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Brain Serotonergic Activity and Plasma Amino Acid Levels in Genetically Obese Zucker Rats[1]

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FINKELSTEIN, J. A., W. T. CHANCE AND J. E. FISCHER. *Brain serotonergic activity and plasma amino acid levels in genetically obese Zucker rats*. PHARMAC. BIOCHEM. BEHAV. 17(5) 939–944, 1982.—In order to test the hypothesis that serotonergic activity is abnormal in the brains of genetically obese Zucker rats, levels of serotonin (5-HT), its amino acid precursor, tryptophan (Trp), and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) were measured in eight brain regions in groups of obese and non-obese male rats. Plasma albumin levels as well as levels of amino acids and related compounds in plasma and in a cortical sample were also determined in the same animals. While Trp was lower in several brain regions of the obese animals, the only region showing a depressed level of 5-HT in the obese group was the mesencephalon. Obese animals also had a lower amount of 5-HIAA in the diencephalon, but no other differences were significant. Both elevations and depressions were observed in cortical amino acid levels in obese animals. The level of plasma albumin was increased in the obese group. Free Trp was decreased in the plasma of obese rats while levels of other amino acids (methionine, leucine, isoleucine, valine and phenylalanine) which compete with Trp for transport across the blood-brain barrier were elevated. Thus the combination of lower plasma free Trp and increased levels of competitive amino acids appears to contribute to decreased levels of Trp in the brain of genetically obese rats.

Genetically obese Zucker rat Brain serotonin Obesity Plasma amino acids Tryptophan

THE genetically obese Zucker rat, fa/fa, is characterized by a number of behavioral, endocrinological and metabolic abnormalities. Shortly after weaning, the obese animals are hyperphagic in comparison to their non-obese littermates, Fa/—. The increase in food intake is reflected in larger meal size, increased meal frequency and an increase in feeding during the light phase of the light:dark cycle [2] in contrast to non-obese rats which eat more during the dark period. The obese animals also display hyperinsulinemia from an early age (5.5 weeks), although blood glucose levels are not abnormal [27]. The amount of adipose tissue is greatly increased in obese animals. Obese rats have heavier inguinal fat pads as early as 7 days of age [5]. The increased adipose tissue is due to increases in both number and size of adipocytes [26,27].

Body weights of genetically obese rats are elevated by the time of weaning [38]; rate of growth is much steeper for the obese animals until about 12–16 weeks of age [38, 44, 46]. Thereafter, the slope of rate of increase in body weight is similar to that of the non-obese animals, although the absolute levels of body weight are significantly greater for the obese rats. A study of food intake per gram body weight has shown that this ratio is significantly greater for obese animals up to 10 weeks of age, not different for obese and lean rats between ages 13 to 18 weeks, and that at 19 weeks of age obese rats eat relatively less per gram body weight than lean animals [18]; in that study, animals were fed a special diet and it is not known if the same results would be obtained if animals were fed standard lab chow.

The locus of the abnormality has not been identified in the fa/fa. Because of the role of central nervous sytem transmitters in the regulation of food intake and body weight in normal animals, abnormalities in brain neurotransmitters have been investigated in genetically obese rats. Cruce *et al.* [11,12] were the first to report differences in catecholamine levels in discrete brain regions of obese rats compared to non-obese animals. They found decreased levels of norephinephrine (NE) in the paraventicular nucleus of the hypothalamus (PVN) and increased levels of NE in the median eminence of four month old female obese rats as com-

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pared to lean animals. In two month old male rats, however, only NE levels in PVN were decreased in obese animals. A more recent study [31] using older animals (seven month) confirmed the observation in PVN, and also showed additional differences in epinephrine (E) and dopamine (DA) levels.

The above studies investigated levels of three different monoamines, NE, DA and E, but to date, no studies of brain concentrations of serotonin (5-HT), another monoamine, in obese rat brains are available. Serotonin, an indoleamine found extensively throughout the neuraxis [34, 39, 40], is thought to play a role in the regulation of food intake and body weight control. For example, intraventricular injection of 5-HT has been reported to reduce food intake in rats [30] as has systemic administration of the 5-HT precursor tryptophan (Trp) [4]. The anorexia-producing drugs fenfluoramine and fluoxetine apparently reduce food intake through direct or indirect stimulation of 5-HT receptors [42]. Furthermore, intracerebroventricular injection of parachlorophenylalanine (a 5-HT depletor) has been reported to induce hyperphagia [6]. Recent experiments have also suggested decreased activity in brain indoleamine systems associated with ventromedial hypothalamic (VMH) lesion induced hyperphagia [9]. Additionally, brain concentrations of 5-HT, Trp and the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) are elevated in cancer-induced anorexia [8,45].

Therefore, in order to gain a more complete picture of central nervous system (CNS) monoamine activity in genetically obese rats, brain levels of Trp, 5-HT and 5-HIAA, were determined. In addition, plasma albumin and amino acid concentrations as well as brain amino acid levels were assayed in the same animals. To allow correlation of biochemical differences with data of consummatory behavior, food intake was also determined.

METHOD

Subjects were eight male genetically obese Zucker rats (fa/fa) [46] and eight male non-obese littermates (Fa/—). Mean age of animals at the time of sacrifice was 13.3 weeks, with a range of 12.5 to 14.3 weeks. The animals were bred in a colony maintained at the Northeastern Ohio Universities College of Medicine and were descendants of rats originally purchased from the Harriet G. Bird Memorial Labs, Stow, MA.

Daily ad lib food intake was measured to the nearest 0.1 g for seven days prior to sacrifice. Animals were decapitated between 1 and 3 p.m. Brains were rapidly removed and dissected into the following regions: cortex (C), hippocampus (HI), corpus striatum (CS), hypothalamus (HYP), remaining diencephalon (DI), mesencephalon (MES), pons-medulla (PM) and cerebellum (CB). These regional brain dissections were conducted similarly to published methods [24] with the following exceptions: (1) mesencephalon and diencephalon were differentiated by a vertical cut just anterior to the superior colliculus, and (2) a smaller hypothalamic area was dissected as defined as tissue taken to a depth of approximately 2.5 mm from the optic chiasma to the posterior mamillary area and bounded laterally by the lateral border of the tuber cinereum. The cortical sample was divided in half. One side was used to determine amino acid levels and the other side was used to determine levels of Trp, 5-HT and 5-HIAA.

Biochemical Analyses

Plasma albumin concentration was determined by the colormetric method of Doumas *et al.* [19]. Plasma free and total Trp were assayed fluorometrically as described by Bloxam and Warren [3], with free Trp being determined in 50 ml of an ultrafiltrate prepared by centrifugation (100 × g, 25 min, 20°C) of 1 ml of plasma (pH=7.4) in a CF 50 Diaflow membrane cone (Amicon Corp., Lexington, MA). Brain 5-HT and 5-HIAA were also determined fluorometrically following acid-butanol extraction, according to the method of Curzon and Green [14], with 5-HT and 5-HIAA being complexed with OPT (Sigma Chem. Co., St. Louis, MO) and read on an Aminco-Bowman fluorometer. Brain Trp was extracted along with 5-HT and assayed fluorometrically according to the procedures utilized for plasma Trp.

Levels of amino acids and related compounds were measured in plasma and half of the cortex on a Beckman 121-MB automated amino acid analyzer as previously published [29]. The compounds measured and their abbreviations are as follows: Ala: Alanine; Arg: Arginine; Asn: Asparagine; Asp: Aspartic Acid; Cit: Citrulline; Gln: Glutamine; Glu: Glutamic Acid; Gly: Glycine; His: Histidine; Hpro: Hydroxyproline; Ileu: Isoleucine; Leu: Leucine; Lys: Lysine; Meth: Methionine; Orn: Ornithine; Phen: Phenylalanine; Pro: Proline; Ser: Serine; Thr: Threonine; Trp: Tryptophan; Tyr: Tyrosine; Val: Valine.

RESULTS

Food Intake and Body Weights

The genetically obese rats weighed significantly more than the non-obese littermates throughout the seven day period prior to sacrifice. Data on body weight are shown in Fig. 1. An analysis of variance on the body weights revealed a significant group effect, $F(1,14)=26.08; p<0.01$, as well as a day's effect, $F(6,84)=58.03$, $p<0.01$, and a significant group × days interaction, $F(6,84)=4.67, p<0.01$. During the seven day period immediately prior to sacrifice, obese rats gained a mean total of 30 grams or 4.3 grams per day. Lean littermates, on the other hand, gained a mean total of 16 grams or 2.3 grams per day.

Daily food intake is shown in Fig. 2 for the same period. Obese animals consistently ate more than lean littermates. An analysis of variance revealed a significant group effect, $F(1,14)=53.48$, $p<0.01$, a significant day effect, $F(6,84)=8.35$, $p<0.01$, and a significant interaction, $F(6,84)=2.33$, $p<0.05$. Food intake relative to body weight as shown in Fig. 3 was significantly elevated in the obese rats. $F(1,14)=13.6$, $p<0.01$; the days effect was also significant, $F(6,84)=6.96$, $p<0.01$, but the interaction was not significant.

Serotonergic Activity in the Brain

Levels of 5-HT, Trp and 5-HIAA are shown in Fig. 4. Obese rats had significantly decreased levels of Trp in CS, HI, C, HYP and DI. Levels of 5-HT were significantly lower in MES and were significantly elevated in CB of obese animals. Only one brain region, DI, showed decreased levels of 5-HIAA for the obese animals.

Levels of Amino Acids in Brain

In comparison to non-obese littermates, cortical tissue from fa/fa rats showed elevated levels of serine, glycine,

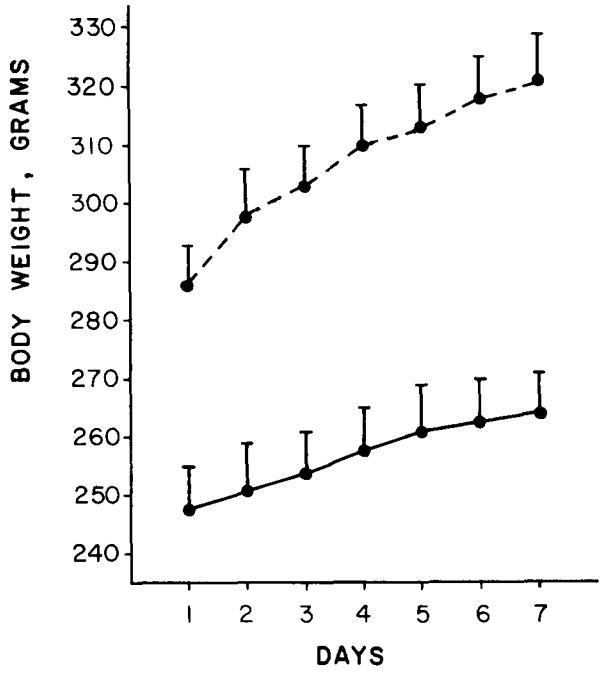

FIG. 1. Daily mean body weight (±S.E.M.) for groups of obese (dashed line) and non-obese rats (continuous line) for seven days prior to sacrifice.

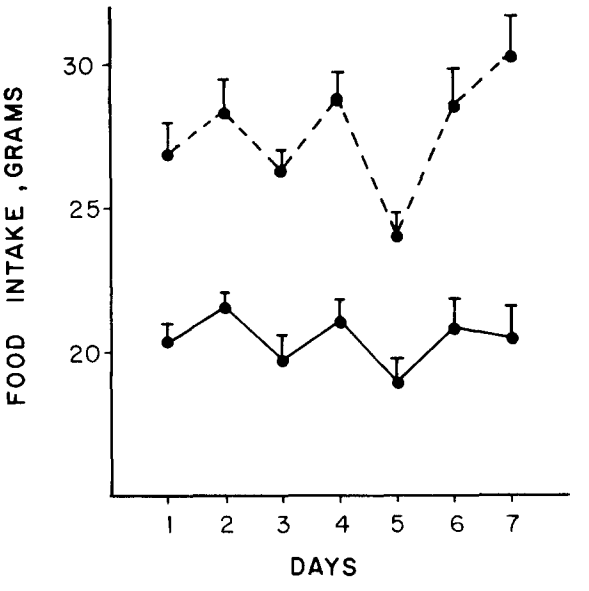

FIG. 2. Daily mean food intake (±S.E.M.) for groups of obese (dashed line) and non-obese rats (continuous line) for seven days prior to sacrifice.

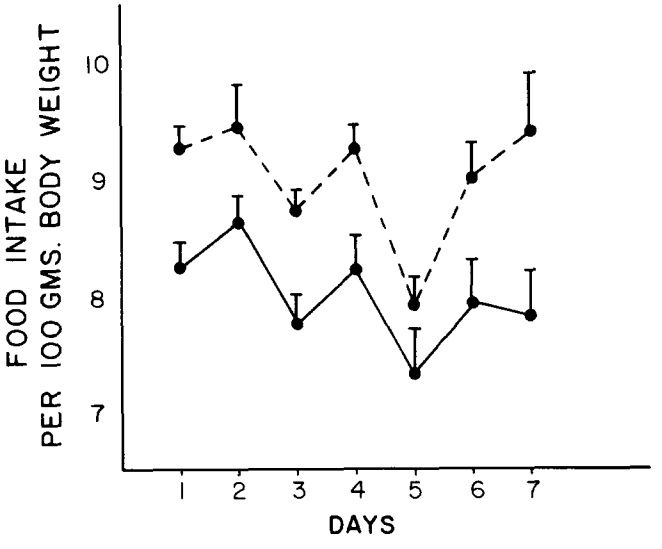

FIG. 3. Daily mean ratios of food intake per 100 grams body weight (±S.E.M.) for groups of obese (dashed line) and non-obese rats (continuous line) for seven days prior to sacrifice.

alanine, valine, ornithine and arginine. On the other hand, significant decreases were seen in the obese rats' brain for the following amino acids: glutamine, methionine, isoleucine, tyrosine and phenylalanine. The data are shown in Table 1.

Levels of Albumin, Amino Acids and Related Compounds in Plasma

As seen in Table 2, albumin levels were significantly ele-

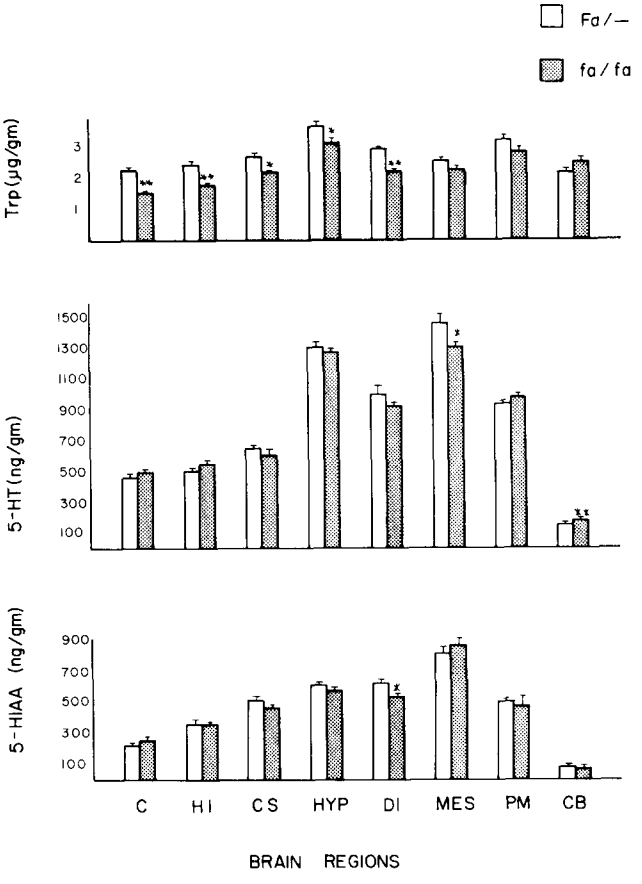

FIG. 4. Mean levels (±S.E.M.) of tryptophan (μg/g), serotonin (ng/g) and 5-hydroxyindoleacetic acid (ng/g) in eight brain regions for groups of obese (fa/fa) and non-obese (Fa—) male rats.

TABLE 1

LEVELS OF AMINO ACIDS AND RELATED COMPOUNDS (nmol/g) (MEAN±S.E.M.) IN CORTICAL SAMPLES FROM GROUPS OF LEAN (Fa/—) AND OBESE (fa/fa) RATS

	Fa/—	fa/fa
Asp	3,852.25 ± 50.31	4,112.38 ± 121.37
Thr	568.13 ± 10.49	619.25 ± 26.19
Ser	805.13 ± 8.21	985.88 ± 18.87†
Glu	11,651.63 ± 144.13	11,819.13 ± 163.37
Gln	4,999.88 ± 109.50	4,651.50 ± 53.09*
Pro	77.63 ± 2.72	81.14 ± 1.72
Gly	656.13 ± 16.00	755.5 ± 20.67†
Ala	618.13 ± 11.14	689.75 ± 6.47†
Cit	20.25 ± 1.53	18.00 ± 1.18
Val	72.75 ± 1.92	100.38 ± 1.67†
Meth	40.50 ± 1.31	35.75 ± 1.38*
Ileu	33.00 ± 0.50	25.63 ± 2.15†
Leu	74.88 ± 1.31	75.88 ± 2.03
Tyr	68.88 ± 3.72	42.75 ± 2.39†
Phen	44.63 ± 2.79	31.00 ± 1.96†
Orn	13.38 ± 0.83	16.13 ± 0.77*
Lys	163.50 ± 5.12	162.88 ± 5.28
His	54.50 ± 2.51	52.75 ± 1.60
Arg	89.75 ± 3.10	104.25 ± 3.39†

*Significantly different from Fa/—, $p<0.05$.
†Significantly different from Fa/—, $p<0.01$.

TABLE 2

LEVELS OF ALBUMIN (g/dl) AMINO ACIDS AND RELATED COMPOUNDS (nmol/ml) (MEAN±S.E.M.) IN PLASMA FROM GROUPS OF LEAN (Fa/—) AND OBESE RATS (fa/fa)

	Fa/—	fa/fa
Albumin	3.66 ± 0.1	4.15 ± 0.1†
Total Trp	129.86 ± 6.71	158.11 ± 13.51
Free Trp	26.83 ± 5.39	14.40 ± 2.25†
Asp	29.63 ± 2.59	20.75 ± 1.44†
Hpro	40.50 ± 2.49	33.13 ± 2.56
Thr	276.25 ± 11.93	356.63 ± 28.18*
Ser	225.13 ± 13.45	189.25 ± 11.23
Asn	85.25 ± 6.46	98.25 ± 9.80
Glu	114.88 ± 2.79	115.50 ± 4.99
Gln	578.38 ± 40.13	426.00 ± 7.33†
Pro	221.38 ± 7.00	327.38 ± 32.34†
Gly	329.50 ± 13.65	187.75 ± 13.41†
Ala	457.75 ± 24.69	586.75 ± 32.14†
Cit	62.88 ± 2.63	68.75 ± 2.35
Val	196.00 ± 7.51	331.00 ± 19.81†
Meth	57.13 ± 1.97	71.13 ± 5.43*
Ileu	93.75 ± 2.79	186.71 ± 2.47†
Leu	140.13 ± 10.88	278.88 ± 16.80†
Tyr	88.63 ± 4.29	83.13 ± 5.31
Phen	69.75 ± 3.10	91.00 ± 3.75†
Orn	84.25 ± 10.52	83.25 ± 7.54
Lys	491.63 ± 23.99	427.38 ± 16.31*
His	64.38 ± 3.88	60.75 ± 2.89
Arg	162.50 ± 17.43	158.50 ± 12.00

*Significantly different from Fa/—, $p<0.05$.
†Significantly different from Fa/—, $p<0.01$.

vated in obese rats. Free (unbound) Trp was significantly decreased in plasma of obese animals in comparison to lean littermates, although the level of total Trp was not significantly different. Free Trp represented 9% of total Trp in obese animals and 21% of total Trp in leans. Significantly elevated levels of threonine, proline, alanine, valine, methionine, isoleucine, leucine, and phenylalanine were present in obese animals. In contrast, obese rats had decreased levels of aspartic acid, glutamine, glycine and lysine.

DISCUSSION

The data on food intake and body weight confirm previous studies [10,44] in showing significant elevation of both measures in fa/fa rats. Relative to body weight, the genetically obese animals also ate significantly more, which would suggest they are in a stage of very rapid growth at this age of 13 weeks. Dilettuso and Wangsness [18] studied food intake relative to body weight in two separate experiments with groups of obese and non-obese rats. In one experiment they found no difference between the two groups at 10 weeks of age or later and in other experiments, obese animals did not show elevated food intake to body weight ratios after seven weeks of age; the authors related the differences to dietary composition and palatibility. In the present study we have demonstrated that even at 13 weeks of age, obese rats show elevated ratios of intake to body weight. Such differences may be related to the diet eaten. We used Purina lab chow whereas Dilettuso and Wangsness [18] used either ground lab chow supplemented with oil or a special semipurified diet. Caution should be exercised when comparing results obtained from animals being maintained on different diets.

Examination of the growth curves published by Zucker and Zucker [46], Powley and Morton [38], and Vasselli *et al.* [44] reveals that the fa/fa goes through an earlier "dynamic" growth stage followed by a more "static" growth phase much as do normal adult rats made obese by lesions in the ventromedial hypothalamic nuclei [25]. The data from the present experiment suggests that at 13 weeks of age obese animals are still in their "dynamic" stage of growth. Recent behavioral work [44] has shown that obese rats work harder for their food than lean rats at 16 weeks but not at 20 weeks of age; these authors feel that the rapid growth stage lasts up to 16 weeks of age. Thus, the data obtained in this study would apply to obese Zucker rats during their rapid growth phase.

This study was conducted to investigate whether regional changes in brain 5-HT and 5-HIAA may be associated with the hyperphagia of the genetically obese, Zucker rat. Since brain levels of the 5-HT precursor, Trp, are directly related to plasma Trp levels [21], peripheral biochemical pertubations affecting Trp transport into the brain may have an effect on 5-HT synthesis. The transport of Trp into the brain is complex, being affected by several variables. Tryptophan is unique among amino acids in that a large portion of plasma stores is bound to albumin [33]. The binding of Trp is furthermore influenced by plasma free fatty acids and insulin, with increased free fatty acids displacing Trp from albumin [15] and elevated insulin levels increasing Trp binding [17], by lowering plasma free fatty acids. The transport of Trp into

the brain is also affected by competition among the neutral amino acids (NAA: Tyr, Phen, Val, Leu, Ileu, Meth and His) which share a common carrier mechanism [35,37]. The situation is further complicated by the effect of increased levels of insulin to reduce plasma levels of these NAA [22].

Adding to these complexities is the controversy of whether free or total Trp best predicts transport into the brain [28, 32, 37]. In the present study, plasma levels of albumin were elevated in the obese rats, resulting in lower levels of free Trp. Although we did not measure plasma levels of free fatty acids or insulin, published reports indicate that both of these substances are increased in the genetically obese rat [27,47]. Since plasma concentrations of many of the NAA were increased rather than decreased, the importance of elevated levels of insulin in Trp transport in the genetically obese rat is questionable. These data suggest that the obese rats may be relatively insulin resistant. The calculated competitor ratios [20] of free Trp/Σ NAA are 0.038 and 0.013 for lean and obese animals, respectively; these data represent a 66% decrease for the obese group. On the other hand, the calculated competitor ratios [20] of total Trp/Σ NAA are 0.183 and 0.143 for lean and obese animals, respectively; these data represent a 22% decrease for the obese group. Thus, the 22% decrease in Trp transport calculated using total Trp corresponds to the actual levels of Trp measured in the various brain regions (mean of 22% decrease for the five regions showing significant differences). Therefore, an explanation of the lowered levels of brain Trp in the obese rats appears to be decreased transport of Trp due primarily to increased concentrations of competing NAA and secondarily to decreased levels of free Trp. These changes in plasma amino acid patterns in the genetically obese rat are particularly interesting considering the reported elevations in several of the NAA in obese humans [1]. Thus, the utility of the genetically obese rat as a model of human obesity is reinforced in respect to peripheral biochemical parameters.

These peripheral biochemical alterations in the obese rats appear to have resulted in lowered levels in brain (cortical) Meth, Ileu, Tyr, Phen and Trp. Thus, the increased competition among the NAA for transport into the brain apparently reduced brain levels of several of them. Lower levels of the 5-HT precursor, Trp, were particularly widespread. Five forebrain regions, representing virtually the entire forebrain (C, HI, CS, HYP and DI) exhibited decreased levels of Trp.

Since levels of brain 5-HT are determined largely by precursor availability [21], the observed changes in 5-HT in the mesencephalon and 5-HIAA in the diencephalon may be secondary to the previously-described aberrations in peripheral biochemistry. Conversely, the widespread presence of Trp-hydroxylase [7] suggests that synthesis of 5-HT can occur in each of the regions exhibiting decreased Trp. Therefore, depressed levels of Trp in several brain areas accompanied by a lack of change in 5-HT and 5-HIAA indicate that the synthesis of 5-HT is being maintained or turnover is reduced in the genetically obese rat. The availability of precursor in maintaining normal levels of 5-HT may not be as important if neural activity of these systems is decreased. Thus, if 5-HT neurons do mediate some aspect of satiety [4, 6, 30], one might expect neural activity of these systems to be decreased in an animal model of hyperphagia. The significant decrease of diencephalic 5-HIAA favors such an interpretation. Although hypothalamic levels of 5-HIAA were not significantly different in obese rats, previous reports [11, 12, 31] have demonstrated significantly decreased concentrations of NE in some hypothalamic regions. Since decrease in catecholamine activity is usually accompanied by increase in indole concentrations [41] NE-5-HT interaction may have a role in maintaining elevated indoleamine concentrations.

In addition, Trp and 5-hydroxytryptophan have been reported to reduce firing rates in mesencephalic raphe neurons [43]. The relative, though not statistically significant, reduction in mesencephalic Trp may have increased the firing rate of these cells, with the reduced precursor availability leading to lower levels of 5-HT in this brain area. An increased firing rate and release of 5-HT at the synapses in rostral brain areas [16,36] would also tend to maintain normal levels of 5-HIAA in these various regions. Alternatively, the reduction in brain Trp may not have been severe enough to significantly influence levels of 5-HT and 5-HIAA. In a previous report [13] larger reductions (30–40%) of brain Trp were observed following the injection of branched-chain amino acids (Val, Leu, Ileu), with minimal effects on regional 5-HT. Levels of 5-HIAA, however, were reduced to a greater extent (20–30%) than obeserved in the present paper. Therefore, the overall changes in brain 5-HT activity in genetically obese rats may result from complex regional interactions of modest decreases in precursor availability, neuronal activity of putative 5-HT satiety systems and a possible interaction with catecholamine neurons.

The present results can be compared with the results of Coscina *et al.* [9] concerning 5-HT depletion after ventromedial hypothalamic lesions, which also produced obesity. These authors observed reduced levels of 5-HT in hypothalamus, hippocampus, thalamus and midbrain, while in the present study 5-HT was lowered only in the mesencephalon (midbrain). Thus, the hyperphagic-ventromedial hypothalamic-lesioned rat exhibits more wide-spread changes in static 5-HT levels. In another study of the role of brain 5-HT systems in obesity, Garthwaite *et al.* [23] found that ob/ob mice have elevated levels of brain 5-HT, plasma total Trp and plasma free Trp. Although their results are the opposite to ours, the animals used in their study were 4–5 months of age and may have been in a more static phase of weight gain. Replication of our present experiment using older animals would be instructive in clarifying this discrepancy.

Therefore, the present experiment suggests that CNS 5-HT activity may be depressed in the mesencephalon and diencephalon of genetically obese rats. Furthermore, these changes in CNS 5-HT and 5-HIAA levels may result from peripheral biochemical changes affecting Trp transport into the brain. Whether these changes in CNS 5-HT activity are the cause or the result of the hyperphagia of the obese rats is a question that remains to be answered.

REFERENCES

1. Adibi, S. A. Influence of dietary deprivations and plasma concentration of free amino acids of man. *J. appl. Physiol.* **25:** 52–57, 1968.
2. Becker, E. E. and J. Grinker. Meal patterns in the genetically obese Zucker rat. *Physiol. Behav.* **18:** 685–692, 1977.
3. Bloxam, D. L. and W. H. Warren. Error in the determination of tryptophan by the method of Dencla and Dewey. A revised procedure. *Analyt. Biochem.* **60:** 621–625, 1974.

4. Blundell, J. E., E. Tombros, P. J. Robers and C. J. Latham. Behavioral analysis of feeding: Implications for the pharmacological manipulation of food intake in animals and man. *Prog. Neuropsychopharmac.* **4:** 319–326, 1980.
5. Boulangé, A., E. Planche and P. Degasquet. Onset of genetic obesity in the absence of hyperphagia during the first week of life in the Zucker rat (fa/fa). *J. Lipid Res.* **20:** 857–864, 1979.
6. Breisch, S. T., F. P. Zemlan and B. G. Hoebel. Hyperphagia and obesity following serotonin depletion by intraventricular p-chlorophenylalanine. *Science* **192:** 382–385, 1976.
7. Brownstein, M. J., M. Palkovits, J. M. Saavedra and J. S. Kizer. Tryptophan hydroxylase in the rat brain. *Brain Res.* **97:** 163–166, 1975.
8. Chance, W. T., M. VonMeyenfeldt and J. E. Fischer. Increased brain serotonergic activity associated with cancer anorexia. *Soc. Neurosci. Abstr.* **7:** 27, 1981.
9. Coscina, D. V., J. J. Warsh and G. H. Anderson. Brain regional serotonin depletion in hypothalamic obesity: Lesion vs. overeating as contributory factors. *Soc. Neurosci. Abstr.* **6:** 783, 1980.
10. Cruce, J. A. F. (Finkelstein), M. R. C. Greenwood, P. R. Johnson and D. Quartermain. Genetic versus hypothalamic obesity: Studies on intake and dietary manipulations in rats. *J. comp. physiol. Psychol.* **87:** 295–301, 1974.
11. Cruce, J. A. F. (Finkelstein), N. B. Thoa and D. M. Jacobowitz. Catecholamines in the brains of genetically obese rats. *Brain Res.* **101:** 165–170, 1976.
12. Cruce, J. A. F. (Finkelstein), 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.
13. Cummings, M. G., P. B. Soeters, J. H. James, J. M. Keane and J. E. Fischer. Regional brain indoleamine metabolism following chronic portacaval anastomosis in the rat. *J. Neurochem.* **27:** 501–509, 1976.
14. Curzon, G. and A. R. Green. Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. *Br. J. Pharmac. Chemother.* **37:** 689–697, 1970.
15. Curzon, G., J. Friedel and P. J. Knott. The effect of fatty acids on the binding of tryptophan to plasma protein. *Nature* **242:** 198–200, 1973.
16. Dählstrom, A. and K. Fuxe. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta physiol. scand.* **62:** Suppl. 232, 1–55, 1964.
17. Daniel, P. M., E. R. Love, S. R. Moorhouse and O. E. Pratt. The effect of insulin upon the influx of tryptophan into the brain of the rabbit. *J. Physiol.* **312:** 551–562, 1981.
18. Dilettuso, B. A. and P. J. Wangsness. Effect of age on hyperphagia in genetically obese Zucker rat. *Proc. Soc. exp. Biol. Med.* **154:** 1–5, 1977.
19. Doumas, B. T., W. A. Watson and H. J. Biggs. Albumin standards and the measurement of serum albumin with bromcresol green. *Clinica chim. Acta* **31:** 87–96, 1971.
20. Fernstrom, J. D. and D. V. Faller. Neutral amino acids in brain: Changes in response to food ingestion. *J. Neurochem.* **30:** 1531–1538, 1978.
21. Fernstrom, J. D. and R. J. Wurtman. Brain serotonin content: Physiological dependence on plasma tryptophan levels. *Science* **173:** 149–152, 1971.
22. Fernstrom, J. D. and R. J. Wurtman. Elevation of plasma tryptophan by insulin in rat. *Metabolism* **21:** 337–342, 1972.
23. Garthwaite, T. L., R. K. Kalkhoff, A. R. Guansing, T. C. Hagen and L. A. Menahan. Plasma free tryptophan, brain serotonin and an endocrine profile of the genetically obese hyperglycemic mouse of 4–5 months of age. *Endocrinology* **105:** 1178–1182, 1979.
24. Glowinski, J. and L. L. Iversen. Regional studies of catecholamines in the rat brain—I. The disposition of [^{3}H] norepinephrine, [^{3}H] dopamine and [^{3}H] dopa in various regions of the brain. *J. Neurochem.* **13:** 655–669, 1966.
25. Hetherington, A. W. and S. W. Ranson. Hypothalamic lesions and adiposity in the rat. *Anat. Rec.* **78:** 149–172, 1942.
26. Johnson, P. R., L. Zucker, J. A. F. (Finkelstein) Cruce and J. Hirsch. The cellularity of adipose depots in the genetically obese Zucker rat. *J. Lipid Res.* **12:** 607–614, 1971.
27. Johnson, P. R., J. S. Stern, M. R. C. Greenwood and J. Hirsch. Adipose tissue hyperplasia and hyperinsulinemia in Zucker obese female rats: A developmental study. *Metabolism* **27:** 1941–1954, 1978.
28. Knott, P. J. and G. Curzon. The relationship between plasma free tryptophan and brain tryptophan metabolism. *Nature* **239:** 452–453, 1972.
29. Krause, R., J. H. James, C. Humphrey and J. E. Fischer. Plasma and brain amino acids in Walker 256 carcinosarcoma-bearing rats. *Cancer Res.* **39:** 3065–3069, 1979.
30. Kurk, Z. L. Inhibition of feeding in rats by dopamine and 5-HT. *Nature* **246:** 52–53, 1973.
31. Levin, B. and A. C. Sullivan. Catecholamine levels in discrete brain nuclei of seven-month-old genetically obese rats. *Pharmac. Biochem. Behav.* **11:** 77–82, 1979.
32. Madras, B. K., E. L. Cohen, R. Messing, H. N. Munro and R. J. Wurtman. Relevance of free tryptophan in serum to tissue tryptophan concentrations. *Metabolism* **23:** 1107–1116, 1974.
33. McMenamy, R. H. and J. L. Onclay. Specific binding of tryptophan to serum albumin. *J. biol. Chem.* **233:** 1436–1447, 1958.
34. Palkovits, M., M. Brownstein and J. M. Saavedra. Serotonin content of the brainstem nuclei in the rat. *Brain Res.* **80:** 237–249, 1974.
35. Pardridge, W. H. Kinetics of competitive inhibition of neutral amino acid transport across the blood-brain barrier. *J. Neurochem.* **28:** 103–108, 1977.
36. Parent, A., L. Descarries and A. Beaudet. Organization of ascending serotonin systems in the adult rat brain. *Neuroscience* **6:** 115–138, 1981.
37. Pérez-Cruet, J., T. N. Chase and D. L. Murphy. Dietary regulation of brain tryptophan metabolism by plasma ratio of free tryptophan and neutral amino acids in humans. *Nature* **248:** 693–695, 1974.
38. Powley, T. L. and S. A. Morton. Hypophysectomy and regulation of body weight in the genetically obese Zucker rat. *Am. J. Physiol.* **230:** 982–987, 1976.
39. Saavedra, J. M., M. Palkovits, M. J. Brownstein and J. Axelrod. Serotonin distribution in the nuclei of the rat hypothalamus and preoptic region. *Brain Res.* **77:** 157–165, 1974.
40. Saavedra, J. M., M. Brownstein and M. Palkovits. Serotonin distribution in the limbic system of the rat. *Brain Res.* **79:** 437–441, 1974.
41. Samanin, R. and S. Garattini. The serotonergic system in the brain and its possible functional connections with other aminergic systems. *Life Sci.* **17:** 1201–1210, 1975.
42. Samanin, R., T. Mennini and S. Garattini. Evidence that it is possible to cause anorexia by increasing release and/or directly stimulating postsynaptic serotonin receptors in the brain. *Prog. Neuropsychopharmac.* **4:** 363–369, 1980.
43. Trulson, M. E. and B. L. Jacobs. Raphe neurons: Depression of activity by L-5-hydroxytryptophan. *Brain Res.* **97:** 350–355, 1975.
44. Vasselli, J. R., M. P. Cleary, K.-L. C. Jen and M. R. C. Greenwood. Development of food motivated behavior in free feeding and food restricted Zucker fatty (fa/fa) rats. *Physiol. Behav.* **25:** 565–573, 1980.
45. Von Meyenfeldt, M., W. T. Chance and J. E. Fischer. Changes in brain indoleamine metabolism correlate with onset of anorexia in rats. *Am. J. Surg.* **143:** 133–138, 1982.
46. Zucker, L. M. and T. F. Zucker. Fatty, a new mutation in the rat. *J. Hered.* **52:** 275–278, 1961.
47. Zucker, T. F. and L. M. Zucker. Hereditary obesity in the rat associated with high serum fat and cholesterol. *Proc. Soc. exp. Biol. Med.* **110:** 165–171, 1962.