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Temporal, regional and cellular selectivity of neonatal alteration of the thyroid state on neurochemical maturation in the rat

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Summary. The effects of alteration of thyroid state on neurochemical maturation have been studied in rats made hypothyroid by daily injections of methimazole or hyperthyroid by daily supplementation with thyroid hormone (T3) from postnatal days 1 to 27. Biochemical assays on seven brain regions plus the spinal cord were carried out on 14 and 28 day-old rats as well as in adult rats after at least 40 days of recovery. 2',3'cyclic nucleotide phosphohydrolase (CNPase), a specific marker for oligodendrocytes and myelination was significantly decreased in all regions except the spinal cord of hypothyroid rats. The astrocytic marker glutamine synthetase (GS) was slightly increased in the hippocampus of hypothyroid rats. Choline acetyltransferase (ChAT), a specific marker for cholinergic neurons, was decreased in the prefrontal and visual cortices, the striatum and the superior colliculus and increased in the cerebellum of hypothyroid rats; in addition, the enzyme activity was increased in the prefrontal cortex and striatum and decreased in the cerebellum of hyperthyroid rats. Acetylcholinesterase (AChE) activity was decreased in the prefrontal cortex and in the striatum of hypothyroid rats while 3H-quinuclidynil benzilate (QNB) muscarinic binding was decreased in all cortical areas and in the hippocampus of hypothyroid rats. Glutamate decarboxylase (GAD), a specific marker for GABAergic neurons, was decreased in the cortical areas of hypothyroid rats. Aromatic amino acid decarboxylase (AAD), a general marker for monoaminergic neurons, was unaffected. Alteration of neurochemical parameters was never observed in the spinal cord. Under our experimental conditions, the effects of alteration of thyroid state appeared graded and selective with respect to temporal, regional and cellular parameters.

Key words: Dysthyroidism – Neurochemical maturation – Neurons – Astrocytes – Oligodendrocytes – Rat

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Introduction

The effects of neonatal dysthyroidism on brain maturation have been the subject of numerous investigations (Legrand 1983; Dussault and Ruel 1987; Vaccari 1988). A general conclusion of these studies in the rat is that nerve tissue is particularly sensitive to the action of thyroid hormone during a temporally restricted postnatal period which roughly corresponds to the time of acquisition of specific morphological, functional and biochemical properties from the various cell types. This notion is also supported by the fact that nuclear triiodothyronine (T3) receptors in the rat brain peak at an early postnatal stage and then decrease substantially towards adult values (Valcana and Timiras 1978; Dussault and Ruel 1987).

In addition to anatomical and behavioural studies concerning the effects of thyroid hormone on brain maturation, interest has been recently focussed on the relationship between neonatal dysthyroidism and acquisition of biochemical markers specific for maturation of neuronal and glial cell types. This aspect is currently under active investigation both in vivo and in vitro (Legrand 1983; Dussault and Ruel 1987; Vaccari 1988).

From some of the in vivo studies, it appears that not only are the effects of neonatal dysthyroidism temporally restricted but also that these effects may be specific with respect to the brain region under examination and to the various cellular types. For neurons, in particular, the selectivity of the effects may be in some way related to the different neurotransmitters used by different neuronal populations (Kalaria and Prince 1985,1986; Patel et al. 1987a, b). The above mentioned suggestions come from a variety of studies dealing with various neurochemical markers in few, and sometimes relatively large, brain subdivisions and employing different schedules of antithyroid treatments or thyroid hormone supplementation.

The aim of the present study was to investigate the

effects of postnatal alteration of thyroid state on a number of neuronal and glial chemical markers in several discrete brain regions as well as in the spinal cord. Selected brain regions were: the prefrontal, frontal sensory-motor and occipital visual cortices, hippocampus, striatum, superior colliculus and cerebellum. The neurotransmitter markers studied were related to cholinergic and cholinoceptive neurons (choline acetyltransferase, ChAT; acetylcholinesterase, AChE; ^{3}[H]-quinuclidinyl benzilate, QNB, muscarinic binding), GABAergic neurons (glutamate decarboxylase, GAD) and monoaminergic neurons (aromatic amino acid decarboxylase, AAD). Glial markers were: glutamine synthetase (GS), a selective marker for astrocytes (Patel et al. 1983; Fonnum 1985) and 2'3'-cyclic nucleotide phosphohydrolase (CNPase), a specific marker for oligodendrocytes and myelin (Gould 1985). The temporal span of the effect was monitored by measuring the neurochemical parameters at midway and at the end of the experimental treatment as well as in adults animals which were allowed to recover for at least 40 days.

Methods

Animals

Non primiparous Wistar female rats were caged together with adult males of the same strain. Pregnant females were individually caged until partum and during lactation.

Rats were kept at constant 22–24° C temperature under a 12 hour light/12 hour dark cycle (illumination lasting from 7 a.m. to 7 p.m.). Starting from postnatal day 1 (i.e. the day after birth) each litter was reduced to six pups (usually three males and three females). Two pups of each litter were injected daily with the anti-thyroid drug methimazole (Aldrich, 20 mg/kg dissolved in saline, s.c.) (Vaccari et al. 1983); two pups were injected daily with L-triiodothyronine (Aldrich, 20 μg/kg dissolved in 0.1 N NaOH and brought to pH 7–7.4 with HCl before use, s.c.); the remaining two pups were used as control for the experimental conditions: they were removed from the dam and manipulated in a way similar to that of experimental pups but they did not receive any injection. Pups belonging to the different experimental groups were marked with non-toxic colours on their back. Rats were weaned on day 23. The treatment lasted from postnatal day 1 to postnatal day 27 (injections being made between 4–6 p.m. of each day) and resulted in negligible mortality. Doses were selected from data available in the literature (Rastogi et al. 1976; Vaccari et al. 1983) after preliminary experiments which resulted in substantial mortality of pups for higher drug doses (in particular for higher doses of T3). Every experimental procedure adopted to provoke dysthyroidism, and in particular the use of goitrogen drugs, has its disadvantages and unfavourable side effects (see Vaccari 1988, for a comprehensive discussion). The use of individual injections of the anti-thyroid drug methimazole (Rastogi et al. 1976; Vaccari et al. 1983; Gottesfeld et al. 1987), instead of the more widely used method of feeding the lactating mother with propylthyouracil (Patel et al. 1980; 1987a, b) does not make the mother itself hypothyroid and has the additional advantage of allowing the comparison between animals belonging to the same litter. It is also noteworthy to recall that recent data (Sherer and Bull 1989) suggest that the doses of propylthyouracil commonly used (50 mg/dam/day) are several times higher than those which result in a maximal decrease of circulating thyroid hormone, thus increasing the possible occurrence of unwanted side effects. A possible problem with the use of individual injections of methimazole or T3 to selected pups of the same litter may reside in nutritional differences, the hyperthyroid pups showing a more active and aggressive behaviour. To minimize such differences we have used experienced (non primiparous) mothers and we have reduced to six the number of the pups. Under these conditions it is very unlikely that nutritional differences have occurred since feeding competition is practically abolished. Neurochemical determinations were carried out on 14 and 28 day-old rats as well as on adult rats (67–70 day-old males and 72–78 day-old females) which had been allowed to recover for at least 40 days after the last treatment. Experimental groups at 14 and 28 days of age were composed of approximately the same number of males and females while adult experimental groups were exactly composed of the same number of males and females.

Neurochemical assays

For each experiment, groups of animals from the same litter were killed by decapitation, the brain and the spinal cord were quickly removed and kept in ice. The thoracocervical spinal cord was homogenized in 0.32 M sucrose; the brain was weighed and the cerebellum was dissected and homogenized in 0.32 M sucrose. The rest of the brain was cut in the cold room in transverse slices (400–500 μm thick) with a Sorvall tissue chopper and selected regions were obtained by microdissection under the stereomicroscope. Each region was homogenized in 0.32 M sucrose (50–70 mg/ml). Aliquots of the homogenates were added with Triton X–100 (0.5% final concentration) and used for the determination of ChAT (Fonnum 1975), AChE (Ellman et al. 1961), GAD (Fonnum et al. 1974), AAD (McGeer et al. 1973) and GS (Berl 1966), modified with the addition of 1 mM ouabain to the incubation medium (Patel et al. 1983). For CNPase determination, homogenates were treated with sodium deoxycholate (0.4% final concentration) and samples were incubated at 30° C (Prohaska et al. 1973). For QNB binding the homogenates were diluted with 0.32 M sucrose in order to obtain an approximate final concentration of 50 μg protein/sample. The assay was essentially run as described by Coyle and Yamamura (1976) using 1 hour incubation at 25° C with a fixed concentration of 2 nM 3H–QNB (NEN) and 100 μM oxotremorine as a cold displacer. Separation was made by filtration with a 12 channel Skatron cell harvester. Proteins were determined by the method of Lowry et al. (1951).

Statistical analysis

Results were subjected to analysis of variance with two way classification, the two criteria of classification being the experimental treatments and the ages at which observations had been made. Since a relationship of direct proportionality existed between the absolute values of the means and their respective variances at the different ages, the data were subjected to logarithmic transformation before the analysis of variance was performed (Snedecor and Cochran 1982). The F value obtained for the second criterion of classification, namely age, was always highly significant, while the corresponding F value using treatment as the criterion was only significant for some neurochemical markers in specific brain regions as illustrated in the tables and/or mentioned in the Results section. In these cases the common standard error obtained by the residual variance of the analysis of variance was used to evaluate the statistical significance of the hyperthyroid and hypothyroid treatments by means of Duncan's t-test.

Results

Assessment of treatment efficacy

Several growth and behavioural parameters were altered in hypothyroid and hyperthyroid rats as compared to

Table 1. Effect of postnatal alteration of thyroid state on brain and body weight

	Body weight (g)	*Brain weight (g)*
14 days		
C (21)	30.7±0.5	1.142±0.013
TX (21)	27.2±0.8**	1.122±0.015
T3 (19)	29.6±0.7	1.084±0.011**
28 days		
C (17)	70.7±2.9	1.454±0.018
TX (18)	41.2±2.0***	1.314±0.030***
T3 (18)	71.5±3.1	1.358±0.022**
Adults		
C (14)	267±13	1.829±0.028
TX (14)	222±10*	1.713±0.023***
T3 (14)	250±14	1.718±0.031**

Values are the mean ±S.E. of the number of animals reported in brackets. C: control animals; TX: hypothyroid animals; T3: hyperthyroid animals. Statistical significance of the differences with respect to controls (Student's t-test): * $p<0.05$; ** $p<0.01$; *** $p<0.005$

controls, in a way similar to that reported by others for comparable experimental conditions (Balazs et al. 1971; Rastogi et al. 1976; Vaccari et al. 1983; Kalaria and Prince 1986; Gottesfeld et al. 1987). Eyelid opening was advanced by about 3 days in hyperthyroid pups while it was delayed by 3–5 days in hypothyroid ones. Body growth was severely retarded (up to −42%) in hypothyroid rats (Table 1). A substantial recovery took place in adults, although the body weight of hypothyroid rats was still significantly lower (by 17%) as compared to control rats of the same sex and age. The brain weight of 28 day-old hypothyroid rats was reduced in comparison to controls but to an extent much lower than the reduction in body weight (−11%). As a consequence, the brain/body weight ratio was significantly higher in hypothyroid than in control rats at this stage. In adults, the brain weight of hypothyroid rats remained slightly but significantly reduced in comparison to controls (−6%). The brain weight of hyperthyroid rats was slightly decreased in comparison to controls at every age (−10% to −6%).

Methimazole-treated rats showed other characteristic features of hypothyroid condition, particularly evident between the second and the fourth week of age: they were obviously of much smaller size with a shorter and round body, a shorter tail and snout and abnormal subcutaneous accumulation of fat. Behaviourally, hypothyroid rats were considerably less active than controls while hyperthyroid rats showed a greater excitability and motor activity.

In order to obtain additional information about the extent of thyroid deficiency in methimazole-treated rats, serum levels of T3 and T4 hormones were measured by radioimmunoassay in pups at 14 days of age, approximately 16–18 hours after the last administration of methimazole. Both T3 and T4 levels were significantly lower in methimazole-treated pups (T3: Control, 51±1.5; Methimazole-treated, 23±0.1 ng/100 ml. T4: Control, 3.35±0.12; Methimazole-treated, 2.01±0.02 μg/100 ml. Means±S.E. of 4–5 determinations).

Theoretically, a rigorous experimental design would have required two control groups of rats, one injected with the vehicle used for T3 and the second with the vehicle used for methimazole. In order to keep down the number of animals to be used and to allow the comparison of hypothyroid and hyperthyroid rats with the same controls, we have used a single control group which did not receive any vehicle injection. Control pups, however, were manipulated in a way similar to that of experimental animals, in order to minimize differences due to the stress caused by the experimental procedure.

Glial cell-related markers

All the values for neurochemical markers are expressed as specific activity/unit of protein weight. No significant differences in protein content/unit of weight were measured at the different developmental stages in the various regions examined (data not shown).

Analysis of variance showed that CNPase levels were significantly affected by dysthyroidism in all the regions examined (Table 2) with the exception of the spinal cord (not shown). Enzyme activity was decreased by a maximum of 30–45% in hypothyroid rats at 14 and 28 days, while some recovery was apparent in adults. Hyperthyroidism did not significantly affect CNPase levels at any stage.

Table 2. Effect of alteration of thyroid state on a specific markers for oligodendrocyter 2',3'-Cyclic Nucleotide 3'-phosphohydrolase (mmoler/g prot/h)

Age	Prefrontal-cortex			Frontal-cortex			Visual-cortex			Hippocampus			Striatum			Colliculus sup.			Cerebellum		
	C	TX	T3	C	TX	T3	C	TX	T3	C	TX	T3	C	TX	T3	C	TX	T3	C	TX	T3
14 days (9)	15.6	11.1[a]	16.5	24.0	15.3[b]	25.8	18.4	11.3[b]	17.7	37.7	23.0[a]	41.8	29.5	19.7[a]	38.1	21.9	15.8[a]	27.3	56.9	43.9	61.2
28 days (8)	30.6	17.7[a]	34.4	58.3	31.8[b]	63.8	59.1	33.3[b]	59.0	135	117	128	100	83.4	98.3	144	104[a]	141	108	76.3	104
Adults (6)	47.5	34.9	42.8	106	84.1	99.7	94.8	70.9	92.1	154	160	154	131	118	130	196	193	191	121	122	111
Common S.E.	2.9			4.3			5.3			8.7			7.0			9.8			11.2		
Treatment	$p<0.01$			$p<0.01$			$p<0.01$			$p<0.01$			$p<0.01$			$p<0.01$			$p<0.01$		
Interaction	n.s.			n.s.			n.s.			$p<0.05$			n.s.			n.s.			n.s.		

Results are the mean of experiments carried out on the number of animals reported in brackets.
C control rats; TX hypothyroid rats; T3 hyperthyroid rats.
[a] $p<0.05$; [b] $p<0.01$

Table 3. Effect of postnatal alteration of thyroid state on cholinergic neurons. Choline Acetyltransferase (μmoles/g prot/h)

Age	Prefrontal-cortex			Frontal-cortex			Visual-cortex			Hippocampus			Striatum			Colliculus sup.			Cerebellum		
	C	TX	T3	C	TX	T3	C	TX	T3	C	TX	T3	C	TX	T3	C	TX	T3	C	TX	T3
14 days (8)	13.8	10.1[b]	17.4[b]	16.9	13.2[b]	18.1	11.6	8.73[b]	12.6	18.3	15.8[a]	21.0[a]	89.7	50.0[b]	105[a]	19.5	16.6[a]	20.8	5.18	7.57[b]	3.70[a]
28 days (9)	35.5	32.7	38.6	29.3	30.0	29.4	29.1	28.2	29.8	35.9	38.1	37.0	200	163[b]	219	44.1	42.0	46.3	3.66	4.22	4.62
Adults (12)	45.3	44.1	48.0	35.2	35.1	34.7	36.4	34.5	35.1	46.3	47.5	45.0	259	218[a]	261	53.2	54.2	52.2	4.18	4.55	4.57
Common S.E.	1.2			1.2			1.2			1.5			3.7			1.7			0.39		
Treatment	$p<0.01$			n.s.			$p<0.01$			n.s.			$p<0.01$			$p<0.05$			$p<0.01$		
Interaction	$p<0.01$			$p<0.01$			$p<0.01$			$p<0.01$			$p<0.01$			$p<0.05$			$p<0.01$		

Results are the mean of experiments carried out on the number of animals reported in brackets. All indications are the same of Table 2

While a slight increase in GS activity was observed in some brain regions of hypothyroid rats at 14 days, the overall activity of this enzyme was not significantly affected by hypothyroidism, except in the hippocampus where an increase of 32% was measured at 14 days. No alterations of GS related to hyperthyroid conditions were observed (not shown).

Neurotransmitter-related markers

The cholinergic/cholinoceptive markers were differentially affected. Overall significant differences were recorded in the prefrontal and visual cortices, the striatum, the superior colliculus and the cerebellum for ChAT (Table 3), in the prefrontal cortex and striatum for AChE (not shown), and in the cortical regions and hippocampus for QNB binding (not shown). Individual comparison of each experimental treatment at the various stages with respect to controls (Table 3), indicated in most cases a significant decrease of activity in hypothyroid rats (up to a maximum of −45% for ChAT in the striatum at 14 days) with the exception of the cerebellum where ChAT activity was increased in 14 day-old hypothyroid rats (+46%). Only in the prefrontal cortex and the striatum was there a significant increase (up to +26% at 14 days) of ChAT levels in hyperthyroid rats in comparison to controls. In contrast, ChAT levels decreased by 29% in the cerebellum of hyperthyroid rats at 14 days of age (Table 3). In the striatum, AChE was decreased to a maximum of 19% in hypothyroid rats while it was increased to the same extent in hyperthyroid rats at 14 days of age (not shown). The decrease of QNB binding was only apparent at 14 days of age in hypothyroid rats and reached a maximum in the prefrontal cortex (−37%) as compared to controls. No effect of the hyperthyroid conditions on this binding was noticed (not shown). AAD was unchanged at every developmental stage in all the brain regions examined (not shown). GAD was significantly affected in the three cortical areas, the effect being due to decrease of enzyme activity in hypothyroid condition (up to −26% in the frontal cortex at 14 days). No effects were noticed for the spinal cord in either hypothyroid or hyperthyroid conditions.

The value of the interaction parameter between treatments and ages given by the analysis of variance, was not significant in most cases with the relevant exception of ChAT activity for which a significant interaction was demonstrated in all the regions with the exception of the spinal cord (Table 3). Furthermore a significant interaction was noticed for CNPase activity in the hippocampus (Table 2).

Discussion

Our systematic study confirms and adds new data to previous evidence (Rastogi et al. 1976; Patel et al. 1980; Legrand 1983; Vaccari et al. 1983; Kalaria and Prince 1985, 1986; Dussault and Ruel 1987; Patel et al. 1987a; 1989a) that neonatal dysthyroidism differentially affects glial and neuronal chemical markers depending on the brain region and the neurochemical marker considered. Under our experimental conditions, these neurochemical alterations were almost exclusively linked to thyroid hypofunction since supplements of exogenous T3 produced only marginal and transitory effects on the biochemical maturation of the brain. Previous data (Rastogi and Singhal 1976; Ito et al. 1977; Smith et al. 1980; Vaccari et al. 1983) suggest that higher doses of T3 or administration of T4 instead of T3 may result in more significant alterations of some neurochemical markers in hyperthyroid rats. In our hands, as stated before, higher doses of T3 (40–100 μg/kg body weight) resulted in high mortality. For this reason, we selected a dose (20 μg/kg) wich resulted in practically no mortality while producing somatic effects clearly related to the hyperthyroid conditions (advanced eyelid opening, hyperactivity, decreased brain weight). Furthermore, our hyperthyroid rats showed in a limited number of cases significant increases of some neurochemical parameters (see below and Tables 3 and 4).

Among the glial cell-related markers examined in the present study, CNPase activity proved to be highly sensitive to thyroid hormone deprivation since it was significantly reduced in all regions, except the spinal cord, of hypothyroid rats. This fits with the well-known impairment of myelination in hypothyroid rats (Legrand 1983; Dussault and Ruel 1987) and confirms that oligodendrocytes are a preferential target for thyroid hormone during development, consistent with the recent finding of nuclear receptors for T3 in oligodendrocytes, at least under in vitro culture conditions (Sarlieve et al. 1988). The presence of a statistically significant interaction between treatments and ages for the hippocampus, suggests that

the early effect of hypothyroidism on myelination is reversed in this region at later developmental stages.

After completion of the present study a paper on the effect of hypothyroidism on CNPase levels in the rat cortex, hippocampus and cerebellum has been published (Patel et al. 1989a). The results are in general agreement with our findings with some minor differences, possibly due to the different method to provoke experimental hypothyroidism.

At variance with oligodendrocytes, astrocytes seem to be rather insensitive to dysthyroidism as suggested by the relative stability of the specific marker GS. Only in the hippocampus, hypothyroidism resulted in significant increase of GS. This may result from indirect stimulation of astrocyte metabolism following a reduction in the maturation rate of neurons (Patel et al. 1989a). The possibility of direct effects of thyroid hormone on astrocytes cannot, however, be ruled out (Madarasz 1987). Previous reports on the effect of hypothyroidism on astrocytic markers in the rat hippocampus had given conflicting results (Rami and Rabie, 1988; Patel et al. 1989a).

Among neurotransmitter-related markers, AAD was not affected by dysthyroidism, whereas cholinergic and GABAergic markers showed region-specific alterations.

The cholinergic innervation of several brain regions appeared particularly sensitive to neonatal hypothyroidism and in at least two cases (the prefrontal cortex and the striatum) also to the hyperthyroid condition. Patel et al. (1987a) have recently described a transient decrease of ChAT in the cortex and hippocampus of hypothyroid rats. The same authors found, in addition, a permanent decrease of ChAT in a large "basal forebrain" region which included the thalamus, striatum and septum in addition to the basal telencephalon proper. These findings, together with the demonstration of a synergistic effect of thyroid hormone and nerve growth factor (Hefti et al. 1986; Hayashi and Patel 1987; Patel et al. 1988a; Patel et al. 1989b), may suggest that thyroid hormone deprivation during development does preferentially affect some of the neurons of the basal forebrain which are responsible for cortical and hippocampal cholinergic innervation and which are known to be a major target for endogenous nerve growth factor (Seiler and Schwab 1984; Mobley et al. 1986). It is, however, unlikely that this effect is permanent since cholinergic levels return to normal in the cortex and hippocampus of adults (Patel et al. 1987a and present results). In agreement with that, a recent immunohistochemical study (Gould and Butcher, 1989) has shown accelerated maturation of cholinergic cells in the basal forebrain of hyperthyroid rats and delayed maturation in hypothyroid rats during development without permanent effects in the adult. The permanent decrease found by Patel et al. (1987a) in their "basal forebrain" samples may be primarily due to the permanent decrease occurring in the striatum (Kalaria and Prince, 1985 and the present results) and possibly in other regions not yet individually tested, such as the thalamus. It is relevant to stress the fact that analysis of variance of our data on ChAT, which is the most specific marker for cholinergic innervation, has demonstrated the existence of a significant interaction between treatments and ages in all regions except the spinal cord. This suggests that the effect of experimental dysthyroidism is more effective at earlier developmental stages and it is then counteracted by the developmental modifications of some endogenous factor. In view of the sensitivity of brain cholinergic neurons to nerve growth factor and of the synergistic action between thyroid hormone and nerve growth factor (Seiler and Schwab 1984; Hefti et al. 1986; Mobley et al. 1986; Hayashi and Patel 1987; Patel et al. 1988a; Patel et al. 1988b), it is tempting to speculate about the possibility that a developmental increase of nerve growth factor production may be able partially or totally to counteract the earlier dependence of cholinergic neurons on thyroid hormone. Available data on NGF accumulation in some brain regions of the rat during development are in apparent agreement with this possibility since NGF levels in the cortex, hippocampus and basal forebrain dramatically increase between the 10th and the 20th postnatal days (Large et al. 1986; Auburger et al. 1987).

The contrasting pattern of dysthyroidism-induced alterations on cerebellar cholinergic markers has been previously noticed by others (Patel et al. 1980; Patel et al. 1987b) and the explanation is likely to reside in the unique developmental profiles of cholinergic markers in the cerebellum (Coyle and Yamamura 1976; Virgili et al. 1990). The highest cholinergic levels are indeed measured in the rat cerebellum at early postnatal stages (Coyle and Yamamura 1976; Virgili et al. 1990) and they thereafter decrease towards adult values, the effect being ascribed to the fact that the early-developing archicerebellum is richer in cholinergic innervation than the late-developing paleo- and neocerebellum (Patel et al. 1980). Since the main effect of thyroid hormone is to accelerate cerebellar maturation, the process of natural decrement of cholinergic levels is delayed in hypothyroid rats while it is accelerated in hyperthyroid condition.

The results for AChE activity only in part paralleled those obtained for ChAT, presumably reflecting the fact that AChE is not only a marker for cholinergic neurons but also for neurons receiving cholinergic synapses but using different neurotransmitters at their own terminations (Bradford 1986).

The differences recorded for muscarinic binding in the cortices and hippocampus, are probably due to the impaired development of dendritic trees which is one of the most obvious structural alterations of cortical and hippocampal neurons in cases of thyroid hypofunction (Legrand 1983; Ipina and Ruiz-Marcos 1986; Rami et al. 1986).

GAD was only affected in the cortical regions, the effect being clearly due to a decrease in hypothyroid condition.

The comparison of the results obtained for markers of different neurotransmitter systems may suggest some sort of specificity of developmental dysthyroidism for neurons utilizing given neurotransmitters. The striatum seems a good example for this sort of specificity of thyroid hormone. Both cholinergic and GABAergic neurons are intrinsic to the striatum, are largely generated during

an overlapping medium-to-late prenatal period (Bayer 1984; Fibiger et al. 1987) and their postnatal neurochemical maturation curves are essentially comparable (Coyle and Yamamura 1976; Coyle and Enna 1976; Virgili et al. 1990). In spite of these common features, the cholinergic striatal neurons are sensitive to postnatal alteration of the thyroid state while GABAergic neurons seem to be totally unaffected (see also Kalaria and Prince 1985). These, and additional differences like the apparent lack of effect of thyroid hormone on glutamatergic neurons and on monoaminergic neurones (Kalaria and Prince 1985; Patel et al. 1987b and the present results on AAD), may actually suggest that differential sensitivity to thyroid hormone is in some way related to the neurotransmitter used by a given neuron. This conclusion, however cannot have a general validity since, for example, GABAergic neurons of the cortex are affected by thyroid hypofunction (present results) and the same holds true for GABAergic neurons of the basal forebrain (Patel et al. 1988a).

Finally, it must be stressed that the effects of dysthyroidism seem to be strictly related to the degree of maturity reached by a given region at the time when the alteration of thyroid function starts. In this context, it is interesting to remark that we have never observed significant effects of neonatal dysthyroidism on the spinal cord in which functional and neurochemical maturation is considerably more advanced than in the brain at birth (Virgili et al. 1990).

Brain region-, time- and cell-specific effects of neonatal dysthyroidism seem to be related to a differential sensitivity of populations of developing neural cells to thyroid hormone at different stages of their maturation. A possible explanation for this differential sensitivity may reside in different receptors density in specific cell types at critical times of development. Regional heterogeneity of T3 receptor density in the brain has been documented for adult animals (Valcana and Timiras 1978; Valcana 1979; Dozin and De Nayer 1984; Ruel et al. 1985). No similar data are available during development. It is, however, conceivable that these differences are even more pronounced and more relevant physiologically during the critical postnatal period when both maximal expression of T3 receptors and maximal dependence on thyroid hormone do occur. Future studies focussed on this aspect may be helpful for better understanding the action of thyroid hormone on brain development.

In conclusion the present study provides a systematic survey on the effects of neonatal disthyroidism on neuronal and glial chemical markers in several different regions of the CNS. In addition to new data which can give better insight into the mechanism of action of thyroid hormone, the results may constitute an useful framework for studies wanting to address the effect of thyroid hormone on specific neurochemical markers in some brain regions at selected developmental stages.

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References

Auburger G, Heumann R, Hellweg R, Korsching S, Thoenen H (1987) Developmental changes of nerve growth factor and its mRNA in the rat hippocampus: comparison with choline acetyltransferase. Dev Biol 120:322–328

Balazs R, Kovacs S, Cocks WA, Johnson AL, Eayrs JT (1971) Effect of thyroid hormon on the biochemical maturation of rat brain: postnatal cell formation. Brain Res 25:555–570

Bayer SA (1984) Neurogenesis in the rat neostriatum. Int J Dev Neurosci 2:163–175

Berl S (1966) Glutamine sinthetase: determination of its distribution in brain during development. Biochemistry 5:916–922

Bradford HF (1986) Chemical neurobiology. W.H. Freeman and Company, New York

Coyle JT and Enna SJ (1976) Ontogenesis of GABAergic neurons in the rat brain. Brain Res 111:119–132

Coyle JT and Yamamura HI (1976) Neurochemical aspects of the ontogenesis of cholinergic neurons in the rat brain. Brain Res 118:429–440

Dozin B, De Nayer (1984) Triiodothyronine receptors in adult rat brain: topographical distribution and effect of hypothyroidism. Neuroendocrinology 39:261–266

Dussault JH, Ruel J (1987) Thyroid hormones and brain development. Ann Rev Physiol 49:321–334

Ellman GL, Courtney KD, Andres V and Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

Fibiger HC, Semba K, Vincent SR (1987) Time of origin of immunohistochemically identified interneurons in the rat striatum. Soc Neurosci Abstr 13:1575

Fonnum F (1975) A rapid radiochemical method for the determination of choline acetyltransferase. J Neurochem 24:407–409

Fonnum F (1985) Determination of transmitter amino acid turnover. In: Boulton AA, Baker JB, Wood JD (eds) Neuromethods, Vol 3. Amino acids. Humana Press, Clifton, New Jersey, pp 201–237

Fonnum F, Grofova I, Rinvik E, Storm-Mathiesen J, Walberg F (1974) Origin and distribution of glutamate decarboxylase in the substantia nigra of the cat. Brain Res 71:77–92

Gottesfeld Z, Garcia CJ, Chronister RB (1987) Perinatal, not adult, hypothyroidism suppresses dopaminergic axon sprouting in the differentiated olfactory tubercle of adult rat. J Neurosci Res 18:568–573

Gould MR (1985) Myelin development. In: Wiggins RC, McCandless DV, Enna SJ (eds) Developmental neurochemistry. University of Texas Press, Austin, pp 47–99

Gould E, Butcher LL (1989) Developing cholinergic basal forebrain neurons are sensitive to thyroid hormone. J Neurosci 9:3347–3358

Hayashi M, Patel AJ (1987) An interaction between thyroid hormon and nerve growth factor in the regulation of choline acetyltransferase activity in neuronal cultures derived from the septal-diagonal band region of the embryonic rat brain. Dev Brain Res 36:109–120

Hefti F, Hartikka J, Bolger MB (1986) Effect of thyroid hormone analogs on the activity of choline acetyltransferase in cultures of dissociated septal cells. Brain Res 375:413–416

Ipina SL, Ruiz-Marcos A (1986) Dendritic structure alterations induced by hypothyroidism in pyramidal neurons of the rat visual cortex. Dev Brain Res 29:61–67

Kalaria RN, Prince AK (1985) Effects of thyroid deficiency on the development of cholinergic, GABA, dopaminergic and gluta-

mate neuron markers and DNA concentrations in the rat corpus striatum. Int J Dev Neurosci 3:655–666
Kalaria RN, Prince AK (1986) Decreased neurotransmitter receptor binding in striatum and cortex from adult hypothyroid rats. Brain Res 364:268–274
Large TH, Bodary SC, Clegg DO, Weskamp G, Otten U, Reichardt L (1986) Nerve growth factor gene expression in the developing rat brain. Science 234:352–355
Legrand J (1983) Hormones thyroidienne et maturation du systeme nerveux. J Physiol (Paris) 78:603–652
Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
Madarasz E (1987) Effects of triiodothyronine on the development of GFAP-immunoreactivity and ChAT-activity in monolayer cultures of embryonal forebrain cells. Int J Dev Neurosci 3:255–262
McGeer EG, Fibiger HC, McGeer PL, Brooke S (1973) Temporal changes in amine synthetizing enzymes of rat extrapiramidal structures after hemitransection or 6-hydroxydopamine administration. Brain Res 52:289–300
Mobley WC, Rutkowski JL, Tennekon GI, Gemski J, Buchanan K, Johnston MV (1986) Nerve growth factor increases choline acetyltransferase activity in developing basal forebrain neurons. Mol Brain Res 1:53–62
Patel AJ, Smith RM, Kingsbury AE, Hunt A, Balazs R (1980) Effect of thyroid state on brain development: muscarinic acetylcholine and GABA receptors. Brain Res 198:389–402
Patel AJ, Hunt A, Tahourdin CSM (1983) Regional development of glutamine synthetase activity in rat brain and its association with the differentiation of astrocytes. Dev Brain Res 8:31–37
Patel AJ, Hayashi M, Hunt A (1987a) Selective persistent reduction in choline acetyltransferase activity in basal forebrain of the rat after thyroid deficiency during early life. Brain Res 422:182–185
Patel AJ, Hunt A, Hayashi M (1987b) Effect of thyroid deficiency on the regional development of glutaminase, a glutamatergic neuron marker, in rat brain. Int J Dev Neurosci 5:295–303
Patel AJ, Hayashi M, Hunt A (1988a) Role of thyroid hormone and nerve growth factor in the development of choline acetyltransferase and other cell specific marker enzymes in the basal forebrain of the rat. J Neurosci 50:803–811
Patel AJ, Kiss J, Hunt A (1988b) Effects of thyroid hormone (TH) and nerve growth factor (NGF) on the development of neural cells. Int J Dev Neurosci 6:(Suppl)36
Patel AJ, Hunt A, Kiss J (1989a) Neonatal thyroid deficiency has differential effects on cell specific markers for astrocytes and oligodendrocytes in the rat brain. Neurochem Int 15:239–248
Patel AJ, Hunt A, Kiss J (1989b) Thyroid hormones and neurotransmitter system: an interaction with nerve growth factor in the regulation of cholinergic neurones. In: DeLong GR, Robbins J, Condliffe PG (eds) Iodine and the brain. Plenum, New York, pp 139–149
Prohaska JR, Clark DA, Wells WW (1973) Improved rapidity and precision in the determination of 2'3'-cyclic 3'-phosphohydrolase. Analit Biochem 56:275–282
Rami A, Patel AJ, Rabie A (1986) Thyroid hormone and development of the rat hippocampus: morphological alterations in granule and pyramidal cells. Neuroscience 19:1217–1226
Rami A, Rabie A (1988) Effect of thyroid deficiency on the development of glia in the hippocampal formation of the rat: an immunocytochemical study. Glia 1:337–345
Rastogi RB, Singhal RL (1976) Influence of neonatal and adult hyperthyroidism on behavior and biosynthetic capacity for norepinephrine, dopamine and 5-hydroxytryptamine in rat brain. J Pharmacol Exp Ther 198:609–618
Rastogi RB, Lapierre Y, Singhal RL (1976) Evidence for the role of biogenic amines in depressed motor activity seen in chemically thyroidectomized rats. J Neurochem 26:443–449
Ruel J, Faure R, Dussault JK (1985) Regional distribution of nuclear T3 receptor in rat brain and evidence for preferential localization in neurons. J Endocrin Invest 8:343–348
Sarlieve LL, Barakat I, Luo M, Besnard F, Droz B (1988) Both types of myelin producing cells: oligodendrocytes (CNS) and Schwann cells (PNS) express nuclear triiodothyronine receptors. Int J Dev Neurosci 6: (Suppl.) 37
Sherer TT, Bull RJ (1989) Traditional propylthiouracil doses used for inducing hypothyroidism are too high. Soc Neurosci Abstr 15:1372
Seiler M, Schwab ME (1984) Specific retrograde transport of nerve growth factor (NGF) from neocortex to nucleus basalis in the rat. Brain Res 300:33–38
Smith RM, Patel AJ, Kingsbury AE, Hunt A, Balazs R (1980) Effect of thyroid state on brain development: β-adrenergic and 5'-nucleotidase activity. Brain Res 198:375–387
Snedecor GW, Cochran WG (1982) Statistical methods, 7th edn, Chap 15. The Iowa State University Press
Vaccari A (1988) Teratogenic mechanisms of dysthyroidism in the central nervous system. Progr Brain Res 73:71–86
Vaccari A, Biassoni R, Timiras PS (1983) Selective effects of neonatal hypothyroidism on monoamine oxidase activities in rat brain. J Neurochem 40:1019–1025
Valcana T (1979) The role of triiodothyronine (T3) receptors in brain development. In: Meisami E, Brazier MAB (eds) Neural growth and differentiation. Raven Press, New York, pp 39–58
Valcana T, Timiras PS (1978) Nuclear triiodothyronine receptors in the developing rat brain. Mol Cell Endocrinol 11:31–41
Virgili M, Barnabei O, Contestabile A (1990) Regional maturation of neurotransmitter-related markers during postnatal development in the rat. Int J Dev Neurosci 8:159–166