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Glucose and Lactate Utilization by the Amygdala of Male and Female Rats

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Gonadal hormones appear to modulate brain energy metabolism, and morphological and functional sexual differences are found in the amygdaloid complex (AC) of rats. Our aim was to study the CO_2 production and lipid synthesis, measured by the rate of L-[U-^{14}C]lactate or D-[U-^{14}C]glucose utilization (in pmol.hr^{-1}.mg^{-1}), by AC slices *in vitro* of male and female rats. Lactate was more used than glucose as energy substrate ($p < 0.01$) but no sex-related difference was observed in glucose or lactate oxidation to CO_2 ($p > 0.05$) or on lipid synthesis obtained from both substrates ($p > 0.05$). In addition, there was no effect of the estrous cycle on lactate oxidation to CO_2 by the AC of females ($p > 0.05$). Based on the present data, it appears that the endogenous normal levels of gonadal hormones are not able to promote sex-related differences in the *in vitro* glucose or lactate utilization by the AC of rats.

KEY WORDS: Amygdaloid complex; brain metabolism; oxygen consumption.

INTRODUCTION

Gonadal hormones modify neuronal morphology and neurochemistry, modulate neuroendocrine function, and affect the occurrence of reproductive and non-reproductive behaviors in rats (1–3). The morphology of astrocytes and the amount or the distribution of the astrocytic marker glial fibrillary acidic protein can also be altered by the level of the gonadal hormones (4). These sex steroids contribute to the development of sexual dimorphisms that became evident in the structure and function of specific central nervous system (CNS) areas in males and females (3,5,6). The rat amygdaloid complex (AC) is composed of several nuclei (7–10) and it is interesting to note that its medial nucleus (MeA) and posteromedial cortical nucleus are examples of sexually dimorphic areas in the rat brain (1,6,11).

Androgen- and estrogen-concentrating neurons are found in the AC of rats (12–13). These gonadal hormones appear to modulate the expression of some peptides, the concentration of monoamine neurotransmitters, and the biochemical activity within AC of rodents (14–16). Moreover, males appear to have a greater area of the MeA than females (6,17). It is likely that the MeA neuropil is one of the major targets for the effects of gonadal hormones, with sex steroids influencing its cellular functioning and synaptic circuitries (1).

The energy metabolism in the nervous system is very active and one of its main energy-demanding functions is the maintenance of the ionic concentrations inside the cells (18,19). Glucose is the obligatory energy substrate of the nervous system, although glycogen

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forms a limited energy store in the brain (18,19). Glucose is almost entirely oxidized to CO_2 and H_2O by glycolysis, the tricarboxylic acid cycle, and the associated oxidative phosphorylation (18). In addition to glucose entering the metabolic routes that form neurotransmitters, such as glutamate and γ-aminobutyric acid, it is also an essential constituent of glycolipids and glycoproteins that compose neural cells (18). Brain glucose can be converted to lactate (19–21) and it was suggested that lactate released by astrocytes may serve as a metabolic substrate for neurons (18). Sonnenwald *et al*. (22) and McKenna *et al.* (23) showed that the extracellular glutamate levels regulate its metabolism in astrocytes, mainly oxidized in the Krebs cycle and converted to lactate via malic enzyme. Both glutamate and glucose are nutrients to neurons that express LDH-1 isoform (24). Moreover, it is noteworthy that the basal rate of glucose utilization by astrocytes is higher than in neurons (18).

In the present study we determined the utilization of lactate and glucose as energy sources and as precursors of lipid synthesis by brain slices containing the AC obtained from adult male and female rats. Animals were normal adult male and regularly cycling female rats used to study the possible occurrence of sex steroid modulation of amygdaloid metabolism. Our first step was to evaluate the consumption of glucose and lactate and lipid synthesis by the whole AC and compare the data between males and females, irrespective of the phase of the estrous cycle. Next, the utilization of lactate by the AC of females studied in the different phases of the normal estrous cycle was determined. For this last purpose, females were studied when the endogenous levels of the ovarian hormones were at their cyclic lower and higher levels, that is, in diestrous, proestrous, and metaestrous.

EXPERIMENTAL PROCEDURE

Chloroform and methanol were obtained from Merck SA, Porto Alegre, RS, Brazil. Hyamine hydroxide was from J. T. Baker Chemical Company, Phillipsburg, NJ, USA, and D-[U-^{14}C] glucose and L-[U-^{14}C] lactate from the Sigma Chemical Company, St. Louis, MO, USA.

Adult male ($N = 6$) and female ($N = 17$) Wistar rats, 3–5 months old, were used. Animals were bred locally and housed in groups with free access to food and water, temperature around 22°C, and a 12-hr light-dark cycle. The estrous cycle phases were determined by vaginal smears inspection.

In the morning of the experiment the animals were killed by decapitation. Brains were quickly removed from the skull, the areas of interest were dissected, weighed, and sliced (0.3 mm) using a McIlwain tissue chopper. Initially, the whole AC was obtained from males ($N = 6$) and females ($N = 5$) irrespective of the phase of the estrous cycle. Next, the AC of females in the diestrous ($N = 3$), proestrous ($N = 4$), and metaestrous ($N = 5$) were dissected according to the coordinates provided by a rat brain atlas (25). As depicted in Fig. 1, in the present study the "whole AC" was basically defined as the tissue that was located from 1.80 to 3.30 mm posterior to bregma (which corresponds to plates 25 to 31, data according to 25), lateral to the optic tract but medial to the external capsule, and near the ventral surface of the brain. Both sides of the brain were used. Thus, for the purpose of this study, AC included the following amygdaloid nuclei: basolateral, basomedial, central, cortical, intercalated, lateral, and medial (Fig. 1). It is important to mention that part of the tissue in the vicinity of the AC was inevitably included in the preparation and this included the bed nucleus of the accessory olfactory tract and intraamygdaloid division of the bed nucleus of the stria terminalis. Therefore, the term "whole AC" does not preclude that the adjacent tissue was also included in tissue preparation, but it is highly likely that the amygdaloid nuclei were the most prominent component of the tissue sample (Fig. 1).

For the measurement of CO_2 production and lipid synthesis (26,27), 8–31 mg of the female AC and 24–32 mg of the male AC were incubated in 1 mL Krebs Ringer bicarbonate buffer, pH 7.4, containing 10 mM L-lactate and 0.2 μCi L-[U-^{14}C] lactate, and with 1 mL Krebs Ringer bicarbonate buffer, pH 7.4, containing 5 mM D-glucose and 0.2 μCi D-[U-^{14}C] glucose. The lactate and glucose concentrations used were saturating both for lipid synthesis and for CO_2 production. The contents of the flasks were gassed with 95% O_2/5% CO_2 for 1 min and then sealed with rubber caps.

The slices containing the AC were incubated at 35°C for 1 hr (28). Incubation was stopped by adding 0.2 mL 50% TCA through the rubber cap. Then 0.2 mL of 1 M hyamine hydroxide was injected into the center wells. The flasks were shaken for a further 30 min at 35°C to trap CO_2, after which the center well content was transferred to vials and assayed for CO_2 radioactivity in a liquid-scintillation counter. The flasks contents were homogenized and transferred to tubes, and after centrifugation the precipitate was washed three times with 10% TCA and the lipids extracted with chloroform:methanol (2:1). The chloroform:methanol phase was evaporated in vials, and radioactivity was measured.

Data were grouped for the statistical analysis, as follows: (1) CO_2 production from lactate and glucose, and lipid synthesis, obtained from the whole AC of males and females, irrespective of the estrous cycle phase; and (2) CO_2 production from lactate obtained from the whole AC of females studied in the diestrous, proestrous, and metaestrous phases of the estrous cycle. Results from 1 were analyzed statistically by the unpaired Student *t* test, whereas results from 2 were analyzed by an one-way ANOVA. In all cases, the level of significance was set at $p < 0.05$.

RESULTS

The consumption of radiactive glucose or lactate by the AC *in vitro* (mean ± SEM of pmoles of substrates oxidized to $CO_2.hr^{-1}.mg^{-1}$ tissue) was 712.1 ± 37.2 and 4717.1 ± 257.1 in males and was 764.4 ± 66.1 and 4453.2 ± 220.5 in females, respectively. Differences between male and female rats on glucose and lactate oxidation to CO_2 by AC slices were not significant

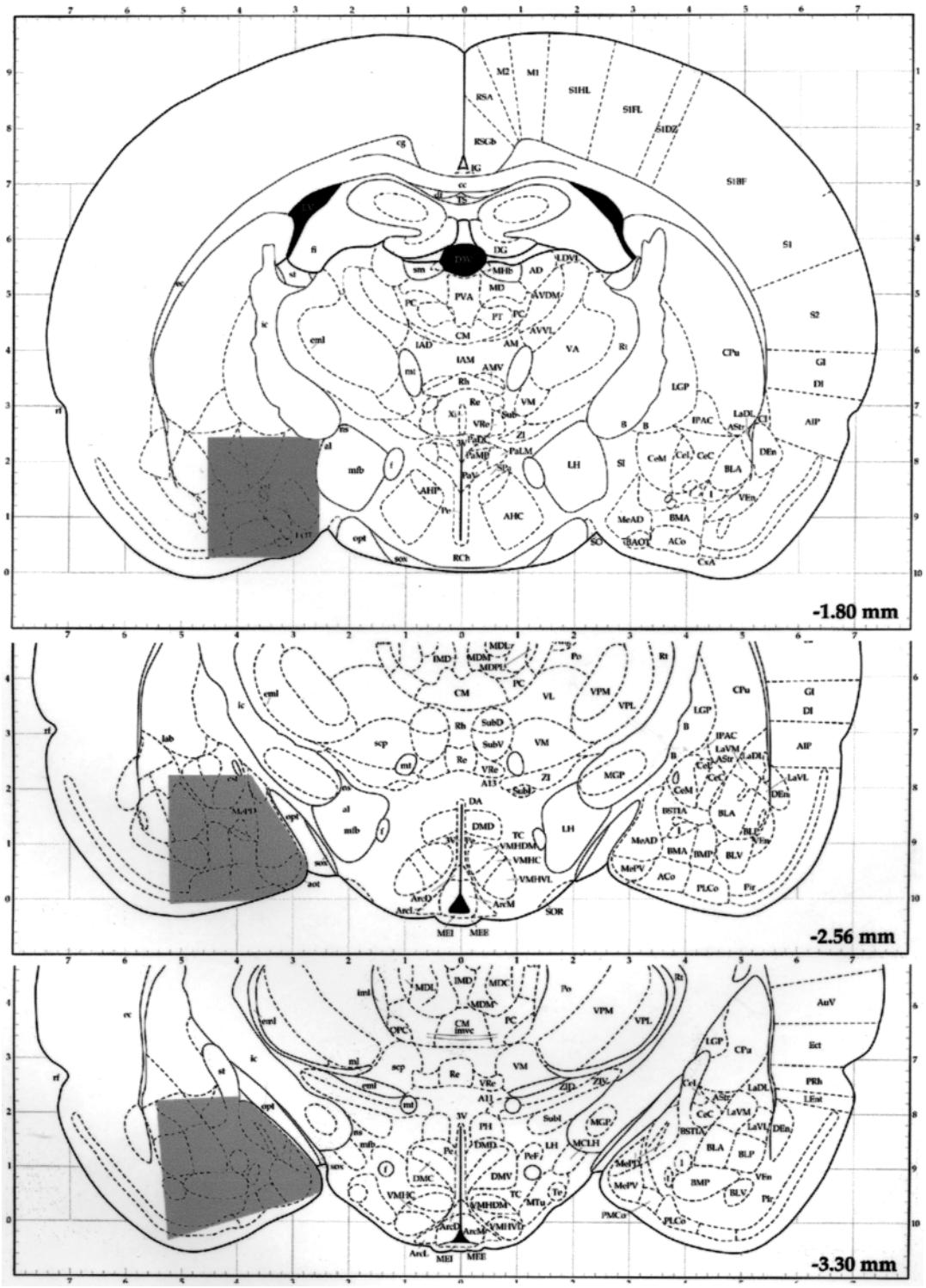

Fig. 1. Schematic drawing (adapted from the atlas of Paxinos and Watson, 25) showing the amygdaloid complex (AC) of rats from which sample tissue was obtained for metabolic study (black box). Sampling extended from the anterior part of the AC (1.80 mm posterior to the bregma, top), passed through its intermediate part (2.56 mm posterior to the bregma), and ended in the AC posterior part (3.30 mm posterior to the bregma, bottom). Nuclei that were included in the study are the following: ACo, anterior cortical amygdaloid nucleus; BAOT, bed nucleus of of the accessory olfactory tract; BLA, anterior part of the basolateral amygdaloid nucleus; BLP, posterior part of the basolateral amygdaloid nucleus; BLV, ventral part of the basolateral amygdaloid nucleus; BMA, anterior part of the basomedial amygdaloid nucleus; BMP, posterior part of the basomedial amygdaloid nucleus; BSTIA, intraamygdaloid division of the bed nucleus of the stria terminalis; CeC, capsular part of the central amygdaloid nucleus; CeL, lateral part of the central amygdaloid nucleus; CeM, medial part of the central amygdaloid nucleus; I, intercalated nuclei of the amygdala; LaVM, ventromedial part of the lateral amygdaloid nucleus; LaVL, ventrolateral part of the lateral amygdaloid nucleus; MeAD, anterodorsal part of the medial amygdaloid nucleus; MePD, posterodorsal part of the medial amygdaloid nucleus; MePV, posteroventral part of the medial amygdaloid nucleus; PLCo, posterolateral cortical amygdaloid nucleus; PMCo, posteromedial cortical amygdaloid nucleus. Coordinates are in mm.

($p = 0.490$ and $p = 0.491$ for glucose and lactate, respectively). Nevertheless, in both sexes, oxidation of lactate to CO_2 was higher than glucose oxidation to CO_2 ($p < 0.01$ for males and females).

Lipid synthesis derived from the utilization of radiactive glucose or lactate by the AC *in vitro* (mean ± SEM of pmoles of substrates converted to lipids. $hr^{-1} \cdot mg^{-1}$ tissue) was 89.2 ± 7.6 and 199.9 ± 27.2 in males and 125.2 ± 17.3 and 240.7 ± 15.5 in females, respectively. Differences between males and females were not significant ($p = 0.251$ and $p = 0.099$ for lactate and glucose, respectively). Again, lipid synthesis from lactate by the AC of both sexes was greater than lipid synthesis from glucose ($p < 0.02$).

Based on these results, lactate oxidation to CO_2 was selected for testing if part of the variability in the data obtained from the AC of females in the above-mentioned experiments could rely on the endogenous cyclic variations of gonadal hormones. The results (mean ± SEM of pmoles of substrates oxidized to $CO_2.hr^{-1}.mg^{-1}$ tissue) of lactate oxidation to CO_2 by AC *in vitro* from female rats in the diestrous, proestrous, and metaestrous phases of estrous cycle were 3073.0 ± 105.9, 2857.8 ± 568.0, and 3502.3 ± 255.3, respectively. No effect of the different phases of the estrous cycle on this parameter was found (F (2,9) = 0.877, $p = 0.449$).

DISCUSSION

The results suggest that sexual dimorphism or estrous cycle effect are not found in the glucose or lactate utilization *in vitro* by slices of the whole AC of the rat. On the other hand, it has been described that lactate may serve as a metabolic substrate for brain cells functioning besides glucose (18, but see 29). Based on the method employed here, lactate was more oxidized to CO_2 than glucose in the AC of rats.

The finding that no sex-related or estrous phase-related differences in glucose or lactate oxidation or lipid synthesis is somewhat surprising because some morphological and functional differences have been described for the amygdala of male and female rats (10, 14,30,31). Moreover, brain cerebral glucose utilization is changeable (34–36) and enzymes of the oxidative and energy metabolism may be affected by gonadal hormones (30–32,37). For example, the activities of hexokinase, lactic dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, isocitric dehydrogenase, and malic dehydrogenase were measured in the pituitary, the basomedial hypothalamus, and the amygdala of ovariectomized female rats (30). In brain samples that included the medial, cortical, central, and part of the basal amygdaloid nuclei, isocitric dehydrogenase and malic dehydrogenase were elevated by substitute estrogen treatment in castrated females (30). Moreover, the metabolic activity of the amygdala of females in proestrous and estrous appears to be higher than that of male rats (32), whereas lactate dehydrogenase activity decreases following proestrous in the amygdala of female rats (31).

The main differences among the above-mentioned experiments and the present one may well be related to the different experimental approaches used. It must be mentioned that neither did we castrate male or female rats nor inject sex hormones as hormonal substitutive therapy. Rather, we used intact animals and their natural hormonal milieu. And we did not look for the activity of specific enzymes that are part of a sequential route for metabolic utilization of a specific energy source. We decided to study the final product of glucose and lactate utilization by the AC of adult male and female rats. The model used here was the same already described for the measurement of CO_2 production and lipid synthesis by rat cerebellum and hippocampus slices (26,27). On the other hand, the data described here refer to an *in vitro* energy utilization, and caution should be taken to extrapolate to what occurs *in vivo*. In fact, *in vivo* studies using 2-deoxyglucose and an autoradiographic method would contribute to reinforcing the present findings and would provide another source of data to compare with the present ones.

It could also be argued that a sexual dimorphism can be found in more restricted areas of the AC, such as the MeA or the posteromedial cortical nucleus (1,6, 11,17). Moreover, not all cells in a specific brain region possess receptors for gonadal hormones (1,13) and it is clear that the AC is not a homogeneous structure (for a discussion, see 7–10,38,39). In fact, the MeA participate in a sex steroid-responsive and sexually dimorphic circuit that integrates chemosensory information and hormonal signals for the occurrence of distinct behaviors of males and females (5,39,40). And, in spite of the fact that male rats have more dendritic spines (1), females have a higher immunoreactivity for GFAP, a protein that constitutes intermediate filaments of mature astrocytes, in the MeA (41).

Although the metabolic activity of the various subnuclei of the AC is not highly heterogeneous, it was recently shown that high levels of phosphorylase α reactivity can be found in all parts of the MeA (33). This last finding would reflect astrocytic glycogenolysis (33) and it was already reported that glycogen stored

in astrocytes can be released as lactate for use in neighboring cells (42). These data would further suggest that the metabolic activity of the MeA neuropil would be different in males and females. However, in another set of experiments, that used the same methodological approach as reported here and in which the MeA was selectively dissected from the rest of the AC, no evidence for sex difference was found in the glucose or lactate utilization by the MeA of male and female rats (data not shown). Possible MeA sex-related metabolic differences *in vivo*, after neuronal and glial stimulation, still deserve future experiments.

In conclusion, based on the present data, a sexual dimorphism or an estrous cycle effect cannot be found in the glucose or lactate utilization *in vitro* by slices of the whole AC of the rat, as assessed by the *in vitro* production of CO_2.

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