Altered brain cholinergic enzymes activity in the genetically obese rat[1]

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Summary. Genetically obese male Zucker rats (*fa/fa*) and their lean littermates (*Fa/–*) were used in this experiment. Fourteen-week-old obese and lean littermates were sacrificed and choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) enzymes were assayed in specific brain regions. The assays of these enzymes indicate that obese animals had a significantly lower ChAT activity in the cerebellum, pons, and cerebral cortex and a significant increase in ChAT activity in the thalamus and hypothalamus. Meanwhile, the cerebral cortex, cerebellum, midbrain, thalamus and hypothalamus of the obese animals showed significantly higher AChE activity than their lean littermates. It was concluded from this study that obesity may be associated with changes in the enzymes of the brain cholinergic system.
Key words. Choline acetyltransferase; acetylcholinesterase; obesity.

Obesity is a complex disorder associated with numerous physiological abnormalities, like hormonal and biochemical dysfunctions[3,4]. These abnormalities contribute largely to the development and accumulation of excess adipose tissue. Moreover, inspite of the existence of several forms of obesity exhibiting different etiologies, it is apparent that most forms possess similar pathophysiological conditions[4].

It is known that the obese rats (*fa/fa*) present several dysfunctions of the CNS[5–7]. Several neurotransmitters such as norepinephrine (NE)[8], dopamine (DA)[9], serotonin (5-HT)[8,10] and endogenous opioids[11,12] have been implicated in obesity. However, the cholinergic involvement as a possible factor in the etiology of obesity has not been explicitly documented. Although hypothalamic acetylcholine (ACh) has been shown to inhibit food intake[8], others have provided evidence that ACh has an excitatory effect on food intake[13]. Moreover, serum cholinesterase (pseudocholinesterase, ChE) has been proposed to be associated with fatty acid and lipoprotein metabolism[14]. An increase in the ChE activity in the serum and low density lipoprotein fraction were observed in hyperlipidemic obese patients[15]. In view of these findings, investigators have attempted to correlate serum ChE level with the high and low density lipoprotein cholesterol as high risk factors for cardiovascular diseases.[16].

Hyperinsulinemia is one of the major hormonal abnormalities which contribute to obesity in the genetically obese Zucker rats[17]. The increased plasma insulin levels are likely to play an important role in the occurrence and persistance of abnormal fat deposition in these animals as well as their evolution toward insulin resistance[18]. In obesity induced by lesions of the ventromedial hypothalamus (VMH), it has been reported that hyperinsulinemia was detectable a few minutes after the lesions were made and this effect was mediated via the vagus nerve as it could be normalized by vagotomy or administration of atropine[19,20]. Moreover, it is well known that the electrical stimulation of the vagus nerve of normal rats induced insulin secretion which was inhibited by atropine[21,22]. It was proposed that VMH lesions brought about alterations in the CNS homeostasis which were responsible for the increase in the activity of the efferent vagus nerve that influences the endocrine pancreatic function[23,24] leading to insulin oversecretion.

Meager data are available that relate the cholinergic enzymes activity in the CNS with obesity. The availability of genetically obese rats with known changes in the brain neurochemistry provided an excellent model to study obesity and cholinergic function. Therefore, our aim in this investigation was to study the effect of obesity on choline acetyltransferase (ChAT, E.C. 2.3.1.6) and acetylcholinesterase (AChE, E.C. 3.1.1.7) activity in specific brain regions of the genetically obese Zucker rat.

Materials and methods

Twenty 14-week-old obese (*fa/fa*) and their lean (*Fa/–*) littermate Zucker rats weighing 590–710 and 350–430 g, respectively, were purchased from Harlan Sprague-Dawley (Blackthorn Bicester, Oxon, England) and used in this study. Animals were housed in a controlled environmental chamber at 21 ± 1 °C with a light period automatically timed to last from 06.00 to 18.00 h followed by a 12-h dark period. Food (Purina Lab Chow, Purina, St. Louis, MO) and water were provided ad libitum up until 5 h prior to sacrificing the animals.

In this experiment, both groups of animals were decapitated between 11.00 and 12.00 h, trunk blood was collected and plasma glucose level was measured with the glucose oxidase method using the Beckman Glucose Analyzer II (Beckman Instruments, Springfield, CA). Brain regions were dissected on ice into cerebral cortex, cerebellum, midbrain, medulla oblongata, hypothalamus, thalamus, and pons.

Acetylcholinesterase activity was measured using a spectrophotometric method[25]. In this procedure, each tissue was homogenized (1% w/v) in 0.1 M ice-cold phosphate buffer (pH 7.4) containing 0.1% Triton X-100 (Sigma). The AChE activity in each homogenate was immediately determined and expressed as nanomoles of substrate

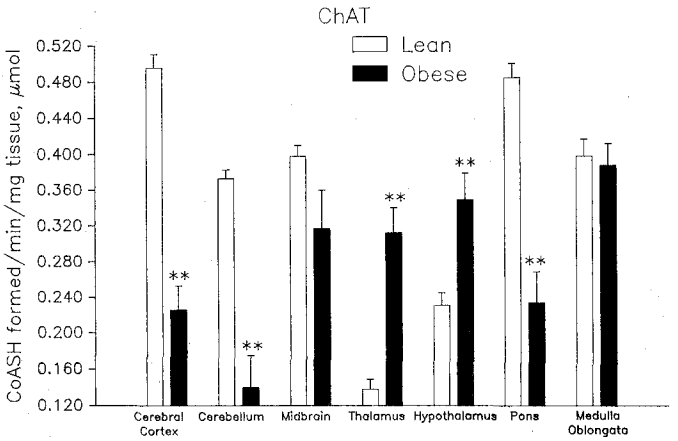

Figure 1. ChAT activity in lean and obese littermate Zucker rat brain regions. Each bar represents the mean ± SEM. **p ≤ 0.01 for 10 rats.

(acetylthiocholine iodide, Sigma Chemical Comp., St. Louis, MO) hydrolyzed per min per mg of tissue.

For the ChAT activity, each brain region was homogenized (1% w/v) in 0.5 M ice-cold phosphate buffer (pH 7.0). The ChAT activity was then determined using a spectrophotometric method[26] and was expressed as micromoles of acetyl CoA sulfhydril (CoASH) formed per minute per milligram of tissue.

Data were statistically analyzed using one-way analysis of variance with the significance level set at $p \leq 0.05$.

Results

The plasma glucose levels were 122.6 ± 1.3 mg/dl for the lean rat and 141.5 ± 3.3 mg/dl for the obese animals. These values represent the mean ± SEM of 10 animals per group. The obese animals exhibited a significantly higher ($p \leq 0.05$) plasma glucose level as compared to their lean littermates.

Figure 1 shows ChAT activity in various brain regions of the Zucker obese rats and their lean littermates. The obese animals show a significant increase ($p \leq 0.01$) in ChAT activity in the hypothalamus and thalamus while a significant decrease ($p \leq 0.01$) was observed in the cerebral cortex, cerebellum and pons.

In figure 2, the obese animals exhibited a significant increase ($p \leq 0.01$) in AChE activity in the hypothalamus, thalamus, cerebral cortex, cerebellum and in the midbrain ($p \leq 0.05$).

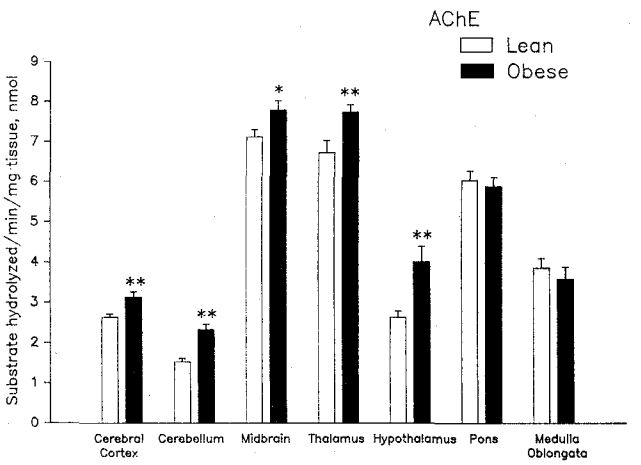

Figure 2. AChE activity in lean and obese littermate Zucker rat brain regions. Each bar represents the mean ± SEM. **p ≤ 0.01 for 10 rats.

Discussion

The results of this study indicate that obesity is associated with changes in the activity of the cholinergic enzymes in most of the brain regions studied. The diencephalon of the Zucker obese rat showed a significant increase ($p \leq 0.01$) in both ChAT and AChE activity which may reflect an increase in acetylcholine turnover rate. It is postulated that the increase in the turnover rate of ACh is probably a cause of obesity rather than a consequence of obesity. Neuroanatomical mapping studies have demonstrated neuronal interconnections between the ventromedial hypothalamus and the vagus nuclei[27] and fibers of the sympathetic nervous system[28]. These findings suggest that the close proximity of each system possess the capability to modulate the neuronal activity of the other.

The genetically obese Zucker rats have several alterations of neurotransmitter concentration and/or metabolism in the CNS[29] and they also exhibited hyperinsulinemia[17]. It is postulated that this type of obesity associated with changes in the cholinergic system of the brain would lead in part, to the increase in insulin secretion observed in this strain of rats. A defect in the nervous system, resulting in an increase in the parasympathetic activity has been proposed to explain the hyperinsulinemia of genetically obese rats[30] and in animals made obese by lesioning of ventromedial hypothalamus (VMH)[31].

Parasympathetic fibers innervating the pancreas have been suggested to play a regulatory role in the development of hyperinsulinemia[32], a common feature of obesity[18]. In obese animals, Berthoud and Jeanrenaud[20] have demonstrated that vagotomy caused a prompt cessation of hyperinsulinemia. Moreover, a complete subdiaphragmatic vagotomy was found to reverse the obesifying effects of preexisting ventromedial hypothalamus lesions[33]. Inspite of the controversy that exists over the extent to which vagotomy attenuate hypothalamic obesity, Sawchenko and Gold[34] demonstrated that severing the coeliac and/or hepatic vagal branches played a significant role in reversing this syndrome. Several investigators have proposed that obesity is manifested by a defect in the autonomic nervous system, resulting in increased parasympathetic and decreased sympathetic activity[8,18]. The present results show a marked increase in AChE activity in all of the brain regions studied in the obese Zucker rat except for the pons and the medulla, which indicate an increase in the neuronal activity. Furthermore, significant changes in ChAT activity, a specific marker for cholinergic neurons[35], were observed.

The data provided from our results indicate that differences in the central cholinergic system occur between the obese Zucker rat and its lean littermate. It may therefore be postulated that one possible etiology of obesity in the Zucker rats is related to the changes in the brain cholinergic function, which might lead to the increase in the vagus nerve activity, which in turn leads to both hyperinsulinemia and thus obesity.

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Administration of D-alanine did not cause increase of D-amino acid oxidase activity in mice

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Abstract. D-amino acid oxidase (DAAO) activity was not altered in the liver and kidney by oral administration of D-alanine to adult mice. The enzyme was apparently not induced by the enteric microflora either, since the enzyme activity in the liver and kidney of germ-free mice was not different from that of specific-pathogen-free mice. The times of appearance of DAAO activity and of free D-amino acids in the kidney were elucidated using suckling mice. DAAO activity started to increase 7 days after birth, and reached almost the adult level by 28 days. The content of free neutral D-amino acids also increased with age, in a similar fashion. A possible conclusion is that the enzyme activity normally increases during this period, to eliminate the free D-amino acids which have increased with age in the suckling mice. Consequently, the administration of D-alanine had no further effect in increasing enzyme activity.
Key words. D-amino acid oxidase; D-amino acids; D-alanine; microflora; induction; germ free.

D-amino acid oxidase (DAAO, EC 1.4.3.3) is a flavoprotein that catalyses the oxidative deamination of neutral free D-amino acids to the corresponding 2-oxo acids. The presence of DAAO has been reported in peroxisomes of many organs such as kidney, liver, brain, etc., but its physiological function is still unclear [1]. We have suggested that the physiological role of DAAO is, in part, to eliminate D-amino acids present in the body. This sug-