Neuroscience Letters 507 (2012) 62–66

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Effects of dehydroepiandrosterone (DHEA) and lactate on glucose uptake in the central nervous system

Danielle Kaiser de Souza [a,*], Maria Flávia Marques Ribeiro [b], Luiz Carlos Rios Kucharski [b]

[a] Faculty of Ceilandia, University of Brasilia, Brasilia, Brazil
[b] Physiology Department, Basic Health Sciences Institute at Federal University of Rio Grande do Sul, Porto Alegre, Brazil

ARTICLE INFO

Article history:
Received 12 June 2011
Received in revised form 6 November 2011
Accepted 26 November 2011

Keywords:
Brain metabolism
Lactate
DHEA
Glucose uptake
Lactate oxidation

ABSTRACT

Dehydroepiandrosterone (DHEA) prevents brain aging, enhances the cerebral metabolism and interacts with energy substrates. The interaction between lactate and DHEA on glucose uptake and lactate oxidation by various nervous structures was investigated and results demonstrate that the 2-^{14}C-deoxiglucose (2-^{14}C-Dglucose) uptake was stimulated by 10 mM lactate in the hypothalamus and olfactory bulb, inhibited in the cerebral cortex and cerebellum, and unaffected in the hippocampus. We also show that, in both the cerebral cortex and hypothalamus, ^{14}C-lactate oxidation was higher than ^{14}C-glucose oxidation ($p \leq 0.001$), demonstrating a relevant role for lactate as energy substrate. The interaction of lactate and 10^{-8} M DHEA was tested and, although DHEA had no significant effect on uptake in the cerebellum, hippocampus, or hypothalamus, 10^{-8} M DHEA increased the 2-^{14}C-Dglucose uptake in the cerebral cortex in the presence of lactate ($p \leq 0.001$), and in the olfactory bulb in the absence of lactate ($p < 0.05$). However, DHEA had no significant effect on ^{14}C-lactate oxidation. We suggest that DHEA improves glucose uptake in specific conditions. Thus, DHEA may affect CNS metabolism and interact with lactate, which is the most important neuronal energy substrate, on glucose uptake.

1. Introduction

Dehydroepiandrosterone (DHEA) is a neurosteroid produced by neural tissue [3] that is able to modulate neuronal excitability, neurogenesis, cell survival, neurotransmitter receptors, and metabolism [8,17]. DHEA enhances mitochondrial oxidative capacity [27] and restored Na^+–K^+ ATPase activity of aging rat brains [32].

The brain is a highly oxidative organ that depends on a continuous glucose supply *in vivo* [12] although lactate is the most important ATP source for neurons during neuronal excitation [23,29]. It was described previously that astrocytes take up glucose, synthesize lactate, and transport the lactate to neurons, demonstrating the complex relations among cells and energy substrates in central nervous system (CNS) [22]. This anaerobical metabolism of CNS is the lactate shuttle hypothesis [22,23,28].

In vitro, lactate can decrease glucose uptake differently in specific regions of CNS [25]. So, as observed previously there are metabolic differences between structures of CNS [5,10,30]. Corroborating with these results, lactate is a preferential substrate for oxidation and it suppresses glucose oxidation by neurons in culture [14].

Age-related neurological disorders like Alzheimer's disease and endocrine diseases like Type 2 diabetes mellitus are conditions related to progressive accumulation of detrimental changes in the brain structure and function [2,11]. Memory disturbances in the elderly and in the initial stages of the disease of Alzheimer's disease patients are related to hypoxia, reduction in blood supply, and glucose hypometabolism in the cerebral cortex, hippocampus, and olfactory bulb [1,34,35,38]. It was postulated that the hypometabolism of neurodegenerative diseases could be reversed or minimized by DHEA, however this has not been clearly established in humans [2,13].

Therefore we tested the hypothesis that DHEA can alter the glucose metabolism of nerve tissues (cerebral cortex, hippocampus, cerebellum, hypothalamus, and olfactory bulb), and the possible interaction between DHEA and lactate, the most important metabolic substrate of neurons, on glucose uptake and lactate oxidation by various central nervous system structures.

2. Materials and methods

2.1. Materials

The reagents utilized in the experiments were analytical grade and were obtained from Merck SA, Porto Alegre, Brazil. Other

Abbreviations: CNS, central nervous system; DHEA, dehydroepiandrosterone; 2-^{14}C-Dglucose, 2-deoxi-1-^{14}C-glucose; KRb, Krebs Ringer bicarbonate; TCA, trichloroacetic acid; SNK, Student–Newman–Keuls.

* Corresponding author. Tel.: +55 61 3107 2926; fax: +55 61 3107 2926.
E-mail address: dany.kaiser@gmail.com (D.K. de Souza).

0304-3940/$ – see front matter.
doi:10.1016/j.neulet.2011.11.052

reagents were purchased as follows: hyamine hydroxide from Baker Chemical Company; L-[U-^{14}C] lactate (108.3 mCi/mmol), 2-deoxi-1-^{14}C-glucose (55 mCi/mmol), and D-[U-^{14}C] glucose (3.0 mCi/mmol), all from Amersham; DHEA from Calbiochе; and 2-hydroxypropyl-β-cyclodextrin (Fluka). DHEA was dissolved in 10% of β-cyclodextrin. All solutions were prepared on the same day they were used.

2.2. Animals

Experiments were performed with adult male Wistar rats, weighing 250–300 g. Animals were housed in groups with free access to food and water, room temperature of approximately 22 °C, and a 12:12 h light–dark cycle. Rats were killed by decapitation and their brain was quickly removed and placed on a petri plate containing a humid filter paper with buffer at 4 °C. Different structures of CNS (cerebral cortex, hippocampus, cerebellum, hypothalamus, and olfactory bulb) were dissected, weighed, and sliced within 2 min. External and visual neuroanatomical landmarks were utilized as reference for dissection and both sides of the brain were used. Dissected tissues were randomly distributed in experimental groups.

The animals were not anesthetized prior to sacrifice. During all the experimental procedures, the animals were treated according to the Guidelines for Care and Use of Animals in Research issued by the National Institutes of Health. All efforts were made to reduce both animal suffering and the number of animals used. Animal experimentation protocols were approved by the Ethics Committee of the University.

2.3. The 2-deoxi-1-^{14}C-glucose (2-^{14}C-Dglucose) uptake

To measure 2-deoxi-1-^{14}C-glucose (2-^{14}C-Dglucose) uptake, tissue slices obtained from the various CNS structures (200 mg/tube) were incubated in (1) 0.5 mL Krebs Ringer bicarbonate (KRb) buffer pH 7.4 containing 0.15 μCi 2-^{14}C-Dglucose; (2) 0.5 mL KRb containing 0.15 μCi 2-^{14}C-Dglucose + 10 mM lactate; (3) 0.5 mL KRb containing 0.15 μCi 2-^{14}C-Dglucose + 10^{-8} M DHEA or 10^{-12} M DHEA; or (4) 0.5 mL KRb containing 2-^{14}C-Dglucose + 10 mM lactate + 10^{-8} M DHEA or 10^{-1} M DHEA [described and modified from [18]]. The 2-^{14}C-Dglucose is a non-metabolized glucose analog. Control groups (without lactate) were incubated with β-cyclodextrin at 10^{-6} M. The contents of the tubes were gassed with 95% O_2/5% CO_2 for 1 min and then closed. Tissues were incubated in a Dubnoff incubator with constant shaking at 37 °C for 1.5 h. After incubation, tissues were withdrawn, rinsed in cold incubation buffer (three times) and blotted with filter paper. Glucose uptake was immediately measured [21] and the results were expressed as tissue/medium (T/M) ratio, i.e., dpm/mL tissue fluid per dpm/mL incubation medium. Analysis of data was done in duplicate.

Time-course curve was performed using 2-^{14}C-Dglucose in cerebellar slices at 30, 60, 90 (1.5 h) and 120 min (data not shown). The peak occurred at 1.5 h of incubation then this time was chosen for the others experiments.

2.4. The D-[U-^{14}C] glucose or L [U-^{14}C] lactate oxidation

To measure glucose oxidation (CO_2 production), slices of brain structures (200 mg/tube) were incubated in tubes containing 1.0 mL KRb pH 7.4, plus: (1) 0.15 μCi [U-^{14}C] glucose + 5 mM glucose + 10 mM lactate + 10^{-6} M β-cyclodextrin; (2) 0.15 μCi L [U-^{14}C] lactate + 10 mM lactate + 10^{-6} β-cyclodextrin; (3) 0.15 μCi L [U-^{14}C] lactate + 10 mM lactate + DHEA 10^{-8}; or (4) 0.15 μCi L [U-^{14}C] lactate + 10 mM lactate [described previously by 7; [18]]. Contents of the tubes were gassed with 95% O_2/5% CO_2 for 1 min and then sealed with rubber caps. Slices were incubated in a Dubnoff incubator with constant shaking at 37 °C for 1.5 h. Incubation was stopped by adding 0.2 mL 50% TCA (trichloroacetic acid) through the rubber cap. Then, 0.2 mL of 1 M hyamine hydroxide was injected into the center of the tube. The tubes were left overnight at 25 °C to trap CO_2, after which the content was transferred to vials and assayed for CO_2 radioactivity in a liquid-scintillation counter [7]. Analysis of data was done in one replicate.

Table 1
Lactate effect on [2-^{14}C] deoxi-glucose uptake of rat brain structures.

Structure	Control	Lactate	p (control versus lactate)
Cerebellum	2.10 ± 0.23 (5)	1.21 ± 0.24 (6)	≤0.001
Cerebral cortex	3.35 ± 0.77 (6)*	1.61 ± 0.52 (8)	0.002
Hippocampus	2.32 ± 1.27 (4)	3.04 ± 0.46 (7)#	0.199
Hypothalamus	2.94 ± 1.64 (4)	7.24 ± 0.81 (5)&	0.001
Olfactory bulb	1.69 ± 0.14 (5)	2.88 ± 0.38 (5)#	≤0.001

Values are expressed as mean ± standard deviation (SD). Value for each n is represented in parentheses. Results are expressed as tissue/medium (T/M) ratio (that is, dpm/mL tissue fluid per dpm/mL incubation medium). See Section 2 for details. For structures incubated with the same medium: control (without lactate) or lactate (10 mM lactate) groups. One-way ANOVA was used to compare among structures in control or lactate groups, and for control versus lactate Student's t test was used. Differences between each nervous structure and inside the same group (control or lactate).

& Differences among all structures ($p \leq 0.001$).

Differences between hippocampus and olfactory bulb versus cerebellum and cerebral cortex ($p \leq 0.001$).

* Difference between cerebral cortex and olfactory bulb ($p = 0.041$).

2.5. Statistical analysis

Student's t-test was utilized to analyze lactate influence on glucose uptake by each CNS structure and in oxidation experiments. Data were analyzed statistically by One Way ANOVA and by Student–Newman–Keuls (SNK) multiple-range test to verify the differences in glucose uptake among tissues. Two-way ANOVA and SNK multiple-range test were utilized to indentify interactions between DHEA doses, incubation time, and lactate influence. Level of significance was set at $p < 0.05$ and data are presented as mean ± standard deviation (SD). All tests were performed using Sigma Stat software.

3. Results

3.1. Lactate effect on 2-^{14}C-Dglucose uptake by CNS structures

First, glucose (2-^{14}C-Dglucose) uptake was tested and compared between all CNS structures in a medium with or without lactate (Table 1). Note that in all tissues except the hippocampus, 2-^{14}C-Dglucose uptake was affected by presence of lactate. Next, we compared 2-^{14}C-Dglucose uptake of all structures incubated with lactate and with their respective controls. In the absence of lactate, 2-^{14}C-Dglucose uptake was the same among almost all structures and the only significantly increase value found was between cerebral cortex when compared to olfactory bulb ($p = 0.041$). However, the hypothalamus showed a great increase in 2-^{14}C-Dglucose uptake ($p \leq 0.001$) in the presence of lactate compared to the other structures. Cerebellum and cerebral cortex showed the lowest uptake values.

3.2. Substrate oxidation by CNS structures

The ^{14}C-lactate oxidation by the CNS structures studied was compared to ^{14}C-glucose oxidation in the presence of 10 mM of lactate (Table 2). No difference in ^{14}C-glucose or ^{14}C-lactate