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α_1- and β-Adrenergic receptors in brown adipose tissue of lean (*Fa/?*) and obese (*fa/fa*) Zucker rats

Effects of cold-acclimation, sucrose feeding and adrenalectomy

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1. The populations of α_1- and β-adrenergic receptors in brown adipose tissue (BAT) of genetically obese Zucker rats (*fa/fa*) were studied with [^{3}H]prazosin and [^{3}H]CGP-12177 respectively. 2. The density of α_1-adrenergic receptors in BAT was significantly lower in obese than in lean Zucker rats, both at 2–4 months of age and at 6 weeks of age. The density of β-adrenergic receptors was identical in BAT of lean and obese 6-week-old Zucker rats. 3. Cold-acclimation increased the α_1-receptor density significantly in BAT of both lean and obese Zucker rats, and the number of β-receptors was also somewhat increased. 4. Sucrose feeding did not affect the density of α_1-receptors in BAT of lean or obese Zucker rats, but it increased β-receptor density. 5. Adrenalectomy restored the density of α_1-adrenergic receptors in BAT of obese Zucker rats to the value observed in lean rats. 6. It is concluded that there is a direct correlation between α_1-receptor density and tissue recruitment, and that α_1-receptor density is thus positively correlated with sympathetic activity. β-Receptor density is apparently better correlated with feeding conditions.

INTRODUCTION

A defect in brown adipose tissue (BAT) function is involved in the development of genetic obesity in the obese *ob/ob* and *db/db* mice (Himms-Hagen & Desautels, 1978; Trayhurn, 1984) and in *fa/fa* Zucker rats (Holt & York, 1982; Holt *et al.*, 1983; Triandafillou & Himms-Hagen, 1983). Several abnormalities in BAT are associated with genetic obesity [for reviews see Bray & York (1979), Himms-Hagen (1983) and Trayhurn (1984, 1986)]. GDP binding to BAT mitochondria is decreased in young obese *fa/fa* rats (Holt & York, 1982) and there is also a decreased amount of thermogenin (Ashwell *et al.*, 1985). Noradrenaline content and turnover are also decreased (Levin *et al.*, 1983; York *et al.*, 1985). Obese Zucker rats have, however, a normal response to cold when measured as the cold-induced increase in the amount of GDP binding to BAT mitochondria (Holt *et al.*, 1983) and as the increase in noradrenaline turnover (shorter half-life) in the tissue (York *et al.*, 1985).

Adrenalectomy is a unique treatment in preventing the further development of genetic, dietary or hypothalamic obesity (Bruce *et al.*, 1982; Rothwell *et al.*, 1984; Marchington *et al.*, 1986).

The sympathetic nervous system, via noradrenaline release, directly regulates the heat production in BAT, i.e. the acute thermogenic response [for reviews see Nedergaard & Lindberg (1982) and Trayhurn & Nicholls (1986)]. A chronic noradrenaline stimulation is also, at least partly, responsible for the recruitment of the tissue in the chronically activated states (Barnard *et al.*, 1980). It is therefore of interest to study the relationship between adrenergic receptors and the degree of recruitment of the tissue.

An increased density of α_1-adrenergic receptors is found in BAT from cold-acclimated hamsters and rats (Raasmaja *et al.*, 1985) and in cafeteria-fed rats (Raasmaja *et al.*, 1984), i.e. in situations where BAT is very active and is physiologically recruited (Cannon & Nedergaard, 1986). An enhanced function of the α_1-adrenergic pathway (Nedergaard *et al.*, 1986) could have an important role in BAT hyperplasia, growth and differentiation (i.e. the physiological recruitment of the tissue). It would therefore be interesting to extend the generality of this concept of a relationship between α_1-receptor density and tissue recruitment to an involuted inactive physiological situation, such as that seen in the obese rats.

A contrasting pattern of responses has been reported for β-receptors. Cold-acclimation has been reported to decrease slightly the density of β-receptors in rat BAT (Bukowiecki *et al.*, 1978; Kurahashi & Kuroshima, 1981), or to have no effect on the β-receptor number in hamster BAT cells (Svartengren *et al.*, 1982), although a functional desensitization [i.e. a decreased coupling between the β-receptor and adenylate cyclase activation, cellular cyclic AMP contents, and thermogenesis (O_2 consumption)] could be seen even in hamsters (Nedergaard, 1982; Svartengren *et al.*, 1982, 1984; Svoboda *et al.*, 1984*a*,*b*). A cafeteria diet, however, apparently slightly increases the density of β-receptors in brown fat of rats (Rothwell *et al.*, 1986).

We have postulated that the ratio α_1/β receptors varies in relation to the degree of recruitment of the tissue (Raasmaja *et al.*, 1984, 1985; Nedergaard *et al.*, 1986). We have here determined both α_1- and β-adrenergic receptors from BAT crude membranes of lean and obese (*fa/fa*) Zucker rats.

Abbreviation used: BAT, brown adipose tissue.

We conclude that the changes in α_1-receptor density found are in accordance with a simple relationship between α_1-receptor density and tissue recruitment state, whereas the changes in β-receptor density seem to be better correlated with the feeding status of the animals.

EXPERIMENTAL

Animals

Male and female lean (*Fa/Fa* or *Fa/fa*) and obese (*fa/fa*) Zucker rats were bred from heterozygote (*Fa/fa*) parents in the animal facility at the University of Southampton. All animals were fed with rat chow (Christopher Hill, Poole, Dorset, U.K.) and water *ad libitum*. Adult rats at 2–4 months of age were used for the initial experiments. Further studies were made with rats of 4–5 weeks of age. The identification of phenotypes was based on visual morphological characteristics: already at a young age, genetically obese rats have a slightly rounder body shape (owing to increasing fat deposits on their sides) and a shorter tail than their lean littermates.

The rats were divided into three groups: control, sucrose-fed and cold-acclimated. In addition to normal water, the sucrose-fed rats had free access to a 35% (w/v) sucrose drinking solution. Control and sucrose-fed animals were housed at 21 ± 2 °C and cold-acclimated animals at 4 ± 1 °C. Sucrose feeding and cold-acclimation were continued for 2 weeks. The animals were then killed by cervical dislocation between 09:00 and 11:00 h, and interscapular, subscapular and cervical BAT was removed, cleaned and minced with scissors.

Bilateral adrenalectomies or sham operations were performed as described by Holt & York (1982) on lean and obese rats at 4 weeks of age. Adrenalectomized rats were maintained on a 0.9% (w/v) NaCl drinking solution. Animals were killed after 1 week for the preparation of BAT membranes.

Preparation of BAT crude membranes

Tissue pieces from four to ten rats were pooled in the preparation buffer (0.25 M-sucrose/10 mM-Tris/HCl/1 mM-EDTA, pH 7.4). The tissue was homogenized in a Potter–Elvehjem homogenizer with a Teflon pestle in 10 vol. of preparation buffer. The homogenate was filtered through one layer of silk cloth (Joymar Scientific, New York, NY, U.S.A.) and the filtrate was centrifuged for 30 min at 100000 *g* in a Beckman high-speed ultracentrifuge. The pellet was rehomogenized, washed and centrifuged additionally two or three times as above. The final pellet was resuspended in 50 mM-Tris/HCl/10 mM-$MgCl_2$ (pH 7.4) and filtered again in order to remove any possible non-homogenized tissue pieces. The crude membranes were frozen at a concentration of 8–10 mg of protein/ml and stored at −60 °C until used (we have not observed a systematic effect of freezing on the number of adrenergic binding sites). Samples were generally prepared in parallel from a full series of lean and obese control, sucrose-fed and cold-acclimated rats.

Protein was determined by the method of Lowry *et al.* (1951), with fatty-acid-free bovine serum albumin fraction V (Boehringer Mannheim) as standard.

Measurement of [^{3}H]prazosin and [^{3}H]CGP-12177 binding

Equilibrium-binding studies were performed with various concentrations of 7-methoxy[^{3}H]prazosin (0.04–5.5 nM) for α_1-receptors (Agrawal & Daniel, 1985) (60 Ci/mmol; Amersham) or [^{3}H]CGP-12177 (0.06–6.2 nM) for β-receptors (Affolter *et al.* (1985) (30 or 36 Ci/mmol; Amersham). BAT crude membranes (0.25 mg for α_1- or 0.75 mg for β-receptor studies) were incubated for 60 min in 0.32 ml of incubation buffer (50 mM-Tris/HCl, 10 mM-$MgCl_2$, 0.2 mM-ascorbic acid, pH 7.4) at room temperature (20 ± 2 °C) in an 8 × 12-well micro-plate on a shaking plate (120 rev./min). The reaction was terminated by filtration of the incubations through a prewetted single Whatman glass microfibre filter GF/B under water-pump vacuum. A semi-automatic Skatron cell harvester 7019 (Skatron AS, Lier, Norway), with a suction unit and tubings for simultaneous harvesting of 12 individual wells, was used. The wells and filters were washed with 8 ml of the incubation buffer (diluted 1:10)/well. This wash volume was enough to minimize background values. The filters were dried overnight, and the radioactivity was determined in 5 ml of Beckman Readysolve scintillation mixture in an Intertechnique or Philips scintillation counter.

The total binding (B_T) was measured as above. The non-specific binding (B_{NS}) was determined by parallel incubations with a 1000-fold excess of phentolamine methanesulphonate (Ciba–Geigy) for [^{3}H]prazosin and a 100-fold excess of (−)-alprenolol (Sigma) for [^{3}H]CGP-12177. The specific binding (B_S) was estimated as the differences between the total and the non-specific binding. The data were analysed as described by Scatchard (1949). All binding assays for either [^{3}H]prazosin or [^{3}H]CGP-12177 were performed in parallel for each preparation series of BAT crude membrane and in duplicates.

Table 1. Adult rats: body weights, protein yields, 5′-nucleotidase activities and receptor densities in BAT from 2–4-month-old lean and obese Zucker rats

A unit of enzyme activity is 1 μmol of AMP hydrolysed/min. Results are means for 21 rats (body weights) or for four preparations in each group. $^*P < 0.05$, $^{*(*)}P < 0.02$, $^{**}P < 0.01$ and $^{***}P < 0.001$, compared with lean group, by Student's t test; ns, not significant.

Rats ...	Lean		Obese
Body wt. (g)	278 ± 14	***	436 ± 17
Protein yield (mg/animal)	15.9 ± 2.0	**	5.4 ± 0.8
5′-Nucleotidase (munits/mg of protein)	3.8 ± 0.0	**	8.0 ± 0.8
[^{3}H]Prazosin			
K_D (nM)	0.04 ± 0.01	ns	0.06 ± 0.01
$B_{max.}$ (fmol/mg of protein)	17.2 ± 1.7	ns	15.7 ± 2.5
$B_{max.}$ (fmol/munit)	4.6 ± 0.4	**	2.0 ± 0.3
[^{3}H]CGP-12177			
K_D (nM)	0.32 ± 0.04	ns	0.40 ± 0.03
$B_{max.}$ (fmol/mg of protein)	14.7 ± 0.8	*(*)	25.3 ± 3.0
$B_{max.}$ (fmol/munit)	3.9 ± 0.2	*	3.1 ± 0.2
Ratio of α_1/β receptors	1.2		0.6

Measurement of 5′-nucleotidase activity

The 5′-nucleotidase assay was a modification of the method of Avruch & Wallach (1971). The incubation

Table 2. Young rats: effects of cold acclimation and sucrose feeding on body weights and BAT of 6-week-old lean and obese Zucker rats

Values are means ± S.E.M. for the indicated numbers of animals, except for protein yields (amount of protein after last centrifugation; see the Experimental section), which are based on the indicated numbers of preparations of pooled material. 5′-Nucleotidase activities were measured in all preparations; units are μmol of AMP hydrolysed/min. $^{(*)}P < 0.1$, $^{*}P < 0.05$, $^{*(*)}P < 0.02$, $^{**}P < 0.01$, $^{***}P < 0.001$ and ns $P > 0.1$ indicate differences between lean and obese animals. (†), † etc. and NS indicate effects of sucrose feeding, cold acclimation (or adrenalectomy) versus the corresponding control group according to the same system. This statistical notation is used in all subsequent tables.

	Control			Cold-acclimated			Sucrose-fed		
	Lean		Obese	Lean		Obese	Lean		Obese
Final body wt. (g)	129 ± 4	*(*)	140 ± 5	93 ± 4 †††	(*)	104 ± 4 †††	146 ± 4 ††	**	165 ± 5 ††
n	54		50	16		16	33		32
BAT wet wt. (mg/animal)	244 ± 9	***	440 ± 27	430 ± 15 †††	***	825 ± 59 †††	442 ± 36 †††	**	627 ± 47 †††
n	41		36	20		20	9		10
Protein yield (mg/animal)	7.7 ± 1.0	ns	6.5 ± 0.7	18.3 ± 2.2 ††	ns	18.9 ± 2.0 †††	13.8 ± 1.6 †††	**	6.2 ± 1.1 NS
n	9		9	4		4	6		6
5′-Nucleotidase (munits/mg of protein)	4.61 ± 0.49	ns	5.74 ± 0.8	4.29 ± 0.71 NS	ns	4.51 ± 0.39 NS	4.82 ± 0.44 NS	ns	5.39 ± 0.94 NS

was carried out with 0.05 mg of protein (100 μl) of BAT crude membranes in a total volume of 350 μl of incubation buffer {containing 57 μM-[^{3}H]AMP (11.2 Ci/mmol; New England Nuclear), 143 mM-Tris/HCl (pH 8.0) and 0.5 mM-$MgCl_2$} for 15 min at 37 °C in a shaking water bath. The reaction was stopped by addition of 200 μl of 0.25 M-$ZnSO_4$, and 330 μl of 0.15 M-$Ba(OH)_2$ solution (Sigma) was added to precipitate protein and unhydrolysed [^{3}H]AMP. The mixture was centrifuged for 1 min at maximal speed (4000 rev./min) in a Hettich Universal micro-centrifuge, and 400 μl of the supernatant (with the released [^{3}H]adenosine) was counted for radioactivity in a scintillation mixture of toluene/Triton X-100 (2:1, v/v) and 2,5-diphenyloxazole (5 g/l), containing 17% water.

RESULTS

Equilibrium-binding experiments were performed with [^{3}H]prazosin and [^{3}H]CGP-12177 to measure the densities of α_1- and β-adrenergic receptors in crude membranes from the BAT of Zucker rats. Initial results (see below) indicated an abnormal α_1- and β-adrenergic-receptor density in BAT of grossly obese Zucker rats of 2–4 months of age. To eliminate the possibility that the observed effects were secondary to obesity as such, we decided to investigate more thoroughly the α_1- and β-receptor characteristics in rats of 6–7 weeks of age, i.e. before the obesity became severe. Further, we have examined the effect of a sucrose diet, cold-acclimation and adrenalectomy.

Body weights and BAT characteristics

Adult rats. The body weight was of course higher in obese than in lean rats (Table 1). The amount of BAT crude membranes obtained (as mg of protein yield) was, however, lower in obese than in lean animals. The specific activity of 5′-nucleotidase was twice as high in obese as in lean rats, probably because more mitochondria are present in the lean animals and the plasma-membrane components thus are more diluted.

Young rats. In young rats the final body weight was only about 10% higher in the obese than in the lean rats under control conditions (Table 2). In both genotypes the final body weight was decreased owing to cold-acclimation and increased by sucrose feeding. The BAT wet weight was higher in obese than in lean animals in all three groups. BAT wet weight was increased both by cold-acclimation and by sucrose feeding, but this increase was relatively lower in sucrose-fed obese rats than in sucrose-fed lean rats.

The protein content of the crude membrane preparations was not higher than in the lean control rats, indicating that in these young animals the obesity-induced BAT atrophy was not so pronounced as in the adults (Table 1). The amount of protein obtained was increased in cold-acclimated lean and obese rats, and in sucrose-fed lean rats, but there was no effect of sucrose feeding on the protein yield of obese rats. This difference indicates a lack of activation of some BAT functions owing to diet in the obese Zucker rats, and suggests that the increase in BAT weight in sucrose-fed obese rats merely reflects an increase in lipid deposition. These general observations are consistent with previous demonstrations, which indicate a decreased response to diet in obese Zucker rats, whereas their response to cold seems to be normal (Holt *et al.*, 1983; Triandafillou & Himms-Hagen, 1983).

Determination of [^{3}H]prazosin-binding sites (α_1-receptors) in BAT

Fig. 1 presents the result of a typical experiment showing a saturable high-affinity ligand binding, with a K_D of 0.05 nM and a $B_{max.}$ of 36 fmol/mg of protein. The non-specific binding (B_{NS}) was 10–50% of the total binding (B_T). The Scatchard plots of the specific binding were always linear, indicating that only one binding site

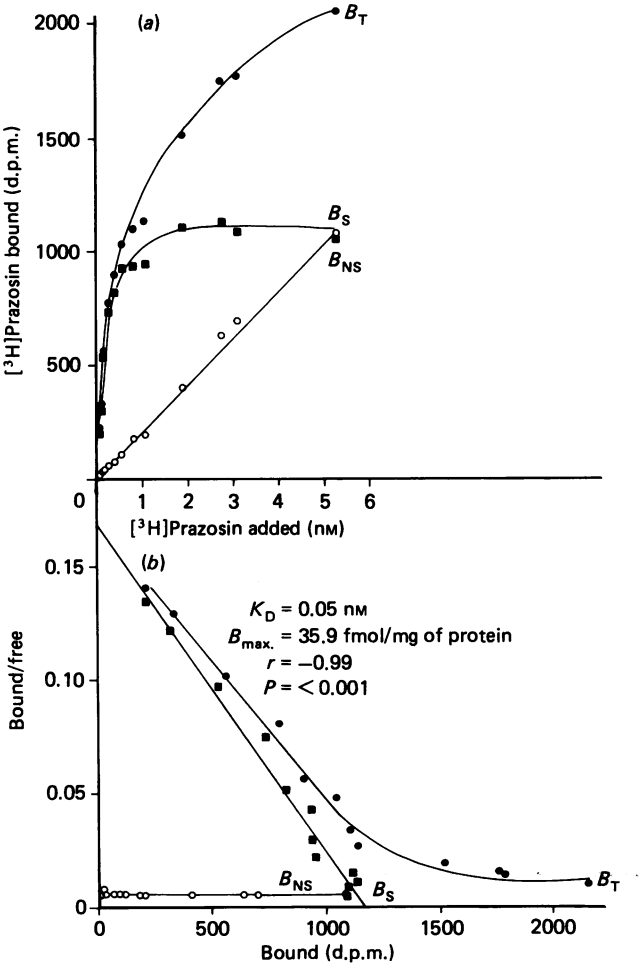

Fig. 1. Binding of [^{3}H]prazosin to BAT crude membranes of lean control Zucker rats

(*a*) Total (B_T), specific (B_S) and non-specific (B_{NS}) binding, as a function of radioligand concentration. The equilibrium binding assays were performed as described in the Experimental section. Each value is the mean of duplicate determinations. Ligand concentrations given are calculated from the actual radioactivity found in the incubations and the specific radioactivity given by the manufacturers. (*b*) Scatchard plots of [^{3}H]prazosin binding. The data from the binding curves in (*a*) were analysed by the method of Scatchard (1949). The slope of the line ($-1/K_D$) was determined by linear regression analysis. The maximum binding capacity ($B_{max.}$) was obtained as the intercept with the abscissa.

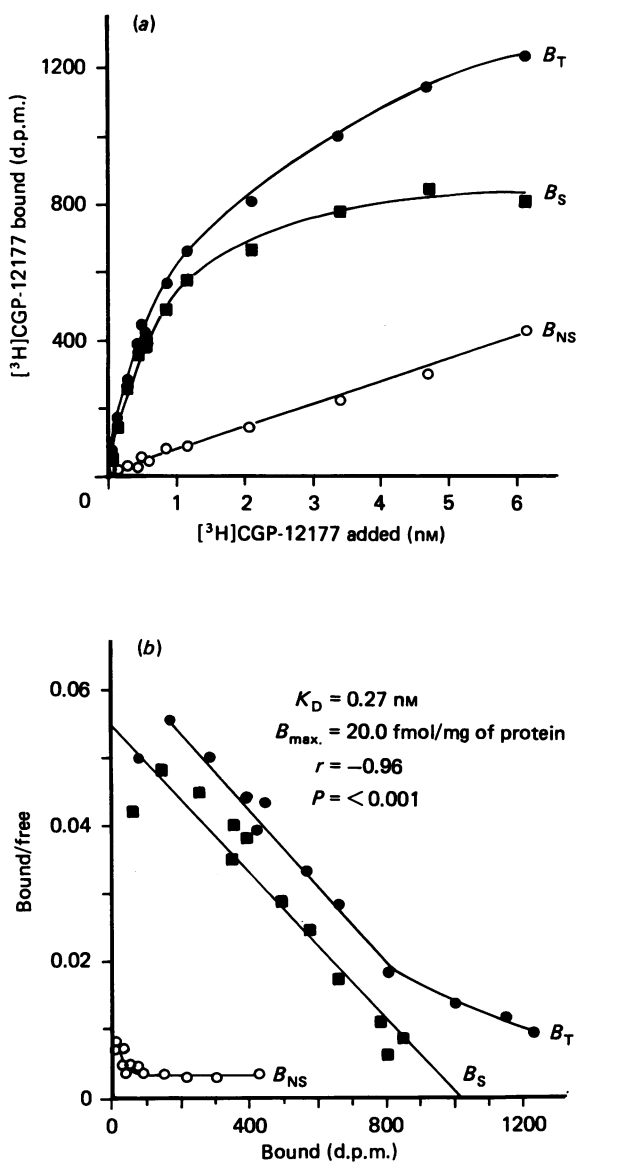

Fig. 2. Binding of [^{3}H]CGP-12177 to BAT crude membranes of obese cold-acclimated Zucker rats

(*a*) Total (B_T), specific (B_S) and non-specific (B_{NS}) binding as a function of radioligand concentration. The equilibrium binding assays were performed as described in the Experimental section. Each value is the mean of duplicate determinations. Ligand concentrations given are those calculated from the actual radioactivity found in the incubations and the specific radioactivity given by the manufacturers. (*b*) Scatchard plots of [^{3}H]CGP-12177 binding. The data from the binding curves in (*a*) were analysed by the method of Scatchard (1949). The slope of the line ($-1/K_D$) was determined by linear regression analysis. The maximum binding capacity ($B_{max.}$) was obtained as the intercept with the abscissa.

was detectable. The affinity of [^{3}H]prazosin was not changed in the different experimental situations (Tables 1 and 3).

Adult rats. In adult Zucker rats the total amount of prazosin-binding sites ($B_{max.}$) per mg of membrane protein appeared to be similar in BAT of lean and obese rats, but when expressed per unit of 5′-nucleotidase activity (i.e. per plasma-membrane unit) the $B_{max.}$ of BAT from obese animals was significantly lower than that of lean animals (Table 1).

Young rats. The total number of [^{3}H]prazosin-binding sites ($B_{max.}$) was always higher in BAT crude membranes of lean than of obese rats, either when expressed per mg of protein or when expressed per unit of 5′-nucleotidase activity (Table 3).

Cold-acclimation led to a significant increase in $B_{max.}$ for [^{3}H]prazosin in BAT from both genotypes. Relatively speaking, this increase was even higher in obese than in

Table 3. α_1-Receptors: comparison of dissociation constants (K_D) and total number of [^{3}H]prazosin-binding sites ($B_{max.}$) in BAT crude membranes from 6-week-old control, cold-acclimated and sucrose-fed lean and obese Zucker rats

The data have been derived from Scatchard analysis of [^{3}H]prazosin-equilibrium-binding experiments, performed as described in the Experimental section. The results are means ± S.E.M. for four to six different crude membrane fractions; units are μmol of AMP hydrolysed/min by the 5′-nucleotidase membrane marker. For statistical notations, see the legend to Table 2.

	Control			Cold-acclimated			Sucrose-fed		
	Lean		Obese	Lean		Obese	Lean		Obese
K_D (nM)	0.12±0.04	ns	0.14±0.06	0.14±0.16 NS	ns	0.11±0.03 NS	0.09±0.02 NS	ns	0.13±0.03 NS
$B_{max.}$ (fmol/mg of protein)	28.0±1.9	**	17.6±2.4	56.3±5.7 ††	ns	42.8±2.9 †††	29.2±3.8 NS	*	17.4±2.5 NS
$B_{max.}$ (fmol/munit)	6.4±0.5	**	3.4±0.5	14.1±2.4 ††	ns	10.0±1.7 ††	5.9±0.3 NS	***	2.6±0.6 NS

Table 4. β-Receptors: comparison of dissociation constants (K_D) and total number of [^{3}H]CGP-12177-binding sites ($B_{max.}$) in BAT from 6-week-old control, cold-acclimated and sucrose-fed lean and obese Zucker rats

The data have been derived from Scatchard analysis of [^{3}H]CGP-12177 equilibrium-binding experiments, performed as described in the Experimental section. The results are means ± S.E.M. for three to seven different crude membrane fractions; units are μmol of AMP hydrolysed/min by 5′-nucleotidase. For statistical notations, see the legend to Table 2.

	Control			Cold-acclimated			Sucrose-fed		
	Lean		Obese	Lean		Obese	Lean		Obese
K_D (nM)	0.34±0.03	ns	0.32±0.05	0.25±0.01 (†)	ns	0.26±0.01 NS	0.35±0.06 NS	ns	0.43±0.04 NS
$B_{max.}$ (fmol/mg of protein)	10.3±1.7	ns	10.7±1.5	15.2±2.1 NS	ns	17.5±2.4 †	18.0±1.5 ††	ns	17.7±8.3 NS
$B_{max.}$ (fmol/munit)	2.2±0.4	ns	1.9±0.2	3.9±0.4 (†)	ns	4.3±1.5 †(†)	3.8±0.7 †	ns	4.0±0.8 †

Table 5. Adrenalectomy: comparison of dissociation constants (K_D) and total number of [^{3}H]prazosin-binding sites in BAT from sham-operated and adrenalectomized Zucker rats

The data have been derived from Scatchard analysis of [^{3}H]prazosin equilibrium-binding experiments, performed as described in the Experimental section. The results are means ± S.E.M. for three to five different crude membrane preparations, units are μmol of AMP hydrolysed/min. For statistical notations, see the legend to Table 2.

	Sham-operated			Adrenalectomized		
	Lean		Obese	Lean		Obese
Final body wt. (g)	120±6	ns	135±8	118±11	ns	124±8
BAT protein yield (mg/animal)	7.6±0.8	*	4.7±1.0	7.2±1.1 NS	ns	8.5±1.7 NS
Nucleotidase (munits/mg)	4.6±1.4	*	12.8±2.0	9.6±0.6 †	ns	10.1±0.6 NS
K_D (nM)	0.09±0.01	*	0.05±0.01	0.03±0.00 ††	ns	0.06±0.03 NS
$B_{max.}$ (fmol/mg of protein)	10.3±1.9	ns	9.7±1.8	26.6±3.3 †(†)	ns	20.2±2.4 †
$B_{max.}$ (fmol/munit)	2.4±0.2	**	0.8±0.2	2.7±0.3 NS	ns	2.1±0.4 †

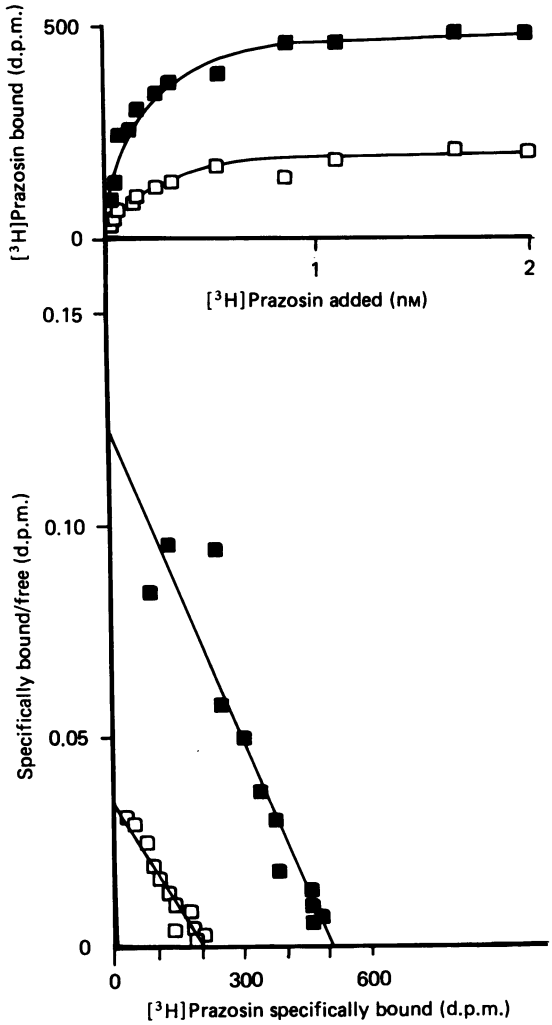

Fig. 3. Specific [^{3}H]prazosin binding in sham-operated and adrenalectomized obese *fa/fa* rats at 6 weeks of age

The equilibrium binding assays were performed on crude BAT membranes prepared from obese rats 1 week after adrenalectomy (■) or sham operations (□), as described in the Experimental section. Other details of the analysis of the data are as described in the legend to Fig. 1, except that only data for specific binding are shown. Data from the experiment shown yielded the following values.

	Sham-operated	Adrenalectomized
$B_{max.}$ (fmol/mg)	6.22	15.5
K_D (nM)	0.05	0.03
r	−0.96	−0.94

lean animals; thus, although the density of α_1-adrenergic receptors in BAT of cold-acclimated Zucker rats was still slightly lower in obese than in lean animals, this difference was now insignificant.

Sucrose feeding did not affect the number of [^{3}H]-prazosin-binding sites ($B_{max.}$) in BAT from lean or from obese Zucker rats.

Determination of [^{3}H]CGP-binding sites (β-receptors) in BAT

In Fig. 2, a typical experiment with CGP-12177 is depicted, showing a K_D of 0.27 nM and $B_{max.}$ of 20 fmol/mg of protein for binding. The binding of [^{3}H]CGP-12177 was also a saturable and high-affinity reaction. The non-specific binding (B_{NS}) was 10–35% of the total binding (B_T). Scatchard analysis showed a linear (one-site) binding in all experiments. The affinity of [^{3}H]CGP-12177 was practically unchanged in all experimental situations (Tables 1 and 4).

Adult rats. The $B_{max.}$ of [^{3}H]CGP-12177 binding per mg of membrane protein was apparently higher in BAT crude membranes of obese than of lean Zucker rats, but this difference was reversed when expressed per unit of 5′-nucleotidase activity.

Young rats. In young Zucker rats, the $B_{max.}$ of [^{3}H]-CGP-12177 was similar in BAT crude membranes from lean and obese Zucker rats in all conditions (Table 4). Cold-acclimation increased the number of [^{3}H]CGP-12177 binding sites in BAT of both lean and obese Zucker rats, and so did sucrose feeding (Table 4).

Effect of adrenalectomy

The effect of adrenalectomy on the density of [^{3}H]-prazosin-binding sites in BAT of obese rats is shown in Table 5 and Fig. 3. After adrenalectomy the decreased number of α_1-receptors in the obese rat was elevated to the value observed in lean control and lean adrenalectomized rats.

DISCUSSION

Equilibrium-binding experiments with [^{3}H]prazosin and [^{3}H]CGP-12177 have here been performed to characterize and measure the populations of α_1- and β-receptors in BAT crude membranes of lean and obese Zucker rats. As the crude membrane preparation used in these studies would also include mitochondria, and since the tissue mitochondrial population will vary with diet, cold, and obese genotype (see, e.g., Holt *et al.*, 1983), the binding values have been expressed per unit of 5′-nucleotidase activity, a plasma-membrane marker enzyme. This mode of expression is likely to provide a more accurate value for the true plasma-membrane receptor density than would data expressed per mg of protein in the relatively impure membrane fractions used in these studies, and in most previous studies of adrenergic receptor density in BAT.

α_1-Adrenergic receptors

There are high concentrations of both α_1- and β-receptors on brown adipocytes (Svoboda *et al.*, 1979; Mohell *et al.*, 1983*a*; Raasmaja *et al.*, 1985). Although most of the thermogenic response to noradrenaline is thought to be β-receptor-mediated, up to 20% of the increase in O_2 consumption can be specifically blocked by the α-antagonist prazosin (Mohell *et al.*, 1983*b*).

An increase in the α_1-receptor density has been found in BAT of cold-acclimated (Raasmaja *et al.*, 1985) and cafeteria-fed rats (Raasmaja *et al.*, 1984), which has led to the suggestion that the α_1-receptor increases may be linked to the adaptive hyperplasia, growth and differentiation (i.e. recruitment) of the tissue. This hypothesis is supported by the observations reported in the present paper of a decrease in the concentration of α_1-receptors in BAT crude membranes of the obese Zucker rat, compared with lean littermates, and the decreases in α_1/

Table 6. Comparison of the ratios of α_1/β-receptors in BAT crude membranes

These are based on mean values from Tables 1, 3 and 4; 1 indicates the reference value for comparisons.

	5–6-week-old rats						3-month-old rats	
	Control		Cold-acclimated		Sucrose-fed		Control	
	Lean	Obese	Lean	Obese	Lean	Obese	Lean	Obese
α_1/β $B_{max.}$ ratio	2.7	1.7	3.7	2.5	1.6	1.0	1.2	0.6
Relative difference	1	0.6	1	0.7	1	0.6	1	0.5
Relative changes	1	1	1.4	1.5	0.6	0.6	–	–

β ratio found with increasing age and with obesity (see below). The BAT of obese rats has a low mitochondrial content, a decreased mitochondrial GDP binding and a decreased content of thermogenin (Holt *et al.*, 1983; Ashwell *et al.*, 1985; J. Allars, S. Holt, R. French & D. A. York, unpublished work), all expressions of a diminished sympathetic stimulation and consequent involution of the tissue. Our observations are thus consistent with previous results, which have shown an increased α_1-adrenergic-receptor density in BAT crude membranes when the sympathetic activity is high (Raasmaja *et al.*, 1984, 1985).

The increase in thermogenic activity in BAT of obese rats after adrenalectomy is shown in the present study to be associated with an increase in α_1-receptor density up to that present in the BAT of lean rats. This is thus another example in which recruitment and activation of BAT is parallelled by an increase in α_1-receptor number. Mitochondrial thermogenin content is also normalized in obese rats after adrenalectomy (J. Allars, S. Holt & D. A. York, unpublished work).

The increase in α_1-receptor density in BAT membranes of cold-acclimated rats previously reported (Raasmaja *et al.*, 1985) was confirmed in the present studies in both lean and obese rats. Young obese rats have been shown previously to have a normal response to cold-acclimation, with an increased noradrenaline turnover, mitochondrial GDP binding and thermogenin content (Holt *et al.*, 1983; Ashwell *et al.*, 1985; York *et al.*, 1985). These obese rats are, however, characterized by an inability to respond to dietary signals (Holt *et al.*, 1983; Marchington *et al.*, 1983), and the sympathetic stimulation of BAT is low (York *et al.*, 1985).

In the present study, sucrose feeding did not affect the number of [^{3}H]prazosin-binding sites in BAT in either lean or obese rats. These results are apparently in contrast with previous reports in which cafeteria feeding in normal rats was associated with an increase in α_1-receptor density (Raasmaja *et al.*, 1984). This difference may reflect the differing sympathetic responses to these dietary manipulations, since there was not an increased sympathetic activity in BAT in sucrose-fed rats [as estimated by noradrenaline half-life (Young *et al.*, 1982; York *et al.*, 1985)], whereas BAT noradrenaline half-life was shortened after cafeteria-feeding (Young *et al.*, 1982).

β-Adrenergic receptors

The density of β-receptors was similar in the BAT crude membrane fractions prepared from young lean and obese rats, and was similarly increased after sucrose feeding or cold-acclimation of both phenotypes.

Our results obtained from adult rats showed a tendency to a decreased number of β-adrenergic receptors per unit of 5′-nucleotidase activity, principally in agreement with Levin *et al.* (1982), who found a decreased number per cell.

In normal cold-acclimated rats, previous results with [^{3}H]dihydroalprenolol have indicated a decreased β-receptor density (Bukowiecki *et al.*, 1978; Kurahashi & Kuroshima, 1981; Rothwell *et al.*, 1986). One explanation for the difference could be that the results in the present paper are from young rats at 6 weeks of age, and the previous results are from adult rats.

As sucrose feeding does not increase the sympathetic-nervous-system activity (when estimated as noradrenaline half-life) (Young *et al.*, 1982; York *et al.*, 1985), the results presented here of an increased β-receptor density in sucrose-fed rats could indicate that the regulation of β-receptor number is not under sympathetic control.

The ratio between α_1- and β-adrenergic receptors in BAT

The ratios between α_1- and β-receptors, calculated from the [^{3}H]prazosin and [^{3}H]CGP-12177 binding studies reported above, are compiled in Table 6. We do not necessarily ascribe a functional role to this ratio as such (although the two pathways may interfere intracellularly), but a clear pattern can be expressed via the calculation of this ratio.

The density of α_1-receptors was as high as, or higher than, that of β-receptors. The α_1/β ratio was lower in adult than in young rats, perhaps in agreement with a lower recruitment of BAT in the adult stage. In the obese animals, the α_1/β ratio was very consistently only about 50% of that in the lean animals.

Sucrose feeding decreased the ratio in both lean and obese animals, mainly owing to the increase in β-receptor numbers. In contrast, in BAT of cold-acclimated rats, the ratio of α_1/β-receptors was always much increased, and, relatively speaking, this increase was equally high in lean and obese animals. The response of adrenergic receptors in BAT to sucrose and cold seems thus to be inverse.

Regulation of adrenoreceptor density

It is not possible to interpret the changes in either α_1- or β-receptor numbers observed in these studies as a classical 'up' or 'down' regulation in response to the altered extents of sympathetic stimulation caused by diet,

cold or genotype. In the traditional view, an increased sympathetic stimulation would be expected to 'down-regulate' the receptor number. This is not the case in BAT. For example, α_1-receptor number was decreased in BAT of obese rats (which lack sympathetic drive), but it was increased in cold-acclimated animals (when sympathetic stimulation of BAT is enhanced). In the same vein, it was observed that β-receptor number was not depressed by cold-acclimation, nor was it increased in the obese rats, despite the changes in sympathetic activity. Thus the mere existence of opposing changes in the densities of α_1- and β-receptors, and the ensuing change in α_1/β-receptor ratio in different conditions, suggests that alternative mechanisms to 'up' or 'down' regulation must operate to control these receptor populations.

Further studies are thus required to understand the mechanism of the regulation of the receptor populations *in vivo* and to explore the background for the low α_1-adrenergic-receptor density in obese rats and its role in the defective BAT function of these animals.

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REFERENCES

Affolter, H., Hertel, C., Jaeggi, K., Portenier, M. & Staehelin, M. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 925–929

Agrawal, D. K. & Daniel, E. E. (1985) J. Pharmacol. Exp. Ther. **233**, 195–203

Ashwell, M., Holt, S., Jennings, G., Stirling, D. M., Trayhurn, P. & York, D. A. (1985) FEBS Lett. **179**, 233–237

Avruch, J. & Wallach, D. F. H. (1971) Biochim. Biophys. Acta **233**, 334–347

Barnard, T., Mory, G. & Nechad, M. (1980) in Biogenic Amines in Development (Parvez, S. & Parvez, H., eds.), pp. 391–439, Elsevier/North-Holland, Amsterdam

Bray, G. A. & York, D. A. (1979) Physiol. Rev. **59**, 719–809

Bruce, B. K., King, B. M., Phelps, G. R. & Veita, M. C. (1982) Am. J. Physiol. **243**, E152–E159

Bukowiecki, L., Follea, N., Vallieres, J. & LeBlanc, J. (1978) Eur. J. Biochem. **92**, 189–196

Cannon, B. & Nedergaard, J. (1986) Proc. Int. Nutr. Congr. 13th, 315–319

Himms-Hagen, J. (1983) Nutr. Rev. **41**, 261–267

Himms-Hagen, J. & Desautels, M. (1978) Biochem. Biophys. Res. Commun. **83**, 628–634

Holt, S. & York, D. A. (1982) Biochem. J. **208**, 819–822

Holt, S., York, D. A. & Fitzsimons, J. T. R. (1983) Biochem. J. **214**, 215–223

Kurahashi, M. & Kuroshima, A. (1981) Biomed. Res. **2**, 126–133

Levin, B. E., Comai, K., O'Brien, R. A. & Sullivan, A. C. (1982) Am. J. Physiol. **243**, E217–E224

Levin, B. E., Triscari, J. & Sullivan, A. C. (1983) Am. J. Physiol. **245**, E87–E93

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275

Marchington, D., Rothwell, N. J., Stock, M. J. & York, D. A. (1983) J. Nutr. **113**, 1395–1402

Marchington, D., Rothwell, N. J., Stock, M. J. & York, D. A. (1986) Am. J. Physiol. **250**, E362–E366

Mohell, N., Svartengren, J. & Cannon, B. (1983*a*) Eur. J. Pharmacol. **92**, 15–25

Mohell, N., Nedergaard, J. & Cannon, B. (1983*b*) Eur. J. Pharmacol. **93**, 183–193

Nedergaard, J. (1982) Am. J. Physiol. **242**, C250–C257

Nedergaard, J. & Lindberg, O. (1982) Int. Rev. Cytol. **74**, 187–286

Nedergaard, J., Mohell, N., Nånberg, E., Connolly, E., Raasmaja, A. & Henschen, L. (1986) in Living in the Cold (Heller, H. C., ed.), pp. 83–91, Elsevier, New York

Raasmaja, A., Mohell, N. & Nedergaard, J. (1984) Biosci. Rep. **4**, 851–859

Raasmaja, A., Mohell, N. & Nedergaard, J. (1985) Eur. J. Pharmacol. **106**, 489–498

Rothwell, N. J., Stock, M. J. & York, D. A. (1984) Comp. Biochem. Physiol. **78A**, 565–569

Rothwell, N. J., Stock, M. J. & Sudera, D. K. (1986) Eur. J. Pharmacol. **125**, 313–323

Scatchard, G. (1949) Ann. N.Y. Acad. Sci. **51**, 660–672

Svartengren, J., Svoboda, P. & Cannon, B. (1982) Eur. J. Biochem. **128**, 481–488

Svartengren, J., Svoboda, P., Drahota, Z. & Cannon, B. (1984) Comp. Biochem. Physiol. **78C**, 159–170

Svoboda, P., Svartengren, J., Snochowski, M., Houstek, J. & Cannon, B. (1979) Eur. J. Biochem. **102**, 203–210

Svoboda, P., Svartengren, J., Naprstek, J. & Jirmanova, I. (1984*a*) Mol. Physiol. **5**, 197–210

Svoboda, P., Svartengren, J. & Drahota, Z. (1984*b*) Mol. Physiol. **5**, 211–220

Trayhurn, P. (1984) Clin. Endocrinol. Metab. **13**, 451–474

Trayhurn, P. (1986) in Brown Adipose Tissue (Trayhurn, P. & Nicholls, D. G., eds.), pp. 299–338, Edward Arnold, London

Trayhurn, P. & Nicholls, D. G. (eds.) (1986) Brown Adipose Tissue, Edward Arnold, London

Triandafillou, J. & Himms-Hagen, J. (1983) Am. J. Physiol. **244**, E145–E150

York, D. A., Marchington, D., Holt, S. J. & Allars, J. (1985) Am. J. Physiol. **249**, E299–E305

Young, J. B., Saville, E., Rothwell, N. J., Stock, M. J. & Landsberg, L. (1982) J. Clin. Invest. **69**, 1061–1071

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