Control of obesity in A^{vy}/a mice by 5α-androstan-17-one

T.T. Yen, J.A. Allan, D.V. Pearson and J.M. Acton[1]

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis (Indiana 46206, USA), 20 March 1978

Summary. Treating VY/WfL-A^{vy}/a mice with 5α-androstan-17-one, a mammalian glucose-6-phosphate dehydrogenase inhibitor, prevented the mice from becoming obese. The weight difference between treated and control A^{vy}/a mice was mainly due to a decreased accumulation of triacylglycerol. The compound did not suppress appetite, had no detectable toxicity and did not affect the lipogenesis rates in the liver and carcass. The weight-controlling effect of 5α-androstan-17-one in A^{vy}/a mice was reversible upon withdrawal of treatment.

We have reported previously that dehydroepiandrosterone, a mammalian glucose-6-phosphate dehydrogenase inhibitor, prevented A^{vy}/a mice from becoming obese[2]. The compound was active by parenteral injection as well as oral administration. The difference in weight between treated and control A^{vy}/a mice could largely be accounted for by the decreased accumulation of triacylglycerol. The compound did not suppress appetite and had no detectable toxicity that could account for the weight change. The prevention of obesity in A^{vy}/a mice was reversed by the withdrawal of treatment. Hepatic lipogenesis rate in dehydroepiandrosterone-treated mice was lower than that in control mice. Based on these observations, it is speculated that dehydroepiandrosterone may play a physiological role in weight regulation by inhibiting glucose-6-phosphate dehydrogenase thereby limiting the supply of NADPH available for fatty acid synthesis. To verify this hypothesis, another glucose-6-phosphate dehydrogenase inhibitor, 5α-androstan-17-one[3], was studied in A^{vy}/a mice. The results are presented in this report. Preliminary results of the study were published elsewhere[4].

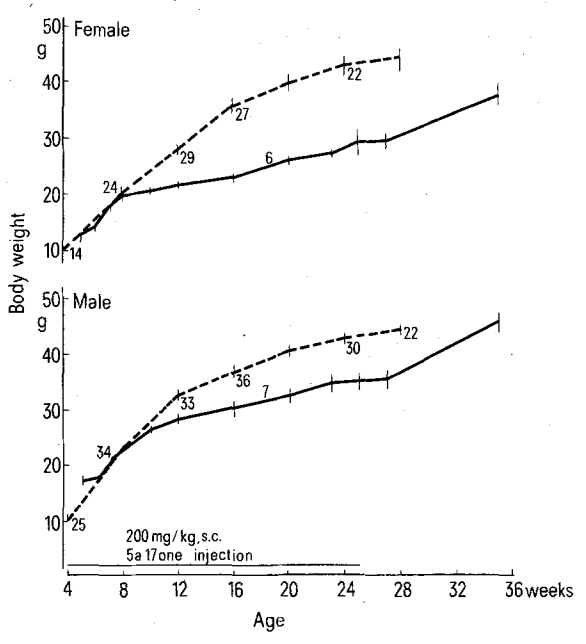

Fig. 1. B.wt of female (top panel) and male (bottom panel) A^{vy}/a mice given 5α-androstan-17-one, 200 mg/kg, s.c., in saline containing 5% Emulphor (EL-620) 3 times weekly starting from 10 to 21 days of age through the 25th week after birth (solid line). At 26 weeks of age, the treatment was discontinued. The A^{vy}/a mice serving as controls were contemporary untreated mice combined with mice treated with sesame oil (dotted line). Purina Laboratory Chow and water were available ad libitum. The curves illustrate mean b.wt ± SE. The numbers on the curves indicate the number of mice for each point. If no number is shown, the number of mice for that point is the same as that for the previous point.

Materials and methods. Inbred VY/WfL-A^{vy}/a mice from our own colony were used in this study. The viable yellow gene (A^{vy}) is an allele at the agouti locus. A^{vy}/a mice that are not 100% agouti-colored are destined to become obese. Some of the metabolic characteristics of A^{vy}/a mice have been described previously[5,6].

Mice were fed Purina Laboratory Chow and given access to water ad libitum. Mice sacrificed for analysis had feed and water available until the time tritiated water was injected. The animal room was lighted from 06.00 h to 18.00 h.

Procedures for analyzing triacylglycerol content and for measuring in vivo lipogenesis rates with tritiated water have been defined elsewhere[6]. Chemicals and standards for all assays were from the same suppliers as described before[6]. 5α-androstan-17-one was purchased from Steraloids, Pawling, N.Y. Emulphor EL-620 (General Aniline and Film, New York), a polyoxyethylated vegetable oil,

Table 1. Food consumption of 5α-androstan-17-one- and vehicle-treated A^{vy}/a mice

Treatment	No. of mice	Food consumption (g/mouse/day)
Control	8 ♂ and 4 ♀	3.8±0.1 (42)*
Control	5 ♂	3.5±0.2 (25)
10 mg/kg, i.p.	8 ♂	3.8±0.2 (8)

* Mean ± SE (No. of measurements).

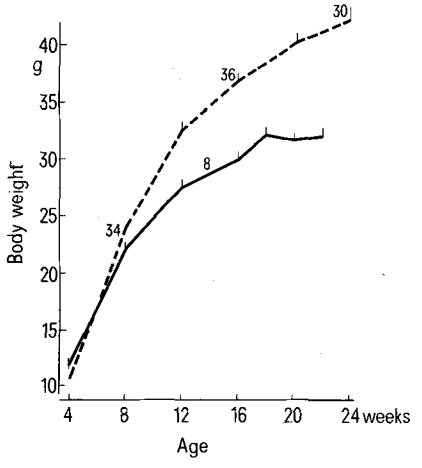

Fig. 2. B.wt of male A^{vy}/a mice given 5α-androstan-17-one, 10 mg/kg, i.p. in saline containing 5% Emulphor (EL-620) 3 times weekly (solid line). Injections of the compound were started at 4 weeks of age and lasted the entire period shown in the graph. The A^{vy}/a mice serving as controls were contemporary untreated mice combined with mice treated with sesame oil (dotted line). Purina Laboratory Chow and water were available ad libitum. The curves illustrate mean b.wt ± SE. The numbers on the curves indicate the number of mice for each point. If no number is shown, the number of mice for that point is the same as that for the previous point. At 4 weeks of age, N = 25 for the control group and N = 8 for the treated group.

Table 2. Triacylglycerol (TG) content and incorporation of ^3H_2O into total lipid* in liver and carcass of male A^{vy}/a mice**

Treatment	N	Body weight (g)	μmoles TG/ g b.wt	Liver weight (g)	μmoles TG/ g liver	μmoles ^3H_2O incorporated into total lipid in carcass/g b.wt	μmoles ^3H_2O incorporated into total lipid in liver/g liver wt
Control	10	40.0±1.7*	278±25	1.62±0.09	39±10	3.5±0.3	31±3
5α-androstan-17-one 10 mg/kg, i.p.	8	31.7±0.8***	144±15***	1.39±0.05***	12± 1***	3.0±0.1	33±1

*Mean±SE. **Both control and treated mice were 5–6 months of age at the time of sacrifice. ***$p<0.05$.

was used to facilitate the suspension of 5α-androstan-17-one in saline.

Results and discussions. Male and female A^{vy}/a mice given 5α-androstan-17-one, 200 mg/kg, s.c., 3 times weekly were prevented from becoming obese (figure 1). Like dehydroepiandrosterone[2], the effect of treatment was reversible upon withdrawal of 5α-androstan-17-one injections (figure 1). At 10 mg/kg, i.p., the compound also controlled the weight gain of male A^{vy}/a mice (figure 2).

The effect of 5α-androstan-17-one on the weight of A^{vy}/a mice was not due to appetite suppression (table 1). Treated mice that were autopsied revealed no pathological changes that would account for the weight difference between treated and control mice[1]. The only finding that was significant was an increase of eosinophilic cells in the ovary of 4 of the 5 female A^{vy}/a mice treated with the compound at 200 mg/kg, s.c. The meaning of this change is not clear to us.

The concentration of triacylglycerol in the carcass and livers of treated A^{vy}/a mice was substantially less than that of age-matched control A^{vy}/a mice (table 2). This difference in triacylglycerol content amounts to 63% of the difference in weight between control and treated A^{vy}/a mice, suggesting that the compound acts on the metabolism of triacylglycerol. However, unlike dehydroepiandrosterone-treated mice, which had lower hepatic lipogenesis rates[2], the 5α-androstan-17-one-treated mice had lipogenesis rates not significantly different from those of control mice (table 2). This suggests that 5α-androstan-17-one prevents A^{vy}/a mice from becoming obese through a mechanism other than the inhibition of lipogenesis.

Alternatively, it is possible that the inhibition of triacylglycerol synthesis by 5α-androstan-17-one is masked in younger mice. Previous studies on dehydroepiandrosterone[2] were done on older mice which probably had a higher rate of triacylglycerol synthesis than the younger mice used in the present study. Since we were measuring the incorporation of ^3H_2O into all lipid species, changes in the rate of triacylglycerol synthesis in older mice should be easier to detect because triacylglycerol synthesis represents a higher proportion of their total lipid synthesis.

Regardless of the mechanism, this study shows that in addition to dehydroepiandrosterone, a second, structurally similar mammalian glucose-6-phosphate dehydrogenase inhibitor, namely 5α-androstan-17-one, can also prevent A^{vy}/a mice from becoming obese.

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Oxytocin analogs effective as noncompetitive inhibitors in uterotonic test

M. Lebl, V. Bojanovska, T. Barth and K. Jošt

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, CS-166 10 Prague 6 (Czechoslovakia), 4 April 1978

Summary. Deamino-l-carba-oxytocin analogs with a chemically reactive group in position 4 were demonstrated to act as noncompetitive oxytocin inhibitors in the assay on isolated rat uterus.

Even though numerous oxytocin (**Ia**) inhibitors are known[1], no compounds with a specific irreversible effect have so far been reported. Our efforts to obtain this type of compound by substitution of the primary amino group of the cysteine in position 1, have resulted merely in the preparation of products showing a competitive inhibitory effect[2,3].

In this study we have been able to synthesize a series of analogs derived from deamino-oxytocin[4] whose disulfide bond was replaced by a thioether group; these analogs have different reactive groups at the γ-carbon of the glutamic acid in position 4.

Materials and methods. As starting material [4-glutamic acid] deamino-l-carba-oxytocin[5] (**Ib**) was used. Analog **Ic** was prepared by condensation of the latter with S-benzyl-cysteine methyl ester, followed by removal of the protecting group by sodium in liquid ammonia. Compound **Id** was obtained by the reaction of analog **Ib** with sec-butyl chloroformate in the presence of N-ethylmorpholine. The common intermediary product for the preparation of 3 other analogs was [4-glutamic acid-γ-hydrazide]deamino-l-carba-oxytocin prepared by carbodiimide condensation of compound **Ib** with Boc-hydrazine and subsequent removal of