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Catecholamines in Discrete Areas of the Hypothalamus of Obese and Castrated Male Rats[1]

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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(3) 287–289, 1978. – Levels of norepinephrine (NE) and dopamine (DA) were measured in eight discrete regions of the hypothalamus in three groups of male rats: genetically obese (fafa), non-obese (FaFa) and castrated non-obese (FaFa). DA levels showed no significant differences among the groups in any of the regions. NE levels in the paraventricular nucleus (PVN) were significantly lower in the obese and castrated animals than in the normal animals. In the median eminence (ME), NE levels were significantly decreased for the castrated group. None of the other regions sampled showed significant differences in NE levels.

Genetic obesity Norepinephrine Dopamine Hypothalamus Castration

IN a previous study of catecholamine (CA) levels in the brains of Zucker rats [3], two discrete regions were found to show significant differences in norepinephrine (NE) levels between the obese animals (fafa) and the non-obese controls (FaFa and Fa–). Certain unanswered questions led to the present study. First, the previous report used female rats of approximately four months of age. In the present study observations were extended to male animals of a younger age (2 months old). Secondly, since abnormal reproductive function has been reported in Zucker rats [19] in order to determine any direct relationship between gonadal function and brain CA levels, a group of non-obese castrated rats of the same strain was also included.

METHOD

The animals were genetically controlled male rats of the Zucker strain [25]. Three groups were used: (1) non-obese (FaFa) control animals, (2) genetically obese (fafa) rats or fatties, (3) non-obese (FaFa) castrated animals. All animals were approximately the same age (± 5 days). The animals in group 3 were castrated at approximately 5½ weeks of age; the time between castration and sacrifice was 22 days.

All animals were sacrificed on the same day by decapitation and the brains were removed and rapidly frozen on dry ice. Brains were sectioned at a thickness of 300 μm in a cryostat at −7°C. The frozen sections were placed on cold slides, slightly thawed and quickly refrozen on dry ice. The slides were placed on a cold plate under a stereomicroscope. Microdissection of brain regions was performed according to the method of Palkovits [17]. The coordinates and size of cannulae used for microdissection were the same as those previously described [3].

For assay of CA, samples of brain regions of each animal were blown from the dissecting cannula into 100 μl of ice cold 0.1 N perchloric acid and homogenized by sonification [6]. A 10 μl aliquot was taken for protein analysis according to the micromethod of Lowry *et al.* [14]. The remaining samples were frozen and stored in the deep freeze until assayed for NE and dopamine (DA). After the frozen samples were thawed, vortexed and centrifuged at 8000 × g for 30 sec, 25 μl aliquots were assayed for CA [2]. The results are reported as nanograms of CA per mg of protein.

RESULTS

Results for NE levels in eight regions are shown in Table 1. Two areas with substantial amounts of DA are shown in Table 2. Analyses of variance were performed on the regions in which the largest differences among the means were observed, and the individual means were compared with each other by using the q-statistic [24]. Five analyses were done as follows: NE in the arcuate (ARC), paraventricular (PVN), dorsomedial nuclei (DM), median eminence (ME) and DA in the median eminence. Neither the F-value

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TABLE 1

NOREPINEPHRINE LEVELS (NG/MG PROTEIN) IN BRAIN REGIONS OF THREE GROUPS OF RATS

Region	Control (fafa)	Fatty (fafa)	Castrated(FaFa)
Arcuate nucleus (ARC)	22.26 ± 2.16	21.14 ± 2.23	15.37 ± 2.59
Median eminence (ME)	24.03 ± 2.76	20.86 ± 3.10	14.14 ± 1.98*
Paraventricular nucleus (PVN)	57.55 ± 6.37	33.72 ± 7.66*	30.88 ± 7.45*
Periventricular nucleus (PERI)	24.13 ± 2.65	24.13 ± 1.85	26.91 ± 2.46
Ventromedial nucleus (VMN)	13.37 ± 0.66	11.56 ± 1.53	10.29 ± 1.53
Medial forebrain bundle (MFB)	12.21 ± 2.47	13.05 ± 1.90	13.96 ± 2.12
Dorsomedial nucleus (DM)	18.76 ± 2.03	27.34 ± 5.90	37.51 ± 6.38
Anterior hypothalamic nucleus (AH)	10.67 ± 0.98	11.19 ± 0.76	9.9 ± 0.59

The values represent mean ± SEM for 6–8 animals.
*Significantly different from control group, $p<0.05$.

TABLE 2

DOPAMINE LEVELS (NG/MG PROTEIN) IN BRAIN REGIONS OF THREE GROUPS OF RATS

Region*	Control (FaFa)	Fatty (fafa)	Castrated (FaFa)
ARC	9.40 ± 1.32	11.53 ± 4.04	8.94 ± 1.91
ME	43.97 ± 6.61	29.19 ± 2.21	27.34 ± 3.93

The values represent mean ± SEM for 6–8 animals.
*Abbreviations as in Table 1.

nor any comparison between means was significant for NE in the ARC $F(2,19) = 2.51$, $p>0.10$, NE in the DM, $F(2,18) = 2.85$, $p>0.05$, or for DA in the ME, $F(2,18) = 3.52$, $p>0.05$.

The overall analysis of variance for NE in PVN was significant, $F(2,17) = 3.82$, $p<0.05$. Results of comparison between the means showed that the control group was significantly different from both castrated and fatty rats ($p<0.05$) and that these two latter groups were not significantly different from each other. Although the overall analysis of variance for NE in the ME was not significant, $F(2,20) = 3.32$, $p>0.05$, the difference between the control and castrated animals was significant ($p<0.05$); neither control nor castrated groups, however, was significantly different from the fatties.

DISCUSSION

The results obtained in the present study using two month old male rats (young males) can be compared with those of a previous report [3] in which four month old females (old females) were employed. In a comparison of CA levels in the brains of obese vs non-obese animals, the young males and old females show one area of agreement and one area of disagreement. The area of agreement is the PVN; NE levels in the PVN of old fatty females and young fatty males show significant decreases as compared to the respective control group. In the region of the ME, however, a contrast in results was found; whereas old fatty females have increased NE levels in the ME as compared to controls, the NE levels in ME of young fatty males are not significantly different from non-obese controls of the same age. This sex difference may be related to the difference in male-female patterns of gonadotropin secretion. It is of interest to note that while gonadectomy results in obesity in female rats, it does not alter the body weight of male rats [23]. In the present study no significant difference in body weight was observed between the non-obese non-castrated animals and the non-obese castrated group.

In the prior study of obese female rats [3], an increase in the NE content of the median eminence suggested a possible correlation with an abnormal secretion of pituitary hormones (gonadotropins, etc.) and/or gonadal hormones. Lofström [11] has reported a decreased NE turnover in the median eminence during diestrus and estrus when gonadotropic and ovarian hormones are relatively low, while an increase in NE turnover occurred during proestrus, at the time of maximum secretion of these hormones [21]. A persistent increase in NE (decrease in turnover) in the median eminence suggests an altered hormonal balance which could lead to the reduced fertility observed in female fatty rats [19]. The increase in the concentration of luteinizing hormone and follicle-stimulating hormone observed in the pituitary of fatty rats [1] is a reflection of abnormal hormone balance and correlates with altered levels of hypothalamic catecholamine [3, 12, 13].

In the non-obese male, castration results in a lowered NE concentration in the median eminence and PVN which suggests an increase in turnover of the noradrenergic transmitter. Löfström *et al.* [10] studied catecholamine turnover in samples of median eminence from groups of intact and castrated rats. Although the difference between these two groups did not reach significance statistically, the castrated animals had higher rates of turnover of catecholamine. A higher rate of turnover would be compatible with the present finding of a decrease in absolute levels in the median eminence. Since castration removes the inhibitory effects of gonadal hormones on gonadotropin release, an increase in NE turnover in the castrated male median eminence supports the postulated role of NE as the mediator which transmits a stimulatory effect on the release of gonadotropins following castration [16]. These results are consistent with the observations that castration increases tyrosine hydroxylase activity in the median eminence, although no change was observed in the PVN [7]. Furthermore, it was suggested that the change in enzyme activity was a reflection of increased dopamine turnover in the median eminence [8]. However, since the tyrosine hydroxylase content of noradrenergic nerves in the median eminence and PVN is quite small, a change in the activity of this enzyme cannot accurately reflect noradre-

nergic nerve activity. A 39% decrease of the dopamine concentration in the median eminence in the present study, although not statistically significant, is consistent with an increase in the dopamine turnover and tyrosine hydroxylase activity [7]. Other authors, however, did not observe changes in activity of DA neurons in castrated animals [5].

The most striking observation in the present communication is the fact that NE levels in the PVN decreased in both castrated and fatty rats. A decrease in NE concentration was also noted in the PVN of fatty females reported previously [3]. This is interesting in light of the fact that fatties show a decreased testicular size [4], which could result in a reduced amount of circulating androgens and would, in effect, mimic castration. Furthermore, the PVN is a target tissue for androgen (and estrogen) uptake as demonstrated by autoradiographic studies [20,22]. That the PVN may be critical for the production of follicle stimulating hormone-releasing factor (FSH-RF) is suggested by the observation that lesions of the PVN cause a decrease in the concentration of FSH-RF [15]. The above observations suggest that the reproductive impairment of fatty rats may be linked to gonadal hormones, the PVN and gonadotropic release from the pituitary gland. Such a link is further supported by studies showing that hypophysectomy abolishes the eating response elicited by injection of small doses of NE into the PVN [9]. In addition, hypophysectomy has recently been shown to abolish postoperative weight gain in fatty rats [18]. How the change in NE in the PVN relates to further changes in the pituitary gland and to the etiology of obesity is a matter of future investigations.

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