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Metabolic and Sympatho-Adrenal Abnormalities in the Obese Zucker Rat: Effect of Chronic Phenoxybenzamine Treatment

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LEVIN, B. E., K. COMAI AND A. C. SULLIVAN *Metabolic and sympatho-adrenal abnormalities in the obese Zucker rat: Effect of chronic phenoxybenzamine treatment* PHARMAC. BIOCHEM. BEHAV. **14**(4) 517–525, 1981.—The obese Zucker rat manifests a number of physiologic and metabolic abnormalities which are controlled or modulated by the sympatho-adrenal system. The interrelationship of these was examined by subjecting 3–4 month old male, homozygous lean and obese Zucker rats to various stresses which are known to activate the sympatho-adrenal system, and by chronic (16–19 days) phenoxybenzamine (PBZ) treatment to block α-adrenergic receptors. Both obese and lean PBZ treated rats gained only 1% and 10% of the body weight of their respective control rats during the treatment period, while only the lean rats had a significant reduction (20%) in food intake. Control obese rats failed to maintain rectal temperature after 4 hr at 7°C and their relative output of plasma catecholamines (CA) to cold stress, as measured from indwelling atrial cannulae, was decreased. PBZ treatment did not alter this rectal temperature response although it was associated with increased baseline norepinephrine levels (at ambient temperature 21–22°C) and relative output of CA in the obese rats, suggesting that sympathetic neural activity was increased under these circumstances. No abnormalities of sympatho-adrenal function, as reflected in plasma CA levels, were found in treated or control obese rats after immobilization for 1 hr followed by decapitation. Simultaneously obtained baseline plasma glucose levels were similar in untreated lean and obese rats, but insulin and glycerol levels in the obese rat were 1350% and 213% of lean values, respectively. During sequential stresses, the obese rats became markedly hyperglycemic and hyperglycerolemic compared to the lean rats, while insulin levels were depressed more in the obese than lean rats (12–15% versus 34–35% of controls, respectively). PBZ affected insulin levels only in the obese rats, reducing their baseline levels by 4-fold and stress induced levels to those seen in the lean control rats. These results suggest that some of the metabolic and physiologic abnormalities of the obese Zucker rat which are modulated by the sympatho-adrenal system can be normalized by procedures which increase sympatho-adrenal activity.

Plasma catecholamines Insulin Glucose Glycerol Zucker rat Sympatho-adrenal system
Phenoxybenzamine Obesity Thermogenesis Stress

THE obese Zucker rat has disorders of both central and peripheral catecholamine (CA) metabolism. Brain levels of CA [10, 11, 32] and their synthetic enzymes [31] are abnormal, and baseline and stress-induced plasma levels of CA are also abnormal in the 7–8 month old male obese Zucker rat [33]. Many of the metabolic and physiologic abnormalities found in the obese Zucker rat are in systems which are modulated by the peripheral sympatho-adrenal system. Thermogenesis [23,35], carbohydrate [15, 40, 43, 50] and lipid [1, 19, 45, 49] metabolism all depend on the sympatho-adrenal system, and the obese Zucker rat has well described defects in thermogenesis [20, 33, 47], oxygen consumption [4,26], carbohydrate [13, 44, 48, 53], and lipid metabolism [5, 9, 46]. All of these defects may, therefore, be potentially linked to abnormalities in sympatho-adrenal functions in the Zucker rat.

The present study was undertaken to investigate the interrelationship between the sympatho-adrenal system and some of the other systems which function abnormally in the obese Zucker rat. Several physiologic stresses were used to activate the peripheral sympatho-adrenal system. Plasma norepinephrine (NE) levels were used as an indicator of

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sympathetic neural activity, epinephrine (E) as an indicator of adrenal-medullary activity, and dopamine (DA) as an indicator of the activity of both systems [27,37]. Simultaneous determinations of plasma glucose and insulin levels were used as an index of carbohydrate metabolism, and glycerol as an index of lipolysis [6,39].

Further manipulation of the sympatho-adrenal system was carried out using phenoxybenzamine (PBZ), a haloalkylamine which irreversibly blocks α-adrenergic receptors [24]. In acute experiments PBZ increases the stimulus evoked outflow of NE [8, 12, 24, 29, 30], presumably causing increased sympathetic activity. While PBZ acts primarily on the post-synaptic α_1 receptor [14], it also has less specific presynaptic effects [8, 12, 24], as well as effects on serotonin and acetylcholine receptors [38] and prostaglandin E synthesis [25] It was our intention that through the use of various procedures which increase sympatho-adrenal activity we would improve some of the abnormalities of the obese Zucker rat.

METHOD

Animals

Groups of 3–4 month old male Zucker homozygous, lean (Fa/Fa) and obese (fa/fa) rats were individually housed on a 12 hr light-dark cycle at room temperatures of 21–22°C for the duration of the study. All animals were fed a synthetic diet composed of corn oil (10%), glucose (60%) and protein (23%) [9]. Different groups of 10 obese and lean animals each were fed either the diet (control) or a dietary admixture of phenoxybenzamine (PBZ: 40 mg/kg body weight, kindly supplied by Smith-Kline and French Pharmaceuticals). Animals were treated for an average of 16–19 days prior to testing and food intake was measured every 2–3 days over the first 10 days of treatment.

Catheters were implanted in the right atrium of all rats through a jugular vein 2 days prior to testing and brought to the outside of individual cages through stainless steel springs [33]. All experiments began between 0730–0900 hr; rats were randomly selected so that at least one rat from each treatment group was tested on each day. Animals were allowed free access to the control diet or PBZ admixture and water throughout the test period.

Stress Experiments

Blood samples (2.0 ml) were drawn from undisturbed animals followed by measurement of their rectal temperatures (Electro-therm Digital Thermometer, Cole-Palmer) at an ambient temperature of 21–22°C. These and subsequent samples were placed in heparinized tubes on ice and the cannulae flushed with 0.5 ml heparinized saline (10 U/ml) [33]. Rats were then placed in the cold (7°C) for 4 hr after which 2.0 ml of blood and rectal temperatures were taken. Animals were then restrained at an ambient temperature of 21–22°C for 60 min in the supine position [33] and an additional 0.5 ml and 2.0 ml of blood were obtained at 5 and 60 min, respectively. Rats were then decapitated and 3–4 ml of trunk blood collected All red cells were resuspended in normal saline equal to the amount of plasma removed, and reinfused after each sampling period (except the last) to maintain blood volume [33]

Assay of Plasma Constituents

Plasma was separated from all blood samples, the protein precipitated with perchloric acid, and the supernatant frozen at −70°C until assayed within 1–3 weeks. Catecholamines (CA) were assayed in 50 μl aliquots of the acid soluble supernatant of plasma by radioenzymatic method [33].

Plasma insulin levels were determined by radioimmunoassay [51] and plasma glucose and glycerol levels by automated procedures

Statistics

All treatment groups were compared by unpaired Student's t-test (two-tailed). Changes between tests within the same rat were compared by paired t-test (two-tailed) and correlations were performed using a Pearson's correlation coefficient (r) test.

RESULTS

Food Consumption and Body Weight Change

Two separate groups of lean and obese, control and treated rats were tested, 1 month apart. One group of rats was 12–14 days older than the other so that initial body weights (410 ± 16 g, obese, 379 ± 5 g, lean) were approximately 100 g higher in the older rats for all groups than for the younger rats (292 ± 19 g, obese; 265 ± 13 g, lean). There were also minor differences in food intake between the two groups although weight change figures were virtually identical, as were all other parameters measured (plasma constituents, temperature responses and carcass composition). Figure 1 represents the data from the older rats and shows that the lean control rats consumed 8% less food than the obese controls ($p<0.1$). PBZ produced a significant decrease of 20% in food intake in only the lean rats after 10 days of treatment (Fig 1A), while weight gain was severely retarded in both the obese (1% of control) and lean rats (10% of control) after 16–19 days of treatment. Therefore, obese treated rats failed to gain weight normally without any change in food intake, while lean rats did show a significant decrease in food intake associated with their weight loss. Although food intake was measured during only the first 10 days of PBZ treatment, there was no alteration in the rate of changes in body weight during the final 6–9 days of treatment. This suggests that no significant alteration in PBZ intake occurred during this later period.

Temperature Control

All groups of rats had comparable baseline rectal temperatures at ambient temperatures of 21–22°C (Fig. 2). However, after 4 hr at 7°C both treated and control obese rats decreased their body temperatures significantly below baseline. While there were no significant changes in rectal temperature induced by cold stress in treated or control lean rats (Fig. 2A), the PBZ treated rats did have a significantly higher rectal temperature than controls after 4 hr at 7°C However, when expressed as percent change from baseline, PBZ did not significantly affect the temperature response of lean rats to cold stress. As previously described in older (7–8 month) male obese Zucker rats [33], there appeared to be two groups with respect to defense of body temperature (Fig. 2B) Approximately one-third had little or no significant change after 4 hr of cold exposure ($\leq$1.4°C decrease in rectal temperature), while two-thirds had a much greater change (>1.4°C). This finding was not altered by PBZ treatment

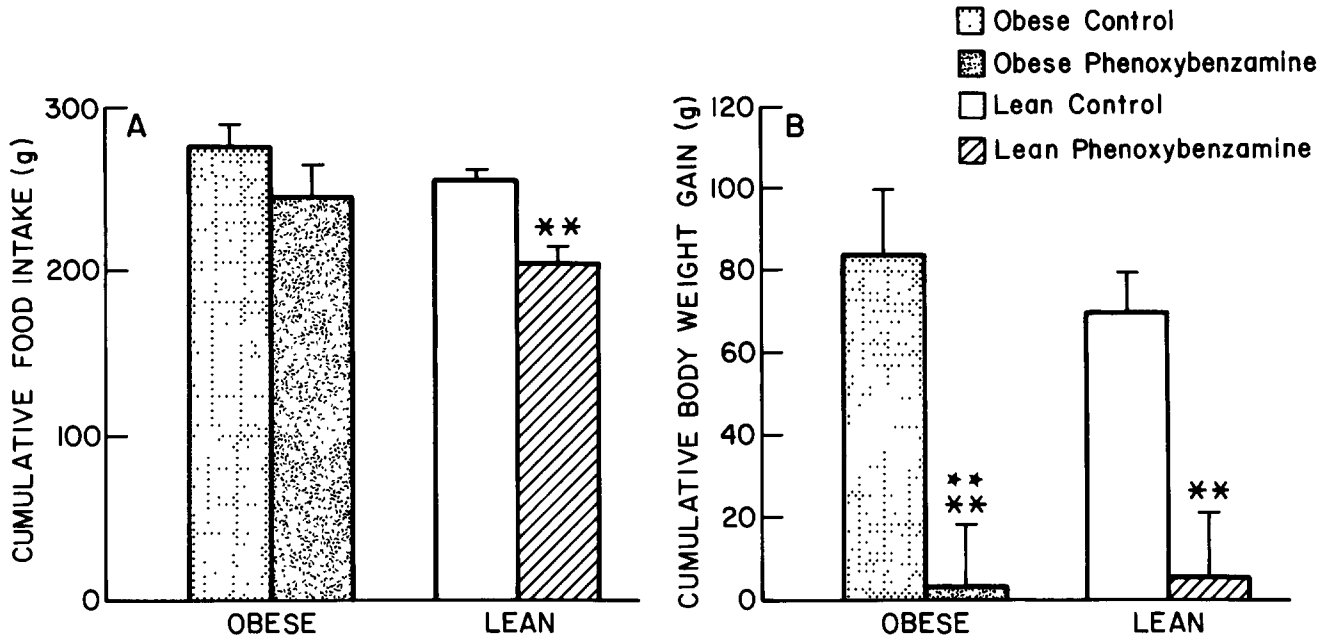

FIG. 1 Food consumption and change in body weight Lean and obese, PBZ (40 mg/kg body weight) treated or control rats in groups of 5 rats each had their food intake (mean grams ± SE) measured over the first 10 days of treatment (Panel A), and change in body weight (mean grams ± SE) was determined over the entire 14–17 day treatment period (Panel B) **=$p<0.01$ when control rats were compared to treated rats of the same genotype ★★=$p<0.01$ when obese treated rats were compared to lean control rats

Plasma Catecholamines

Baseline levels of plasma CA were similar in the obese and lean control rats, while PBZ treated obese rats had significant elevations of plasma NE levels compared to both obese and lean controls (Fig 3). Plasma CA were generally higher than baseline levels for all subsequent stresses in both lean and obese rats. After 4 hr of cold stress both obese control and treated rats had significantly higher levels of plasma NE and E when compared to the lean rats. Also, PBZ treated obese rats had significantly higher NE levels than their obese controls and also tended to have higher E and DA levels.

There was a linear relationship (Table 1) between rectal temperatures and plasma CA levels for all control rats except for E levels in the lean rats. The slopes of these lines represent the relative levels of CA reached per degree of decline in rectal temperature. For NE, the obese rats showed a relative deficit in levels reached, i.e., the slope was 4.6-fold higher in the obese than lean rats, indicating that a much greater drop in temperature was required in the obese rats to produce comparable levels of NE in the lean rats. This ratio was 9.7 for DA levels in the obese versus lean rats, while there was no correlation between E levels and temperature in the lean rats. PBZ treatment tended to correct this deficit for all CA in the obese rats. NE levels reached were 57% greater in treated than obese control obese rats, while E levels were 33%, and DA levels were 57% greater than obese control rats. Therefore, while PBZ treatment did not improve the ability of obese rats to withstand cold stress, it did increase the levels of CA reached in response to cold. Furthermore, PBZ eliminated the linear relationship between NE and DA and rectal temperature in the lean rats primarily

TABLE 1

CORRELATION OF RECTAL TEMPERATURE AND PLASMA CATECHOLAMINES

	Lean Control	Lean PBZ	Obese Control	Obese PBZ
NE				
slope	−0 57	−0.05	−2 65	−1 14
intercept	37 1°	37 0°	38.8°	38 3°
r	−0 77	−0 19	−0.70	−0 90
p	<0 001	NS	<0.025	<0 05
E				
slope	−0 89	0.20	−6 60	−4 40
intercept	35 9°	36 9°	38.1°	37 3°
r	−0 18	0.12	−0.61	−0.98
p	NS	NS	<0.05	<0 001
DA				
slope	−0.89	−0 90	−8.57	−3.68
intercept	36.9°	37 2°	38 6°	36.9°
r	−0 62	−0.42	−0.60	−0.62
p	<0.025	NS	<0.05	<0.05

Rectal temperatures and simultaneous plasma CA levels were measured in groups of 7–8 rats at ambient temperatures of 21–22°C and after 4 hr of exposure to 7°C. A linear regression of these points was calculated and the slopes given as °Cng^{-1} ml^{-1}, and intercepts as °C. r=Pearson's correlation coefficient and *p* represents the probability of the data falling on a given line where NS is $p>0.05$

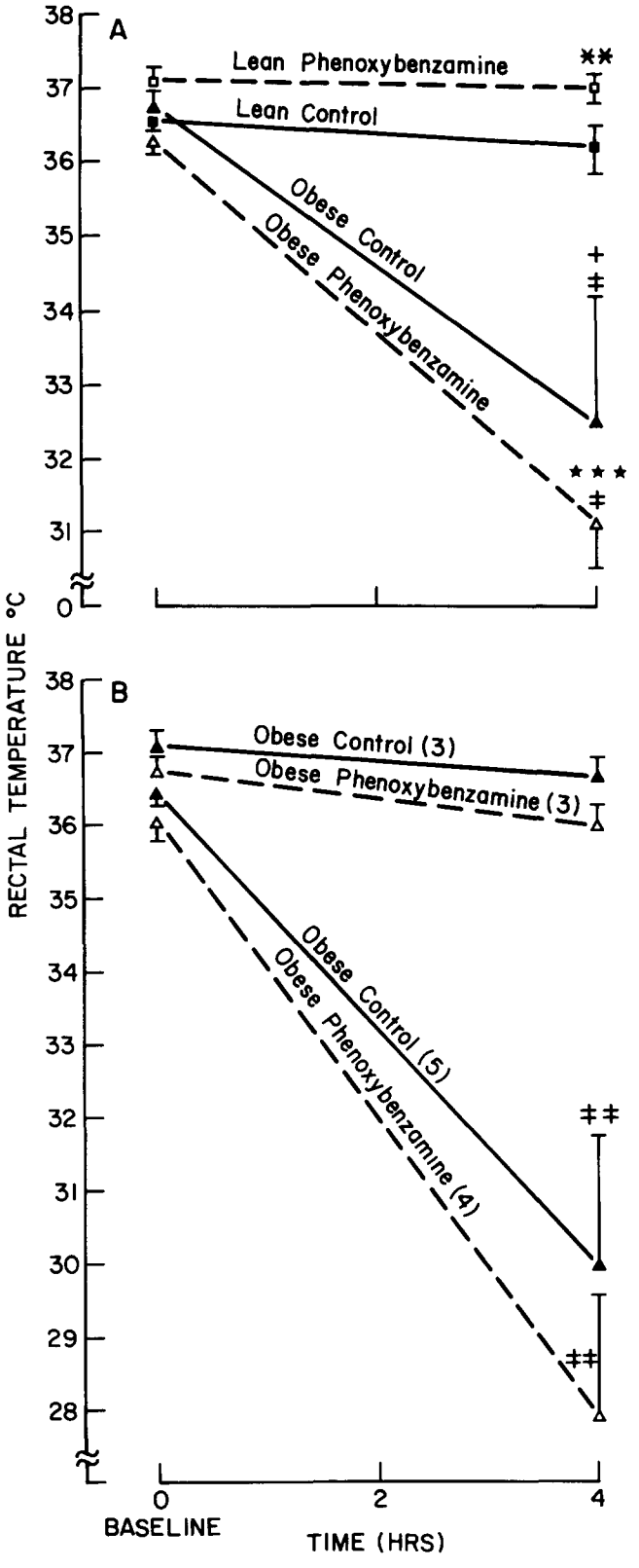

FIG 2 Rectal temperatures before and after cold stress. Four groups of 7–8, lean and obese, PBZ treated or control rats had their rectal temperatures (°C ± SE) measured at an ambient temperature of 21–22°C ("Baseline") and again after exposure to 7°C ("4 hr cold"). Panel A compares the results from all 4 groups Panel B compares the temperature responses in those obese rats which had a fall in rectal temperature of less than or equal to 1 4°C (upper 2 lines) and those with a fall of greater than 1 4°C (lower 2 lines) Numbers in parentheses are the number of rats in each group $^{**}p<0.01$ when control rats were compared to treated rats of the same genotype, $+p<0.05$ when obese controls were compared to lean controls, $\star\star\star p<0.001$ when obese treated rats were compared to lean controls, $\ddagger p<0.05$, $\ddagger\ddagger p<0.01$ when baseline values were compared to 4 hr cold values in the same animals

because they had no change in temperature during cold stress.

There were no significant differences between CA levels in the obese and lean control rats after 5 min or 60 min of immobilization or after decapitation. PBZ did not alter these responses, except for the decapitated, PBZ treated lean rats where NE levels were higher than in any of the other groups.

Plasma Glucose and Insulin

Baseline plasma glucose levels were comparable in lean and obese control rats (Fig. 4). However, only the obese rats had significant elevations of plasma glucose (290% of baseline) after 4 hr of cold stress. While lean rats did have significant increases above baseline in plasma glucose after immobilization (54%) and decapitation (92%), the obese rats exhibited two-fold greater increases than the lean rats under both these conditions. This relationship was not changed appreciably by PBZ treatment except that post-decapitation levels of glucose were reduced toward lean control levels in the obese treated rats.

While obese control rats had normal baseline glucose levels, their insulin levels were 1350% of lean control values. Cold stress produced no significant change in insulin levels in lean or obese control rats, but immobilization and decapitation produced significant depressions of insulin levels in the obese (15% and 12% of baseline, respectively) and lean (34% and 35% of baseline, respectively) control values. PBZ treatment reduced baseline insulin levels significantly (by 4-fold) in the obese rats to 318% of lean control values, as compared with 1350% in the untreated obese rats. Stress-induced insulin levels in the obese rats were significantly reduced towards lean control levels by PBZ treatment, while there were no significant changes in the lean rats. Therefore, there was an inverse relationship between plasma glucose and insulin levels during serial stresses in both lean and obese rats, but the obese rats showed an approximately 2-fold greater increase in glucose and 1.3-fold greater decrease in insulin levels than the lean animals. PBZ treatment tended to correct only the hyperinsulinemia in the obese rats

Plasma Glycerol

Baseline glycerol levels in the obese control rats were 213% of those in the lean control rats (Fig. 5). Glycerol levels increased by 122% in the obese control rats after 4 hr of cold stress, but there was no change in the lean controls which maintained glycerol levels at significantly lower levels (23%) than the obese control rats. There were no significant changes with immobilization compared to baseline levels of

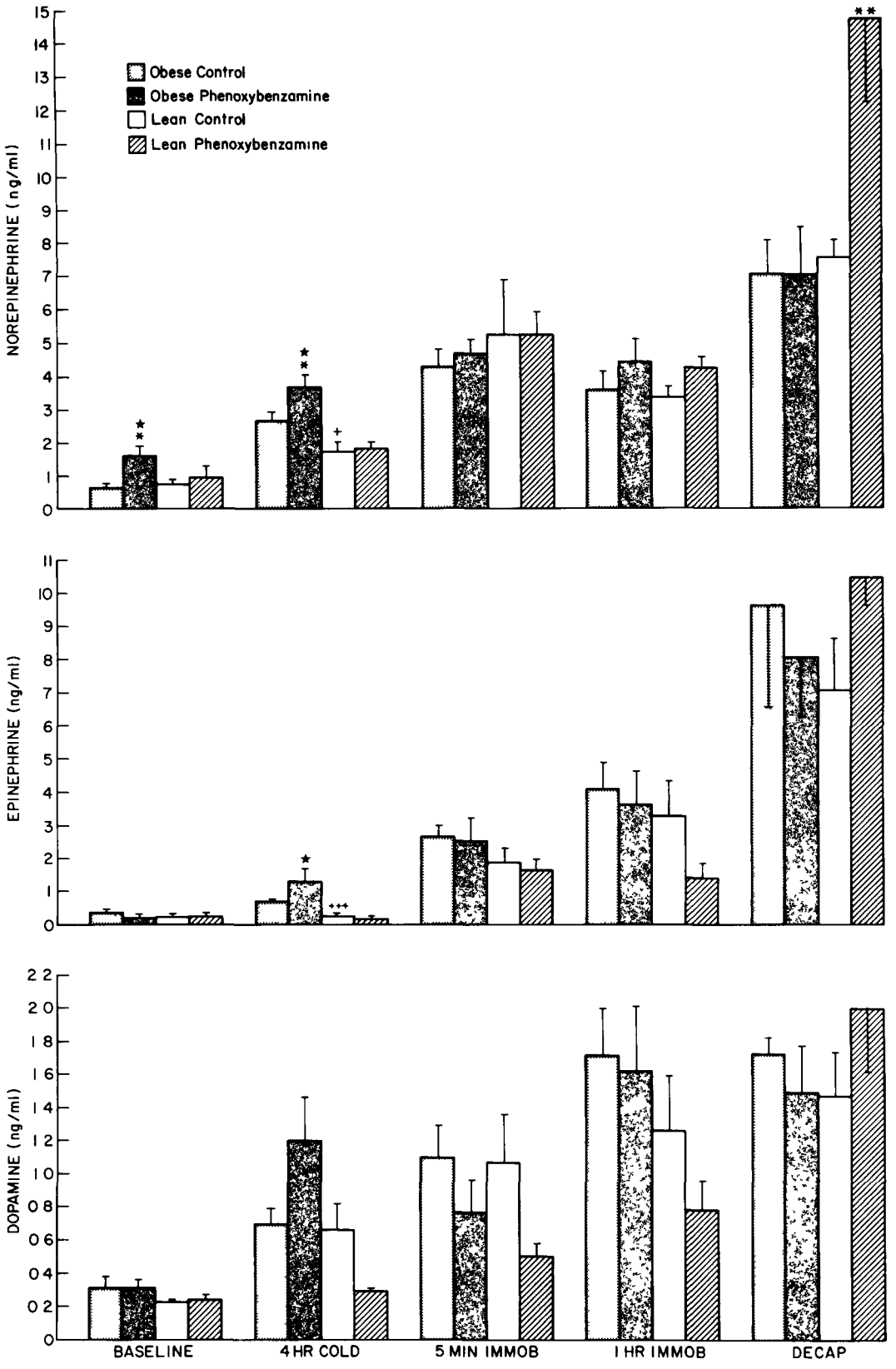

FIG 3. Plasma catecholamine levels. Four groups of 7–8 lean and obese, PBZ treated and control rats had CA measured in plasma drawn from indwelling atrial catheters in ng/ml ± SE (vertical bars). Levels were measured at ambient temperature of 21–22°C in undisturbed animals ("Baseline"), after 4 hr of exposure to 7°C ("4 hr Cold"), 5 min of immobilization ("5 min Immob"), 1 hr of immobilization ("1 hr Immob") and decapitation ("Decap"). *$p<0.01$ when control rats were compared to treated rats of the same genotype, +$p<0$ 05, ++$p<0.01$, +++$p<0.001$ when lean control rats were compared to obese control rats, ★$p<0$ 05 when obese treated rats were compared to lean controls

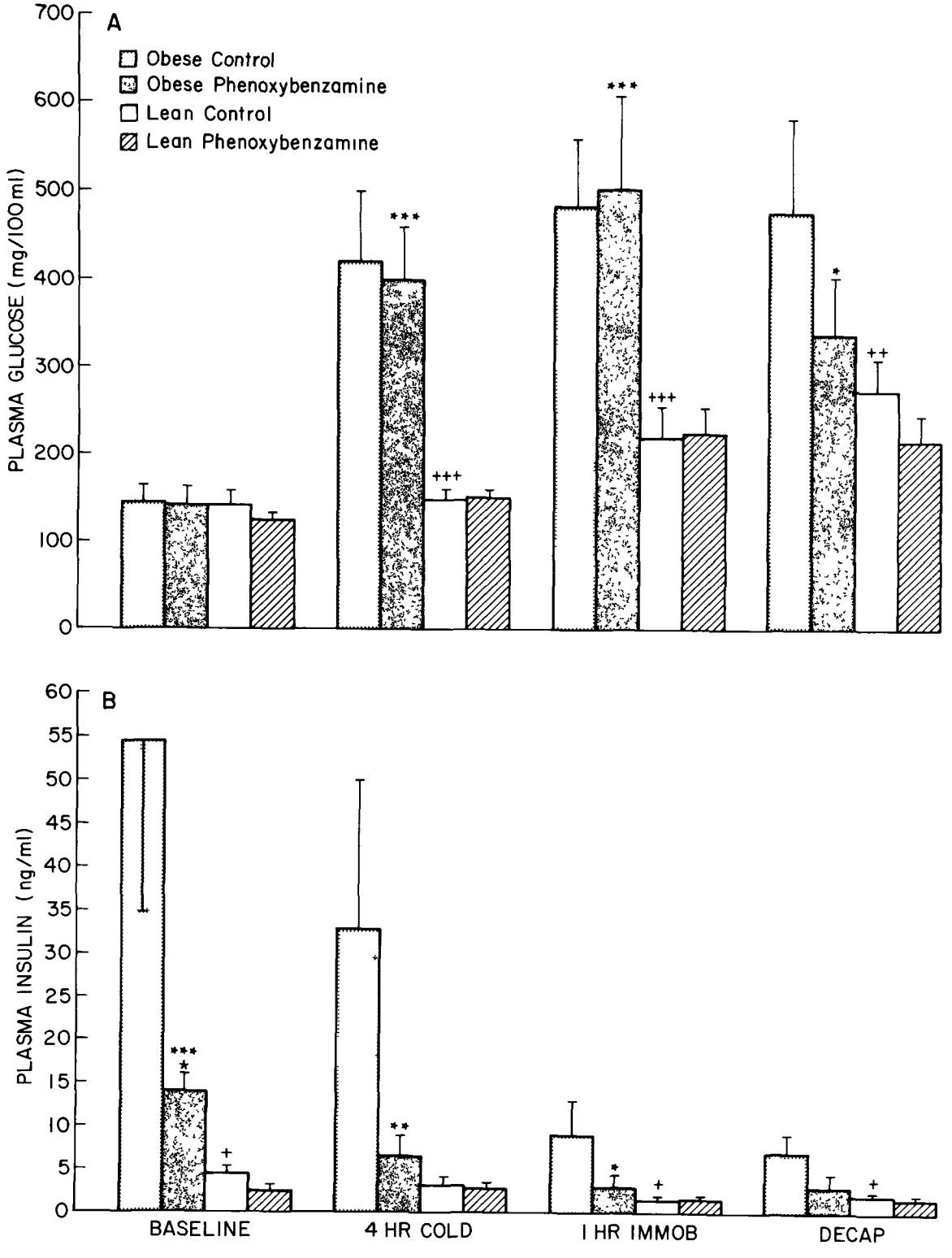

FIG 4 Plasma glucose and insulin levels Four groups of 7–8 lean and obese, PBZ treated and control rats had glucose (mean mg/100 ml ± SE) and insulin (mean ng/ml ± SE) measured in plasma samples from indwelling atrial catheters under varying conditions (see Fig 3) Symbols are the same as Figs 1, 2 and 3

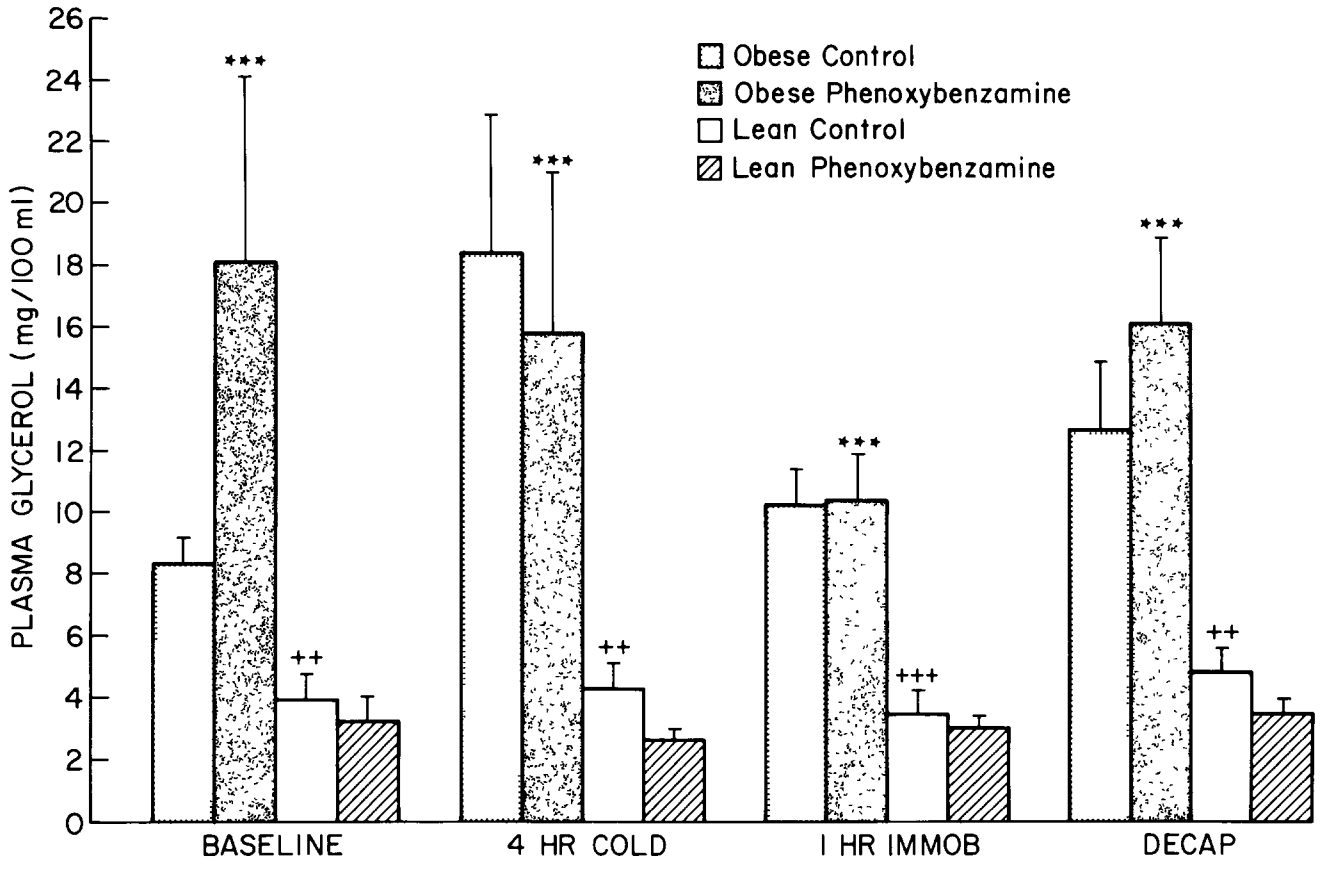

FIG. 5 Plasma glycerol levels. Four groups of 7–8 lean and obese, PBZ treated and control rats had glycerol levels (mean mg/100 ml ± SE) measured in plasma samples from indwelling atrial catheters under varying conditions (see Fig. 3). Symbols are the same as Figs 1, 2 and 3.

glycerol in lean or obese rats, while the obese rats had a 53% increase above baseline after decapitation. Glycerol levels remained higher in the obese rats than lean rats after immobilization (394%) and decapitation (359%). PBZ treatment did not significantly alter these responses although there was a tendency towards higher baseline glycerol levels in the PBZ treated obese rats. Therefore, the obese Zucker rats were hyperglycerolemic under all conditions tested and PBZ treatment did not appreciably affect this metabolic abnormality.

DISCUSSION

This study emphasizes the important interrelationships between sympatho-adrenal function and several of the metabolic and physiologic abnormalities of the obese Zucker rat. The abnormalities in sympatho-adrenal function in the 3–4 month rats used in the current study were related primarily to relative defects in the levels of plasma CA reached during cold stress (Table 1). Unlike the 7–8 month old obese rats previously studied [33], the current group showed no deficits in CA levels after 60 min of immobilization (Fig. 3), nor were CA levels different after 5 min of immobilization. However, preliminary studies in 3–4 month old lean and obese rats fed on standard lab chow have shown lower plasma CA levels in obese rats after 5 min of immobilization (unpublished results). Since rats in the present study were fed a 10% polyunsaturated fat diet, it is possible that this dietary difference might also have affected sympatho-adrenal activity [28]. In an attempt to correlate defects of sympatho-adrenal function with other abnormalities in the obese Zucker rat, we simultaneously determined plasma CA levels and plasma glycerol, glucose and insulin levels during sequential stresses designed to activate the sympatho-adrenal system PBZ was also used because of its known ability to increase NE outflow [8, 12, 24] and because another α-blocker, phentolamine, had been reported to decrease the fat content of epididymal fat pads [3].

We were, however, surprised to find that PBZ virtually eliminated weight gain in both lean and obese rats, while producing no change in food intake of the obese rat (Fig. 1). The reason for this lack of weight gain is uncertain. One possibility is that lowered insulin levels produced this change although it is equally as plausible to assume, conversely, that the weight loss itself was the cause of the decreased plasma insulin levels. PBZ does delay gastric emptying in rats [18] but the expected depression of food intake from this effect was unimpressive in the obese rats. PBZ might also have produced malabsorption of ingested food by reducing the output of the exocrine pancreas via its α blocking effect [38] or via its inhibitory action on pancreatic lipase [42]. However, no increase in fecal fat was found in the treated animals

(unpublished results). A further possibility is that a PBZ induced increase in sympathetic activity might have caused an increased metabolic rate. This coupled with α blockade of skin vasoconstriction, could have caused a net loss of body heat and weight. While Grzanna and Coyle [21] did not find that 11 days of PBZ treatment elevated plasma CA in trunk blood of Sprague-Dawley rats, there was an increase in baseline NE levels in the PBZ treated obese Zucker rats in our study (Fig. 3). Although changes in baseline plasma CA levels do not necessarily imply parallel changes in sympathetic neural activity, increased sympathetic activity as a mechanism for weight loss in the obese rats remains a reasonable possibility.

PBZ did not alter the ability of either the lean or obese rats to withstand cold stress (Fig. 2), but it did increase the relative sympatho-adrenal response to cold stress in the obese rats (Table 1). However, since increasing NE levels by intravenous infusions did not protect 7–8 month old obese Zucker rats against cold stress [33], it is not surprising that a relative increase in plasma CA levels would have had little protective effect on the obese rats in the present study. Furthermore, regulation of body temperature is complex and is modulated by both central α receptors [7] and peripheral α and β receptors [17,23] and PBZ could have acted at either site. Although it is not generally believed that α receptors play an inhibitory role in lipolysis in the rat [1, 45, 49] as they do in man [2], it is interesting that PBZ is strongly concentrated in brown, but not white adipose tissue in the rat [36]. Brown adipose tissue plays an important role in nonshivering thermogenesis in the rat [16], especially the obese [41] and cold adapted rat [16, 22, 23]

Like thermogenesis, glucose, metabolism in the rat is strongly influenced by adrenergic agonists. *In vivo* glucose levels in the rat, such as those determined in this study, reflect a complex series of metabolic steps, many of which are modulated by the sympatho-adrenal system. These include stimulation of hepatic glycogenolysis [34,43] gluconeogenesis, glucose production from muscle lactate, increased glucagon release [15,34], alterations in insulin release under both α(inhibitory) and β(facilatory) [40,50] control and the insulin facilitated uptake of glucose by muscle [13]. Others have reported that obese Zucker rats are euglycemic and hyperinsulinemic as determined from trunk blood [44, 48, 53]. While baseline levels from cannulated rats were compatible with these findings in the present study, we also found that the obese rat had an exaggerated response to stresses which activate the sympatho-adrenal systems. They became markedly hyperglycemic and their insulin levels were markedly depressed, while levels of glucose and insulin in the lean rats were less affected (Fig 4).

PBZ did not change glucose levels but further reduced baseline insulin levels and brought stress-induced insulin levels in the obese rats to those seen in the lean rats. While the glucose and insulin responses in the stressed, untreated obese rats were in the expected direction [50], the changes in insulin levels seen after PBZ treatment were opposite to those expected after release of inhibition of insulin secretion by α blockade [40]. These results are difficult to explain and any explanation would be further complicated by the nonspecific pharmacologic effects of PBZ and by the lack of a similar finding in the lean rats.

Basal, and in particular, stress-induced lipolysis (Fig. 5) as reflected in plasma glycerol levels [6,39], showed exaggerated responses in the obese rat, similar to those seen for glucose and insulin. *In vitro* E induced lipolysis rates generally have been found to be normal or exaggerated in the obese rat [52] suggesting that no defect in this β-adrenergic mediated [1,49] mechanism exists The lack of a significant PBZ effect was not unexpected, therefore, since the rat probably has no α mediated inhibitory receptor in white adipose tissue [1,45]. Nevertheless, the increased basal and stress induced glycerol levels suggest that either some inhibitory mechanism is defective in the obese rat or that this response is an attempt to deplete their elevated lipid stores [52].

In conclusion, the obese Zucker rat has abnormalities of lipid and carbohydrate metabolism which were corrected (hyperinsulenemia), induced (hyperglycemia) or exaggerated (hyperglycerolemia) by stress-induced activation of the sympatho-adrenal systems. Chronic treatment with the α-adrenergic blocking agent, PBZ, appeared to increase baseline sympathetic activity in the obese rat and led to a marked improvement of its hyperinsulinemia. Weight loss, with no decrease in food intake, accompanied these changes in the obese rats, while lean rats had a significant decrease in food intake associated with their weight loss. The present results and others [33] suggest that the sympatho-adrenal system in the obese rat is abnormal and that some of the metabolic abnormalities of the obese rat may be related to this dysfunction.

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REFERENCES

1 Aprille, J. R , R J Lefkowitz and J B Warshaw [^{3}H]-Norepinephrine binding and lipolysis by isolated fat cells. *Biochim biophys. Acta* **373:** 502–513, 1974
2 Arner, P. and J. Ostman Changes in the adrenergic control and the rate of lipolysis of isolated human adipose tissue during fasting and after re-feeding *Acta med. scan* **200:** 273–279, 1976
3 Boshart, C. R., T C Smith, L Will, A Pirre, J Perrine and I Ringler Action of phentolamine on rat adipose tissue *J Pharmac* **143:** 221–229, 1964
4. Bray, G. A. Oxygen consumption of genetically obese rats *Experientia* **25:** 1100–1101, 1969.
5 Bray, G. A , W S. Barry and S Mothon Lipogenesis in adipose tissue from genetically obese rats. *Metabolism* **19:** 839–848, 1970
6. Bray, G A. and C Galton Genetic obesity in rats A fat storage disease. *Clin Res* **14:** 347, 1966
7 Cantor, A and E. Satinoff. Thermoregulatory responses to intraventricular norepinephrine in normal and hypothalamic-damaged rats *Brain Res* **108:** 125–141, 1976.
8 Chan, C-C and S Kalsner An examination of the negative feedback function of presynaptic adrenoceptors in a vascular tissue. *Br J Pharmac* **67:** 401–407, 1979

9 Comai, K., J. Triscari and A. C. Sullivan. Differences between lean and obese Zucker rats the effect of poorly absorbed dietary lipid on energy intake and body weight gain *J Nutr.* **108:** 826–835, 1978.

10 Cruce, J A. F., N. B. Thoa and D M Jacobowitz Catecholamines in the brains of genetically obese rats. *Brain Res* **101:** 165–170, 1976

11. Cruce, J A F., N B Thoa and D M. Jacobowitz Catecholamines in discrete areas of the hypothalamus of obese and castrated male rats *Pharmac Biochem Behav* **8:** 287–289, 1978

12 Cubeddu, L. X , E. M. Barnes, S Z Langer and N. Weiner Release of norepinephrine and dopamine-β-hydroxylase by nerve stimulation I Role of neuronal and extraneuronal uptake and of alpha presynaptic receptors *J Pharmac exp. Ther.* **190:** 431–450, 1974.

13 Czech, M P., D K Richardson, S G. Becker, C G Walters, W. Gitomer and J. Heinrich Insulin response in skeletal muscle and fat cells of the genetically obese Zucker rat *Metabolism* **27:** Suppl. 2, 1967–1981, 1978.

14 Doxey, J. C , C F C. Smith and J M. Walker Selectivity of blocking agents for pre- and postsynaptic α-adrenoreceptors *Br J Pharmac* **60:** 91–96, 1977.

15 Ellis, S. Some explanations for species differences in hyperglycemic responses to adrenergic agents *Life Sci* **22:** 1229–1236, 1978

16 Foster, D O and L. Frydman Brown adipose tissue: the dominant site of nonshivering thermogenesis in the rat In· *Effectors of Thermogenesis*, edited by L. Girardier and J. Seydoux Basel and Stuttgart· Birkhauser Verlag, 1978, pp 147–151.

17 Fregly, M J , F P Field, M J Katovich and C. C Barney Catecholamine-thyroid hormone interaction in cold-acclimated rats *Fedn Proc* **38:** 2162–2169, 1979

18. Giri, S. S and S. Rice Influence of phenoxybenzamine on gastric evacuation in rats *Life Sci* **9:** 1109–1115, 1970

19 Giudicelli, Y , B Agli, D Brulle and R Nordmann Influence of α-adrenergic blocking agents on cyclic AMP, cyclic GMP and lipolysis in hamster white fat cells. *FEBS Lett* **83:** 225–230, 1977

20 Godbole, V , D A. York and D P Bloxham Developmental changes in the fatty (fa/fa) rat· evidence for defective thermogenesis preceding the hyperlipogenesis and hyperinsulinemia *Diabetologia* **15:** 41–44, 1978

21 Grzanna, R and J T. Coyle Absence of a relationship between sympathetic neuronal activity and turnover of serum dopamine-β-hydroxylase *Naunyn-Schmiedeberg's Arch. Pharmac* **304:** 231–236, 1978.

22 Harri, M. N. E Effect of prolonged beta-blockade on energy metabolism and adrenergic responses in the rat *Med Biol* **55:** 268–276, 1977

23. Janský, L Heat production In. *Body Temperature*, edited by P Lomax and E Schonbaum. New York and Basel: Marcel Dekker Inc., 1979, pp. 89–117.

24 Kalsner, S. and C-C Chan. Adrenergic antagonists and the presynaptic receptor hypothesis in vascular tissue *J Pharmac exp Ther* **211:** 257–264, 1979

25. Kandasamy, S B Inhibition of bovine cerebral cortex prostaglandin synthetase by phenoxybenzamine and cyproheptadine *in vitro. Clin exp. Pharmac Phys* **4:** 585–588, 1977

26 Kaplan, M L Consumption of oxygen and early detection of fa/fa genotype in rats. *Metabolism* **28:** 1147–1181, 1979.

27. Kvetnansky, R., V R Weise, N B. Thoa and I J Kopin. Effects of chronic guanethidine treatment and adrenal medullectomy on plasma levels of catecholamines and corticosterone in forcibly immobilized rats *J Pharmac exp Ther.* **209:** 287–291, 1979

28 Landsberg, L and J B. Young. Fasting, feeding and regulation of the sympathetic nervous system *N. Engl J Med* **298:** 1295–1301, 1978.

29 Langley, A. E and N. Weiner. Enhanced exocytotic release of norepinephrine consequent to nerve stimulation by low concentrations of cyclic nucleotides in the presence of phenoxybenzamine. *J Pharmac exp Ther* **205:** 426–437, 1978.

30 Lerner, P , P S Dendel and L. F Major Dopamine-β-hydroxylase in cerebrospinal fluid and plasma effects of α-adrenergic agents. *Brain Res* **189:** 183–191, 1980

31. Levin, B. E. and A C. Sullivan. Catecholamine synthesizing enzymes in various brain regions of the genetically obese Zucker rat *Brain Res* **171:** 560–566, 1979.

32. Levin, B. E. and A C. Sullivan. Catecholamine levels in discrete brain nuclei of seven month old genetically obese rats *Pharmac Biochem. Behav* **11:** 77–82, 1979.

33 Levin, B E , J Triscari and A. C. Sullivan. Abnormal sympatho-adrenal function and plasma catecholamines in obese Zucker rats *Pharmac Biochem Behav* **13:** 107–113, 1980.

34. Lum, B. K. B., Y-S. Lau, R. Buesa, R. H Lockwood and S-H Kuo. Studies on the hyperglycemia and hepatic glycogenolysis produced by alpha and beta adrenergic agonists in the cat *Life Sci* **26:** 1195–1202, 1980.

35. Maickle, R. P., N. Matussek, D. N. Stern and B. B Brodie. The sympathetic nervous system as homeostatic mechanism. I. Absolute need for sympathetic nervous function in body temperature maintenance of cold exposed rats *J Pharmac exp Ther* **157:** 103–110, 1967.

36. Masuoka, D , L-E. Appelgren and E Hansson. Autoradiographic distribution studies of adrenergic blocking agents. I. ^{14}C-phenoxybenzamine (Bensylyt NFN), an α-receptor-type blocking agent. *Acta pharmac tox* **25:** 113–122, 1967

37. Micalizzi, E R and D. T. Pals Evaluation of plasma norepinephrine as an index of sympathetic neuron function in the conscious, unrestrained rat *Life Sci* **24:** 2071–2076, 1979

38 Nickerson, M. and B. Collier. Drugs inhibiting adrenergic nerves and structures innervated by them In· *Pharmacologic Basis of Therapeutics*, edited by L S. Goodman and A Gilman, 5th edition New York. MacMillan Publishing Co., Inc., 1975, pp. 533–540

39 Ostman, J. A procedure for *in vitro* studies on fatty acid metabolism by human subcutaneous adipose tissue. *Acta med scan* **177:** 183–197, 1965.

40. Robertson, R P , J B. Halter and D Porte A role for α-adrenergic receptors in abnormal insulin secretion in diabetes mellitus. *J. clin Invest* **57:** 791–795, 1976.

41. Rothwell, N J and M. J. Stock. A role for brown adipose tissue in diet-induced thermogenesis *Nature* **281:** 31–35, 1979.

42. Santhanam, K , Y K. Yip, S. Ramachandran, D. O Allen and S R. Wagle. Studies on the mechanisms of inhibition of lipolysis by phenoxybenzamine. *Life Sci.* **10:** 437–442, 1971.

43 Sherline, P , A Lynch and W. H Glinsmann Cyclic-AMP and adrenergic receptor control of rat liver glycogen metabolism *Endocrinology* **91:** 680–690, 1972

44 Stern, J., P R Johnson, M R. C. Greenwood, L M Zucker and J Hirsch Insulin resistance and pancreatic insulin release in the genetically obese Zucker rat. *Proc Soc exp Biol Med* **139:** 66–69, 1972

45 Stock, K and T Thomas. Biphasic effect of the α-adrenolytic phentolamine on hormone-stimulated formation of cyclic adenosine-3′,5′-monophosphate in isolated fat cells of rats *Metabolism* **24:** 277–286, 1975

46 Sullivan, A C , J Triscari and H. E. Spiegel. Metabolic regulation as a control for lipid disorders. II Influence of (−)-hydroxycitrate on genetically and experimentally induced hypertriglyceridemia in the rat *Am J clin Nutr* **30:** 777–784, 1977

47 Trayhurn, P., P L Thurlby and W. P. T. James. A defective response to cold in the obese (obob) mouse and obese Zucker (fafa) rat. *Proc Nutr Soc* **35:** 133A, Abstract, 1976.

48. Triscari, J , J. S. Stern, P. R. Johnson and A C. Sullivan. Carbohydrate metabolism in lean and obese Zucker rats. *Metabolism* **28:** 183–189, 1979

49 Wertheimer, E., M. Hamosh and E. Shafrir. Factors affecting fat mobilization from adipose tissue *Am J clin Nutr.* **8:** 705–711, 1960.

50 Wright, P H and I J Malaisse Effects of epinephrine, stress and excercise on insulin secretion by the rat *Am J Physiol* **214:** 1031–1034, 1968.

51 Wright, P H and I J Malaisse Assay of partially neutralized guinea pig antiinsulin serum. *Endocrinology* **81:** 226–234, 1967

52 Zucker, L M Fat mobilization *in vitro* and *in vivo* in the genetically obese Zucker rat "fatty" *J Lipid Res* **13:** 234–243, 1972.

53. Zucker, L. M and H. N. Antoniades Insulin and obesity in the Zucker genetically obese rat "fatty". *Endocrinology* **90:** 1320–1330, 1972