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Succinate Confers Stronger Cytoprotection in Kidney Cells than in Astrocytes Due to Its More Efficient Involvement in Energy Metabolism

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Abstract—Being among the most metabolically active organs, brain and kidneys critically depend on efficient energy metabolism, which primarily relies on oxidative phosphorylation. Acute pathological conditions associated with a lack of metabolic substrates or their impaired utilization trigger signaling cascades that initiate cell death and lead to poorly reversible organ dysfunction. One of the therapeutic approaches to correct the energy deficit is administration of exogenous metabolites of the tricarboxylic acid cycle, such as succinate. In this study, we investigated the effects of exogenous succinate on astrocytes and renal epithelial cells under normal conditions and in serum deprivation-induced injury. Incubation with succinate increased the viability of both cell types under normal and pathological conditions, but a more pronounced cytoprotective effect was observed in renal cells. In injured renal epithelial cells, succinate increased mitochondrial membrane potential, a critical parameter for the maintenance of mitochondrial function and ATP generation. Comparison of respiration and oxidative phosphorylation parameters in astrocytes and renal epithelial cells in the presence of exogenous succinate revealed that epithelial cells exhibited a significantly higher respiratory control and lower proton leak compared to astrocytes, which correlated with the higher cytoprotective activity of succinate for kidney cells. Therefore, succinate showed a noticeable positive effect in the renal epithelium both under normal conditions and after serum deprivation; however, in astrocytes, its effect was less pronounced. This discrepancy might be related to a more efficient succinate utilization by the mitochondria in renal cells and intrinsic bioenergetic differences between astrocytes and epithelial cells. Despite the clinical use of succinate-containing drugs, the determination of optimal dosages and development of effective therapeutic regimens require further investigation. Our results demonstrate cell type-dependent differences in the efficacy of succinate, suggesting that its therapeutic potential may differ significantly depending on the organ-specific bioenergetic and metabolic properties.

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Keywords: brain, astrocytes, kidneys, apoptosis, energy substrates, succinate, mitochondria, oxidative phosphorylation

INTRODUCTION

Severe pathological conditions, such as ischemic disease (including myocardial infarction and ischemic stroke), various forms of shock (septic, hypovolemic, and others), and acute and chronic heart failure in the decompensation stage provoke systemic and local tissue hypoperfusion, resulting in strong deprivation of key energy substrates in organs and tissues. The ensuing energy crisis triggers pathophysiological

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cascades, the central event being dysregulation of cellular energy metabolism. This disrupts the balance between anabolic and catabolic processes and suppresses key metabolic and signaling pathways [1, 2], leading to cell death. The functioning of organs with a high basal energy demand, such as the brain, heart, and kidneys, critically depends on a high rate of adenosine triphosphate (ATP) synthesis, which makes them particularly vulnerable to such disruptions in metabolism. The kidneys are among the most metabolically active human organs [3, 4] due to a high density of mitochondria in the tubular epithelial cells [5, 6]. This ultrastructural feature is directly related to the significant energy expenditure required for ATP-dependent processes, primarily, active tubular reabsorption of electrolytes, glucose, amino acids, and water. The energy expenditure of the brain is also very high. Despite its relatively small mass, the brain consumes approximately 20% of all oxygen and 25% of glucose utilized by the human body at rest [7, 8]. High metabolic demands are characteristic not only of neurons, but astrocytes as well. Astrocytes play a key role in the neurometabolic interactions; they actively capture glucose from the bloodstream, metabolize it glycolytically to lactate, and supply this lactate to neurons via the astrocyte-neuron lactate shuttle as an energy substrate for oxidative phosphorylation in the mitochondria [8-10]. Hence, astrocytes require metabolic substrates both for their own needs and to maintain the energy level in neurons.

A decreased supply of nutrients to tissues leads to impaired mitochondrial function, in particular, inefficient oxidative phosphorylation. The damage to the mitochondria not only reduces ATP production but also induces oxidative stress, ultimately resulting in cell death [2, 11]. It is commonly accepted that the use of additional energy substrates can promote metabolic adaptation and prevent energy collapse by enabling rapid metabolism of tricarboxylic acid (TCA) cycle intermediates and replenishment of reducing equivalents utilized in the electron transport chain [12]. Since mitochondrial dysfunction caused by cell damage leads to extensive cell death, restoration of normal mitochondrial function and maintenance of oxidative phosphorylation and ATP synthesis are considered promising therapeutic approaches in acute conditions [13]. Succinic acid salts have long been proposed as therapeutic agents for this purpose. However, no systematic *in vitro* studies of the effects of succinate salts on the bioenergetic processes in various cell types under physiological and stress conditions have yet been fully conducted.

Here, we investigated the efficacy of disodium succinate in preventing the death of astrocytes and renal epithelial cells in the *in vitro* model of cellular stress induced by serum deprivation (a classic method for inducing cellular damage). We also analyzed its effects on the key parameters of mitochondrial function, including transmembrane potential and cellular respiration. The aim of this study was to compare the molecular mechanisms of succinate-mediated cytoprotection in astrocytes and renal tubular epithelial cells under physiological and stress conditions, with a focus on the mitochondrial function as a potential basis for this protective effect.

MATERIALS AND METHODS

Cell cultures. Experiments were performed in primary astrocyte cultures isolated from the brains of neonatal rats and in cultured normal rat kidney epithelial NRK-52E cells (CRL-1571; ATCC, USA). All animal procedures were carried out in accordance with the guidelines of the Federation of European Laboratory Animal Science Associations (FELASA) and the ARRIVE guidelines. Animal protocols were approved by the Ethics Committee of the Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University (protocol 006-1/2/2024). For the experiments, neonatal rats were humanely euthanized with CO_2. The skull was then opened aseptically, the brain hemispheres were removed, and the meninges were excised. Brain tissue was transferred to 0.05% trypsin-EDTA solution (BioinnLabs, Russia), incubated for 30 min, washed with trypsin-EDTA solution, and resuspended in complete culture medium containing DMEM with 1 g/L glucose (BioinnLabs) and F-12 (BioinnLabs) at a 1 : 1 ratio, supplemented with stable L-glutamine (BioinnLabs) and 10% fetal bovine serum (FBS) (HiMedia, India). The suspension was transferred to a culture flask pre-coated with poly-D-lysine (Sigma-Aldrich, USA). After cells formed a monolayer, they were washed to remove the microglia by shaking at 135 rpm for 16 h. The medium was then removed, and astrocytes were detached with 0.05% trypsin-EDTA solution to be used in the experiments. NRK-52E cells were cultured in a medium containing F-12 and DMEM at a 1 : 1 ratio with 1 g/L glucose and stable L-glutamine supplemented with 10% FBS (HiMedia). The cells were passaged using 0.05% trypsin-EDTA solution. Both astrocytes and renal epithelial cells were incubated until the formation of 50% monolayer, after which the control cells received normal culture medium, while experimental cells were grown in the medium containing disodium succinate (Sigma-Aldrich). Serum deprivation was performed by incubating the cells in the medium containing all components except FBS (with or without succinate) for 48 h, after which the cells were analyzed. For all treatments, medium pH was monitored using phenol red.

Cell viability assay. Astrocytes and renal epithelial cells were seeded in 96-well plates, cultured until 50% confluence, and treated with disodium succinate (12.5, 25, 50, and 100 mM) under normal and serum deprivation conditions. Cell viability was assessed using an MTT assay (Macklin, China). The MTT reagent was dissolved at 5 mg/mL in DMEM/F-12 medium without sodium bicarbonate (GE Healthcare, USA) and incubated with cells for 1 h at 37°C. The cells were then lysed with dimethyl sulfoxide (Macklin, China), and the optical density of formazan was measured at 595 nm with a Zenyth 3100 multimodal plate reader (Anthos Labtec, Austria). Cells incubated with water for 24 h served as a negative control.

Cell death assessment. To study the effect of succinate on cell death, the cells were stained with propidium iodide (PI) according to the manufacturer's instructions (Lumiprobe, Russia). To visualize live cells, the cells were stained with the vital dye Calcein AM as recommended by the manufacturer (Invitrogen, USA) and imaged using an LSM 900 confocal microscope (Carl Zeiss, Germany) at the excitation wavelengths of 543 nm for PI and 488 nm for Calcein AM, with emission recorded at 560-590 nm for PI and 505-530 nm for Calcein AM.

Mitochondrial transmembrane potential measurement. To assess changes in the mitochondrial membrane potential, astrocytes and kidney epithelial cells were incubated with 50 mM disodium succinate for 48 h and stained with 200 nM tetramethylrhodamine ethyl ester (TMRE, Invitrogen) for 20 min at 37°C. TMRE-stained cells were visualized with LSM 900 microscope at the excitation wavelength of 543 nm with emission detected at 616-700 nm. Subsequent image processing was performed with the Fiji/ImageJ software by analyzing the average TMRE fluorescence intensity in the cells.

Cellular respiration analysis. The oxygen consumption rate (OCR) of astrocytes and kidney epithelial cells was measured using a Seahorse XFp analyzer (Seahorse Bioscience, USA). The cells were seeded in the wells of a Seahorse XF8 microplate. The following day before the experiment, the medium in the wells was replaced with a Seahorse XF DMEM base medium (Agilent, USA) supplemented with 2 mM glutamine (BioinnLabs), and the cells were incubated for one hour at 37°C. The plate was then placed in the analyzer, and various stressors (all from Sigma-Aldrich) were added to the indicated final concentrations in the following order: 1) disodium succinate, 50 mM; 2) oligomycin, 4.5 μM; 3) carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), 1 μM; 4) a combination of rotenone (2.5 μM), antimycin A (4 μM), and 2-deoxy-D-glucose (2-DG, 50 mM). In some experiments, the substances were added in a different order: 1) rotenone, 2.5 μM; 2) disodium succinate, 50 mM; 3) antimycin A, 4 μM. The obtained OCR values were normalized to the total protein concentration in each well, measured using the bicinchoninic acid assay (Sigma-Aldrich). After the experiment, the medium from each well was collected in a separate microtube and centrifuged for 10 min at 12,000*g* to precipitate detached cells. Also, 10 μl of 2x RIPA buffer (Millipore, Germany) was added to the bottom of each well to lyse the attached cells. The resulting lysate was combined with the pellet obtained by centrifuging the culture medium from the same well, and the pooled lysate was assessed for protein content. The results of OCR measurements were analyzed using the Seahorse XFp software (Wave 2.6.1, Seahorse Bioscience), according to the recommendations for the Seahorse XF Cell Mito Stress Test Kit (103015-100, Agilent, USA). Basal respiration was calculated as the difference between the OCR values after succinate addition and after addition of a mixture of rotenone, antimycin, and 2-DG. Respiration associated with ATP production was calculated as the difference between the OCR values after succinate addition and addition of oligomycin. Proton leak was calculated as the difference in OCR values after addition of oligomycin and addition of rotenone, antimycin, and 2-DG, while respiratory control was assessed as the ratio of OCR values after addition of FCCP to those after addition of oligomycin. The contribution of succinate-based respiration to the total oxygen consumption was assessed as the ratio of OCR after succinate addition to the basal OCR (before the addition of rotenone).

Statistical analysis of data was performed using the GraphPad Prism 9 software (version 9.5.1). All values are presented as mean ± standard deviation (SD). The data were tested for the normal distribution using the Shapiro–Wilk test. For experiments including two experimental groups, the nonparametric Mann–Whitney U test or Student's *t*-test for normally distributed data were used. For the experiments including four experimental groups, parametric variables were analyzed using the one-way analysis of variance (ANOVA) with the Tukey's multiple comparison test; nonparametric variables were analyzed using the Kruskal–Wallis test with the Dunn's multiple comparison test.

RESULTS

Succinate promotes the viability of renal epithelial cells and astrocytes under both physiological and serum deprivation conditions. We evaluated the effect of a 48 h incubation with succinate on the viability of renal epithelial cells and astrocytes under both physiological and serum deprivation conditions. Under physiological conditions, succinate

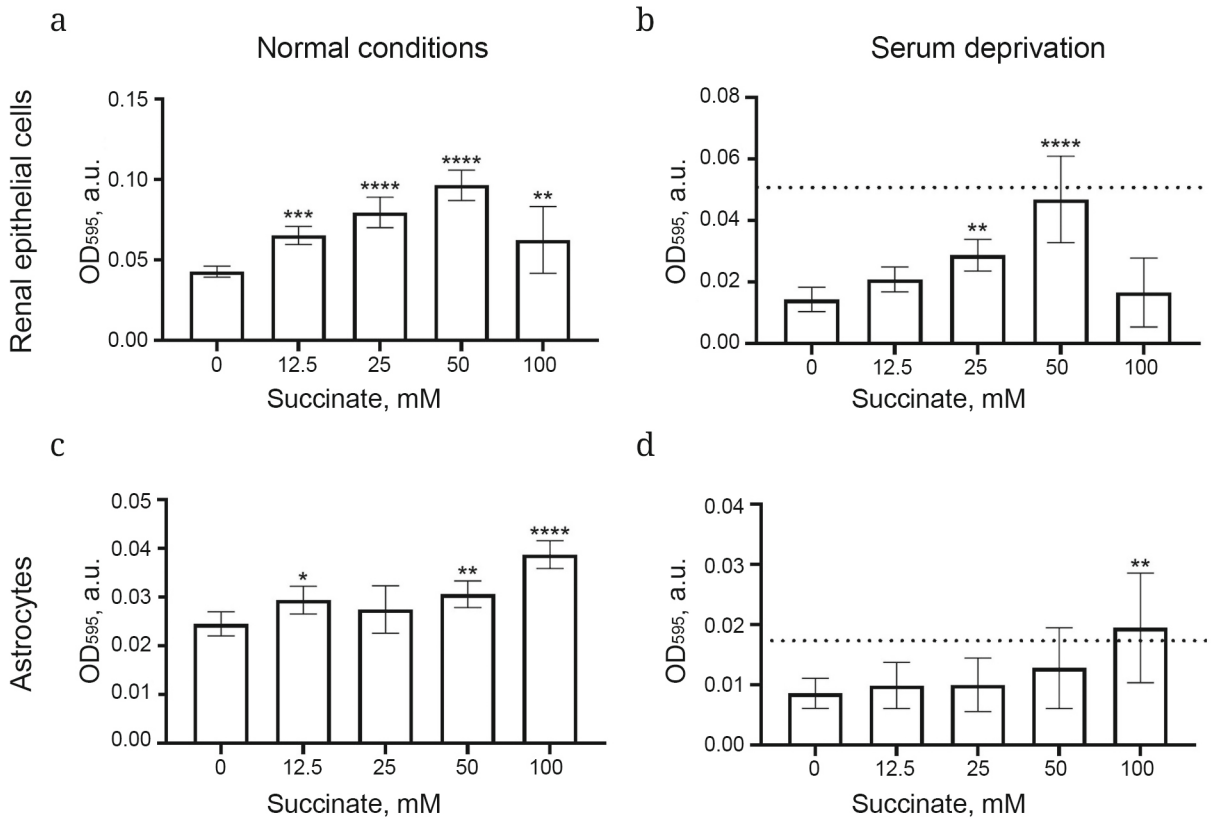

Fig. 1. Effect of succinate on the viability of renal epithelial cells and astrocytes after 48 h of incubation under physiological (a, c) or serum deprivation (b, d) conditions. Cell viability was evaluated using the MTT assay. Data are presented as optical density (OD) at 595 nm for renal epithelial cells (a, b) and astrocytes (c, d); dotted line (b, d) shows the viability of intact cells; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ compared to the control succinate-free group.

significantly increased the viability of renal epithelial cells at all tested concentrations (Fig. 1a). The maximal effect was observed at the succinate concentration of 50 mM, which increased cell viability more than two-fold compared to the untreated control. Serum deprivation for 48 h reduced the viability of renal epithelial cells by 71%. Succinate markedly enhanced cell viability upon serum deprivation; the treatment with 50 mM succinate restored cell viability to the levels comparable to those observed under physiological conditions (Fig. 1b). However, 100 mM succinate exhibited a toxic effect in serum-deprived renal epithelial cells, whereas under normal conditions, the same concentration produced a positive effect (although less pronounced compared to other tested concentrations) (Fig. 1, a, b).

Succinate also improved the viability of astrocytes, albeit to a lesser extent than that of renal epithelial cells. Under physiological conditions, the viability of astrocytes was significantly enhanced at 12.5, 50, and 100 mM succinate (Fig. 1c) (1.25- and 1.5-fold at 50 and 100 mM, respectively). Serum deprivation reduced the viability of astrocytes by ~50%, whereas the treatment with 100 mM succinate completely abolished this detrimental effect (Fig. 1d). Overall, succinate exerted a pro-survival effect in both cell types, but it was more pronounced in renal epithelial cells.

Succinate treatment reduces cell death in renal epithelial cells, but not in astrocytes. The effect of succinate on cell death in renal tubular epithelial cells was further assessed using propidium iodide (PI) and Calcein AM staining (Fig. 2). Serum deprivation markedly increased the proportion of PI-positive cells (Fig. 2, b, d-f), whereas 48-h incubation with succinate significantly reduced the percentage of dead cells (Fig. 2, b, e). In contrast, succinate failed to attenuate the death of serum-starved astrocytes (Fig. 2, d, f). These results supported the data of MTT assay, indicating that reduced viability during serum deprivation was due to cell death, predominantly via necrosis, as well as the ability of succinate to substantially improved the survival of renal tubular epithelial cells under damaging conditions.

Succinate increases mitochondrial transmembrane potential in renal epithelial cells under both physiological and serum deprivation conditions. Mitochondrial transmembrane potential was measured to evaluate the effect of succinate on the status of mitochondria (Fig. 3). A significant increase

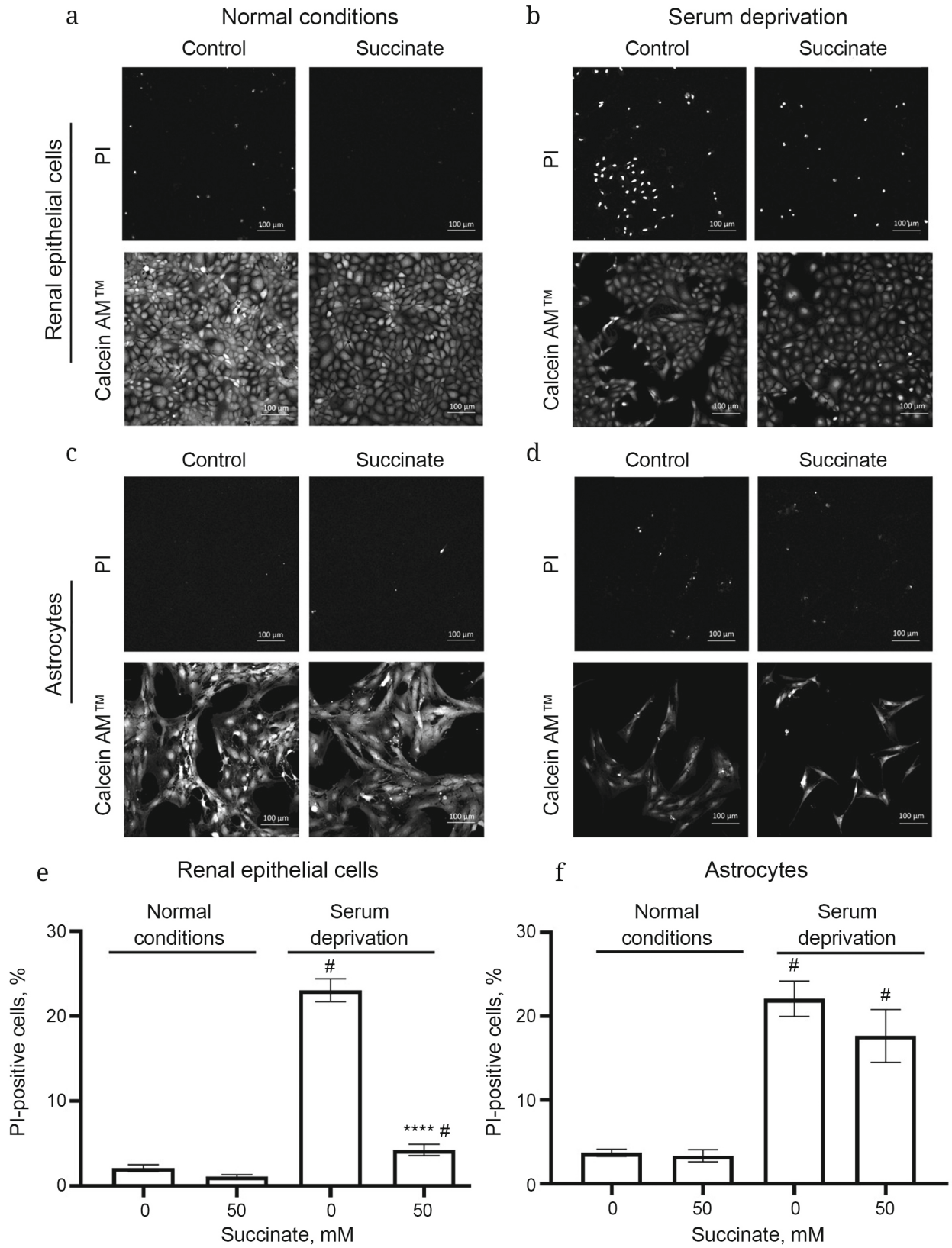

Fig. 2. The effect of succinate on the death of renal epithelial cells and astrocytes under physiological and serum deprivation conditions. Renal epithelial cells (a, b) and astrocytes (c, d) were stained with Calcein AM and PI after 48 hours of incubation with succinate under physiological (a, c) and serum deprivation (b, d) conditions. Quantification of PI-positive renal epithelial cells (e) and astrocytes (f) following succinate treatment; **** $p < 0.0001$ compared to 0 mM succinate within the same experimental group; # $p < 0.05$ compared to the group exposed to physiological conditions.

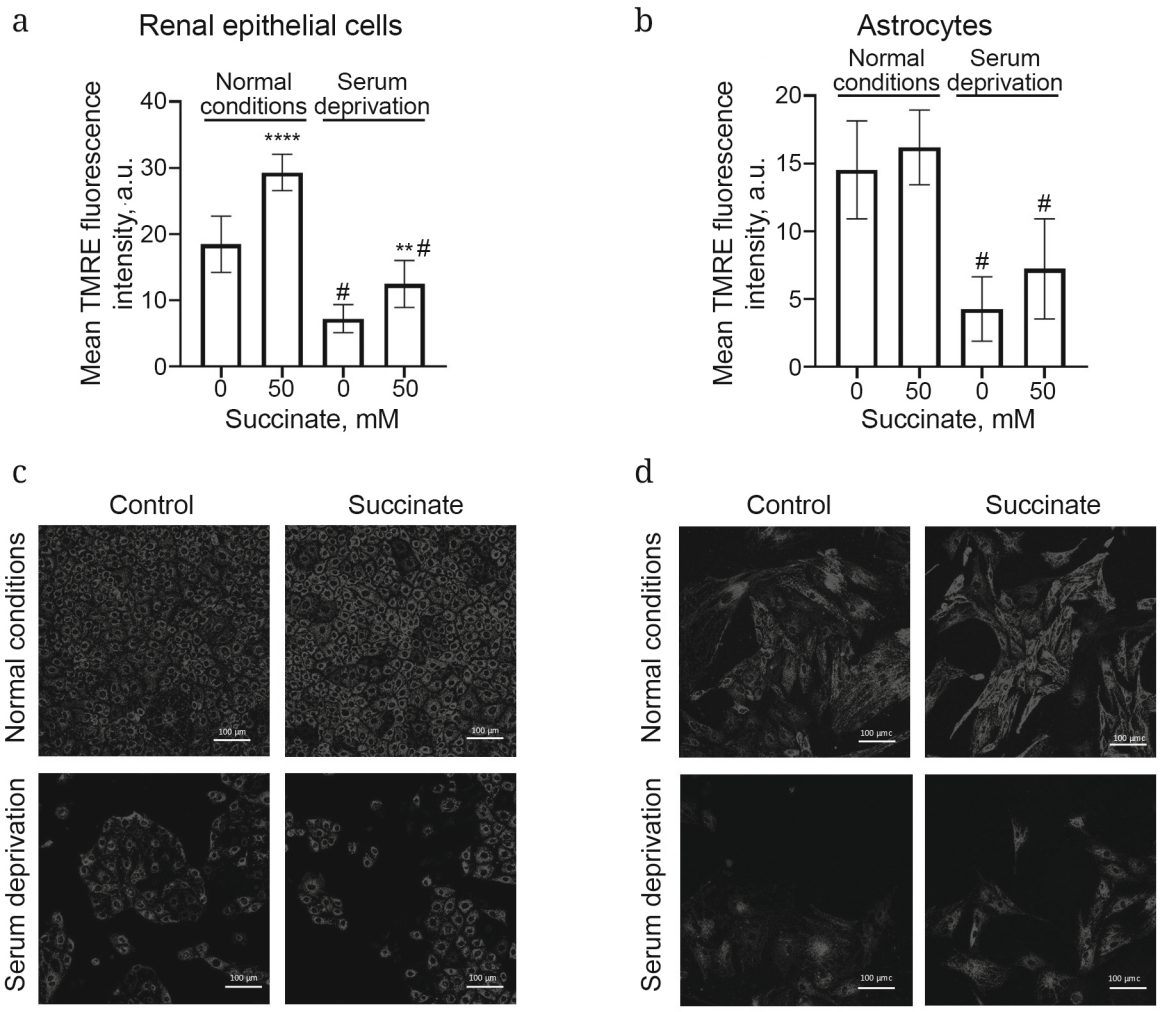

Fig. 3. Changes in the mitochondrial transmembrane potential of renal epithelial cells and astrocytes after succinate treatment under physiological and serum deprivation conditions. Mean TMRE fluorescence in renal epithelial cells (a) and astrocytes (b). Representative confocal images of TMRE-stained renal epithelial cells (c) and astrocytes (d). ** $p < 0.01$; **** $p < 0.0001$ vs. 0 mM succinate under physiological conditions; # $p < 0.05$ vs. corresponding groups under normal conditions.

in the transmembrane potential was observed in renal epithelial cells (Fig. 3, a, c), but not in astrocytes (Fig. 3, b, d), following succinate treatment under control conditions. Serum deprivation for 48 h led to a substantial decrease in the TMRE fluorescence in both renal epithelial cells and astrocytes (Fig. 3). The treatment with 50 mM succinate under serum deprivation conditions improved mitochondrial potential in renal epithelial cells (Fig. 3, a, c), but failed to exert a similar effect in astrocytes (Fig. 3, b, d).

Respiratory response of kidney epithelial cells and astrocytes to succinate treatment. The effects of exogenous succinate on the respiration parameters were studied to identify possible mechanisms underlying the differences in the cytoprotective effect of succinate in kidney epithelial cells and astrocytes (Fig. 4). To measure the OCR, the culture medium was replaced with a reaction medium containing no substrates for the respiration or glycolysis, after which cells were analyzed using a Seahorse XF analyzer after sequential addition of respiratory substrates and inhibitors (see Materials and Methods). The basal respiration rate was significantly higher in astrocytes than in kidney epithelial cells (Fig. 4, a, b). At the same time, renal epithelial cells showed lower proton leak (Fig. 4d) and, therefore, higher respiratory control compared to astrocytes (Fig. 4e). These findings suggest that the higher basal respiratory rate of astrocytes may be due to a greater uncoupling of respiration and oxidative phosphorylation. Nevertheless, both cell types utilized exogenous succinate as a respiration substrate, as demonstrated by the OCR increase following succinate addition after rotenone-mediated inhibition of complex I (Fig. 4f). Notably, succinate utilization was faster in epithelial cells, as indicated by the kinetics of OCR response (Fig. 4f),

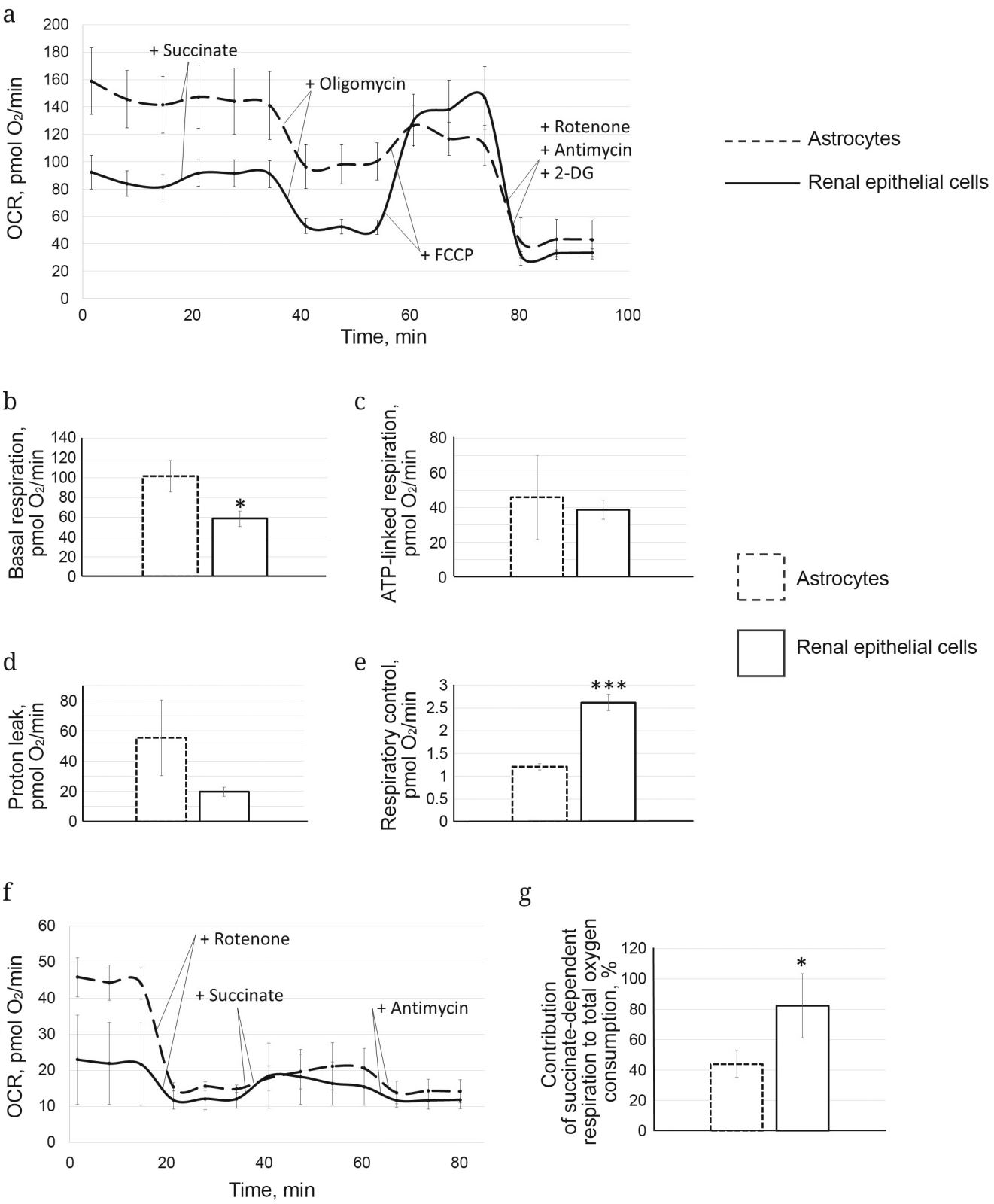

Fig. 4. The effect of exogenous disodium succinate on the respiration of astrocytes and renal epithelial cells. a) OCR curves following addition of disodium succinate to the cells in the absence of complex I inhibition. b-e) Quantitative analysis of mitochondrial respiration parameters calculated from the OCR changes after addition of respiration inhibitors and uncoupler: basal respiration (b), ATP-linked respiration (c), proton leak (d), and respiratory control (e). f) OCR curves showing the response to disodium succinate following rotenone-induced complex I inhibition. g) The contribution of succinate-dependent respiration to the total oxygen consumption in astrocytes and renal epithelial cells; * $p < 0.05$; *** $p < 0.001$.

and its contribution to the oxygen consumption was more significant than in astrocytes (Fig. 4g).

DISCUSSION

The brain and kidneys are among the organs with the highest basal energy metabolism. Numerous studies have shown that the optimal functioning of brain cells depends directly on the activity of mitochondria, while mitochondrial dysfunction contributes to or exacerbates brain pathologies [14]. Although neurons are very metabolically active cells, astrocytes demonstrate a high glycolytic activity in order to supply neurons with metabolic substrates [15]. Nevertheless, mitochondria are considered an important factor in the regulation of astrocyte functions. It has been shown that during ischemic injury, changes in the mitochondrial function of astrocytes are closely associated with impaired brain homeostasis, defects in glutamate and fatty acid metabolism, Ca^{2+} regulation, formation of reactive oxygen species (ROS), and induction of inflammation [16]. The kidneys are also among the most energy-consuming organs, especially renal tubular cells [17, 18]. These cells are particularly vulnerable to the mitochondrial dysfunction, which can impair kidney function [19].

Despite the fact that bioenergetic characteristics of various cell types can differ, mitochondrial dysfunction is a central component of many, if not all, pathological conditions associated with a deficiency of nutrient substrates and oxygen. An insufficient supply of glycolytic and respiratory substrates disrupts oxidative phosphorylation [20], leading to the destabilization of electron transport chain complexes, electron leakage, and excessive ROS generation [21]. This creates a vicious cycle, as oxidative stress potentiates further mitochondrial damage, thus increasing ROS production, critically reducing ATP synthesis [22, 23], and ultimately activating intrinsic apoptotic pathway, which results in cell death and, finally, organ dysfunction. Therefore, strategies aimed at maintaining energy homeostasis, such as replenishing metabolite deficiencies with exogenous substrates, are considered as a promising approach for correcting mitochondrial dysfunction and preventing cell death. In this study, serum (FBS) deprivation was used to induce the damage and death of kidney and brain cells as an *in vitro* model of cell damage and to examine the possibility of metabolic correction of the observed pathological changes with succinate. Low concentrations or absence of FBS in the medium lead to the activation of signaling molecules of the intrinsic mitochondrial apoptotic pathway that involves proteins of the Bcl-2 family [11]. The absence of FBS also causes ROS generation, DNA damage, and activation of apoptotic processes [2]. After serum deprivation, cells consume nutrients in particular, metabolites of the TCA cycle (e.g., succinate) at a higher rate [24]. In the acute phase of injury, maintaining a normal amount of energy substrates is very important, as ROS can impair the functioning of succinate dehydrogenase and other respiratory chain complexes, which may disrupt normal mitochondrial function even after the nutrient supply restoration [25].

In this study, we investigated the effects of disodium succinate on brain astrocytes and renal epithelial cells. Disodium succinate was used to avoid acidification of the culture medium. Although succinate is widely used in clinical practice, its organ-specific effects, optimal dosage, and effective therapeutic regimens for various pathologies have not been fully explored.

Mammalian cells take up exogenous succinate primarily via sodium-dependent dicarboxylate transporters (NaDCs) of the SLC13A family [26]. Two main classes of NaDCs have been identified, that differ in their affinity for succinate: low-affinity transporters with the Michaelis constant (K_M) for succinate of ~0.5 mM, and high-affinity transporters with a K_M of ~25 μM [27]. These transporters are expressed in various tissues, including astrocytes, neurons, kidney cells, hepatocytes, and immune cells, and facilitate the entry of succinate and other dicarboxylates into cells, where these compounds are incorporated into energy metabolism [28].

Disodium succinate increased the viability of renal epithelial cells and astrocytes under both standard conditions and serum deprivation-induced stress. However, the beneficial effect of succinate was more pronounced in renal epithelial cells (Fig. 1). These data are consistent with the previously obtained results showing that succinate improved mitochondrial function in glial cells [29] and reduced neuroinflammation in experimental intracerebral hemorrhage [30]. One of the key biochemical markers of brain injury is the lactate/pyruvate ratio in brain tissue. An increase in this ratio reflects a shift towards anaerobic glycolysis and oxidative stress development. Clinical administration of succinate-containing formulations to patients with traumatic brain injury (TBI) decreased the lactate/pyruvate ratio, indicating improved redox balance and increased glucose utilization efficiency [31-33]. This effect is thought to result from the stimulation of the TCA cycle and oxidative phosphorylation, leading to the increased pyruvate levels and decreased pyruvate reduction to lactate [33]. Furthermore, exogenous succinate improved glutamatergic synaptic transmission in rat hippocampal vital slices, which may be due to the improved energy supply to presynaptic terminals [34]. In contrast to the nervous system, the effects of succinate

on the renal tissue have been studied much less. However, succinate can also improve mitochondrial function by reducing oxidative stress and replenishing deficient energy substrate in the cases of renal proximal tubule cell damage [35].

We demonstrated that exogenous succinate maintained cellular energy status by significantly increasing the mitochondrial transmembrane potential. However, this effect was specific to renal epithelial cells (Fig. 3). To understand why succinate had a more pronounced positive effect on renal epithelial cells vs. astrocytes, we compared the respiration parameters under succinate energization (Fig. 4). The basal bioenergetic characteristics of astrocytes and renal epithelial cells differed significantly. Astrocytes exhibited higher basal respiration rates, likely due to a higher degree of mitochondrial uncoupling rather than to a more active oxidative phosphorylation. Oligomycin-sensitive respiration was similar between the two cell types, whereas the proton leak and respiratory control values (Fig. 4, d, e) indicated the occurrence of the futile cycle in astrocytes. This might reflect the reliance of astrocytes on glycolysis to fuel the astrocyte–neuron lactate shuttle, with an unchanged rate of oxidative phosphorylation. Since the cells under the experimental conditions had only succinate as a respiratory substrate, it can be assumed that it was more efficiently utilized by renal tubular cells. Oxygen consumption values in the case of complex I inhibition by rotenone following addition of succinate, showed that succinate significantly increased the respiration rate (Fig. 4f). This confirms the functional involvement of exogenous succinate in the electron transport chain. Moreover, the increase in the oxygen consumption in tubular epithelial cells after succinate addition occurred more rapidly than in astrocytes (Fig. 4f), and the overall contribution of succinate-dependent respiration in renal epithelial cells was significantly higher than in astrocytes (Fig. 4g). The cell type-specific efficacy of disodium succinate can be explained by both differences in the expression of dicarboxylate transport systems and higher activity of succinate dehydrogenase in renal mitochondria. These features make the energy metabolism of tubular epithelial cells dependent on the availability of succinate [36].

In addition to its direct role in energy metabolism, the protective effect of succinate under the damaging conditions may be mediated by several other mechanisms, such as activation of the SUCNR1 receptor (GPR91) and inhibition of prolyl hydroxylases (PHDs). The SUCNR1 receptor is widely expressed in brain cells, in particular, astrocytes, microglia, and neurons, as well as in renal tubular epithelial cells [33, 37]. SUCNR1 is thought to be involved in tissue adaptation to adverse conditions, including stimulation of cell proliferation, migration, and angiogenesis [38, 39]. Notably, the interaction of extracellular succinate with SUCNR1 can induce expression of SLC13A family genes, which ensures succinate transport into the cells [40]. This creates a positive feedback loop, where extracellular succinate stimulates its own uptake, enhancing both its metabolic and signaling effects. Moreover, after entering the cytosol, succinate can inhibit 2-oxoglutarate-dependent dioxygenases, primarily PHDs [41, 42], which are responsible for the oxygen-dependent degradation of hypoxia-inducible factor 1-alpha (HIF-1α). Inhibition of PHDs stabilizes HIF-1α even under normoxic conditions, enabling its nuclear translocation [43] and activation of genes involved in the adaptation to hypoxia and regulation of mitochondrial function [44]. In particular, this triggers the synthesis of cytochrome oxidase subunit 2 (COX4-2), which replaces the constitutive form COX4-1, thus increasing the efficiency of complex IV at low oxygen concentrations [45]. It is worth noting that, in addition to succinate, other exogenous metabolites and their derivatives with a higher bioavailability are actively studied. One example is diacetoxymethyl succinate (NV118), which shows a pronounced cytoprotective effect due to the improvements in the mitochondrial metabolism in the models of damage induced by carbon monoxide, cyanides, amiodarone, N-methylcarbamate, and metformin [46-52]. Other alternative substrates (e.g., sodium fumarate) reduce cell death during hypoxia, mostly by activating alternative pathways rather than restoring mitochondrial ATP synthesis [53]. Dimethyl fumarate has demonstrated the renoprotective effect against cyclosporine A- and lipopolysaccharide (LPS)-induced toxicity, mediated by suppression of oxidative stress and inflammation, as well as enhancement of the antioxidant defense [54, 55]. Dimethyl fumarate also inhibited pyroptosis via modulation of endoplasmic reticulum stress and the JAK2–STAT3 pathway in a model of contrast-induced nephropathy [56] and improved mitochondrial function in the case of di(2-ethylhexyl)phthalate toxicity [57]. The effects of dimethyl fumarate are most likely explained by the activation of the NRF2 pathway [58], which accounts for its neuroprotective potential [59, 60], including attenuation of neuroinflammation and neuronal cell death after traumatic brain injury [61, 62]. Similarly, monomethyl fumarate acts through NRF2, as it suppresses cell death and oxidative stress, and reduces brain edema in a model of ischemic stroke [63-65]. Taken together, these data suggest that TCA cycle metabolites and their derivatives have a considerable therapeutic potential for ameliorating mitochondrial dysfunction across a broad spectrum of pathologies that require multiorgan protection. However, despite their enhanced membrane permeability, ester derivatives of dicarboxylic acids, including succinate, have

limitations as therapeutic agents. In particular, commonly used alkyl esters, such as dimethyl succinate, are hydrolyzed by cellular esterases with the release of corresponding alcohols (e.g., methanol), which may exert toxic effects. This highlights the critical need for the development of alternative, safe formulations capable of enhancing succinate bioavailability without compromising the safety.

CONCLUSION

This study demonstrated a prominent cytoprotective potential of exogenous disodium succinate in cells derived from two highly metabolically active organs – the brain and kidneys – especially after injury. Succinate significantly increased the viability of both astrocytes and renal epithelial cells under both physiological and stress conditions. However, its protective effect was more pronounced in kidney epithelial cells than in astrocytes. This difference likely reflects a more pronounced ability of succinate to modulate the mitochondrial transmembrane potential in kidney epithelial cells and to support oxidative phosphorylation when other metabolic substrates are limited, suggesting more efficient utilization of succinate in renal epithelial cells. These findings support the hypothesis that succinate, a key TCA cycle intermediate, can alleviate cellular energy deficit and prevent cell death. However, its effects appear to be cell type-dependent, necessitating careful selection of target pathologies. Therefore, succinate represents a particularly promising candidate for the nephroprotective strategies in acute kidney injuries associated with bioenergetic crisis.

Abbreviations

2-DG	2-deoxyglucose
ATP	adenosine triphosphate
FBS	fetal bovine serum
PHD	prolyl hydroxylases
PI	propidium iodide
ROS	reactive oxygen species
TMRE	tetramethylrhodamine ethyl ester

Contributions

N. V. Andrianova and E. Y. Plotnikov developed the concept and supervised the study; M. I. Buyan, K. S. Cherkesova, N. V. Andrianova, A. A. Brezgunova, and I. B. Pevzner conducted the experiments; E. Y. Plotnikov, N. V. Andrianova, M. I. Buyan, K. S. Cherkesova, A. A. Brezgunova, and I. B. Pevzner discussed the study results; M. I. Buyan, K. S. Cherkesova, and N. V. Andrianova wrote the original draft; E. Y. Plotnikov, A. A. Brezgunova, and I. B. Pevzner edited the manuscript.

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Ethics approval and consent to participate

Animal study protocols were reviewed and approved by the Ethics Committee of the Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University (protocol no. 006-1/2/2024). All animal manipulations were performed in accordance with ARRIVE guidelines.

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

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