Mild Uncoupling of Respiration and Phosphorylation as a Mechanism Providing Nephro- and Neuroprotective Effects of Penetrating Cations of the SkQ Family

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Abstract—It is generally accepted that mitochondrial production of reactive oxygen species is nonlinearly related to the value of the mitochondrial membrane potential with significant increment at values exceeding 150 mV. Due to this, high values of the membrane potential are highly dangerous, specifically under pathological conditions associated with oxidative stress. Mild uncoupling of oxidative phosphorylation is an approach to preventing hyperpolarization of the mitochondrial membrane. We confirmed data obtained earlier in our group that dodecylrhodamine 19 (C12R1) (a penetrating cation from SkQ family not possessing a plastoquinone group) has uncoupling properties, this fact making it highly potent for use in prevention of pathologies associated with oxidative stress induced by mitochondrial hyperpolarization. Further experiments showed that C12R1 provided nephroprotection under ischemia/reperfusion of the kidney as well as under rhabdomyolysis through diminishing of renal dysfunction manifested by elevated level of blood creatinine and urea. Similar nephroprotective properties were observed for low doses (275 nmol/kg) of the conventional uncoupler 2,4-dinitrophenol. Another penetrating cation that did not demonstrate protonophorous activity (SkQR4) had no effect on renal dysfunction. In experiments with induced ischemic stroke, C12R1 did not have any effect on the area of ischemic damage, but it significantly lowered neurological deficit. We conclude that beneficial effects of penetrating cation derivatives of rhodamine 19 in renal pathologies and brain ischemia may be at least partially explained by uncoupling of oxidation and phosphorylation.

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Abbreviations: AKI, acute kidney injury; C12R1, dodecylrhodamine 19; DNP, 2,4-dinitrophenol; I/R, ischemia/reperfusion; ROS, reactive oxygen species; SkQ, cationic derivatives of plastoquinone; SkQR1, 10-(6′-plastoquinonyl)decylrhodamine 19; SkQR4, 10-(6′-plastoquinonyl)decylrhodamine B.

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pharmacological treatment. In addition to the strategy of targeted delivery of antioxidants to mitochondria [7, 8], the reduction of the rate of ROS generation in mitochondria due to mild uncoupling [9, 10] seems to be another promising avenue for prevention of oxidative damage to components of cells. It is the positive correlation between mitochondrial membrane potential and ROS production that it the basis for this phenomenon. Even a small increment in membrane potential exceeding the level of approximately 150 mV was shown to increase \( \text{H}_2\text{O}_2 \) generation by mitochondria, this effect being disproportionately high [9, 11, 12]. Accordingly, mild uncoupling, i.e. a slight decrease in membrane potential, which does not lead to a decrease in ATP synthesis in mitochondria, may have a useful antioxidant effect [10]. Since a number of clinically significant diseases of kidney [1, 3, 4] and brain [5, 6] are associated with oxidative stress, it seems reasonable to try to apply the strategy of partial uncoupling in these cases, so as to protect these organs from damage.

Hydrophobic derivatives of rhodamine 19, a representative of a new class of cationic uncouplers, seem to be particularly promising in this respect. As shown in our laboratory [13], such compounds, namely SkQR1 and \( \text{C}_{12}\text{R}1 \) (see Fig. 1), are accumulated in mitochondria due to the membrane potential in these organelles. Their accumulation reduces this potential, a fact preventing further excessive accumulation (and thus uncoupling). In other words, SkQR1 and \( \text{C}_{12}\text{R}1 \) act as “mild” uncouplers — their moderate concentrations can prevent mitochondrial hyperpolarization and the associated ROS generation without the risk of disabling the main mitochondrial function, ATP synthesis, which depends on membrane potential, but does not require very high values of it. It seems quite significant that SkQR4 molecule, where rhodamine 19 is substituted for rhodamine B, does not carry groups with dissociating \( \text{H}^+ \) ion (in contrast to rhodamine 19 and its derivatives), and of itself it has no protonophorous properties and requires free fatty acids for the manifestation of uncoupling activity [14].

In this study we compared the properties of the derivatives of rhodamine 19 (SkQR1 and \( \text{C}_{12}\text{R}1 \)) and rhodamine B (SkQR4), as well as the conventional uncoupler, the protonophore 2,4-dinitrophenol [15] as protective agents in ischemia and reperfusion (I/R) of kidneys, rhabdomyolysis, and experimental stroke.

**MATERIALS AND METHODS**

**Modeling of renal ischemia.** Experiments were performed on male outbred white rats (200-250 g) on an *ad libitum* diet. A 40-min ischemia of the left kidney was conducted as previously described [3]. Right-sided nephrectomy was performed along with the ischemia. Blood samples were taken from the animals on the second day after ischemia. The concentration of urea and creatinine in blood was determined using a CellTac blood analyzer (Nihon Kohden, Italy).

**Modeling of rhabdomyolysis.** Rhabdomyolysis was induced according to a conventional procedure by injecting 50% aqueous solution of glycerol (ICN, USA) into the paw muscles of rats as previously described [4]. Blood samples were taken from the animals on the second day after rhabdomyolysis. The concentration of urea and creatinine in blood was determined.

All the experiments on the ischemia and rhabdomyolysis models were performed on at least eight animals in each group. Data are presented as average ± SEM.

**Modeling of brain ischemia.** Ischemia of rat brain was induced by the introduction of a silicone-coated nylon thread into the middle cerebral artery [16, 17]. The blood flow was occluded for 60 min; then the thread was removed from the vessel, restoring the blood flow in the basin of the middle cerebral artery. The animal’s body temperature was maintained at 37 ± 0.5°C during and after the operation. The sham-operated animals were subjected to the same procedures except for the cutting of blood vessels and introduction of the thread. The area of cerebral infarction was determined on the first day for the studies of neuroprotective effect of the compounds or within 7 days for studies of the dynamics of development of ischemic damage. This was evaluated by morphometric analysis of digital images obtained by magnetic resonance imaging (MRI). All the MRI experiments were performed as previously described [18] on a BioSpec 70/30 instrument (Bruker, Germany) with the magnetic field induction of 7 T and the gradient system 105 mT/m.

Behavioral tests were carried out 1 day before surgery and on the first day after ischemia. A 14-point scale [19] modified as in [20] was used to estimate neurological disorders caused by the occlusion of the middle cerebral artery. The final score is formed in this scale as the sum of the scores in seven tests evaluating the response of the hindlimbs and forelimbs to tactile and proprioceptive stimulation. The following counting system was used to estimate the disturbances in the function of the limbs: 2 points, the rat fully performed the test; 1 point, the rat performed the test with a delay of more than 2 sec or incompletely; 0 points, the rat did not respond to the stimulation of the limb. The animals were treated and all the experiments in accordance with generally accepted international guidelines for experimentation on animals.

The results are expressed as the mean value ± standard error of mean. Normality of the characteristic distribution in the sample was evaluated using the Shapiro–Wilk W-test. The Mann–Whitney U-test was used to compare the data in the behavioral tests (for independent samples). The \( t \)-test with a significance level \( p < 0.05 \) was used to estimate the statistical significance of differences in infarct size.

**Measurement of mitochondrial membrane potential.** Mitochondria were isolated from rat liver by differential...