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Catecholamine Levels in Discrete Brain Nuclei of Seven Month Old Genetically Obese Rats

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LEVIN, B. E. AND A. C. SULLIVAN. *Catecholamine levels in discrete brain nuclei of seven month old genetically obese rats.* PHARMAC. BIOCHEM. BEHAV. 11(1) 77–82, 1979.—Catecholamine levels were measured in microdissected nuclear groups of seven month old, obese and lean, male and female Zucker rats. By analysis of variance, obese rats showed significantly reduced levels of norepinephrine and epinephrine in the paraventricular nucleus and of epinephrine and dopamine in the dorsomedial hypothalamic nucleus. Norepinephrine levels were increased in the median forebrain bundle and caudate nucleus. When compared by sex and genotype (*t*-test), the female obese rats had significant decreases compared to the leans of norepinephrine and epinephrine levels in the paraventricular and dorsomedial hypothalamic nuclei, while norepinephrine was increased in the median forebrain bundle and caudate nucleus. Dopamine was decreased in the dorsomedial and increased in the C2 nuclei. Male obese Zucker rats showed changes only in the dorsomedial and C2 nuclei where dopamine levels were decreased. In general, female obese and lean rats tended to have lower levels of catecholamines in various brain areas than males of the same genotype. Comparisons of these data to previous studies in younger Zucker rats [9,10] suggested that changes also occurred in various nuclei with aging. It is postulated that catecholamine deficits in certain hypothalamic nuclei of the Zucker obese rat may be a contributing factor to the development and/or maintenance of obesity, possibly in association with abnormalities in thermal regulation.

Norepinephrine Epinephrine Dopamine Brain nuclei Hypothalamus Brain Zucker rat Catecholamines Obesity

BRAIN catecholamines (CA) have been implicated in the modulation of numerous physiological functions, many of which have been found to be abnormal in the obese Zucker rat. These animals manifest obesity as an autosomal recessive trait [41], as well as abnormalities of lipid metabolism [26,38], endocrine balance [34,40], thermoregulation [11,39], and ingestive behavior [1,5]. Many of these functions are normally thought to be under the influence of hypothalamic CA which Cruce *et al.* have shown to differ in lean and obese male [10] and female [9] Zucker rats. The latter two studies examined two month old male and four month old female rats so that direct comparison between sexes was not possible. Furthermore, epinephrine (EPI) levels were not measured, nor were brain stem areas containing EPI and norepinephrine (NE) cell bodies. In light of the possible importance of all of these factors in explaining some of the physiological differences between the lean and obese Zucker rat, we have examined NE, EPI, and dopamine (DA) levels in discrete brain areas of seven month old male and female, lean and obese Zucker rats, using a sensitive radioenzymatic essay for CA combined with the microdissection technique of Palkovits [30].

METHOD

Groups of five homozygous obese (fa/fa) and lean (Fa/Fa) male and female rats, 27–29 weeks of age, were housed 1–2 per cage for at least two weeks prior to study. All animals were fed on an ad lib schedule and kept on a 12 hr light-dark cycle. All animals were killed by decapitation between 0730 and 0830 hr and the brains were quickly removed and frozen on dry ice. Cryostat sections (300 μm) were placed on a cold plate under a dissecting microscope and microdissected by the method of Palkovits [30]. Cannula size and approximate coordinates are shown in Table 1.

Brain samples were blown into 150 μl of 0.2N PCA containing 0.1 mM dithiothreitol and homogenized by sonication. Fifty μl aliquots of 11,000 xg supernatant were stored at −70°C for 1–3 days and assayed for CA by modifications of the radioenzymatic assays of Saller and Zigmond [35] and

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TABLE 1
DISSECTION TECHNIQUES

Area	Number of Punches	Cannula Size (mm)	Approximate Coordinates*
Caudate (body and tail)	6	1.0	A 6570-A 5780
Median Forebrain Bundle (MFB)	6	1.0	A 5780-A 5340
Paraventricular Nucleus (PVN)	4	0.5	A 5660; A 5340
Median Eminence (ME)	5	0.5	A 4890-A 3750
Dorsomedial Nucleus (DMN)	4	0.5	A 4380; A 3990
Locus Coeruleus (LC)	8	0.3	P 1.5–P 2.8
C2	8	0.3	P 5.0–P 6.5

*Hypothalamic coordinates according to König and Klippel [17], LC according to Palkovits and Jacobowitz [31]; C2 according to Hökfelt et al. [14].

Peuler and Johnson [33]. This assay is linear from 2.0–5000 pg and has a sensitivity (2×blank) of 1.8 pg for NE, 2.5 pg for EPI and 10.1 pg for DA. There is less than 1% carryover from one CA to another. Protein determinations on 10–20 μl samples were carried out by the method of Lowry *et al.* [25] and units expressed as pg of CA per μg of protein.

RESULTS

Obese Zucker rats of each sex were significantly heavier than their respective lean counterparts and males were heavier than females (Table 2). Female rats showed the most numerous differences in brain CA between the lean and obese animals (Fig. 1). NE and EPI were significantly decreased in the obese females to 51% and 33% of the lean female values, respectively, in the paraventricular nucleus (PVN), and to 47% and 42%, respectively, in the dorsomedial nucleus (DMN). DA also was decreased in the DMN of the obese females to 48% of lean. NE levels in the obese females were increased to 201% of lean in the median forebrain bundle (MFB) and to 213% in the caudate, although the latter values were quite low. DA levels in the C2 area of the medulla were below the limits of sensitivity of the assay for the lean females, but were reliably detectable in the obese. Male obese Zucker rats (Fig. 2) had reduced DA in the DMN (60%) and C2 (43%) areas as their only differences in comparison to the lean animals. Analysis of variance showed significant increases in the obese rats for NE in the caudate, $F(1,16)=8.34$, $p<0.01$, and MFB, $F(1,16)=6.01$, $p<0.025$. NE and EPI were significantly decreased in obese rats in the PVN, $F(1,14)=10.98$, $p<0.005$ and $F(1,16)=6.73$, $p<0.019$, respectively, while EPI and DA were significantly decreased in obese rats in the DMN, $F(1,16)=6.26$, $p<0.024$, and $F(1,15)=12.90$, $p<0.003$, respectively. EPI values in the caudate and C2 areas were below the limits of sensitivity of the assay in all groups.

An interesting, if somewhat tentative comparison to CA values in younger males and females is presented in Table 3 taken from the present study (7 months) and the two studies of Cruce *et al.* [9,10]. While differences in dissection technique, CA assays, as well as other unknown variables must be taken into account, it appears that changes in CA levels did occur with age, as well as with sex and genotype. Another finding was that CA levels in various areas were frequently lower in the female rats than the males of comparable ages. This was most strikingly seen in the 7 month old animals (Figs. 1 and 2) where analysis of variance showed NE to be significantly reduced in female vs male rats in the MFB, $F(1,16)=29.99$, $p<0.001$, PVN, $F(1,14)=33.81$, $p<0.001$, ME, $F(1,15)=43.03$, $p<0.001$, DMN, $F(1,16)=17.66$, $p<0.001$, LC, $F(1,16)=5.88$, $p<0.028$, and C2 areas, $F(1,13)=23.56$, $p<0.001$. EPI was also reduced in the MFB, $F(1,16)=15.71$, $p<0.001$, PVN, $F(1,16)=12.13$, $p<0.003$, and DMN, $F(1,16)=9.26$, $p<0.008$, while DA was reduced in the LC, $F(1,16)=12.35$, $p<0.003$, and C2 area, $F(1,14)=7.55$, $p<0.016$, in the female vs male rats. The studies of Cruce *et al.* [9,10] (Table 3) also suggest generally lower CA levels in the females and similar changes are present in Sprague-Dawley rats at two months of age [7].

TABLE 2
BODY WEIGHTS

Genotype	Sex	Body Weight* (g ± SE)
Obese (fa/fa)	Female	472 ± 15
Lean (Fa/Fa)	Female	269 ± 6
Obese (fa/fa)	Male	591 ± 9
Lean (Fa/Fa)	Male	456 ± 16

*Analysis of variance: Genotype effect (obese versus lean), $F(1,16)=19.2$, $p<0.001$ and sex effect (male versus female), $F(1,16)=157.5$, $p=0.001$.

In summary, female rats generally had lower CA levels in many brain areas when compared to the males, especially at 7 months of age, while the obese rats had increases in NE in the MFB and caudate with decreases in the PVN. EPI was decreased in the obese rats in the PVN and DMN while DA was also decreased in the DMN. The females showed the greater number of significant genotype-related changes.

DISCUSSION

The obese Zucker rat manifests a number of abnormalities of endocrine, ingestive and physiologic function which may be related to differences in hypothalamic CA levels between the lean and obese animal. While it is worthwhile to enumerate these abnormalities and attempt to correlate them with the changes in CA found in this and past studies, a major difficulty arises because of the differences between the age and sex of the Zucker rats used in previous

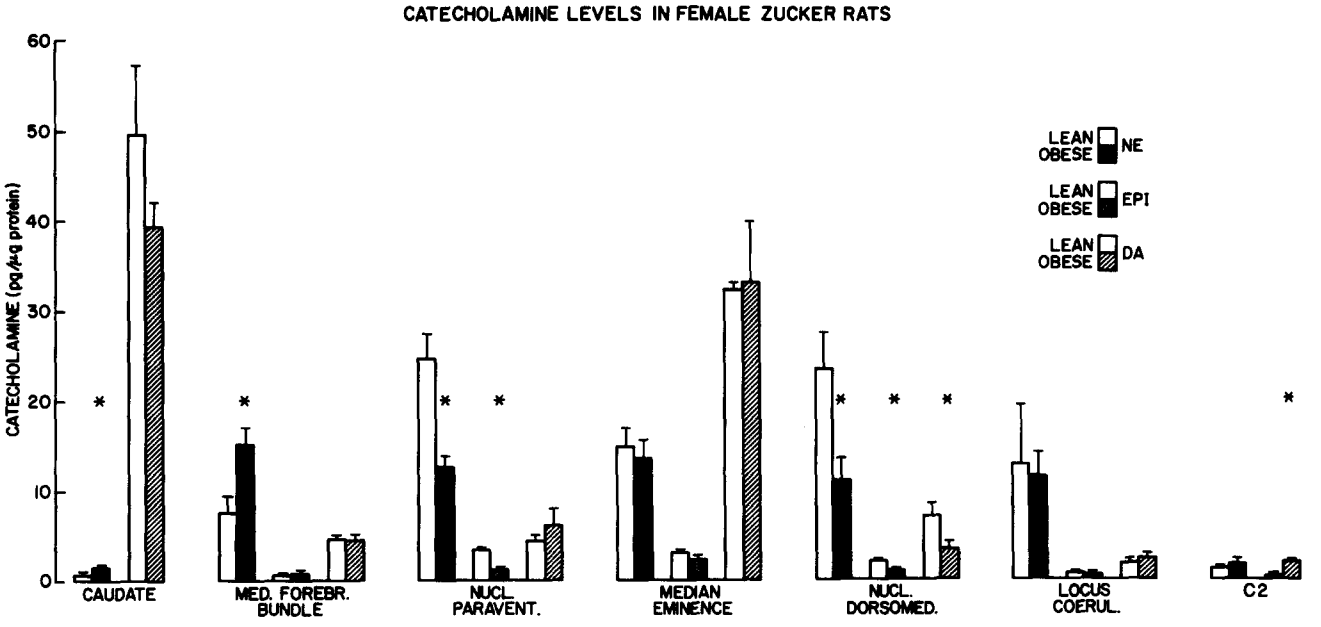

FIG. 1. Catecholamines in discrete brain nuclei of the female Zucker rat. Each pair of bars represent the norepinephrine (NE), epinephrine (EPI) and dopamine (DA) levels for lean and obese rats, as mean pg/μg of protein ± SE (vertical lines) for a given brain area. *=significant differences between the lean and obese rat for the same catecholamine in a given area ($p<0.05$ by two-tailed Student's t-test).

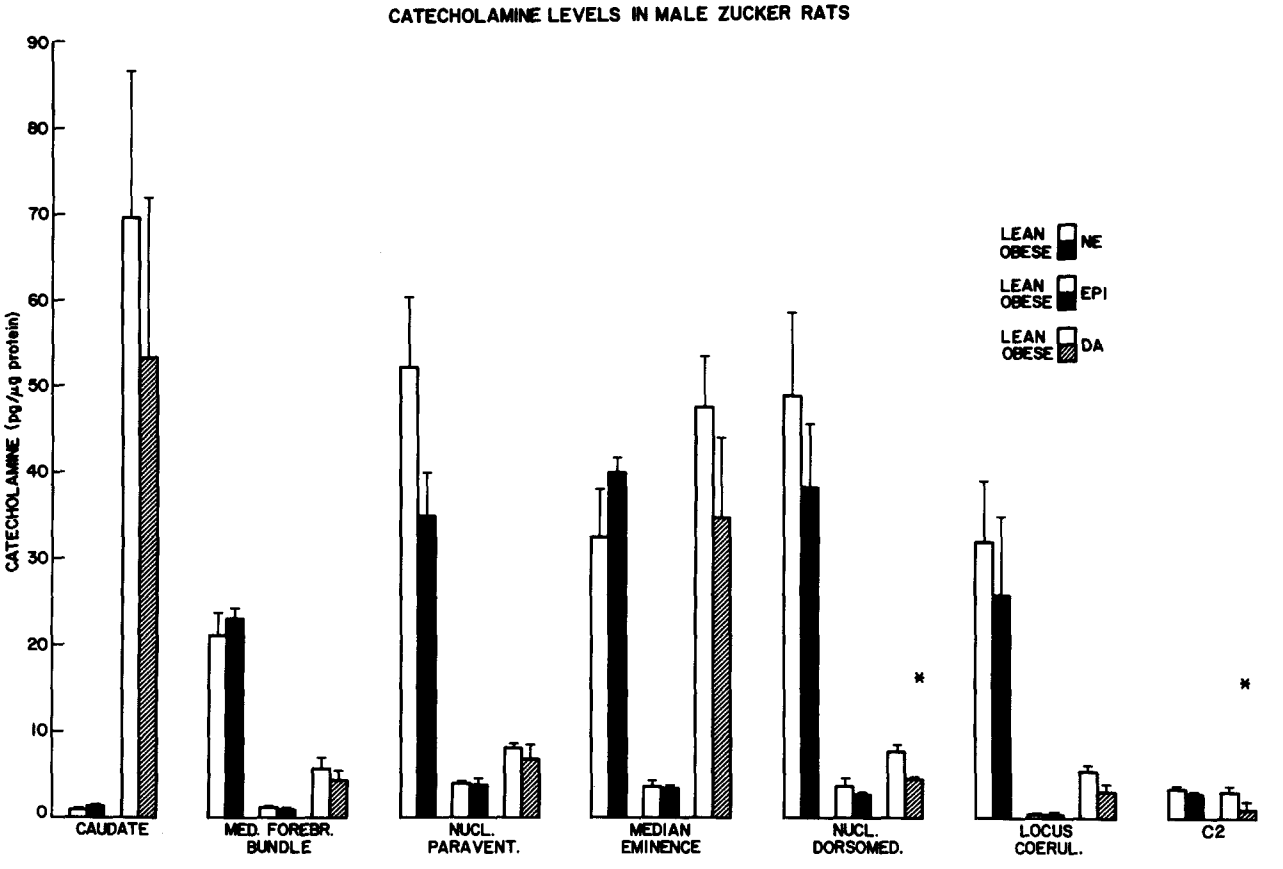

FIG. 2. Catecholamines in discrete brain nuclei of the male Zucker rat. Legend is the same as Fig. 1.

TABLE 3

COMPARISON OF BRAIN CATECHOLAMINES (CA) IN YOUNG AND OLD ZUCKER RATS BY SEX AND GENOTYPE

Area of Brain*	CA†	Male 2 Months‡ Obese	Male 2 Months‡ Lean	Female 4 Months‡ Obese	Female 4 Months‡ Lean	Male 7 Months Obese	Male 7 Months Lean	Female 7 Months Obese	Female 7 Months Lean
ME	NE	20.86 ± 3.10§	24.03 ± 2.76	17.96 ± 2.69¶	8.48 ± 1.17	40.03 ± 1.53	32.61 ± 5.49	13.43 ± 2.05	14.78 ± 2.16
	DA	29.19 ± 2.21	43.97 ± 6.61	51.34 ± 7.23	41.02 ± 3.55	34.75 ± 9.30	47.63 ± 5.79	32.99 ± 6.91	32.27 ± 0.68
PVN	NE	33.72 ± 7.66¶	57.55 ± 6.37	18.04 ± 1.69¶	27.00 ± 2.51	35.24 ± 4.22	52.02 ± 7.21	12.82 ± 1.36¶	24.66 ± 2.60
MFB	NE	13.05 ± 1.90	12.21 ± 2.47	14.63 ± 2.10	14.26 ± 1.44	22.95 ± 1.21	21.08 ± 2.70	15.25 ± 1.75¶	7.59 ± 1.77
DMN	NE	27.34 ± 5.90	18.76 ± 2.03	39.24 ± 6.00	33.33 ± 6.11	38.32 ± 7.30	49.08 ± 9.06	11.04 ± 2.60¶	23.52 ± 3.99

*Abbreviations of the brain areas are the same as in Table 1.

†CA = catecholamine, NE = norephinephrine, DA = dopamine. Units are mean pg catecholamine per μg protein ± SE.

‡Data for two month males from Cruce et al. [10] and from four month females from Cruce et al. [9].

§Data are expressed as mean ± SE.

¶Significantly different ($p < 0.05$) from animals of same age and sex (obese versus lean) by two-tailed Student's t-test.

studies of physiologic function and those used to determine CA levels. This problem is confounded by the fact that differences in CA levels appear to become more numerous with age, particularly in the obese female. These age-related changes in CA levels are also reflected in a number of changes in CA synthesizing enzyme activities in the hypothalamus and brain stem which appear with increasing age [22]. It is therefore difficult to generalize about the relationship of CA levels to functional abnormalities when levels of CA are not consistent between the sexes or with aging. The most numerous changes were found in the female Zucker rat, particularly at 7 months of age in the PVN and DMN. The lack of comparable changes in the PVN of males at 7 months of age raises the possibility that these differences were related to the female reproductive cycle. The PVN shows cyclical changes in NE levels during the normal estrous cycle with low levels during proestrus and estrus and peak levels during the metestrus and diestrus stages [8]. The obese Zucker has both a prolonged estrous cycle and decreased fertility [34] which could be related to a deficiency or lack of rhythmic changes in PVN NE during the estrous cycle. NE levels in the ME were increased in obese females at 4 months of age [9], but by 7 months there was no longer a difference. However, NE in the PVN of obese females was decreased at both 4 months [9] and 7 months of age suggesting that this consistent change might be related to the abnormalities in the estrous cycle.

There is a large body of evidence linking CA to feeding behavior. Leibowitz has reported that injections of EPI in low doses into the perifornical area lateral to the PVN inhibit feeding, while higher doses facilitate feeding [18]. The specific area of MFB sampled in the present study was chosen because it best approximated this perifonical, lateral hypothalamic area. NE but not EPI or DA was found to be increased in the obese rats in this area, while NE and EPI were decreased in the PVN of obese animals. NE injections into the PVN stimulate feeding [12, 19, 27] while lesions of the tractus filiformis, which supplies the PVN with NE fibers from the lateral hypothalamic area, produce transient hypophagia [29]. While differences in CA in this area which is thought to be related to feeding are interesting, levels of CA give no indications of turnover or receptor sensitivity. Also, the observed changes in feeding behavior after CA injections appear to be related to the type of adrenergic receptor stimulated [18]. While the changes in CA found in the present study are in the opposite direction to that which might have been predicted from certain injection studies to result in the hyperphagia seen in obese Zucker rats, a disturbance in the overall balance or turnover of CA in the hypothalamus might still produce these changes in feeding behavior. Furthermore, other factors such as time of day are known to influence the response to injected catecholamines so that the situation is far from clear.

Other abnormalities in endocrine and physiologic function which might be related to defects in CA metabolism include thyroid function and temperature regulation. Since direct comparisons are not possible because of the lack of adequate metabolic data in comparable animals, mention will only be made of possible associations. The obese Zucker rat appears to be both hypothyroid [40] and hypothermic [11]. Hypothalamic CA have been implicated in both thyroid [16] and thermal [3, 4, 6, 15, 28, 32, 37] regulation. Thyrotropin releasing hormone levels in the rat are high in the ME, PVN and DMN [2], while thyroidectomy in the normal animal increases the activity of the CA synthesizing enzyme, tyrosine hydroxylase, in the ME, DMN and other hypothalamic nuclei [16]. Since a decrease in DMN DA levels (acting as a primary transmitter and/or NE precursor) was the only consistent change found in both the 7 month old obese male and female Zucker rats, it is possible that this change may be related to the deficit in thyroid function of the obese animals, especially since this nucleus seems to be involved in thyroid function.

CA also appear to play a role in body temperature regulation through both central and peripheral pathways [3]. The central pathways involve the anterior hypothalamic preoptic area where both NE [28] and DA infusions [6] produce heat loss in response to a warm or neutral environment. NE also appears to act in the more caudal periventricular hypothalamic area where it causes an increase in nonshivering thermogenesis which is: (1) dependent upon ambient, core and skin temperatures, (2) antagonized by warming of the anterior hypothalamic preoptic area, and (3) mediated by α receptors [3]. While this periventricular area has been examined primarily in the guinea pig, evidence exists in the rat that NE also plays a role in temperature regulation which is independent of its effect upon the anterior hypothalamic preoptic area [4]. NE also causes an increase in metabolic

rate and nonshivering thermogenesis via a neurally mediated effect on brown adipose tissue [37], skeletal muscle [15], and other organs. This effect is dependent, in part, upon normal thyroid function [32], which is deficient in the obese Zucker rat [40]. Since CA appear to play an important role in thermal regulation, a central and/or peripheral deficit of CA metabolism might be expected to result in abnormalities of thermogenesis. The present study and those of Cruce *et al.* [9,10] suggest that such central deficits exist in hypothalamic CA. Additional support for a deficit in CA function comes from the study of Hemmes *et al.* [13] in which chronic oral administration of L-dopa, a precursor of CA, produced weight loss in the obese Zucker rat which could not be totally accounted for by reduced food consumption alone. These data suggest that L-dopa might be correcting a defect in the metabolic rate of the obese rat. We propose that the obesity of the Zucker rat might not be due to CA related abnormalities in feeding behavior but rather to CA mediated central (and/or peripheral) deficits in thermal regulation [11,39] and possibly thyroid function [40]. These deficits, possibly related to deficient DMN CA levels, might prevent the obese rat from adequately dissipating calories during its various metabolic activities.

The only genotype related changes in CA seen outside the hypothalamus were an increase in caudate NE and C2 DA levels in the obese female, and a decrease in DA in the C2 area of the obese males versus the lean animals. It is not surprising that there were no major differences in NE or EPI in the region of heavy cell body concentrations of these two CA in the brain stem [14,24]. NE, at least, is synthesized primarily in the nerve terminals from enzymes which are synthesized in the perikaryon and transported to the terminals [20,21]. Also, despite the high levels of phenylethanolamine N-methyl transferase [23], the enzyme which synthesizes EPI from NE, levels of EPI were below the sensitivity of our assay in the rat C2 area in agreement with the low levels reported by Sauter *et al.* [36]. While the presence of DA in this area is compatible with the suggestion that both dopaminergic cell bodies and terminals lie in this region [24], DA levels may reflect the presence of DA as either a precursor in NE or EPI neurons, or as the actual primary transmitter in dopaminergic neurons. It is quite possible that such dopaminergic cells, or in fact, NE or EPI cells, could explain some of the observed physiological differences between the obese and lean Zucker rat via an influence on vagal and/or other medullary centers.

In conclusion, the presence or absence of changes in CA levels in various brain areas gives only an incomplete picture of the status of CA metabolism. While it is commonly suggested that differences in CA levels may be the cause of differences in physiologic function, it is equally as plausible to suppose that such differences in function create the observed changes in CA levels. The present study demonstrates that brain levels of CA in the Zucker rat vary according to sex, genotype and possibly age. Future studies should attempt to correlate these changes with known physiological defects in the obese Zucker rat, especially abnormalities in thermal regulation and possibly thyroid function.

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