Development of thyroxine type II deiodinase activity in brains of Zucker rats

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The present study was undertaken to determine whether the capacity for 3,5,3',5'-tetraiodothyronine (T_4) conversion into 3,5,3'-tri-iodothyronine (T_3), as measured by the activity of thyroxine type II 5'-monodeiodinase (T_4 -5'D), was altered in the brains of young Zucker *fa/fa* rats during the period of intense maturation of the central nervous system (i.e. from 10 to 20 days of life). From 7 to 14 days of age, no difference in brain T_4 -5'D activity could be detected between lean and pre-obese rats; serum free T_4 was not affected by the *fa* gene. During the suckling to weaning transition, T_4 -5'D reached a plateau in brains of lean rats, while it increased by 50% in brains of pre-obese rats; concurrently, serum free T_4 increased in *Fa/fa* rats, whereas it did not change in *fa/fa* rats. The increased capacity

for conversion of T_4 into T_3 observed in brains of pre-obese compared with lean rats could not be ascribed to a variation in the amount of T_4 -5'D, since V_{max} did not differ between the two genotypes; however, it could be totally accounted for by an increased affinity of the enzyme for T_4 . This change may represent an adaptive response to low serum free T_4 in order to maintain the cerebral T_3 concentration in pre-obese rats. These results show that the alteration in T_4 metabolism in brains of *fa/fa* rats is not an early event and thus cannot interfere with maturation of the central nervous system. However, the decreased serum free T_4 which was observed in pre-obese rats after suckling might play a secondary role in development of this genetic obesity.

INTRODUCTION

The obesity of Zucker *fa/fa* rats is characterized, in adulthood, by numerous metabolic abnormalities such as hyperphagia, hyperinsulinaemia, insulin resistance in several tissues and decreased oxygen consumption (Jeanrenaud, 1978; Bray and York, 1979). Different studies have provided evidence that a reduction in energy expenditure is present in *fa/fa* rats before weaning (Kaplan, 1979; Planche et al., 1983; Schmidt et al., 1984; Moore et al., 1985) and that it precedes excessive fat deposition (Planche et al., 1988). Brown adipose tissue (BAT), whose thermogenic efficiency is altered in neonatal *fa/fa* rats (Bazin et al., 1984) could play a role in the impairment of energy expenditure. The report of diminished noradrenaline turnover in several tissues of adult *fa/fa* rats (Levin et al., 1981) has suggested that reduced sympathetic activity in BAT might be of importance in the development of this obesity. More recent data in suckling *fa/fa* pups have reinforced this hypothesis (Krief and Bazin, 1991). However, the thermogenic response to noradrenaline (Bertin et al., 1983; Rothwell et al., 1981; York et al., 1985) and the efferent sympathetic pathways from the hypothalamus (Holt et al., 1987) are normal in *fa/fa* rats, and the alteration probably resides in the hypothalamus or at a higher level of regulation, i.e. in the central nervous system.

We have recently shown (Marie et al., 1992) that a decreased capacity for conversion of T_4 into T_3 was one of the earliest defects detectable in BAT of neonatal *fa/fa* rats, and that serum free T_3 was reduced in pre-obese compared with lean pups. These data suggest that altered production of T_3 could be involved in the onset of this genetic obesity.

Thyroid hormones are important for growth and development (Legrand, 1986), and their absence during fetal and early-neonatal life may cause irreversible biochemical and morpho-

logical abnormalities in various organs, particularly in the brain (Legrand, 1986; Dussault and Ruel, 1987). T_3 , the major active thyroid hormone at the cellular level (Oppenheimer, 1979), is produced from T_4 primarily by 5'-deiodination. In brain, two phenolic ring deiodinases (type I and type II T_4 -5'D) have been identified (Visser et al., 1981, 1982); however, the enzyme of type II is mainly responsible for conversion of T_4 into T_3 (Silva et al., 1982). In this tissue, Silva and Larsen (1982) have demonstrated that in situ T_4 -5'-deiodination is the main source of T_3 in neonatal rats. Moreover, development of type II 5'D parallels that of brain maturation (Kaplan and Yaskoski, 1981), suggesting that this enzyme activity may be of physiological relevance in the development of the central nervous system.

The present study was undertaken to determine whether type II 5'D activity is altered in brains of pre-obese *fa/fa* rats. Since thyroid hormones are essential for normal maturation of the central nervous system during a short critical period (from day 10 to day 20 in the life of the rat; Dussault and Ruel, 1987), enzyme activity was studied at 7, 14, 21 and 28 days of age. In addition, plasma T_4 , which is known to play a predominant role in regulating T_4 -5'D in the central nervous system (Silva and Leonard, 1985), was also measured.

EXPERIMENTAL

The animals used in this study were obtained from obese (*fa/fa*) males and heterozygous lean (*Fa/fa*) females. Pups and their mothers were housed in a temperature-controlled room ($22 \pm 1^\circ\text{C}$) on a 12 h-light/12 h-dark cycle. The dams were fed on a stock diet (M25; Piètrement, Sainte-Colombe, France) *ad libitum*. Pups were weaned at 21 days of age. At 7 days of age the

Abbreviations used: BAT, brown adipose tissue; DTT, dithiothreitol; T_3 , 3,5,3'-tri-iodothyronine; T_4 , 3,5,3',5'-tetraiodothyronine; T_4 -5'D, type II thyroxine 5'-monodeiodinase.

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genotype of the pups was achieved through the study of frequency curves of fat-cell size from inguinal-white-adipose-tissue biopsies, as previously described (Dugail et al., 1986). At 14 days of age and thereafter the genotype was diagnosed by plotting inguinal-fat-pad weight versus body weight (Lavau and Bazin, 1982). Rats were killed by decapitation, and the brain was dissected out. Cerebral hemispheres were separated, weighed and frozen in liquid nitrogen for further utilization.

Activity of type II T_4 -5'D was measured as described by Visser et al. (1982), with the following modifications: tissues were homogenized in 60 vol. (w/v) of ice-cold buffer (0.25 M sucrose/0.2 mM EDTA/1 mM Hepes, pH 7.2) and then centrifuged for 10 min at 1600 *g*. Supernatants (60 μ g of protein) were assayed for enzyme activity with 2 nM T_4 plus 3×10^5 c.p.m. of [125 I] T_4 in the presence of 20 mM DTT. To avoid 5-deiodination of [125 I] T_4 , unlabelled T_3 (50 nM) was added (Courtin et al., 1989). Incubation was for 30 min at 37 °C.

Kinetic parameters of T_4 -5'D were determined in assays with T_4 concentration ranging from 0.15 to 6 nM. Apparent K_m and V_{max} values were calculated from the regression lines of the Lineweaver-Burk plots.

Serum free T_4 concentrations were determined by a radioimmunoassay (RIA-gnost FT4; Behring D-3550, Marburg, Germany). Protein was measured by the method of Bradford (1976). All values are presented as means $\pm$ S.E.M. The significance of the differences was assessed by Student's *t* test.

RESULTS

The results presented in Table 1 show that, in lean pups, the brain weight gain was very important between 7 and 14 days of age (+84%) and then slowed down (+40% between 14 and 28 days of age). In pre-obese rats, a similar developmental pattern was found; however, from 14 days of age, brain weight was significantly reduced as compared with that of lean littermates.

In *Fa/fa* rats, enzyme activity at 2 nM T_4 (a subsaturating concentration), expressed on a per-mg-of-protein basis, increased by 71% from 7 to 14 days of age and then reached a plateau. When expressed on a per-total-weight-of-tissue basis, owing to the increase in brain weight, the activity rose continuously throughout the studied period.

In *fa/fa* rats, enzyme activity, expressed either per mg of protein or per brain, steadily increased throughout the first month of life. Up to 14 days of age, there was no difference in

Table 1 Development of weight and type II T_4 -5'D activity in brains of *Fa/fa* and *fa/fa* rats

T_4 -5'D was measured as described in the Experimental section. *n* is the number of rats examined. A unit of enzyme activity is defined as the amount needed to release 1 fmol of I⁻/h at 37 °C. Values are means $\pm$ S.E.M. ^a*P* < 0.01, ^b*P* < 0.05 between two consecutive ages within the same genotype; ^{**}*P* < 0.01; ^{*}*P* < 0.05 *fa/fa* versus *Fa/fa* of the same age.

Age (days)	Rat	<i>n</i>	Brain weight (mg)	Type II deiodinase	
				(units/mg of protein)	(units/brain)
7	<i>Fa/fa</i>	9	456 $\pm$ 9.7	82 $\pm$ 6.1	1558 $\pm$ 163
	<i>fa/fa</i>	9	455 $\pm$ 6.7	78 $\pm$ 7.5	1355 $\pm$ 86
14	<i>Fa/fa</i>	17	837 $\pm$ 10.5 ^b	141 $\pm$ 7.6 ^a	6781 $\pm$ 376 ^a
	<i>fa/fa</i>	15	794 $\pm$ 16.2 ^{b*}	149 $\pm$ 8.9 ^a	6914 $\pm$ 567 ^a
21	<i>Fa/fa</i>	8	992 $\pm$ 6.1 ^b	126 $\pm$ 13	8178 $\pm$ 909
	<i>fa/fa</i>	8	967 $\pm$ 9.3 ^{b*}	184 $\pm$ 15 ^{b*}	11764 $\pm$ 860 ^{a*}
28	<i>Fa/fa</i>	12	1030 $\pm$ 10.7 ^b	149 $\pm$ 3.2	10837 $\pm$ 362 ^a
	<i>fa/fa</i>	17	969 $\pm$ 11.6 [*]	223 $\pm$ 15.1 ^{**}	16305 $\pm$ 1098 ^{a**}

Table 2 Serum free T_4 concentration and kinetic constants of brain type II T_4 -5'D in 14- and 28-day-old *Fa/fa* and *fa/fa* rats

Free T_4 was measured in serum using a radioimmunoassay (see the Experimental section). *n* is the number of rats examined. A unit of enzyme activity is defined in Table 1. Results are means $\pm$ S.E.M. ^a*P* < 0.01 14 versus 28 days of age within the same genotype; ^{**}*P* < 0.01 *fa/fa* versus *Fa/fa* of the same age.

Age (days)	Rat	<i>n</i>	Serum free T_4 (pg/ml)	<i>n</i>	Type II deiodinase	
					K_m (nM)	V_{max} (units/mg of protein)
14	<i>Fa/fa</i>	21	16.6 $\pm$ 0.85	4	2.1 $\pm$ 0.31	206 $\pm$ 42
	<i>fa/fa</i>	16	16.4 $\pm$ 0.93	4	2.0 $\pm$ 0.45	239 $\pm$ 66
28	<i>Fa/fa</i>	9	25.6 $\pm$ 1.40 ^a	7	7.1 $\pm$ 1.02 ^a	797 $\pm$ 53
	<i>fa/fa</i>	9	15.5 $\pm$ 1.67 ^{**}	12	3.4 $\pm$ 0.47 ^{**}	704 $\pm$ 110

T_4 -5'D activity between lean and pre-obese pups. In the late suckling period (21 days) and thereafter, enzyme activity was significantly higher (+50%) in *fa/fa* than in *Fa/fa* rats.

To gain further insight into the mechanism responsible for the increased brain T_4 -5'D activity of pre-obese rats, we determined enzyme kinetic parameters in 14- and 28-day-old rats of both genotypes. Results presented in Table 2 clearly show that, at 14 days of age, no significant difference between the two genotypes could be detected in respect of either the K_m or the V_{max} of T_4 -5'D. In contrast, at 28 days of age, the K_m was twofold reduced in pre-obese compared with lean rats, whereas V_{max} was not affected by the *fa* gene.

Table 2 also shows that serum free T_4 concentrations were strictly identical in 14-day-old suckling *fa/fa* and *Fa/fa* littermates. From 14 to 28 days of age, a 55% increase in the hormone concentration was observed in *Fa/fa* rats, whereas no change could be detected in their pre-obese *fa/fa* counterparts. Consequently, in 4-week-old rats, the free T_4 level was significantly lower in *fa/fa* than in *Fa/fa* rats.

DISCUSSION

In brains of both *Fa/fa* and *fa/fa* rats, the substantial increase in the specific activity of T_4 -5'D which was observed within the second week of life strongly suggests that the type II deiodinase has a relevant role in the intense maturation of the central nervous system which occurs during this short period (Dussault and Ruel, 1987).

The main finding of the present study was that pre-obese rats developed a hypercapacity for conversion of T_4 into T_3 in the brain during the suckling-to-weaning transition.

In 14-day-old rats, neither activity of type II deiodinase at 2 nM T_4 , a subsaturating concentration, nor the kinetic parameters of the enzyme were affected by the *fa* gene. In lean Zucker rats, as previously reported by Kaplan and Yaskoski (1981) in Sprague-Dawley rats, the suckling-to-weaning transition was characterized by a fall in the affinity of brain deiodinase for T_4 .

In *fa/fa* rats, by contrast with what was observed in lean rats, no significant change in K_m value was observed from 14 to 28 days of age. Thus, in 28-day-old *fa/fa* compared with *Fa/fa* rats, the hyperactivity of deiodinase could be accounted for by the higher affinity of the enzyme for T_4 .

The thyroid status of *fa/fa* rats is not clear, since previous studies have reported that, in obese rats, circulating T_4 was either unchanged (Fletcher et al., 1986), decreased (Martin et al., 1978; Young et al., 1984) or even increased (Katzeff and Selgrad,

1993). This study clearly shows that a decrease in serum free T_4 is present in 1-month-old *fa/fa* rats compared with their lean *Fa/fa* counterparts.

Several authors have reported that T_4 -5'D increased in situations of experimental hypothyroidism coinciding with the decrease in the T_4 serum level (Serrano-Lozano et al., 1993; Obregon et al., 1991; Silva and Matthews, 1984). This increase might be a compensatory mechanism for maintaining the T_3 level in brain, since 80% of T_3 is formed by local T_4 -5'-deiodination (Crantz et al., 1982). However, our model strongly differed from those described in experimental hypothyroidism in which serum levels were dramatically reduced (Silva and Larsen, 1982; Leonard et al., 1981). Thus it cannot be ascertained whether, in obese rats, the decreased serum T_4 totally accounted for the increased capacity of T_4 5'-deiodination observed in brain.

Up to 14 days of age, pre-obese rats are characterized by a sharp decrease in type II deiodinase activity in BAT (Marie et al., 1992). The present study, which establishes that this activity is not impaired in the brain, demonstrates that regulation of this enzyme is highly tissue-specific. Our data confirm that sympathetic activity, which is known to be decreased in *fa/fa* rats (York et al., 1989), regulates deiodinase activity in BAT, but not in the brain (Guerrero et al., 1990).

In conclusion, in suckling *fa/fa* rats, the capacity for T_3 production in the brain was not altered early in life, at a time when thyroid hormones are essential for normal maturation of the central nervous system. However, during the suckling-to-weaning transition, increased activity of T_4 -5'D occurred in brains of pre-obese rats, and was totally accounted for by an increased affinity of the enzyme for T_4 . Such an abnormality could be interpreted as an adaptive response so as to maintain an adequate cerebral T_3 concentration despite the reduced serum free T_4 . In *fa/fa* rat, this mild decrease in circulating T_4 might play a secondary role in the development of obesity.

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