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Up-regulation of 2-oxoglutarate dehydrogenase as a stress response☆

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ABSTRACT

2-Oxoglutarate dehydrogenase multienzyme complex (OGDHC) operates at a metabolic cross-road, mediating Ca^{2+}- and ADP-dependent signals in mitochondria. Here, we test our hypothesis that OGDHC plays a major role in the neurotransmitter metabolism and associated stress response. This possibility was assessed using succinyl phosphonate (SP), a highly specific and efficient *in vivo* inhibitor of OGDHC. Animals exposed to toxicants (SP, ethanol or $MnCl_2$), trauma or acute hypoxia showed intrinsic up-regulation of OGDHC in brain and heart. The known mechanism of the SP action as OGDHC inhibitor pointed to the up-regulation triggered by the enzyme impairment. The animal behavior and skeletal muscle or heart performance were tested to correlate physiology with the OGDHC regulation and associated changes in the glutamate and cellular energy status. The SP-treated animals exhibited interdependent changes in the brain OGDHC activity, glutamate level and cardiac autonomic balance, suggesting the neurotransmitter role of glutamate to be involved in the changed heart performance. Energy insufficiency after OGDHC inhibition was detectable neither in animals up to 25 mg/kg SP, nor in cell culture during 24 h incubation with 0.1 mM SP. However, in animals subjected to acute ethanol intoxication SP did evoke energy deficit, decreasing muscular strength and locomotion and increasing the narcotic sleep duration. This correlated with the SP-induced decrease in NAD(P)H levels of the ethanol-exposed neurons. Thus, we show the existence of natural mechanisms to up-regulate mammalian OGDHC in response to stress, with both the glutamate neurotransmission and energy production potentially involved in the OGDHC impact on physiological performance.

This article is part of a Directed Issue entitled: Bioenergetic dysfunction, adaptation and therapy.

1. Introduction

Multienzyme 2-oxoglutarate dehydrogenase complex (OGDHC)[1] catalyzes a highly regulated step of the mitochondrial tricarboxylic acid cycle, being essential for the organism

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[1] AveRR, the average RR interval in the sample; CHAPS, 3-[(3-cholamidopropyl) dimethyl ammonio]propanesulfonic acid; DMEM, Dulbecco's Modified Eagle Medium; ECG, electrocardiography; FW, fresh weight; GFAP, glial fibrillary acidic protein; HBSS, Hank's buffered salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HRV, heart rate variability; K_d, dissociation constant; MOPS, 3-(N-morpholino)propanesulfonic acid; NBM, neurobasal medium; OGDHC, 2-oxoglutarate dehydrogenase complex; PBS, phosphate buffered saline; RMSSD, parasympathetic, or relaxation, index of the nervous system state; SI, sympathetic, or stress, index of the nervous system state; SP, succinyl phosphonate; ThDP, thiamine diphosphate; V_{max}, maximal catalytic rate.

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development and survival (Bunik and Strumilo, 2009; Bunik and Fernie, 2009). Both the well-known coupling between the carbohydrate and amino acid metabolism through the OGDHC oxidation of the glutamate precursor 2-oxoglutarate and the recent discovery of the OGDHC-mediated sensitivity of the tricarboxylic acid cycle to the fatty acid influx (Wiczer and Bernlohr, 2009) underline indispensable role of OGDHC for metabolic network. A basic physiological parameter, respiration, was shown to directly relate to the OGDHC activity level in the *in situ* systems under increased energy demand, such as perfused rat heart under maximal workload (Cooney et al., 1981) or the ADP-stimulated mitochondria (Araújo et al., 2008; Cheshchevik et al., 2010). We hypothesize that the OGDHC-mediated coupling of respiration to metabolic state is essential to fulfill the multitude of life functions which generally rely on supporting homeostasis under conditions of permanent metabolic perturbations. The OGDHC malfunction may therefore be unnoticeable in a resting or healthy state, but disturb the system ability to properly deal with perturbations in a stimulated or disease state. In particular, this feature may underlie the known association between the reduced activity of brain OGDHC and cognitive deficit in neurodegenerative diseases (Gibson et al., 2005). In the present work, we sought for experimental evidence to support this hypothesis and answer the following questions. Does the directed regulation of mitochondrial OGDHC have a local or global impact on physiology? What are the conditions maximizing the impact? What are molecular mechanisms mediating the OGDHC-dependent physiological responses? To answer these questions, we have chosen experimental systems *in vivo* and *in situ*, which enabled us to correlate certain molecular parameters, such as the OGDHC activity and metabolite levels, with physiological functions of intact organism. Animals were exposed to stresses of different etiology, with an efficient OGDHC inhibitor succinyl phosphonate (SP) applied as a stressor shown to specifically target OGDHC *in vivo* (Bunik and Fernie, 2009). The OGDHC activity in the tissues known to exhibit the highest sensitivity to stresses, *i.e.* brain and heart, was assayed to reveal if/when the OGDHC regulation is used by nature to support the animal reactivity and/or adapt an animal to stresses. In order to relate molecular events associated with the OGDHC function to overall physiology, physiological testing of animals subjected to the OGDHC inhibitor SP was performed along with assays of the brain OGDHC and excitatory neurotransmitter glutamate. Contribution of the OGDHC regulation to the energy production was assessed using a neuronal culture model. The results obtained point to an important role of OGDHC in physiological responses of animal to stress, mediated by the glutamate neurotransmission. Energy-related consequences of the OGDHC malfunction are more obvious when potentiated by metabolic stress.

2. Materials and methods

2.1. Animal treatments

All experiments were performed according to the guidelines of the Helsinki Declaration on the Guide for the Care and Use of Laboratory Animals, defining the conduct of ethical research on laboratory animals. Animals were kept at $21 \pm 2\,^{\circ}C$ on standard ration and 12/12 h light/dark cycle. Wistar rats of 300–330 g (males, the myocardial infarction experiments), 200–250 g (males, the alcohol, SP or thiamine treatment experiments) or 250–300 g (females) were used. Female rats were exposed to hypobaric hypoxia in a decompression (altitude) chamber by decreasing the atmospheric pressure in 1 min to 145 mm Hg, correspondent to 11500 m altitude, as described previously (Trofimova et al., 2010). Pregnant rats were exposed to the hypoxia at the 9–10 day of pregnancy. SP was introduced to animals at 5 and 25 mg/kg by intranasal application of the water solution of the trisodium salt, with 0.9% NaCl substituting for SP in all reference groups. Ethanol (25%, v/v in water) was injected i.p. at a single dose of 4.5 g/kg. In the study of the SP influence on hypoxic or ethanol effects SP was introduced 45–50 min before the treatment with other stressor. Thiamine was introduced as a single i.p. injection of 400 mg/kg. $MnCl_2$ was introduced to animals at 10 mg/kg by four i.p. injections every 12 h, with the four injections of 0.5 mL 0.9% NaCl performed in the reference group. Introduction of $MnCl_2$ to infarcted rats was done according to the same scheme, with the first injection right after the infarction injury. NaCl was not injected to the reference group for the sham-operated rats and those subjected to myocardial infarction only. No difference between the two reference groups, *i.e.* with and without NaCl injections, was detected. Myocardial infarction was modeled through the left thoracotomy (sham operation), followed by ligation of the anterior descending coronary artery (Fishbein et al., 1978; Johns and Olson, 1954) as described earlier (Tylicki et al., 2008).

2.2. Animal electrocardiography

ECG was registered using metal electrodes with the diameter of 9 mm and tip length of 7 mm. The electrodes were implanted subcutaneously on the back of the sacrum (one electrode) and shoulders (two electrodes). Sodium thiopental (60 mg/kg) was used as anesthetic. The signal from the electrodes was transduced to an analog-digital converter E14-440 ("L-Card", Moscow, Russia) connected to a computer. Registration of the analog signal digitized with frequency of 273 Hz was carried out on rats of free behavior, using the software "Iscope" ("Open Science", Moscow, Russia). Balance of the vegetative regulation, representing relative contributions of the sympathetic and parasympathetic components of the nervous system activity, was assessed according to Baevsky et al. (2001) by the following parameters of the heart rate variability (HRV): the average RR-interval in the sample (AveRR), ms; parasympathetic, or relaxation, index of the nervous system state (RMSSD), calculated as the square root of the sum of the differences of consecutive series of RR-intervals $RMSSD = \sqrt{(1/(n-1))\sum_{i=1}^{n-1}(RR_i - RR_{i+1})^2}$, where n is the number of RR-intervals in the sample; sympathetic, or stress, index of the nervous systems state (SI), calculated as $SI = AMo/(2 \times Mo \times \Delta X')$, where Mo is a mode of RR-intervals in the sample, ΔX – dispersion of the RR-interval in the sample, ms ($\Delta X = RR_{max} - RR_{min}$).

2.3. Behavioral parameters

The hole board test (Jackson and Broadhurst, 1982) was used to quantify behavior 4 h after the rats were awaken from narcotic sleep induced by the ethanol injection at 4.5 g/L.

2.4. Estimation of muscular strength

The parameter was measured using Grip Strength Meter (TSE Systems GmbH, Bad Homburg, Germany) with the sensor capacity up to 2000 g and sensitivity of 1 g. On the second day after the ethanol and/or SP treatment, three serial tests were performed for each animal with the three measurements separated by 2 min pause. Both mean and maximal values of the muscular strength were registered.

2.5. Cerebellar granule cell culture

Cerebellar granule cells were prepared as previously described (Bettendorff et al., 1991). Cerebella from 6 to 8 days old rat pups were excised from the freshly extracted brains, placed into

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