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Iodine Deficiency and Brain Development in the Rat

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Abstract

An assessment was made of the influence of low-iodine diet on somatic and brain development at birth (day 0) and 21 days postnatally in the rat. The rat mothers were proven to be iodine-deficient by assay of plasma thyroxine and thyroid stimulating hormone prior to mating, and at 21 days postnatally, when maternal thyroids were removed, weighed and stored for subsequent iodine analysis, along with those of the offspring. There were no significant differences in body weight or brain weight of the offspring at birth, or in the content of DNA or protein. However, at 21 days there was a significant reduction in body weight (21·7%) and whole brain weight (7·9%, $P < 0·02$) which was associated with a significant fall in cholesterol content (12·4%, $P < 0·05$) and protein level (9·6%, $P < 0·01$), while DNA was not significantly affected (6%). The greatest reduction in weight was seen in the cerebellum. The thyroids in these rats were double normal size, showed follicular cell hypertrophy and absence of colloid histologically, and contained 8% of the iodine content of controls.

It is concluded that iodine deficiency retards both somatic and brain development, the change in the latter case being expressed as a reduction in cell size in the cerebral hemispheres and cerebellum, along with reduced myelination throughout the brain.

Extra keywords: hypothyroidism.

Introduction

There have been a significant number of studies on rats, reporting the influence of hypothyroidism on brain development. Balazs (1977), in reviewing this work, concluded that while brain cell number is not significantly affected, cell size is reduced along with weight and myelinogenesis is retarded at a later stage. Where iodine deficiency was used as a means of producing hypothyroidism in the developing rat smaller changes in brain development were observed (Hay *et al.* 1976; Hay 1978).

The ability of commercially available low-iodine diets to achieve severe iodine deficiency and maintain good rat growth has been questioned (Riesco *et al.* 1976; Rosman *et al.* 1978; Van Middlesworth and Norris 1980). By careful selection and preparation of ingredients a diet for rats has been compounded which consistently contains less than 20 ng iodine per gram, while maintaining good growth (McIntosh *et al.* 1980). This diet has been used to rear young females to reproductive maturity, at which stage they were mated following verification of iodine deficiency. This paper reports the results of such a study on rat brain development at birth and 21 days postnatally.

Materials and Methods

Animals

Black Hooded Wistar female rats were placed at weaning on the experimental diets, 12 receiving a low-iodine diet containing 15–20 μg iodine per kilogram (McIntosh *et al.* 1980) and 12 an iodine-supplemented control diet (1 mg iodine per kilogram). Feed and deionized distilled water were provided *ad libitum*. The rats were kept in chrome-plated wire cages as they developed and through mating until 1 week before parturition, when they were transferred to plastic breeding boxes with chrome wire tops and raised stainless steel mesh floors to reduce coprophagy. Mating was commenced when rats were iodine-deficient as assessed by thyroid hormone status using plasma TSH (thyroid stimulating hormone) and T_4 (thyroxine) levels as indicators. Triiodothyronine (T_3) was not assayed, because no changes were observed in iodine-deficient rats in the previous study. No bedding was provided at any stage, as the common materials used had proved to be a rich source of iodine. At birth the pups were sexed and weighed and four offspring of each sex left with the mother. The remainder were removed, killed and dissected. At this stage some mothers were anaesthetized with ether, bled, killed, and thyroids were removed for iodine analysis and histology.

Two male and two female new-born pups from each litter were weighed, killed with chloroform and their thyroids removed. One lobe was fixed for histology and the other stored frozen for iodine assay. The brain was dissected free and sectioned into cerebellum, brainstem and cerebral hemispheres which were weighed and frozen for subsequent DNA and protein analyses. The brain of a fifth pup was taken for moisture and cholesterol analyses.

At 21 days post partum the remaining pups were killed with chloroform, and a blood sample was removed by cardiac puncture for T_4 and TSH assays. The thyroids and brains were then dissected out as with the new-born pups, except that the brain was divided down the midline, and then further subdivided into the same components as at birth. One-half was set aside for protein and DNA assays, the other for moisture and cholesterol.

Table 1. Mean plasma thyroxine (T_4) and thyroid stimulating hormone (TSH) of maternal and 21-day-old offspring on iodine-deficient and control diets

Diet	No. of rats	Plasma $T_4 \pm$ s.e.m. (nmol/l)		Plasma TSH $\pm$ s.e.m. (ng/ml)	
		21-day-old offspring	Mothers	21-day-old offspring	Mothers
Iodine-deficient	6	$35{\cdot}2 \pm 3{\cdot}4$	$8{\cdot}0 \pm 2{\cdot}7$	813 ± 128[A]	1039 ± 39
Control	7	$112{\cdot}4 \pm 5{\cdot}9$	$60{\cdot}2 \pm 6{\cdot}1$	257 ± 22	308 ± 29
Percentage difference		$-68{\cdot}7$	$-86{\cdot}7$	$+316$	$+337$
P		$<0{\cdot}001$	$<0{\cdot}001$	$<0{\cdot}001$	$<0{\cdot}001$

[A] Mean value for 5 rats.

Analytical Methods

Moisture and cholesterol assays were carried out on the same samples of brain tissue, by drying the tissues in glass culture tubes at 100°C for 24 h and then storing in a desiccator until cool enough to weigh. The dried brain segment was extracted by agitating in a chloroform–methanol (2 : 1 v/v) mixture for 5–6 h, then adding 0·25% (v/v) HCl, mixing and standing for a further 6 h. The method for assay of cholesterol was similar to that of Levine and Zak (1964). DNA determinations were carried out by the diphenylamine method of Margolis (1969) and protein assays by the method of Gaunce and D'Iorio (1970), using a Technicon Auto-Analyser. Cholesterol, DNA content and protein to DNA ratio were used as indices of myelin formation, cell numbers and cell size in brain tissue respectively (Cheek 1975). The method for determination of the iodine content of thyroids has been described previously (Potter *et al.* 1980). Plasma T_4 and TSH concentrations were measured by radioimmunoassay procedures, which have been described previously (McIntosh *et al.* 1980).

Table 2. Effect of iodine deficiency on rat brain composition at birth and 21 days postnatally and on thyroid iodine status

Mean values ±s.e.m. are given. A mean of 2–4 offspring was used from each litter, and the total number of litters is given in parentheses

	Body wt (g)	Whole brain Wt (g)	Moisture (%)	Cholesterol (mg)	DNA (μg)	Protein (mg)	Brain wt / Body wt (%)	Thyroid Wet wt (mg)	Thyroid Total I (μg)	Maternal thyroid Wet wt (mg)	Maternal thyroid Total I (μg)
					Day 0, iodine-deficient rats						
	5·36	0·203	89·2	1·13	775	10·48	3·78	2·9	0·004	—	—
	0·09	0·003	1·4	0·14	15	0·23	0·10	0·5	0·001	—	—
	(7)	(7)	(5)	(5)	(7)	(7)	(7)	(7)	(7)	—	—
					Day 0, control rats						
	5·14	0·204	88·8	1·20	756	10·82	3·98	1·8	0·079	—	—
	0·14	0·004	1·2	0·15	12	0·24	0·07	0·4	0·013	—	—
	(8)	(8)	(5)	(5)	(8)	(8)	(8)	(8)	(8)	—	—
Percentage difference	+4·3	−0·4	+0·4	−5·8	−0·1	−3·1	−5·0	+61·1	−95·6	—	—
P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0·001	—	—
					Day 21, iodine-deficient rats						
	26·0	1·060	81·9	18·21	2456	80·1	4·20	9·8	0·116	32·7	0·486
	2·4	0·027	0·4	0·88	73	1·6	0·32	1·3	0·012	1·5	0·063
	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(5)	(5)	(5)
					Day 21, Control rats						
	33·2	1·151	81·8	20·79	2609	89·3	3·58	5·4	1·50	14·1	9·056
	0·8	0·015	0·2	0·66	4·3	1·4	0·05	0·2	0·06	1·0	0·685
	(7)	(7)	(5)	(6)	(7)	(7)	(7)	(7)	(7)	(6)	(6)
Percentage difference	−21·7	−7·9	+0·1	−12·4	−6·0	−9·6	+17·3	+80·8	−92·3	+133	−94·6
P	<0·02	<0·02	n.s.	<0·05	n.s.	<0·01	n.s.	<0·01	<0·001	<0·001	<0·001

The TSH assay had a sensitivity and minimal detectable limit of 20 ng/ml. Statistical analysis of the results was by Student *t*-test.

Results

The influence of an iodine-deficient diet on the iodine status of rats has been reported previously (McIntosh *et al.* 1980). In the present experiment, the degree

Table 3. Effects of iodine deficiency on composition of brain segments of developing rat pups at birth and 21 days postnatally

Mean values ±s.e.m. are given. A mean of 2–4 offspring was used from each litter, and the total number of litters is given in parentheses

	Weight (mg)	Moisture (%)	Cholesterol (mg/g)	DNA (μg)	Protein (mg)
			Cerebellum		
Day 0	8·24	—	—	31·29	0·38
Iodine-deficient rats	0·25	—	—	3·31	0·03
	(7)			(7)	(7)
Control rats	8·10	—	—	30·80	0·41
	0·24	—	—	4·14	0·03
	(8)			(8)	(8)
Percentage difference	+1·7	—	—	+1·6	−7·3
P	n.s.	—	—	n.s.	n.s.
Day 21	141·4	82·3	13·5	1284	10·6
Iodine-deficient rats	6·3	1·3	1·6	69	0·6
	(6)	(6)	(6)	(6)	(6)
Control rats	157·1	83·4	14·8	1404	12·2
	3·0	0·3	0·5	31	0·3
	(7)	(6)	(7)	(7)	(7)
Percentage difference	−10·0	−1·3	−8·8	−8·5	−13·1
P	<0·05	n.s.	n.s.	n.s.	<0·05
			Brainstem		
Day 0	80·5	—	—	164·7	3·52
Iodine-deficient rats	1·6	—	—	5·9	0·10
	(7)			(7)	(7)
Control rats	70·8	—	—	164·1	3·60
	2·7	—	—	6·0	0·13
	(8)			(9)	(8)
Percentage difference	−0·4	—	—	−0·4	−2·2
P	n.s.	—	—	n.s.	n.s.
Day 21	150·6	79·3	26·9	220	12·0
Iodine-deficient rats	6·3	0·80	1·4	7	0·5
	(6)	(6)	(6)	(6)	(6)
Control rats	159·5	78·7	29·5	232	13·2
	2·6	0·6	1·2	4	0·4
	(7)	(6)	(7)	(7)	(7)
Percentage difference	−5·6	+0·8	−8·8	−5·2	−9·1
P	n.s.	n.s.	n.s.	n.s.	n.s.

Table 3 (*continued*)

	Weight (mg)	Moisture (%)	Cholesterol (mg/g)	DNA (μg)	Protein (mg)
		Cerebral Hemispheres			
Day 0	123·5	—	—	559·1	6·58
Iodine-deficient rats	2·6	—	—	9·8	0·17
	(7)			(7)	(7)
Control rats	125·3	—	—	560·7	6·80
	2·6	—	—	12·7	0·21
	(8)			(8)	(8)
Percentage difference	−1·4	—	—	−0·3	−3·2
P	n.s.	—	—	n.s.	n.s.
Day 21	778·3	82·3	16·0	952	58·1
Iodine-deficient rats	17·9	0·3	0·6	17	1·8
	(6)	(6)	(6)	(6)	(6)
Control rats	834·2	81·6	17·1	973	63·8
	10·5	0·3	0·8	12	1·1
	(7)	(7)	(7)	(7)	(7)
Percentage difference	−6·7	+0·9	−6·4	−2·2	−8·9
P	<0·02	n.s.	n.s.	n.s.	<0·05

to which deficiency was achieved and maintained throughout the experiment is shown by the thyroid hormone status (Table 1) and thyroid iodine status (Table 2). At 21 days there was an 87% lower T_4 level in the iodine-deficient mothers than controls, and maternal TSH level was elevated to three times normal. The hormone levels in the 21-day-old offspring did not change as much but were nevertheless significantly affected in the same way. Thyroid iodine status reflected this change, both at day 0 and day 21 in the offspring (4·4 and 7·3% of control values, respectively). Histological changes were observed in the goitrous thyroids of iodine-deficient rats similar to those reported previously (McIntosh *et al.* 1980).

The influence of iodine deficiency upon brain development is shown in Tables 2 and 3. There were no significant changes evident at birth, but by 21 days there was a 21·7% reduction in somatic growth ($P < 0·02$) along with a significant ($P < 0·02$) but smaller reduction (7·9%) in overall brain growth. This reduction in weight was greatest (10%) in the cerebellum ($P < 0·05$), but was present to a lesser degree in the cerebral hemispheres (6·7%, $P < 0·02$) while the change in brainstem weight (5·6%) was not significant statistically. These changes were accompanied by significant reductions in protein in the cerebellum and cerebral hemispheres, but not in the brainstem. DNA was not significantly affected in any of these segments. Cholesterol, as an indicator of myelin present, was significantly lower in the whole brain of 21-day-old iodine-deficient rats (Table 2) relative to controls ($P < 0·05$), but moisture was not significantly affected. When brain data were computed for cell size and number, a decrease of 4% in cell size (n.s.) and a 2·9% increase in cell number (n.s.) were detected. However, there was a significant decrease in cell size in the cerebellum (5·3%, $P < 0·05$) and cerebral hemispheres (6·9%, $P < 0·01$) and an increase in cell number relative to weight in the cerebral hemisphere (7·1%, $P < 0·001$).

Discussion

The results give a clear indication of the influence of iodine deficiency on brain and somatic development in the rat. While high levels of neonatal mortality, such as have been reported in other studies in which hypothyroidism has been induced by other means (Schwark 1977), were not seen in this study, in other respects the changes produced were similar to those reported for hypothyroidism in the rat (Balazs 1977). Gross iodine deficiency was achieved while maintaining a diet sufficient for rat growth and reproduction (McIntosh *et al.* 1980).

The absence of effects measurable at birth is in accord with other observations and supports the hypothesis that the most vulnerable period in the rat is 0–12 days postnatally (Balazs 1977; Oklund and Timiras 1977). While the brain and somatic changes are not as great as have been observed in rats made hypothyroid by other means (Horn 1955; Balazs *et al.* 1968; Oklund and Timiras 1977) there could be other effects, for example those produced by the use of propylthiouracil, ^{131}I irradiation and diets unsuited for growth and reproduction (Van Middlesworth and Norris 1980) which could account for the difference in degree of response observed between this and other studies. Nevertheless, in our experiment there was a significant retardation in brain development at 21 days both in terms of reduced cell size in the cerebellum and cerebral hemispheres and myelination. Cell number did not appear to be as affected. The wet weight of cerebellum was particularly affected, as also were the cerebral hemispheres, while brainstem was least affected. The morphological nature of such changes in the rat cerebellum associated with hypothyroidism has been described in detail (Rosman 1972; Clos and Legrand 1973; Legrand 1979).

While individual rat offspring from the iodine-deficient group showed evidence of delayed skeletal maturation and eye opening, poor locomotor ability and listlessness similar to hypothyroid rats (Schwark 1977), this was not true of all sibs in a litter, nor of all litters. It therefore suggests that some young rats may have had a restriction in general nutrition superimposed on iodine deficiency, by virtue of (1) their inferior social status and therefore access to milk supply or (2) the inability of the mothers to provide adequate nutrition by lactation. However, evidence of the latter was not apparent in this experiment in terms of the brain DNA changes observable with undernutrition. Hypothyroidism has a marked effect on lactation in some other species studied, such as the sheep, where there is failure of normal udder development in the latter stages of gestation.

Although this study has shown a significant effect of iodine deficiency on growth and development of rat offspring, it has not clarified the degree to which brain changes observed at 21 days are due to prenatal events not able to be easily monitored at day 0. The changes are not as great as have been produced by other techniques of inducing hypothyroidism in the rat, nor as great as those produced in the sheep by iodine deficiency (Potter *et al.* 1979), but this could be explained by the greater food intake relative to body weight seen in the rat compared with the sheep, thereby making iodine deficiency in the rat more difficult to achieve. Nevertheless, at the level of iodine deficiency achieved in this study, there is a significant effect upon brain and somatic development in the rat attributable to iodine deficiency, probably having its effect via hypothyroidism.

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