Biochemical Pharmacology, Vol. 29, pp. 1612–1615.

0006-2952/80/0601-1612 $02.00/0

Thyroid status and effects of 3,5,3′ triiodothyroacetic acid and Fenproporex in genetically lean and obese female rats*

(*Received* 19 *June* 1979; *accepted* 10 *January* 1980)

The obese rats described by Zucker [1] inherit their obesity as an autosomal Mendelian recessive trait. Among several defects which have been reported on these rats were hyperphagia inducing hyperlipemia [2] with high levels of phospholipids, cholesterol and triglycerides in plasma [3, 4], impaired reproductive function [5] and alterations in thyroid function [6, 7]. Genetically obese female rats on a low iodine diet showed a decrease in iodine thyroid uptake and turnover rate of radioactive iodine as well as a lower PBI, but serum level of TSH was normal. Bray and York [8] showed that the food intake of Zucker female rats was reduced after treatment by 5-hydroxytryptamine, *d*-amphetamine or iproniazid.

In the present work the thyroid function of genetically obese female rats and of their lean litter mates are examined. The effects on their body weight gain, food intake and serum lipid levels of two drugs, Fenproporex, an anorexigenic agent, and 3,5,3′ tri-iodothyroacetic acid (TRIAC), a metabolite of 3,5,3′ tri-iodo-L-thyronine [9, 10], are also studied.

Fenproporex has been reported to have an anorectic activity similar to that of *d*-amphetamine. This drug is used for the treatment of human obesity [11]. In general, experimental studies on genetically obese animals of effects of anorexigenic agents were performed in *ob/ob* mice. This latter observation led to the present experiments on genetically obese female rats.

The study of the effects of TRIAC was considered after earlier observations [12–15] which indicated that some properties of TRIAC are qualitatively similar to those of the thyroid hormones. In obese patients, thyroxine and triiodothyronine cause a decrease in the body weight gain [16]. Consequently, we investigated the effects of TRIAC in genetically obese female rats in order to examine whether its action on weight gain is similar to that of thyroid hormones.

Genetically obese female rats and their lean litter mates came from C.S.E.A.L.–C.N.R.S., Orléans, France. Animals at 6–8 weeks old were individually maintained in a temperature-controlled room (23°). They were given laboratory chow, including 17 per cent proteins, 3 per cent fat and 60 per cent carbohydrate. Food and water were available *ad lib*.

Three experiments were performed. In the first experiment, 8 genetically obese rats and 8 of their lean litter mates were injected i.p. with approximately 10 μCi of carrier-free ^{131}I (C.E.A.).They were killed 24 hr after the injection. Thyroid glands were dissected and weighted. The thyroid and blood radioactivity was measured with a NaI scintillator detector (Packard). In the second experiment, 8 genetically obese and 8 lean rats were killed and their blood collected. The concentration of thyroxine (T_4) in the serum was determined by competitive protein-binding analysis [17], whereas 3,5,3′ tri-iodo-L-thyronine (T_3) and 3,3,′5′ tri-iodo-L-thyronine (rT_3) were estimated by radioimmunoassay [18] with commercial kits (Ames, Hypolab).

In the third experiment 28 genetically obese rats and 28 of their lean litter mates were used. Each group was divided into 3 subgroups of obese and lean rats. Two subgroups—each one including 8 obese and 8 lean rats—were given either 175 μg of Fenproporex or 20 μg of TRIAC/100 g body wt/day. The third subgroup, composed of 12 obese and 12 lean animals, was used as control. The drugs were dissolved in 0.2 N NaOH–C_2H_5OH–water (1 : 4 : 5 v/v) and given by force feeding with stomach tube for 4 weeks. The control rats were given the same solution free from drug. Food intake and body weight were measured daily. Half the animals in each group were killed 24 hr after the last dose of Fenproporex or TRIAC and their blood collected. Serum cholesterol concentrations were determined by the Lieberman–Burchard reaction in chloroform after extraction with dimethoxy-methane–methanol (4 : 1 v/v) [19, 20]. Triglycerides were determined by an enzymatic method using glycerokinase and lacticodeshydrogenase [21]. Thyroxine was measured as described previously. The remaining animals were killed 2 weeks after the end of the treatment and serum T_4 concentration was determined as previously.

The thyroid uptake of ^{131}I (per cent of administrated dose) is about the same in obese (8.71 ± 0.43) and in lean (8.84 ± 0.48) rats. However, it appears (Table 1) that the serum T_4 levels are significantly lower ($P < 0.01$) in genetically obese rats (44.10 ± 2.90 ng/ml) than in their litter mates (55.10 ± 2.47 ng/ml). In contrast, the rT_3 concentrations are significantly higher ($P < 0.05$) in obese rats (0.33 ± 0.03 ng/ml) than in lean rats (0.24 ± 0.03 ng/ml), but T_3 concentrations are similar in both groups.

The data presented in Fig. 1 illustrate the effect of Fenproporex and TRIAC on body weight gain of rats during 4 weeks of treatment. The body weight gain of lean rats treated with Fenproporex is lower by approximately 20 per cent compared with the controls, while TRIAC has practically no effect. In genetically obese rats, whereas Fenproporex has no effect, TRIAC induces a rapid body weight loss compared with the controls. The inhibiting effect of TRIAC begins during the first week and becomes more and more marked throughout treatment. Thus, after one week, whereas untreated obese rats gain 22 ± 2 g, obese rats treated with TRIAC only gain 16 ± 1 g. After four weeks, obese controls gain 100 ± 3 g and TRIAC-treated obese rats 67 ± 6 g, which represents a decrease in body weight gain of about 30 per cent.

The effects of Fenproporex and TRIAC on the food intake expressed per unit of body weight gain (FI/BWG) and the serum content of various categories of lipids and of thyroxine in obese and lean rats are reported in Table 2. Compared with lean control, obese control rats have consistently higher levels of total cholesterol ($P < 0.01$), total lipids ($P < 0.01$) and triglycerides ($P < 0.01$), confirming previous observations [3, 4]. However, the treatment with Fenproporex or TRIAC has no statistically significant effect on any of these parameters in either obese or lean rats. Serum T_4 concentration, which, as had been previously found, is lower in the obese controls when compared to the mean for the lean controls, appears highly reduced under the action of TRIAC (14.25 ± 1.18 ng/ml in lean rats and 15.75 ± 2.78 ng/ml in obese rats). This

* Sections of this work were reported at the 9th Pan American Congress of Endocrinology, Quito, Ecuador, 27 November–1 December 1978, Abstract No. 14.

Table 1. Serum thyroid hormones concentration in lean and obese female rats*

Animals	No. rats	Body weight (g)	T_4 (ng/ml)	T_3 (ng/ml)	rT_3 (ng/ml)
Lean	8	236 ± 3	55.10 ± 2.47	0.98 ± 0.06	0.24 ± 0.03
Obese	8	398 ± 3§	44.10 ± 2.90‡	0.99 ± 0.06	0.33 ± 0.03†

* Results are expressed as means ± S.E.M.
† $P < 0.05$.
‡ $P < 0.01$.
§ $P < 0.001$.

decrease in T_4 concentration is temporary, since the values return to normal levels in the second week after treatment.

Previous studies indicated that genetically obese female rats had several abnormalities in thyroid function [6]. The present experiments confirm these data. The results show that ^{131}I thyroid uptake is about the same in both obese and lean rats but significant differences in serum iodothyronine concentrations are observed in obese Zucker rats in comparison with their litter mates. The serum T_4 concentration in genetically obese rats is significantly lower than in lean rats. In contrast, rT_3 concentration increases in obese animals. It was previously found that PBI was decreased in obese Zucker rats [6]. The present results support these data, since the T_4 level which is related to the PBI value is reduced in these rats. In spite of low PBI and low T_4 serum concentrations, it is observed that obese rats are slightly hypothyroid. This fact is difficult to explain if we consider the T_4 values only, but if we consider the T_3 values, we find that these are similar in obese and lean rats. Therefore, as T_3 is the most active hormone, it is not surprising that the thyroid effects are almost normal. Nevertheless, as the animals are hyperphagic, more T_3 is needed to utilize the extra food intake. Since serum T_4 concentration is reduced when rT_3 is clearly high, it seems likely that the peripheral deiodination of T_4 in obese rats is impaired, for, instead of giving rise to T_3, the inactive rT_3 is generated. This observation supplements other studies which reveal an abnormal peripheral deiodination of T_4 in obesity [22–26].

Our experiments show the effects of Fenproporex and of TRIAC on the body weight gain and on FI/BWG, serum lipids and thyroxine. It is found that Fenproporex decreases the body weight gain in lean rats but not in obese rats. Our results may be related to those previously obtained by Bray and York [8], who studied the effects of *d*-amphetamine on food intake of obese female and lean rats in acute conditions. They found that amphetamine injected 5 min prior to feeding almost completely inhibited food intake in lean rats but there was only a 50 per cent inhibition in obese ones. In view of the report by Maickel and Zabik [27] on the pharmacology of anorexigenis, it is reasonable to assume that brain biogenic amines which participate in the action of anorexigenic agents are probably inadequately stimulated in obese rats. In contrast, TRIAC, which is without effect on lean rats, reduces the body weight gain in genetically obese rats. However, we find that in fatty rats the food intake expressed per unit of body weight gain is not affected. This result is comparable with data reported by Bray *et al.* [28] who showed that estradiol 17 β treatment of genetically female rats reduced their body weight by 30 per cent without any change in their food intake. We also find that serum lipid levels are not altered by TRIAC. This result may be surprising, but it is known that TRIAC affects both the anabolism and the catabolism of cholesterol.

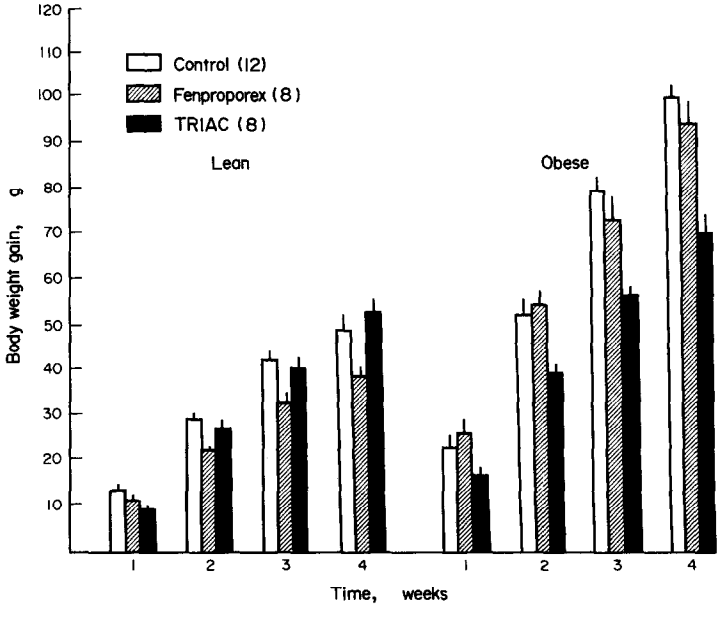

Fig. 1. Effect of Fenproporex and TRIAC on the body weight gain of lean and obese female rats during 4 weeks. Vertical bar represents ± S.E.M. No. of rats in parentheses.

Table 2. Effects of Fenproporex and TRIAC on the body weight, serum T_4 concentration and serum lipid levels in lean and obese female rats after 4 weeks of treatment*

	Lean			Obese		
	Control	Fenproporex	TRIAC	Control	Fenproporex	TRIAC
Initial body weight (g)	119 ± 4 (12)	122 ± 3 (8)	123 ± 3 (8)	155‡ ± 6 (12)¶	169 ± 5 (8)	140 ± 8 (8)
Final body weight (g)	168 ± 3 (12)	160† ± 3 (8)§	174 ± 3 (8)	254‡ ± 7 (12)¶	263 ± 7 (8)	208† ± 11 (8)¶
FI/BWG	9.03 ± 0.73 (12)	10.96 ± 0.43 (8)	8.30 ± 0.65 (8)	5.94‡ ± 0.18 (12)¶	6.57 ± 0.22 (8)	6.66 ± 0.39 (8)
Total cholesterol (g/l)	0.83 ± 0.07 (6)	0.75 ± 0.03 (4)	0.87 ± 0.03 (4)	1.15‡ ± 0.07 (6)‖	1.15 ± 0.16 (4)	1.22 ± 0.06 (4)
Total lipids (g/l)	3.70 ± 0.18 (6)	3.40 ± 0.21 (4)	3.83 ± 0.11 (4)	6.28‡ ± 0.26 (6)¶	6.63 ± 0.66 (4)	6.84 ± 0.24 (4)
Triglycerides (g/l)	0.70 ± 0.09 (6)	0.96 ± 0.13 (4)	0.98 ± 0.08 (4)	2.02‡ ± 0.12 (6)¶	2.56 ± 0.31 (4)	2.26 ± 0.38 (4)
T_4 (ng/ml)						
At the end of treatment	48.33 ± 2.73 (6)	54.75 ± 3.57 (4)	14.25† ± 1.18 (4)¶	41.33‡ ± 2.05 (6)§	50.50 ± 4.93 (4)	15.75† ± 2.78 (4)¶
Two weeks after end of treatment	59.32 ± 4.22 (6)	51.80 ± 9.25 (4)	54.00 ± 4.16 (4)	51.25 ± 4.25 (6)	55.36 ± 3.48 (4)	51.33 ± 4.67 (4)

* Results are expressed as means ± S.E.M. The number of rats is given in parentheses.
† Compared with control rats.
‡ Compared with lean rats.
§ $P < 0.05$.
‖ $P < 0.01$.
¶ $P < 0.001$.

Several mechanisms could explain the lower body weight gain in TRIAC-treated obese female rats. Bray *et al.* [8, 28] showed that genetically obese rats respond abnormally to various diets, cold and several drugs. Thus, lean rats adapt to changes in the caloric density of their diet, but obese rats do not. In a cold atmosphere, the food intake of fatty rats falls, but lean rats compensate for heat by eating more. The response of genetically obese rats to drugs is also different from that of their lean litter mates. We have found out that genetically obese rats are slightly hypothyroid. Thus, it is suggested that TRIAC treatment should probably partly restore this abnormality and thus allow a better degradation of the food intake.

To summarize, the ^{131}I thyroid uptake is about the same in genetically obese female rats as in their lean litter mates. However, the serum thyroxine (T_4) concentration is significantly lower in obese Zucker rats in comparison with lean rats. In contrast, the serum concentration of 3,3'5' triiodo-L-thyronine (rT_3) of genetically obese rats is higher than in lean rats. The serum 3,5,3' triiodo-L-thyronine (T_3) is similar in all the animals. The treatment by Fenproporex causes the body weight gain to decrease in lean rats but not in obese rats. The administration of 3,5,3' tri-iodothyroacetic acid (TRIAC) is without effect on lean rats but rapidly induces an important body weight loss in obese rats. These drugs have no statistically significant effect on the various categories of serum lipids in either obese or lean rats.

Biochimie Cellulaire,
ENSBANA,
Campus Universitaire,
21000 *Dijon,*
and Endocrinologie,
UER des Sciences
Pharmaceutiques
et Biologiques
4, *avenue de l'Observatoire,*
75006 *Paris, France.*

NICOLE AUTISSIER
PAUL DUMAS
ANNIE LOIREAU
RAYMOND MICHEL

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