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Abnormal Sympatho-Adrenal Function and Plasma Catecholamines in Obese Zucker Rats

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LEVIN, B. E., J. TRISCARI AND A. C. SULLIVAN. *Abnormal sympatho-adrenal function and plasma catecholamines in obese Zucker rats.* PHARMAC. BIOCHEM. BEHAV. 13(1) 107–113, 1980.—The functional integrity of the peripheral sympathetic nervous system and adrenal medulla was assessed in homozygous, lean and obese, 7–8 month old male Zucker rats by the changes in plasma catecholamines during cold and immobilization stresses. Five of eight obese, but no lean rats died during a 24 hr cold stress (4–7°C) from hypothermia. While both lean and obese rats had decreased rectal temperatures after 4 hr of cold stress, the obese had lower temperatures, relatively less of an increase of plasma norepinephrine (NE) and epinephrine (E) than the lean rats, and were unable to consistently maintain their temperatures even during intravenous NE infusions. Obese rats had lower rectal temperatures and higher plasma NE and dopamine levels at 21–22°C ambient temperature, a relative failure to increase plasma NE and E levels after 1 hr of immobilization, but normal or supranormal plasma catecholamine levels after decapitation compared to the lean rats. These results suggest that the obese Zucker rat has abnormalities of both peripheral sympatho-adrenal function and thermoregulation, which may play roles in the development and/or maintenance of many of the physiological and metabolic defects in this animal model of genetic obesity.

Norepinephrine Cold stress Epinephrine Immobilization stress Dopamine Genetic obesity Thermogenesis

THE genetically obese Zucker rat has proven to be a useful model of human obesity, particularly of juvenile onset [3,18]. This obesity is inherited as an autosomal recessive trait (fa/fa) [45] and these animals also manifest abnormalities in feeding behavior [3,8], lipid [30,38] and glucose [12, 41, 44] metabolism, and endocrine balance [4, 36, 43]. A defect in thermogenesis [17,40] is also present by the sixteenth day of life and preceeds abnormalities in lipid and glucose metabolism [17]. Abnormalities in catecholamines (CA) [10, 11, 26] and their synthetic enzymes [27] have also been demonstrated in the brains of obese Zucker rats. Since CA play a role in the modulation of several of the systems which are defective in the obese Zucker rat, the hypothesis has been proposed that brain CA may play an important etiological role in the other metabolic abnormalities of these animals [27].

Peripheral CA also play an important role in thermogenesis [24,29], and lipid [1,20] and glucose metabolism [14], yet the function of the peripheral sympathetic system has not been investigated in genetic obesity. We have chosen to evaluate the functional integrity of the peripheral sympathetic nervous system and adrenal medulla in the obese Zucker rat, as reflected in changes in plasma CA levels, during basal conditions and in response to cold and immobilization stresses.

METHOD

Animals

Seven to eight month old, male Zucker rats, which were homozygous for the lean (Fa/Fa) or obese (fa/fa) trait, were housed 2–6 per cage for at least one week prior to study. They were kept on a 12 hr light-dark cycle in a room at 21–22°C and fed ad lib on a standard rat chow diet. Two days prior to study, a jugular vein cannula (PE-50 tubing) was surgically implanted in the right atrium, tunneled subcutaneously up the back of the neck and to the outside of the cage through a stainless steel spring which was anchored to the skull by a screw. The cannulae were flushed twice a day with 0.5 ml heparinized saline (100 units/ml). The animals were studied on the second or third post-operative day by which time plasma CA had reached a stable baseline indicating recovery from surgical stress. All experiments were begun at 0730–0900 hr. Animals had access to water throughout all of the experiments listed below.

Stress Experiments

Baseline plasma levels of CA were obtained from groups of 5–10 lean and obese animals by withdrawing 2.0 ml of whole blood from undisturbed animals, placing this into heparinized tubes and then immediately reinfusing 0.5 ml of

heparinized saline (10 units/ml). Temperatures were then taken by rectal probe using an Electro-therm digital thermometer (Cole-Parmer) and the animals were then placed in a cold room kept at 4–7°C. Red cells were resuspended in a volume of normal saline equal to the amount of plasma removed and this was reinfused within 15 min of being withdrawn. Thus fluid and red cell volume were maintained in this and subsequent studies. Samples were obtained for plasma CA after 1 hr and 4 hr of cold exposure. Rectal temperatures were taken immediately following the 1 hr and 4 hr blood samples.

Animals were then kept at 22°C ambient temperature and immobilized for 1 hr in the prone position with leg restraints and a broad band over the back attached to a board. Blood was sampled for plasma CA at the end of 1 hr. Since experiments were not performed on all animals simultaneously, care was taken to always observe lean and obese rats at the same time in each experimental group. A separate group of six lean and six obese animals, which had no previous surgery or stress testing, was decapitated and the first 5 cc of blood collected from the trunk to determine their CA response to this stress.

Cold Stress and Norepinephrine Infusion

Two separate groups of eight lean and eight obese rats were placed in a cold room at 2°C for 24 hr and rectal temperatures were taken after 1, 4, 6, 8 and 24 hr of cold exposure. A separate group of six obese rats had jugular cannulae implanted and on the second and third post-operative days were placed in a room at 8°C for 8 hr. Rectal temperatures were measured at hourly intervals as they were infused with saline containing either norepinephrine bitartrate (NE:Sigma) plus ascorbic acid (in a ratio of 1/100) or ascorbic acid alone. The rates of NE (free base) infusion were either 10 ng/g body weight$^{0.74}$/min [13] (equivalent to approximately 1.2 μg/min) or 0.65 μg/100 g body weight/min [33] (equivalent to approximately 4.0 μg/min). Animals were tested on two successive days and infused with various sequences of NE or ascorbic acid carrier.

Catecholamine Assay

Plasma CA were measured by a radioenzymatic method modified from Peuler and Johnson [35] and Saller and Zigmond [37]. Heparinized plasma was deproteinized with 5 N perchloric acid in a ratio of 40 μl of perchloric acid to 960 μl plasma, and 50 μl of the supernatant of a 15 min centrifugation at 11,000 g was assayed. To this was added 35 μl of 2 M Tris buffer, pH 8.8, containing (in final concentration): EGTA (9 mM), benzylhydroxylamine (0.1 mM), and dithiothreitol (0.02 mg/ml); 10 μl catechol-0-methyl transferase [43]; 5 μl (2.5 μCi) adenosyl-L-methionine, S[methyl-^{3}H] (10–12 Ci/mmole; New England Nuclear); 10 μl 0.2 N perchloric acid containing $MgCl_2 \cdot 6H_2O$ (27 mM) which also contained 100 pg of norepinephrine bitartrate, epinephrine bitartrate (E) and dopamine HCl (DA) free base (Sigma) in those tubes used as internal standards. Blanks contained 50 μl 0.2 N perchloric acid in place of sample. A timed reaction was run for 60 min at 37°C and stopped by the addition of boric acid (800 mM), pH 11.0, containing EDTA (80 mM) and metanephrine HCl, normetanephrine HCl and 3-methoxytyramine HCl carriers (4 mM free base; Sigma). The [^{3}H] labelled products were extracted into the organic phase of 2 ml of toluene:isoamyl alcohol (3:2) solution and the tubes were frozen in a toluene dry ice bath. The organic phase was decanted into a tube containing 100 μl of 0.1 N acetic acid and the aqueous phase discarded. These tubes were vortexed, centrifuged at 2000 g for 15 min and frozen. The organic phase was discarded and absolute ethanol (100 μl) was added to the aqueous phase containing the [^{3}H]CA metabolites. The total volume (200 μl) was spotted on silica gel plates containing fluorescent indicators and developed for 45 min in chloroform: methanol: 70% ethylamine (80:15:10).

The flourescent spots corresponding to the [^{3}H]labelled products were scraped into small (7 ml) scintillation vials containing 500 μl of 0.05M NH_4OH. Five ml of toluene:isoamyl alcohol:Liquiflour (New England Nuclear) (700:300:50) were added to the tubes containing the [^{3}H] 3-methoxytyramine spots. Periodate cleavage was carried out on [^{3}H]metanephrine and [^{3}H]normetanephrine spots for 5 min with 25 μl of 4% sodium metaperiodate at room temperature and the reaction was stopped with 25 μl of 10% glycerol. Samples were counted by scintillation spectroscopy in a standard toluene based scintillant after the addition of 500 μl of 0.1 N acetic acid. This assay has also been applied to the measurement of tissue levels of CA in the supernatant of a 0.2 N perchloric acid homogenization solution [27]. The assay is linear over a range of 2 pg to 5 ng and has a sensitivity (2×blank) of 2.0 pg for NE, 2.5 pg for E and 10.0 pg for DA. There is less than 1% carryover between CA. The average interassay coefficient of variation is 5–7% and the intraassay coefficient of variation is 7–8%. While samples from various experiments were run separately, 4–6 samples from preceding assays were always run in subsequent ones to insure uniform interassay correlation.

RESULTS

Cold Exposure for 24 Hours

Lean rats in the unoperated, unstressed experimental group were significantly lighter (465 ± 17 g) than the obese rats (721 ± 23 g; $p<0.001$) and also had significantly higher rectal temperatures (37.3 ± 0.1°C) than the obese rats (36.9 ± 0.1°C; $p<0.05$) at ambient temperatures of 21–22°C. These weight and rectal temperature differences were seen throughout all the groups of rats used in the following studies as well. When placed at 2°C, lean rats had no significant change in rectal temperature over 24 hr (Fig. 1). The obese rats had two types of responses (Fig. 1). Five of eight had no change after 1 hr, but thereafter rectal temperatures fell rapidly over the next 7 hr and, by 24 hr of cold exposure, all had died. The three remaining obese rats had no significant change in rectal temperature over the first 8 hr and, although temperatures had fallen significantly below baseline by 24 hr, all survived the entire cold stress period. Rectal temperatures in both groups of obese rats were significantly lower than the lean rats at all time periods except at basal and 1 hr times in the obese cold survivors. Both lean and obese rats appeared to shiver although the obese animals failed to maintain piloerection. Although both the obese survivors and those obese rats that died were significantly heavier than the lean rats, the surviving obese rats were also significantly lighter than those which died (662.7 ± 26.0 g versus 756.6 ± 19.9 g; $p<0.05$). There were, however, no significant absolute correlations between body weights and rectal temperatures in lean or obese rats at either 22°C or 2°C ambient temperatures.

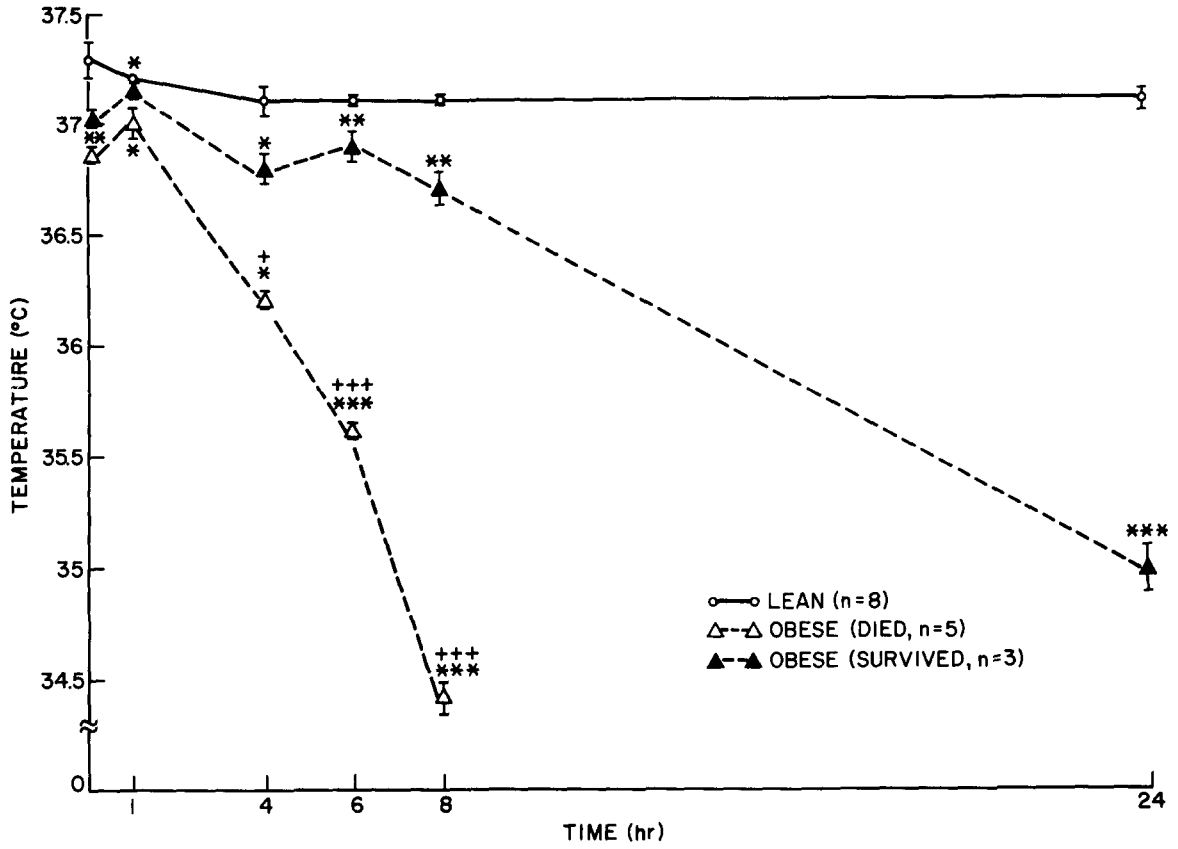

FIG. 1. Rectal temperature responses to cold stress (2°C) for 24 hr in lean and obese Zucker rats. Eight lean and eight obese rats were placed in a room at 2°C for 24 hr and rectal temperatures (°C) were measured at 1,4,6,8 and 24 hr. Figures are given as mean °C ± SE (vertical crossed bars). *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$ compared to lean rats. †=$p<0.05$, ††=$p<0.01$, †††=$p<0.001$ comparing the obese rats who died (n=5) to those who survived (n=3) 24 hr of cold exposure.

Plasma Catecholamine Levels and Stress

A separate group of animals with surgically implanted jugular venous catheters underwent 4 hr of cold stress followed by 1 hr of immobilization. The obese rats had significantly lower rectal temperatures (36.3 ± 0.3°C) than the comparable group of lean rats (37.1 ± 0.2°C; $p<0.05$) at ambient temperatures of 21–22°C. Conversely, their basal levels of plasma NE and DA were 236% and 169%, respectively, of the lean animals, while plasma E levels were not significantly different (Fig. 2). In contrast to the unoperated lean rats in the 24 hr cold study, rectal temperatures fell significantly in these lean animals to 35.9 ± 0.4°C after 1 hr of cold exposure (4–7°C). This was accompanied by significant increases in plasma DA (185% of baseline), but not in NE or E levels. No further decrease in rectal temperature occurred after 4 hr of cold exposure although plasma NE (588%), E (401%) and DA (211%) had all risen significantly above baseline levels.

Rectal temperatures in the obese rats fell significantly below basal levels by 1 hr of cold exposure (35.0 ± 0.4°C) with an accompanying, significant increase in plasma NE (184%) and E (193%), but not DA levels above baseline values (Fig. 2). Plasma NE levels were significantly higher in the obese (194%) than the lean rats at this point but E and DA levels were comparable. By 4 hr, rectal temperatures had fallen further to 33.5 ± 0.6°C in the obese rats and were significantly lower than the lean rats ($p<0.05$). Concomitantly, plasma NE and E rose to significantly higher levels than at 1 hr and were now both significantly higher than basal levels. DA levels remained essentially unchanged. At this time, there were no differences between plasma NE, E and DA levels in lean and obese rats. It should be noted that these cannulated, obese rats could be divided into two groups, as could the previous group of unoperated obese rats, regarding their responses to cold stress. Four of 10 operated obese rats had a 2°C or less drop in rectal temperature to 35.4 ± 0.4°C after 4 hr of cold exposure. Six of 10 had more than a 2°C drop in temperature to 32.3 ± 0.3°C at 4 hr which was significantly lower than the other group ($p<0.005$). Both groups of operated obese rats had significantly lower 4 hr rectal temperatures than the respective groups of surviving and nonsurviving, unoperated obese rats from the previous experiment. Thus, the process of cannulation appeared to make both lean and obese rats less able to withstand cold stress.

There was a significant correlation between rectal temperatures and plasma NE and E levels in both lean and obese rats at 21–22° and 4–7°C (Fig. 3), while plasma DA levels did not correlate with temperature in either lean (Pearson's product correlations, r=−0.274) or obese (r=0.026) rats. Although plasma NE and E levels were comparable in the lean

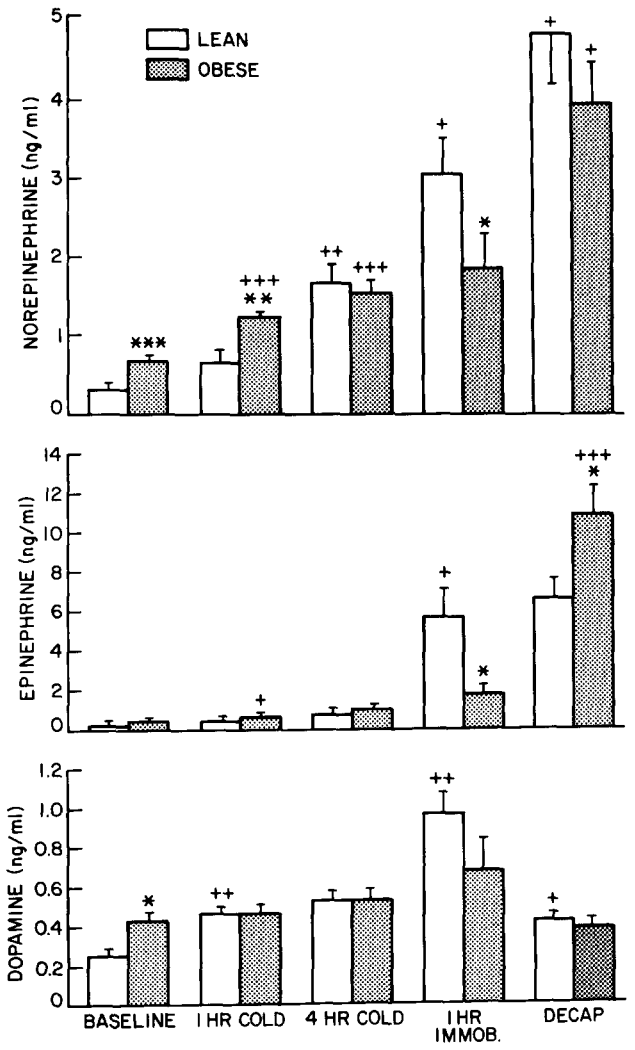

FIG. 2. Plasma catecholamine levels at rest and during cold and immobilization stresses in lean and obese Zucker rats. Groups of 6–10 lean and obese rats with jugular venous cathethers had blood samples taken for plasma CA at an ambient temperature of 22°C ("Baseline"), after 1 hr and 4 hr of cold exposure at 4–7°C ("1 hr cold" and "4 hr cold"), after 1 hr of immobilization ("1 hr immob.") and, in a separate group of rats, after decapitation ("decap."). Levels are expressed as mean CA level (ng/ml) ± SE (vertical crossed bars) for lean (open) and obese (stippled bars) rats. *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$ for lean versus obese plasma levels under the same conditions. †=$p<0.05$, ††=$p<0.01$, †††=$p<0.001$ compared to the levels for the same genotype during the immediately preceding conditions.

and obese rats after 4 hr of cold exposure, comparisons of the slopes of the regression lines plotted in Fig. 3 show that this required a two-fold (NE) and four-fold (E) greater decrease in body temperature in the obese rats to produce the same plasma CA levels as the lean rats.

Immobilization for 1 hr produced significantly lower levels of NE and E in the obese than the lean rats (Fig. 2). This was primarily due to the failure of the obese animals to increase their NE and E levels significantly above the preceding 4 hr cold stress levels, while the lean rats increased their plasma NE levels to 183% and E levels to 832% of the preceding values. DA levels were also increased significantly to 188% of 4 hr cold stress level in the lean rats. Decapitation of a separate group of previously unstressed rats (Fig. 2 "decap") produced significantly higher levels of plasma NE (214%) and E (652%) in the obese, and only of plasma NE levels (155%) in the leans compared to levels observed after 1 hr of immobilization in the previous experimental group. Plasma DA was significantly lower (43%) after decapitation than immobilization in the lean rats. Plasma E levels were significantly higher in the obese than lean animals after decapitation (169%), while there were no significant differences in NE or DA levels in obese compared to lean rats.

Norepinephrine Infusions

Since the obese rats appeared to have defects in thermogenesis and relatively decreased levels of NE reached in response to cold stress, an attempt was made to try and correct the thermogenic abnormality with NE infusion. The intravenous infusion of NE into obese rats kept at 8°C did not consistently prevent a drop in rectal temperature from occurring (Fig. 4). However, there was a tendency for temperatures to be higher in the same rat when treated with NE than with the ascorbic acid carrier. For example, on the first test day, rat A maintained a constant body temperature for 4 hr while being infused with NE and then dropped its temperature when NE was discontinued. However, on the second test day, this animal failed to drop its temperature at all, despite the lack of NE infusion, although rectal temperatures were generally lower than those on the preceding day during NE infusion. Rats B and D had temperatures which were similar at the end of 8 hr of cold exposure with and without NE, and rats E and F had precipitous drops in rectal temperatures despite infusions of NE at maximal calorigenic doses of 0.65 μg/100g/body weight/min (4 μg/min) [33]. Rat C did appear to maintain its temperature more consistently during NE infusion on the first test day than during the ascorbic acid infusion on the second day, and NE appeared to prevent a further drop in temperature seen during carrier infusion on the second day. However, no increases in temperature to the previous day's levels were seen. At best, then, NE may have had a slight effect in preventing a fall in rectal temperature in the obese Zucker rats tested, but this effect was inconsistent and was totally absent in the two rats showing the most severe drops in body temperature.

DISCUSSION

The obese Zucker rat has abnormalities in several metabolic and physiologic systems, many of which are modulated by the sympatho-adrenal system in normal animals. The functional integrity of this system in the Zucker rat was examined using the paradigm of measuring plasma CA. Previous studies have shown that plasma NE levels primarily reflect sympathetic neural activity, and E levels are indicative of adrenal medullary activity [15, 23, 34], while DA is released from both systems [23]. Not only does the obese rat have abnormalities of its central autonomic pathways [10, 11, 26, 27], but we now report that the peripheral sympathetic pathways function defectively as well. Obese rats had both abnormally high baseline levels of plasma NE and DA and a relative abnormality of the NE and E levels reached during cold stress. Since plasma NE and E levels correlated with rectal temperatures and since the obese rats had a much greater fall in temperatures, uniformly higher,

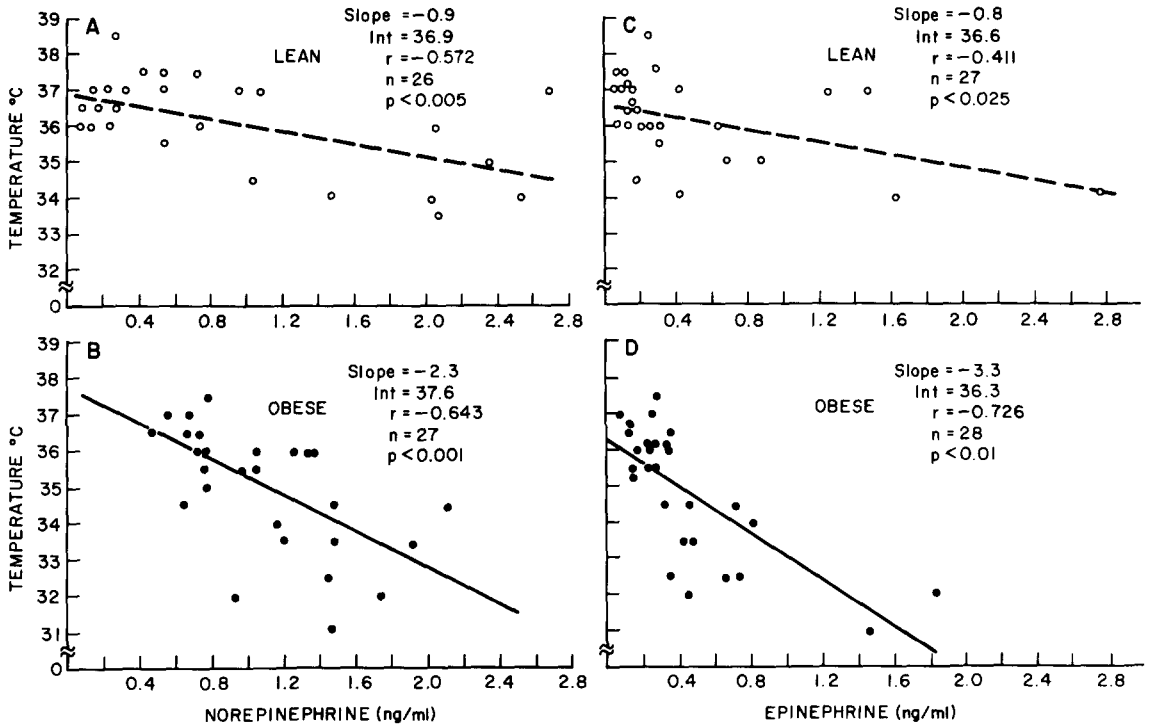

FIG. 3. Correlation of rectal temperatures and plasma catecholamine levels in lean and obese Zucker rats. Plasma CA levels (ng/ml) and rectal temperatures (°C) were determined in the same groups of 5–10 lean and obese rats shown in Fig. 2 at ambient temperatures of 22°C ("Baseline") and after 4 hr at 4–7°C, as described under Methods. Data pairs were compared by Pearson's product correlation (r). Slope, y intercept (Int.) and number of observations (n) are given for lean (○) and obese (●) animals and a regression line plotted for each set of data.

rather than equivalent levels of CA would have been expected for the obese rats. The only place where such higher levels were in fact found was in plasma NE levels after 1 hr of cold stress.

The obese rats also showed an absolute defect in the levels of NE and E reached after 60 min of immobilization stress following cold stress, while reaching NE and DA levels equal to and E levels higher than the lean rats after decapitation. It is possible that the obese rats did have a normal, early response to immobilization and cold, comparable to their normal response to decapitation, but that after 1 hr, levels had fallen below those in the lean rats. In the rat, peak CA levels are usually reached within 5–20 min after immobilization, although they are generally still significantly elevated after 1 hr of stress [22,23]. These results therefore indicate a relatively or absolutely blunted sympatho-adrenal response to various stresses in the living animal with a normal or supranormal response following decapitation. Our data are insufficient to predict whether these differences in plasma CA levels reflect abnormalities in release (as seen in some inbred strains undergoing cold stress [2]), in rates of removal (as seen in other inbred strains undergoing immobilization [32] and humans undergoing spinal cord stimulation [25]), or in defective feedback regulation involving peripheral and central autonomic pathways.

In addition to the finding of defective sympatho-adrenal function, we have confirmed and extended the previous finding of defective thermoregulation in the obese Zucker rat [17,40]. Besides a lowering of body temperature, cold-induced vasoconstriction in the liver and skin have also been found to be abnormal in the obese rat [21]. Peripheral vasoconstriction, shivering, piloerection [21] and nonshivering thermogenesis [16,19] are all critical to maintenance of normal core temperature and the sympatho-adrenal system plays a critical role in all of these parameters allowing the animal to respond to cold stress [24,28]. Thermoregulatory defects similar to those seen in the obese Zucker rat are also seen in sympathectomized [28], adrenalectomized [29] and hypothyroid rats [39]. Since NE infusions in doses adequate to produce maximal calorigenesis in normal rats [33] did not correct the obese rats' thermoregulatory problem, it is unlikely that insufficient levels of plasma NE alone were the primary cause of this problem. One possibility is that the known abnormality in thyroid function in the obese Zucker rat [4,43] may play a critical role in the apparent unresponsiveness of the obese rat to the calorigenic and other peripheral effects of NE. It is also possible that this abnormality represents a central nervous system defect in thermogenesis where either NE or DA are known to produce a hypothermic response [6, 7, 9] which may be related to a reduced metabolic rate in the rat [9] or, as in the cat, a reduction of shivering and concomitant vasoconstriction [6]. Since infused NE would not be expected to cross the blood brain barrier [31], it could not correct such a central defect in CA regulation of thermogenesis, if one were present.

Another potentially important observation is that there appeared to be two distinct groups of obese rats which responded to cold stress in a dissimilar fashion. While both groups had impaired thermoregulatory responses, one group appeared to tolerate and survive prolonged cold stress better. This

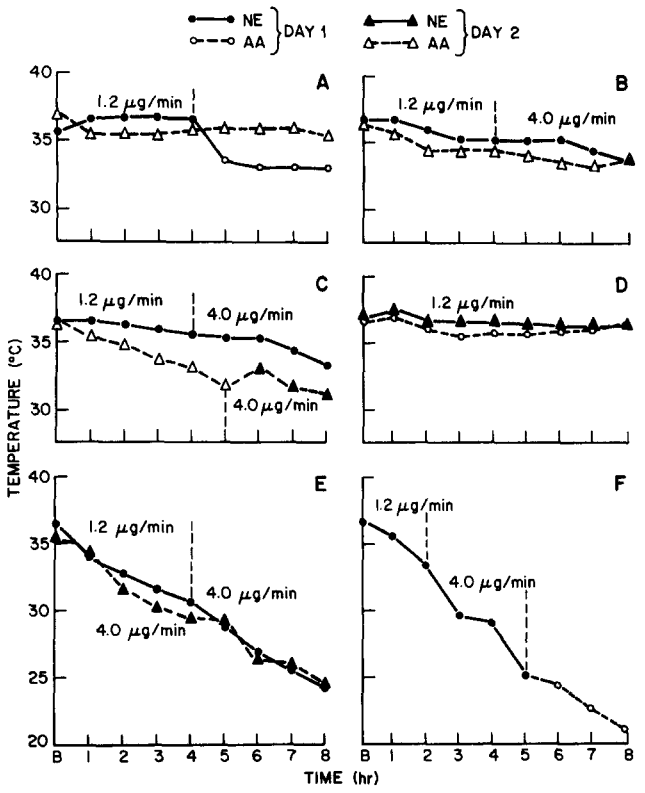

FIG. 4. Effect of intravenous norepinephrine or ascorbic acid infusions on rectal temperature during cold stress in obese Zucker rats. Six obese rats were placed in the cold (8°C) for 8 hr and infused through a jugular venous catheter with norepinephrine (NE) or the ascorbic acid (AA) carrier solution (1:100/NE:AA) in normal saline at rates of 1.2 μg/min (approximately 10 ng free base/g body weight $^{0.74}$/min) or 4.0 μg/min (approximately 0.65 μg/100 g body weight/min) on two successive days. Rectal temperatures (°C) were taken at hourly intervals. Vertical dashed lines represent a change in the rate, type or amount of infused materials on the respective day as shown.

raises the possibility of a genetic inhomogeneity within the obese phenotype and suggests that similar groupings might be present for other physiological and metabolic parameters. Additional comment should be made about the differences in thermoregulatory responses between both nonoperated and operated (cannulated) lean and obese rats. While no differences were obvious at ambient temperatures of 21–22°C, comparable groups of cannulated lean and obese rats clearly had lower rectal temperatures during cold stress despite a slightly warmer (4–7°C versus 2°C) ambient temperature. It seems likely that the combination of post-operative stress and an indwelling venous catheter were responsible for these differences and suggests that the criteria of adequate recovery from operation used (stable baseline plasma CA levels) might not have been adequate as regards temperature regulation. However, by the second post-cannulation day, baseline levels of plasma CA reach levels which remain unchanged for at least 3–4 more days suggesting stabilization of the sympatho-adrenal system by the second day.

What can be said with certainty is that the obese Zucker rat demonstrates several abnormalities in its autonomic nervous system, both centrally and peripherally. Bray and York [5] have proposed that decreased sympathetic and increased parasympathetic activity may play a major role in the pathogenesis of obesity caused by lesions in the ventromedial hypothalamus. Although they felt that genetic obesity might be better explained by an abnormality of endocrine function, our results suggest that defective autonomic nervous system function must also be considered in the pathogenesis of the physiological and metabolic abnormalities found in the obese Zucker rat and also as a possible cause of its obesity.

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