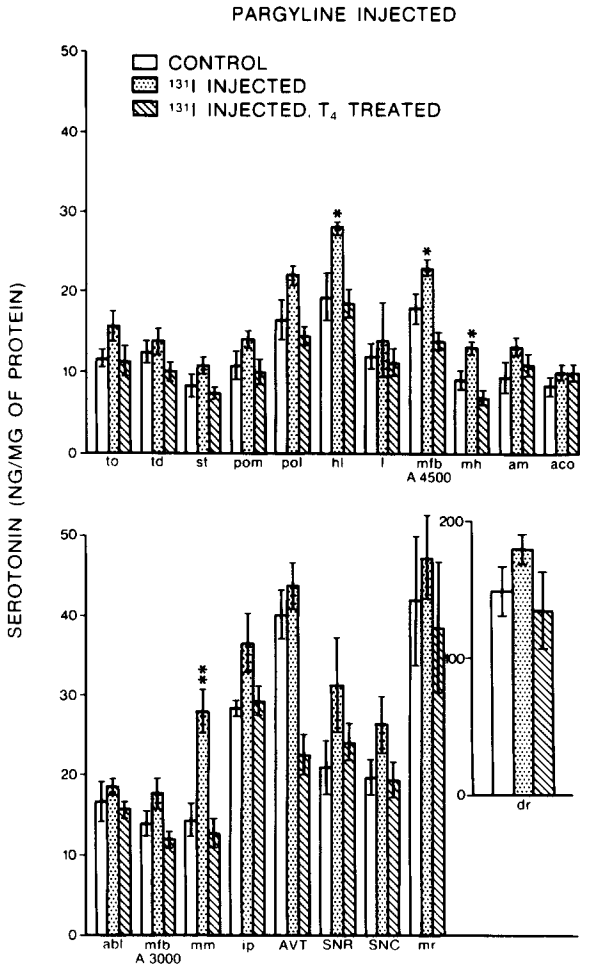


Fig. 5. Changes in the distribution of 5-HT after neonatal thyroidectomy and T_4 replacement therapy in rats having received one injection of pargyline (i.p., 75 mg/kg, body wt.) 2 h before sacrifice. The 5-HT content was measured as described in Materials and Methods. Results shown are the mean ± S.E.M. of 4 groups of 5 animals each. The significance of differences between means was tested by the multiple-range test of Kramer. $*P < 0.05$, $**P < 0.01$ (compared either to control group or ^{131}I- and T_4-treated group). For abbreviations, see list.

These findings suggest that the higher levels of 5-HIAA observed in neonatal hypothyroid rats could reflect a lower rate of disappearance of 5-HT metabolites.

DISCUSSION

The present data indicate that the concentration of the monoamines serotonin and its metabolite 5-HIAA in specific nuclei of the developing rat brain are influenced by low thyroid hormone levels. The

TABLE III

Decline of 5-HIAA: the LOG (Ao/A) of the ratio of 5-HIAA measured before (Ao) and after pargyline injections (A) in euthyroid, neonatal hypothyroid and neonatal hypothyroid rats having received T_4-replacement therapy.

The significance of differences between means was tested by the multiple range test of Kramer. * $P < 0.05$, ** $P < 0.01$ (compared either to control group or ^{131}I- and T_4-treated group). For abbreviations, see list. No significant variations of the ratio was observed in td, I, mfb A4500, aco, abl, mfb A3000 and AVT.

Brain nucleus	*Neonatal rats*		
	Control	*^{131}I-treated*	*^{131}I + T_4-treated*
to	0.82 ± 0.11	0.37 ± 0.16*	1.19 ± 0.21
st	0.73 ± 0.17	0.25 ± 0.15*	0.63 ± 0.14
pom	0.78 ± 0.15	0.23 ± 0.14*	0.99 ± 0.15
pol	0.70 ± 0.19	1.10 ± 0.12**	0.66 ± 0.07
hl	0.45 ± 0.17	0.03 ± 0.08*	0.69 ± 0.14
mh	0.45 ± 0.10	0.12 ± 0.10**	0.81 ± 0.32
am	1.06 ± 0.18	0.53 ± 0.17*	1.17 ± 0.17
mm	1.04 ± 0.14	0.05 ± 0.15**	0.95 ± 0.23
ip	0.70 ± 0.12	0.28 ± 0.10*	0.81 ± 0.14
SNR	0.73 ± 0.14	0.02 ± 0.10**	0.67 ± 0.11
SNC	0.72 ± 0.09	0.06 ± 0.11*	0.69 ± 0.12
dr	1.15 ± 0.12	0.48 ± 0.16**	0.89 ± 0.17
mr	0.60 ± 0.11	0.01 ± 0.08**	0.59 ± 0.15

specificity of this action was corroborated by the correction of all anomalies seen by hormone replacement therapy with low doses of T_4.

In hypothyroid rats, there is an accumulation of 5-HT and 5-HIAA in many discrete brain nuclei. The lower ratio of 5-HT/5-HIAA of these animals as compared to euthyroid or T_4-treated rats suggests that the increase in 5-HT-degratory activity is greater than the increase of 5-HT biosynthetic mechanisms. The use of pargyline or PCPA-treatments can help to differentiate between these two possibilities since following blockage of 5-HT formation, the residual biosynthesis may be evaluated by the rise in 5-hydroxytryptophan or from the decline in 5-HT as a function of time. Similarly, following blockage of 5-HT degradation, 5-HT synthesis may be calculated from the rise in 5-HT level or for 5-HIAA decline.

It has been shown by Tozer et al.[48] that 75 mg/kg, body wt. of pargyline produces an approximately linear accumulation of brain 5-HT and an approximately exponential decline in brain 5-HIAA, for 90 min after its administration. In this study, the lower ratio of 5-HIAA concentration (measured before and after pargyline injection) observed in many discrete brain nuclei of neonatal hypothyroid animals may indicate a slower decline of 5-HIAA than in both euthyroid animals or rats having received T_4-replacement therapy. Note, however, that this observation must remain qualitative since Johnson and Crowley[20] demonstrated, that following pargyline injection, rat brain nuclei exhibit different rates of 5-HT accumulation and of 5-HIAA depletion varying from 30 to 120 min. Thus, the lower ratio of 5-HT/5-HIAA observed in hypothyroid rats could be a result of an accumulation of 5-HIAA. In fact, Atack et al.[2] demon-

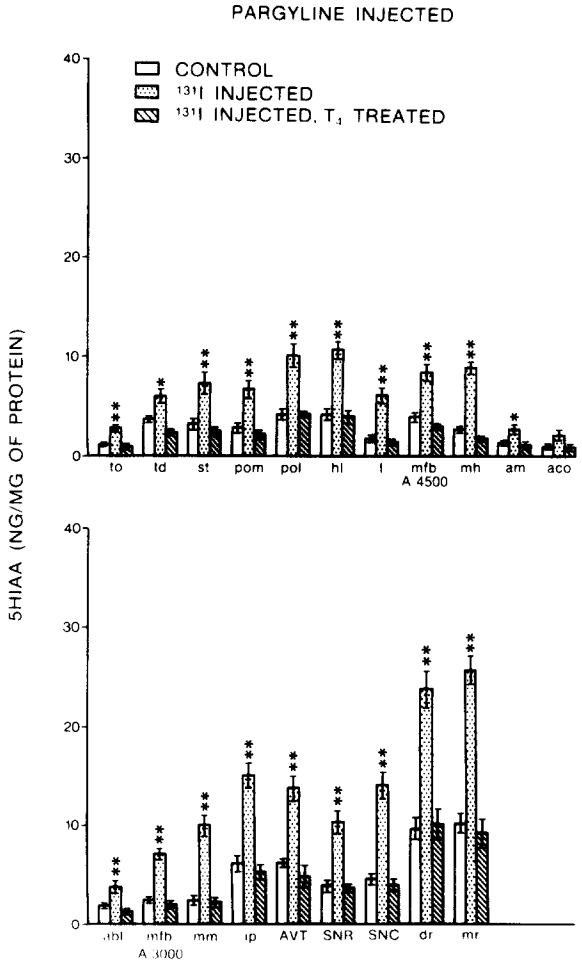


Fig. 6. Changes in the distribution of 5-HIAA after neonatal thyroidectomy and T_4 replacement therapy in rats having received one injection of pargyline (i.p. 75 mg/kg, body wt.) 2 h before the sacrifice. The 5-HIAA content wass measured as described in Materials and Methods. Results shown are the mean ± S.E.M. of 4 groups of 5 animals each. The significance of difference between means was tested by the multiple-range test of Kramer. *$P < 0.05$, **$P < 0.01$ (compared either to control group or ^{131}I- and T_4-treated group). For abbreviations, see list.

strated that the process of elimination of organic acids from the brain of newborn animals is not quite as efficient as in adults. It is not inconceivable that neonatal hypothyroidism, which is known to cause profound morphological and biochemical alterations in the development of the CNS, might have retarded the maturation of the process responsible for the elimination of 5-HIAA.

Following inhibition of tryptophan hydroxylase activity by PCPA, there is a marked depletion in brain 5-HT content. In the median eminence, the dorsal raphe nucleus and the area ventralis tegmenti of neonatal hypothyroid animals, there is a most marked decline of 5-HT compared to that seen in euthyroid or T_4-treated rats, as shown by the higher ratio of 5-HT measured before or after PCPA-treatment. Note that the same treatment abolished the significant increases of 5-HT concentration of neonatal hypothyroid rats in the median raphe nucleus and some other nuclei of the pons medulla, thus suggesting that the hypothyroid-induced accumulation of 5-HT is tryptophan hydroxylase-dependent in these nuclei. However, in neonatal hypothyroid rats, brain nuclei rich in 5-HT fibers and nerve terminals show a slower decline of 5-HT concentration following inhibition of tryptophan hydroxylase in the bed nucleus of the stria terminalis, preoptic nuclei and some amygdaloid nuclei, as indicated by the lower ratio of 5-HT measured before and after PCPA-treatment. This opposite effect of PCPA-treatment on the decline of 5-HT in brain nuclei containing 5-HT soma or 5-HT-nerve terminals could be dependent on both the time required by 5-HT to reach the nerve terminals and the time required by 5-HT to be metabolized. Thus, higher activity of tryptophan hydroxylase in the brain of neonatal hypothyroid rats could be responsible for 5-HT accumulation in many discrete brain nuclei. Neonatal hypothyroidism which is known to retard the normal development of the rat could provoke accumulation of serotonin by increasing the availability of tryptophan in the brain since it has been shown that the amounts of free-unbound tryptophan in blood is higher in young rats[6] and that the tryptophan concentration in brain depends partly on the free-form of the amino acid in the plasma[23].

The use of monoamine oxidase or tryptophan hydroxylase inhibitors to measure 5-HT turnover is subject to criticism since pargyline and PCPA are not specific for the 5-HT system. The effect of PCPA on serotonin and norepinephrine levels has already been documented by Koe and Weismann[24] in the caudate, thalamus and hypothalamus of the dog and in several brain regions of the rat by Miller et al.[34]. Reader[43] confirmed this non-specific effect of PCPA on norepinephrine levels, and extended them to the other catecholamines. Since many interactions between the catecholaminergic and the serotoninergic systems have been described[36,39,52] a part of the PCPA effects on the 5-HT concentration in brain could be related to modifications of the catecholamine system. On the other hand, inhibition of monoamine oxidase activity by pargyline may affect other aspects of monoamine metabolism such as inhibition of reuptake, stimulation of the amine release process, and inhibition of 5-hydroxytryptophan decarboxylation. The possibility of end-product feedback inhibition of tryptophan hydroxylase activity which may result from intraneuronal accumulation of 5-HT, could be responsible for the disappearance of the hypothyroid-induced 5-HT accumulation following pargyline treatment. The direct measurement of monoamine oxidase and tryptophan hydroxylase activity in discrete brain nuclei could be the subject of further investigations but the freezing of brains, a step necessary for microdissection, and the little quantity of tissue extracts are factors limiting the kinetic studies of these enzymes. Recently, however, Luine and co-workers[32,33] used this method with succes in the study of monoamine oxidase activity in rat brains.

Evidence has already been presented that alterations in thyroidal status produce marked changes in the metabolism of several biogenic amines in developing brain[46]. In whole brain, neonatal hypothyroidism decreased the norepinephrine, the dopamine and the 5-HT concentrations, as well as the tyrosine hydroxylase and the tryptophan hydroxylase activity, and increased the acetylcholine and the 5-HIAA content without modification of the acetylcholinesterase and monoamine oxidase activity[46]. In macrodissected brain, following neonatal hypothyroidism, there was a decrease of 5-HT levels and an increase of 5-HIAA contents in cerebellum, midbrain and striatal regions whilst in the pons-medulla, there was an accumulation of 5-HT and 5-HIAA[42]. Monoamine oxidase was increased in midbrain and de-

creased in the hypothalamus[46]. Our results contrast with those of Rastogi and Singhal[42] with exception of the pons-medulla. These discrepancies may by studies on whole brain or large brain structures masking the effect elicited in specific brain regions. On the other hand, in agreement with Rastogi and Singhal[40,42] we observed an accumulation of 5-HIAA in the brain of hypothyroid rats and results obtained after pargyline injection suggest that hypothyroidism could affect the process of organic acid elimination from brain.

Steinbusch[47] has described the distribution of 5-HT cell bodies and terminals in the CNS of the rat. Moreover, Parent et al.[38] studied the organization of ascending 5-HT systems in the rat brain and described two major subsystems of ascending 5-HT projections, one, transtegmental, issues mainly from the nucleus raphe dorsalis and, to a lesser extent, from nucleus raphe medianus. The other, periventricular, appears to originate from the rostral pole of nucleus raphe dorsalis. Neurons of the periventricular systems were shown to terminate mainly in the periventricular hypothalamus, arcuate nucleus and internal layer of the median eminence[3], while neurons of the transtegmental system spread all over the brain[38]. Van de Kar and Lorens[50] suggested, by lesion studies, that the median raphe nucleus is the principal origin of 5-HT fibers innervating the medial preoptic and the anterior hypothalamic areas. Modification of thyroid status does not seem to differentially affect the two putative 5-HT pathways since hypothyroidism induces accumulation of 5-HT and/or 5-HIAA in many nuclei of the forebrain, the midbrain and the hindbrain. We cannot exclude differences in the maturation and the differentiation of neurons caused by the absence of thyroid hormone at birth, resulting in our observations on the serotonergic system.

The demonstrated differences in endogenous 5-HT and 5-HIAA levels in hypothyroid animals are difficult to interpret in terms of neuronal activity, because transmitter levels are the net result of synthesis and degradation as well as release and reuptake processes. However, evidence has been given that chemically provoked neonatal hypothyroidism markedly decreases the behavioral syndrome believed to reflect activation of central 5-HT receptors after treatment of rats with L-5-hydroxytryptophan, the immediate precursor of 5-HT[49]. This demonstration of serotonergic hypofunction would be of relevance in the etiology of mental retardation occurring in neonatal and juvenile hypothyroidism.

Since cretinism is an irreversible condition the modifications of the CNS associated with this neonatal state might also be irreversible, suggesting that thyroid hormone is required for the maturation process of 5-HT-containing neurons in rat brain.

In conclusion, neonatal radiothyroidectomy causes the accumulation of 5-HT and 5-HIAA in many rat brain nuclei, probably by increasing the tryptophan hydroxylase activity and by moderating the disappearance of 5-HIAA out of the neurons. This modification of the 5-HT system may have an extremely important role in the development of hypothyroid-induced impairment of CNS function.

LIST OF ABBREVIATIONS USED IN FIGURES AND TABLES*

abl	nucleus amygdaloideus basalis, pars lateralis
aco	nucleus amygdaloideus corticalis
am	nucleus amygdaloideus medialis
AVT	area ventralis tegmenti (Tsai)
dr	nucleus dorsalis raphes
hl	nucleus lateralis (hypothalami)
I	infundibulum
ip	nucleus interpeduncularis
mfbA4500	medial forebrain bundle
mfbA3000	medial forebrain bundle
mh	nucleus medialis habenulae
mm	nucleus mamillaris medialis, pars medialis
mr	nucleus medianus raphes
pom	nucleus preopticus medialis
pol	nucleus preopticus lateralis
SNC	substantia nigra, zona compacta
SNR	substantia nigra, zona reticulata
st	nucleus interstitialis striae terminalis
td	nucleus tractus diagonalis (Broca)
to	tuberculum olfactorium

* Nomenclature is based on the sixth edition of Nomina Anatomica, revised by the International Anatomical Nomenclature Committee (König, J. R. F. and Klippel, R. A., 1967).

REFERENCES

1 Agrawal, H. C., Elisson, S. N., and Himwich, W. A., Changes in monoamines of rat brain during postnatal ontogeny, *Biochim. biophys. Acta,* 130 (1966) 511–513.

2 Atack, C., Bass, N. H. and Lundberg, P., Mechanisms for the elimination of 5-hydroxyindoleacetic acid from brain and cerebrospinal fluid of the rat during postnatal development, *Brain Research,* 77 (1974) 111–120.

3 Aznitia, E. C., The serotonin-producing neurons of the midbrain medial and dorsal nuclei. In L. L. Iversen, S. Iversen and S. H. Snyder (Eds.), *Handbook of Psychopharmacology, Vol. 9,* Plenum Press, New York, 1978, pp. 233–314.

4 Baker, P. C., Hoff, K. M. and Goodrich, C. A., Changes in 5-HT and 5-HIAA stores in maturing mouse brain following MAO blockade with pargyline, *Gen. Pharmacol.,* 12 (1981) 493–495.

5 Bennett, D. S. and Giarman, N. J., Schedule of appearance of 5-hydroxytryptamine (serotonin) and associated enzymes in the developing rat brain, *J. Neurochem.,* 12 (1965) 911–918.

6 Bourgoin, S., Faivre-Bauman, A., Benda, P., Glowinski, J. and Hamon, M., Plasma tryptophan and 5-HT metabolite in the CNS of the newborn rat, *J. Neurochem.,* 23 (1974) 319–327.

7 Degushi, T. and Barchas, J., Regional distribution and developmental change of tryptophan hydroxylase activity in rat brain, *J. Neurochem.,* 19 (1972) 927–929.

8 Di Paolo, T., Dupont, A., Savard, P. and Daigle, M., Determination of 5-hydroxytryptophan, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in 20 rat brain nuclei using liquid chromatography with electrochemical detection, *Canad. J. Physiol. Pharmacol.,* 61 (1983) 530–534.

9 Dupont, A., Dussault, J., Rouleau, D., Di Paolo, T., Coulombe, P., Gagné, B., Mérand, Y., Moore, S. and Barden, N., Effect of neonatal thyroid deficiency on the catecholamine, substance P and thyrotropin-releasing hormone contents of discrete rat brain nuclei, *Endocrinology,* 108 (1981) 2039–2045.

10 Dussault, J. H. and Labrie, F., Development of the hypothalamo-pituitary thyroid axis in the neonatal rat, *Endocrinology,* 97 (1975) 1321–1327.

11 Ford, D. H. and Cramer, E. B., Developing nervous system in relation to thyroid hormones. In G. D. Grave (Ed.), *Thyroid Hormones and Brain Development,* Raven Press, New York, 1977, pp. 1–18.

12 Fowler, C. J., Oreland, L. and Calligham, B. A., The acetylenic monoamine oxydase inhibitors chlorgyline, deprenyl, pargyline and J-508: their properties and applications, *J. Pharm. Pharmacol.,* 33 (1981) 341–347.

13 Gal, E. M. and Millard, S. A., The mechanism of inhibition of hydroxylases in vivo by *p*-chlorophenylalanine: the effects of cycloheximide, *Biochim. biophys. Acta,* 227 (1971) 32–41.

14 Gal, E. M., Roggeveen, A. E. and Millard, S. A., DL-[2-^{14}C]*p*-chlorophenylalanine as an inhibitor of tryptophan 5-hydroxylase, *J. Neurochem.,* 17 (1970) 1221–1235.

15 Hamburgh, M., Mendoza, L. A., Burkart, J. F. and Weil, F., Thyroid-dependent processes in the developing nervous system. In M. Hamburgh and E. J. Barrington (Eds.), *Conference on Hormone in Development,* Appleton-Century-Crofts, New York, 1971, pp. 403–415.

16 Hedner, Th. and Lundberg, P., Serotoninergic development in the postnatal rat brain, *J. neural Transm.,* 49 (1980) 257–279.

17 Hetzel, B. S. and Hay, I. D., Thyroid function, iodine nutrition and fetal brain development, *Clin. Endocrinol.,* 11 (1979) 445–460.

18 Jacobowitz, D. M. and Maclean, P. D., A brainstem atlas of catecholaminergic neurons and serotonergic perikarya in a pygmy primate, *J. comp. Neurol.,* 177 (1978) 397–416.

19 Jécquier, E., Lovenberg, W. and Sjoerdsma, A., Tryptophan hydroxylase inhibition: the mechanism by which *p*-chlorophenylalanine depletes rat brain serotonin, *Mol. Pharmacol.,* 3 (1967) 274–278.

20 Johnson, M. D. and Crowley, W. R., Serotonin turnover in individual brain nuclei: evaluation of three methods using liquid chromatography with electrochemical detection, *Life Sci.,* 32 (1982) 589–595.

21 Karki, N. I., Kuntzman, R. and Brodle, B. B., Norepinephrine and serotonin levels at various stages of ontogenic development, *Fed. Proc.,* 19 (1960) 282-300.

22 Karki, N. I., Kuntzman, R. and Brodie, B. B., Storage, synthesis and metabolism of monoamines in the developing brain, *J. Neurochem.,* 9 (1962) 53-58.

23 Knott, P. J. and Curzon, C., Free tryptophan in plasma and brain tryptophan metabolism, *Nature (Lond.),* 239 (1972) 452-453.

24 Koe, K. and Weissman, A., *p*-Chlorophenylalanine: a specific depletor of brain serotonin, *J. Pharmacol. exp. Ther.,* 154 (1966) 499–516.

25 König, J. R. F. and Klippel, R. A., *The Rat Brain: a Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem,* Krieger, New York, 1967.

26 Kramer, C. Y., Extension of multiple-range tests to group means with unequal numbers of replications, *Biometrics,* 12 (1956) 307–310.

27 Lan, C. and Slatkin, T. A., Accelerated development of rat sympathetic neurotransmission caused by neonatal triiodothyronine administration, *J. Pharmacol. exp. Ther.,* 208 (1979) 485–490.

28 Lan, C. and Slatkin, T. A., Maturation of sympathetic neurotransmission in the rat heart. II. Enhanced development of presynaptic and postsynaptic components of noradrenergic synapses as a result of neonatal hyperthyroidism, *J. Pharmacol. exp. Ther.,* 212 (1980) 126–130.

29 Lan, C. and Slotkin, T. A., Maturation of sympathetic neurotransmission in the rat heart. VII. Slowed development of noradrenergic synapses resulting from hypothyroidism, *J. Pharmacol. exp. Ther.,* 220 (1982) 629-636.

30 Lidov, H. G. W. and Molliver, M. E., An immunohistochemical study of serotonin neuron development in the rat: ascending pathways and terminal fields, *Brain Res. Bull.,* 8 (1982) 389–430.

31 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with the Folin phenol reagent, *J. biol. Chem.,* 193 (1951) 265-275.

32 Luine, V. N. and Fischette, C. T., Inhibition of lordosis behavior by intrahypothalamic implants of pargyline, *Neuroendocrinology,* 34 (1982) 237-244.

33 Luine, V. N. and Paden, C. M., Effects of monoamine oxidase inhibition on female sexual behavior, serotonin levels and type A and B monoamine oxidase activity, *Neuroendocrinology,* 34 (1982) 245-251.

34 Miller, F. P., Cox, R. H. Jr., Snodgraas, W. R. and Maickel, R. P., Comparative effects of *p*-Chlorophenylalanine, chloroamphetamine and *p*-chloro-N-methylamphetamine

on rat brain norepinephrine, serotonin and 5-hydroxyindole-3-acetic acid, *Biochem. Pharmacol.*, 19 (1970) 435–442.

35 Nicholson, J. L. and Altman, J., Effects of early hypo- and hyperthyroidism on the development of rat cerebellar cortex. 1. Cell proliferation and differentiation, *Brain Research*, 44 (1972) 13–23.

36 Nicolaou, N. M., Garcia-Munoz, M., Arbuthnott, G. W. and Eccleston, D., Interactions between serotonergic and dopaminergic systems in rat brain demonstrated by small unilateral lesions of the raphe nuclei, *Europ. J. Pharmacol.*, 57 (1979) 295–305.

37 Palkovits, M., Isolated removal of hypothalamic or other brain nuclei of the rat, *Brain Research*, 59 (1973) 443-455.

38 Parent, A., Descarries, L. and Beaudet, A., Organization of ascending serotonin systems in the adult rat brain. A radioautographic study after intraventricular administration of [^{3}H]5-hydroxytryptamine, *Neuroscience*, 6 (1981) 115–137.

39 Pujol, J. F., Keane, P., MeRa, A., Lewis, B. D. and Renaud, B., Biochemical evidence fo serotonergic control of the locus coeruleus. In S. Garattini, J. F. Pujol and R. Samanin (Eds.), *Interactions Between Putative Neurotransmitters in the Brain*, Raven Press, New York, 1978, pp. 410–419.

40 Rastogi, R. B. and Singhal, R. L., Thyroid hormone control of 5-hydroxytryptamine metabolism in developing rat brain, *J. Pharmacol. exp. Ther.*, 191 (1974) 72–81.

41 Rastogi, R. B. and Singhal, R. L. Alterations in brain norepinephrine and tyrosine hydroxylase activity during experimental hypothyroidism in rats, *Brain Research*, 81 (1974) 253–266.

42 Rastogi, R. B. and Singhal, R. L., The effect of thyroid hormone on serotonergic neurons: depletion of serotonin in discrete brain areas of developing hypothyroid rats, *Arch. Pharmacol.*, 304 (1978) 9–13.

43 Reader, T. A., Catecholamines and serotonin in rat frontal cortex after PCPA and 6-OHDA: absolute amounts and ratios, *Brain Res. Bull.*, 8 (1982) 527–534.

44 Saavedra, J. M., Distribution of serotonin and synthesizing enzymes in discrete areas of the brain, *Fed. Proc.*, 36 (1977) 2134–2141.

45 Schmidt, M. J. and Sanders-Buch, E., Tryptophan hydroxylase activity in developing rat brain, *J. Neurochem.*, 18 (1971) 2549–2551.

46 Singhal, R. L., Rastogi, R. B. and Hrdina, P. D., Brain biogenic amines and altered thyroid function, *Life Sci.*, 17 (1975) 1617–1626.

47 Steinbusch, H. W. M., Distribution of serotonin-immunoreactivity in the central nervous system of the rat cell bodies and terminals, *Neurosciences*, 6 (1981) 557–618.

48 Tozer, T. N., Neff, N. H. and Brodie, B. B., Application of steady stage kinetics to the synthesis rate and turnover time of serotonin in the brain of normal and reserpine-treated rats, *J. Pharmacol. exp. Ther.*, 153 (1966) 177–182.

49 Vaccari, A., Decreased central serotonin function in hypothyroidism, *Europ. J. Pharmacol.*, 82 (1982) 93–95.

50 Van De Kar, L. D. and Lorens, S. A., Differential serotonergic innervation of individual hypothalamic nuclei and other forebrain regions by the dorsal and median midbrain raphe nuclei, *Brain Research*, 162 (1979) 45–54.

51 Van Loon, G. R., Shum, A. and Sole, M. J., Decreased brain serotonin turnover after short-term (two-hour) adrenalectomy in rats: a comparison of four turnover methods, *Endocrinology*, 108 (1981) 1392–1402.

52 Waddington, J. L. and Crow, T. J., Rotational responses to serotonergic an dopaminergic agonists after unilateral dihydroxytryptamine lesions of the medial forebrain bundle: co-operative interactions of serotonin and dopamine in neostriatum, *Life Sci.*, 25 (1979) 1307-1314.