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Burst of succinate dehydrogenase and α-ketoglutarate dehydrogenase activity in concert with the expression of genes coding for respiratory chain proteins underlies short-term beneficial physiological stress in mitochondria☆

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ABSTRACT

Conditions for the realization in rats of moderate physiological stress (PHS) (30–120 min) were selected, which preferentially increase adaptive restorative processes without adverse responses typical of harmful stress (HST). The succinate dehydrogenase (SDH) and α-ketoglutarate dehydrogenase (KDH) activity and the formation of reactive oxygen species (ROS) in mitochondria were measured in lymphocytes by the cytobiochemical method, which detects the regulation of mitochondria in the organism with high sensitivity.

These mitochondrial markers undergo an initial 10–20-fold burst of activity followed by a decrease to a level exceeding the quiescent state 2–3-fold by 120 min of PHS. By 30–60 min, the rise in SDH activity was greater than in KDH activity, while the activity of KDH prevailed over that of SDH by 120 min. The attenuation of SDH hyperactivity during PHS occurs by a mechanism other than oxaloacetate inhibition developed under HST.

The dynamics of SDH and KDH activity corresponds to the known physiological replacement of adrenergic regulation by cholinergic during PHS, which is confirmed here by mitochondrial markers because their activity reflects these two types of nerve regulation, respectively. The domination of cholinergic regulation provides the overrestoration of expenditures for activity. In essence, this phenomenon corresponds to the training of the organism. It was first revealed in mitochondria after a single short-time stress episode.

The burst of ROS formation was congruous with changes in SDH and KDH activity, as well as in *ucp2* and *cox3* expression, while the activity of SDH was inversely dependent on the expression of the gene of its catalytic subunit in the spleen. As the SDH activity enhanced, the expression of the succinate receptor decreased with subsequent dramatic rise when the activity was becoming lower.

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

Abbreviations: ACH, acetylcholine; ADR, adrenaline; CBCh, cytobiochemical; CS, citrate synthase; HST, harmful stress; ISC, isocitric acid; MAL, malonate; NBT, nitroblue tetrazolium; OAA, oxaloacetate; PNMT, phenylethanolamine *N*-methyltransferase; PES, psychoemotional stress; PHS, physiological stress; qRT-PCR, quantitative real time PCR; ROS, reactive oxygen species; *sdha*, SDH catalytic subunit; SUC, succinate; SDH, succinate dehydrogenase; *gpr91*, succinate receptor; *cox3*, third subunit of cytochrome oxidase; *ucp2*, uncoupling protein 2; KGL, α-ketoglutarate; KDH, α-ketoglutarate dehydrogenase.

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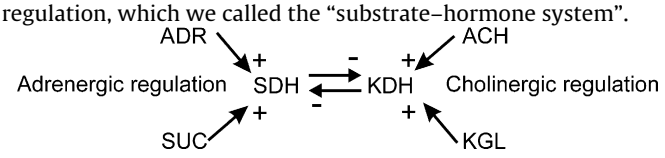
1. Introduction

In experimental and clinical investigations the name stress is used to label different functional states of the organism, leading to uncertainty concerning the state being examined (Sabban et al., 2006; Kvetansky and Mikulay, 1970). Since the pioneering studies of Selye, severe experimental models of stress have approached the states close to physiological conditions and a new term was proposed: "physiological stress" (PHS) (Arshavsky, 1979, 1982). In contrast to damaging pathogenic stress, PHS stimulates adaptation and development of the organism. Currently, the most widely used stressor is mild, a painless physical restraint of rats or mice in a narrow perforated plastic box (Wang et al., 2002; Kapitonova et al., 2010; Yin et al., 2000; Sheridan et al., 1998). Such restraint stress is thought to be largely psychological in nature. It was shown that it induces the production of various immunosuppressive mediators.

Adrenaline (ADR) is known as a starting hormone of stress response. In the course of response ADR control is followed by acetylcholine (ACH) regulation and only later by corticosteroid influence. ADR provides the acceleration of energy supply, while ACH stimulates restorative processes. It was shown in previous studies by our group that ADR regulation involves the activation of the most powerful enzyme succinate dehydrogenase (SDH), while ACH regulation activates α-ketoglutarate dehydrogenase (KDH). The activity of these enzymes and their substrates serve as a link between vegetative regulation and mitochondria as shown in the scheme and considered elsewhere (Kondrashova et al., 2009; Section 3.2).

We consider these links as a particular physiological system of regulation, which we called the "substrate–hormone system".



Besides the well-known parasympathetic system, intracellular regulation by non-neuronal ACH is found in lymphocytes (Kawashima and Fujii, 2000). The presence of ACH in lymphocytes favors our investigations of its effect on the KDH activity. The existence of this system was supported by finding receptors for only SUC and KGL, substrates namely for SDH and KDH (He et al., 2004). The dominance of SDH in energy supply is known for more than a century and was most convincingly demonstrated by the discovery of SUC-dependent reversed electron transfer (Chance and Hollunger, 1961). More recently physiological functions of SDH were demonstrated by the key role of its stability in prevention of diseases, including tumors (Kondrashova, 1976; Selak et al., 2005; Pollard et al., 2003; Rustin et al., 2002).

The goal of the present study was to determine whether mitochondria are involved in the development of PHS from the activity of the two dehydrogenases: SDH, which is responsible for the energy supply in the active state, and KDH, which is supposed to be crucial for restoration. The activity of these enzymes also provides information on adrenergic and cholinergic regulation in the organism.

To gain insight into the mechanisms of SDH activity changes, we investigated the nature of SDH inhibition and expression of SDH catalytic subunit and SUC receptor genes.

The formation of reactive oxygen species (ROS) inherent in any stress (Lee et al., 2006; Oishi and Machida, 2002), including the regulation of their level by expression of *ucp2* and *cox3* genes, was also studied.

2. Materials and methods

2.1. Animal treatment

As a model of PHS, painless restraint of movement of a rat in a narrow perforated plastic box for the short periods of time (30, 60, 120, 180 min) was used.

Juvenile rats before maturation (six-week old) were investigated as they are more sensitive than adults. Wistar males bred in the vivarium of our institute were used. A single experiment included a simultaneous investigation of a group of animals ($n = 6–8$), which were previously selected so that they were as identical as possible. The animals were habituated to each other and to investigator. Any excitation of animals was avoided. Blood was taken after euthanasia in CO_2 from the total blood obtained by decapitation. Routine investigations of a group of individuals are not really simultaneous, because samples of blood or mitochondrial suspensions are taken sequentially and change during several hour storage. Preparation of mitochondria in a blood smear, which in this case are stable through this time permits one to carry out really simultaneous measurements, starting the reaction at the same moment for all samples by immersion into solution. These experimental details considerably diminish the data dispersion.

According to this protocol we obtain results identical for different animals in the same state. Therefore, high statistical validity of the results is provided by computation of a multitude of objects as well as by a careful design of experiment, which prevents most of the so-called "uncontrolled" dispersion of data. Examples of identical results are presented below.

2.2. Immune activity and mitochondrial energetics. Systemic test

Very sensitive test for evaluation the state of immune system within conditions compatible with life was used; lymphocyte to neutrophil ratio (L/N), lymphocyte index. Two or three thin blood smears were made from whole blood. The smears were air-dried and stained with May–Grunwalds–Giemsa solutions. Slides were viewed under a light microscope for differential white cell determination. Two or three hundreds cells per each slide including neutrophils, basophils, eosinophils, monocytes and lymphocytes, were counted manually under a microscope at 1000 magnification. Total white blood cells were counted after erythrocyte lysis in 3% acetic acid. White blood cells were counted with Gentian violet under microscope using a glass count chamber.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.biocel.2012.07.003.

Energy functions of mitochondria were measured by Ca^{2+} accumulation in concentrated rat liver homogenate, which better retains physiological responses than isolated mitochondria (Kondrashova et al., 2001b). The calcium retention capacity of mitochondria was estimated from the Ca^{2+} concentration necessary for the irreversible decrease in the membrane potential in the course of successive additions of $CaCl_2$, each increasing its final concentration per 50 μM. Rat liver homogenate was incubated in standard KCl-based medium containing 120 mM KCl, 1.5 mM KH_2PO_4, 15 mM HEPES (pH 7.25), and 1 μM TPP^+ supplemented with 4 mM succinate.

Simultaneous measurements of the mitochondrial functions and the activity of immune blood cells allow one to evaluate the state of two key systems that underlie biological resistance: the intracellular energetics and the immune system. We call the above-described protocol the "systemic test" of the organism's state.