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Review

The possible role of cytochrome *c* oxidase in stress-induced apoptosis and degenerative diseases

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Abstract

Apoptotic cell death can occur by two different pathways. Type 1 is initiated by the activation of death receptors (Fas, TNF-receptor-family) on the plasma membrane followed by activation of caspase 8. Type 2 involves changes in mitochondrial integrity initiated by various effectors like Ca^{2+}, reactive oxygen species (ROS), Bax, or ceramide, leading to the release of cytochrome *c* and activation of caspase 9. The release of cytochrome *c* is followed by a decrease of the mitochondrial membrane potential $\Delta\Psi_m$. Recent publications have demonstrated, however, that induction of apoptosis by various effectors involves primarily a transient increase of $\Delta\Psi_m$ for unknown reason. Here we propose a new mechanism for the increased $\Delta\Psi_m$ based on experiments on the allosteric ATP-inhibition of cytochrome *c* oxidase at high matrix ATP/ADP ratios, which was concluded to maintain low levels of $\Delta\Psi_m$ in vivo under relaxed conditions. This regulatory mechanism is based on the potential-dependency of the ATP synthase, which has maximal activity at $\Delta\Psi_m = 100-120$ mV. The mechanism is turned off either through calcium-activated dephosphorylation of cytochrome *c* oxidase or by 3,5-diiodo-L-thyronine, palmitate, and probably other so far unknown effectors. Consequently, energy metabolism changes to an excited state. We propose that this change causes an increase in $\Delta\Psi_m$, a condition for the formation of ROS and induction of apoptosis.

Keywords: Mitochondrial membrane potential hyperpolarization; Cytochrome *c* oxidase; Apoptosis; 3,5-diiodo-L-thyronine; Palmitate; Calcium-activated protein dephosphorylation; cAMP-dependent phosphorylation

1. Mammalian cytochrome *c* oxidase

Prokaryotic cytochrome *c* oxidase consists of three to four subunits. In contrast, the mammalian enzyme is composed of 13 subunits. The three largest subunits (I–III), encoded by mitochondrial DNA, are homologous to subunits I–III from bacteria. The 10 additional subunits are encoded by the nuclear genome and occur as tissue-specific isoforms [1]. In addition to the muscle- and non-muscle-specific expression of subunits VIa, VIIa and VIII, a lung-specific isoform was identified for subunit IV (IV-2) [2]. Recently, an isoform of subunit VIb was found to be expressed exclusively in mammalian testes [3].

The structures of the three catalytic centers of the bacterial and mammalian enzymes, however, are almost identical [4,5]. In both enzymes subunit II contains the two-copper center Cu_A, the binding site for cytochrome *c*. Heme a and the oxygen binding heme a_3/Cu_B centers are located in subunit I. Accordingly, both enzymes have the same catalytic functions [6,7]: (1) transfer of electrons from ferrocytochrome *c* to oxygen, accompanied by the vectorial uptake of protons for the formation of water, forming a membrane potential $\Delta\Psi_m$, and (2) outward translocation of protons, associated with the formation of a pH gradient and $\Delta\Psi_m$ (predominantly). The catalytic properties of the enzymes from *Paracoccus denitrificans* and bovine heart are similar, when measured with the isolated enzymes under standard conditions [8,9]. Under more physiological conditions, however, large differences in the catalytic activities are found between the two. The differences in electron transport and proton pumping are related to the additional subunits of the mammalian enzyme. A decrease of the H^+/e^- stoichiometry (from 1.0 to 0.5) of the heart-type enzyme (subunit VIaH) at high matrix ATP/ADP-ratios [10], and of

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the liver-type enzyme (subunit VIaL) at low concentrations of palmitate [11], was found. The mechanism of proton pumping in cytochrome *c* oxidase is still debated [12], and different views exist for the mammalian [13] and bacterial enzymes [14]. Therefore, mechanisms on how the H^+/e^- stoichiometry can be modulated remain to be elucidated.

2. Regulation of mitochondrial respiration by cytochrome *c* oxidase

The rate and efficiency of electron transport in the respiratory chain of mammalian mitochondria must be controlled according to the cellular needs for ATP and heat production. Applying "metabolic control analysis" to isolated mitochondria, a "control strength" of 0.15–0.2 was determined for cytochrome *c* oxidase, indicating little rate-limiting control by the terminal enzyme of the respiratory chain [15]. When the same method is applied to cultured cells, however, cytochrome *c* oxidase was shown to represent the rate-limiting step of the mitochondrial electron transport chain [16]. The control of respiration by the utilization of ATP is named "respiratory control", and is explained by the chemiosmotic theory [17]: the free energy of substrate oxidation is partly stored in a proton gradient across the inner mitochondrial membrane (mainly $\Delta\Psi_m$). At high ATP utilization the rate of ATP synthase, driven by the consumption of $\Delta\Psi_m$, increases, resulting in a decrease of $\Delta\Psi_m$. A lowered $\Delta\Psi_m$ stimulates respiration because the three proton pumps of the respiratory chain (complexes I = NADH dehydrogenase, III = cytochrome *c* reductase, and IV = cytochrome *c* oxidase) are inhibited only at high $\Delta\Psi_m$ (180–200 mV).

A second mechanism of respiratory control was discovered based on inhibition of cytochrome *c* oxidase at high intramitochondrial ATP/ADP ratios [18,19]. This allosteric ATP-inhibition of cytochrome *c* oxidase, verified by sigmoidal kinetics with a Hill coefficient of 2 [18], is independent of $\Delta\Psi_m$, and due to high-affinity binding of ATP (or ADP) to the matrix domain of subunit IV ([20], see also Ref. [2]). At low ATP/ADP ratios in the mitochondrial matrix (half-maximal inhibition at ATP/ADP = 28), the enzyme becomes maximally active in a non-allosteric manner (hyperbolic kinetics). We postulated that in vivo the ATP-inhibition normally keeps mitochondrial membrane potential low ($\Delta\Psi_m$ = 80–140 mV), due to feedback control by ATP [21], based on saturation and maximal rates of ATP synthesis by the ATP synthase at $\Delta\Psi_m$ = 100–120 mV [22]. The ATP-inhibition, however, is only effective when cytochrome *c* oxidase is phosphorylated, perhaps at Ser441 of subunit I of the bovine enzyme [21]. In mitochondria the phosphate group can be reversibly cleaved off by a calcium-activated protein phosphatase in the intermembrane space [23,24]. When cytochrome *c* oxidase is dephosphorylated, and the control of respiration (and thus of $\Delta\Psi_m$) by the ATP/ADP ratio is abolished, $\Delta\Psi_m$ is controlled by the potential-dependency of the three proton pumps, leading to inhibition of respiration only at high $\Delta\Psi_m$ values (140–200 mV).

In addition to dephosphorylation of cytochrome *c* oxidase, the allosteric ATP-inhibition is switched off by 3,5-diiodo-L-thyronine [25], which is produced in cells from triiodo-L-thyronine by a deiodinase. Furthermore, free palmitate was found to specifically switch off the allosteric ATP-inhibition of reconstituted bovine heart cytochrome *c* oxidase with half-maximal effect at 0.3 μM (Shahla Hammerschmidt, Diplomarbeit, Fachbereich Chemie, Philipps-Universität Marburg, 1998). From these results a molecular–physiological hypothesis was proposed, suggesting, in living cells, a reversible switch from relaxed to excited state of energy metabolism by stress, with the consequence of hyperpolarization of $\Delta\Psi_m$ and reactive oxygen species (ROS) production (Fig. 1) [21,26–28]. The regulation of energy metabolism by the allosteric ATP-inhibition of cytochrome *c* oxidase was recently reviewed [29].

In the following sections, important cellular responses that involve an increased mitochondrial membrane potential are reviewed. These findings are discussed in the light of cytochrome *c* oxidase regulation, which might provide a molecular mechanism to explain changes in $\Delta\Psi_m$.

3. Cellular stress

Cellular stress is caused by multiple factors including injury, inflammation, hypoxia, hormones, high concentrations of metabolites or xenobiotics, and excessive muscle work. "Metabolic stress" is created when the activity of a cell is stimulated, and could result in "oxidative stress" when ROS are produced intracellularly, particularly in mitochondria which participate in type 2 apoptosis [30–32]. Furthermore, xenobiotics and exogenous oxidants can generate "cytotoxic stress" that threatens survival of the cell. ROS represent the reaction products of various oxidases and are produced in peroxisomes, endoplasmatic reticulum and plasma membrane of lymphocytes, where they participate in eliminating bacteria. Small amounts of ROS are probably produced at the plasma membrane of all cells, where they participate as "second messenger" in receptor-mediated signal transduction [33]. Deleterious amounts of ROS are produced at the respiratory chain in mitochondria by transfer of an unpaired electron from ubisemiquinone (at complexes I and/or III) to dioxygen leading to the production of the superoxide radical anion $O_2^{-}\cdot$. The superoxide radical anion is only produced at higher membrane potentials ($\Delta\Psi_m$>140 mV) [34,35], but not in mitochondria of resting cells, which posses a low $\Delta\Psi_m$. The regulation of the $\Delta\Psi_m$ level in living cells, however, is not completely understood.

Previous studies demonstrated a decrease of the mitochondrial membrane potential $\Delta\Psi_m$ after induction of type 2 apoptosis by various compounds in different cells, including

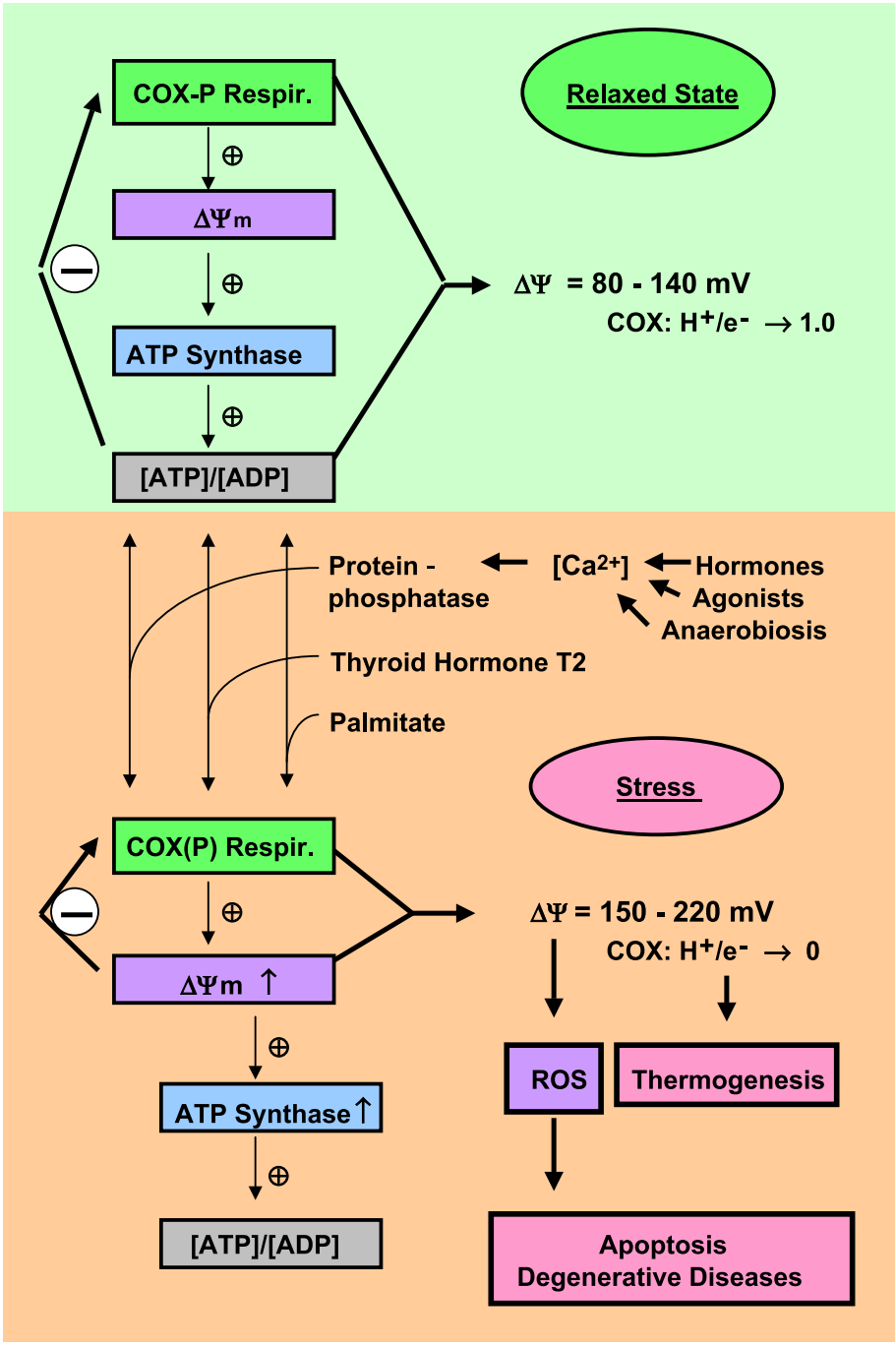

Fig. 1. The relaxed state of cellular energy metabolism, characterized by a low mitochondrial membrane potential $\Delta\Psi_m$ (80–140 mV), is proposed to be based on feedback inhibition of cytochrome *c* oxidase (COX-P, respiration) by high matrix ATP/ADP ratios, which are produced by the ATP synthase. Oxidative stress in mitochondria is proposed to originate from turning off the allosteric ATP-inhibition of cytochrome *c* oxidase either by calcium-activated dephosphorylation (COX), induced by hormones, agonists and anaerobiosis, or without dephosphorylation (COX(P)) by 3,5-diiodo-L-thyronine (T2) and palmitate. As a consequence, $\Delta\Psi_m$ increases to high values (150–220 mV), because respiration is no longer controlled by the ATP/ADP ratio but by $\Delta\Psi_m$. At high $\Delta\Psi_m$ values (>140 mV), ROS are produced which could induce apoptosis and degenerative diseases. High $\Delta\Psi_m$ values also decrease the H^+/e^- stoichiometry of cytochrome *c* oxidase, thus increasing thermogenesis and the rate of oxidative phosphorylation under stress [29].

the glutamate cytotoxicity in neurons. In contrast, more recent publications indicate a transient increase of $\Delta\Psi_m$ (hyperpolarization) during apoptotic cell death. Absolute values of $\Delta\Psi_m$ were rarely determined in living cells, because most fluorescence dyes only indicate a relative increase or decrease of $\Delta\Psi_m$. Wan et al. [36] determined absolute $\Delta\Psi_m$ values in perfused rat hearts ranging from 100 to 140 mV, depending on the work load and the supplied substrate. These low values obtained from intact organs contrast with the high $\Delta\Psi_m$ values of 180–220 mV,

measured with isolated mitochondria [17] or reconstituted cytochrome *c* oxidase [37]. In a recent report, Zhang et al. [38] verified the low $\Delta\Psi_m$ values in intact cells with a newly developed method and measured 105 ± 0.9 mV in cultured fibroblasts and 81 ± 0.7 mV in neuroblastoma cells.

4. Hyperpolarization of $\Delta\Psi_m$ during apoptosis

Two pathways of programmed cell death are known. Type 1 is initiated from the cell surface death receptors (Fas, TNF-family receptors) and involves activation of caspase-8, a cysteine protease cleaving at the C-side of aspartate [31]. Type 2 is triggered by changes of mitochondrial integrity and involves release of cytochrome *c* into the cytosol, which activates procaspase-9 to caspase−9 via a complex with Apaf-1 and dATP (apoptosome). Subsequently the mitochondrial permeability transition pore is formed with consequent decrease of $\Delta\Psi_m$. The mitochondrial pathway can be initiated by Ca^{2+}, Bax, ROS, p53, or ceramides, and is inhibited by Bcl-2 or Bcl-xL [30,32,39]. The death receptor-initiated apoptosis (type 1) can also lead to release of cytochrome *c* via cleavage of Bid to truncated Bid (tBid) by caspase−8. Recently, the release of cytochrome *c* was explained by formation of a pore in the outer mitochondrial membrane, composed of tBid/Bax and cardiolipin [40]. This release of cytochrome *c* would not involve opening of the mitochondrial permeability transition pore [41] or decrease of $\Delta\Psi_m$ [32,42]. In contrast to the observed depolarization of $\Delta\Psi_m$ in previous studies, the following more recent publications describe a primary and transient increase of $\Delta\Psi_m$ (hyperpolarization) after induction of apoptosis by various stress parameters. The possible involvement of cytochrome *c* oxidase in the hyperpolarization of $\Delta\Psi_m$ during the apoptotic pathway, via turning off the allosteric ATP-inhibition, will be discussed. Other mechanisms regulating $\Delta\Psi_m$ have been recently reviewed [29].

4.1. Transient increase of $\Delta\Psi_m$ in apoptotic cell death

A transient increase of $\Delta\Psi_m$ after induction of apoptosis in human Jurkat T cells by Anti-Fas antibody, staurosporine, or IL-3 depletion of FL5.12 cells was first described by Vander Heiden et al. [43]. Subsequently, a transient increase of $\Delta\Psi_m$ in different cells after induction of apoptosis by a variety of compounds has been reported in many publications, for example the cytostatica cisplatin [44], thymitaq [45], cyclic hydroxamates [46], and camptothecin [47], by the organophosphorus compounds parathion, tri-o-tolylphoshate, and triphenylphosphit [48], and by areca nut and arecoline [49]. Furthermore, oxidized low density lipoprotein (LDL) [50], overexpression of transglutaminase [51], and stimulation of peripheral blood lymphocytes with IL-3, IL-10, TGF-beta(1), IFN-gamma or co-stimulation with CD3/CD28 [52] lead to transient hyperpolarization of $\Delta\Psi_m$. Finally, general stress factors like H_2O_2 [53], alkalosis (high pH in the medium) [54], irradiation with a Helium-Neon laser [55], and gamma-irradiation [56] caused a transient hyperpolarization of $\Delta\Psi_m$, followed by apoptosis. The mechanisms leading to hyperpolarization of $\Delta\Psi_m$ are unknown. We propose a decrease of $\Delta\Psi_m$ due to abolition of the allosteric ATP-inhibition of cytochrome *c* oxidase, either directly via binding of some of the above affectors (similarly as observed for 3,5-diiodo-L-thyronine and palmitate) or indirectly by dephosphorylation of cytochrome *c* oxidase via a calcium-activated protein phosphatase. While control of respiration by the ATP/ADP ratio via phosphorylated cytochrome *c* oxidase is proposed to keep $\Delta\Psi_m$ low, the dephosphorylation of the enzyme causes an increase in respiration, because now proton pump activity is only limited by high values of $\Delta\Psi_m$ (when substrate supply is high and $\Delta\Psi_m$ consumption low).

4.2. Apoptosis via calcium-activated increase of $\Delta\Psi_m$

Induction of apoptosis in Jurkat T-cells by the common fungicide tributyltin was preceded by an immediate increase of cytosolic [Ca^{2+}] accompanied by hyperpolarization of $\Delta\Psi_m$ (maximal at 1 min after addition), followed by loss of $\Delta\Psi_m$, release of mitochondrial cytochrome *c* and activation of type 2 caspases [57]. During ischemia in heart and brain, ATP is synthesized by glycolysis instead of oxidative phosphorylation. The accumulation of lactic acid and decrease of pH result in inhibition of calcium pumps and increase of cytosolic [Ca^{2+}] [58]. The glutamate cytotoxicity in neurons is based on apoptosis via increased [Ca^{2+}] and mitochondrial ROS production [59]. A decrease of $\Delta\Psi_m$ by mitochondrial uncouplers prevented ROS formation and neuronal apoptosis [59,60], indicating that hyperpolarization of $\Delta\Psi_m$ causes ROS production. Recently, $\Delta\Psi_m$ was measured in hippocampal neurons after ischemia−reperfusion. Ischemia for 30 min decreased $\Delta\Psi_m$, but $\Delta\Psi_m$ increased after reoxygenation to hyperpolarizing values (200%). After prolonged ischemia for 90 min, $\Delta\Psi_m$ values remained low and did not recover [61]. We hypothesize that calcium accumulation during ischemia leads to dephosphorylation of cytochrome *c* oxidase. After reperfusion, $\Delta\Psi_m$ increases to hyperpolarizing values and ROS are produced which, depending on the amount, initiate apoptosis. Prolonged ischemia leads to irreversible damage of mitochondria (e.g. opening of the permeability transition pore [41]) and cell death.

The ischemia−reperfusion injury in heart is generally believed to be related to oxidative stress during reoxygenation, which finally could lead to apoptotic cell death [62]. ROS formation in the heart might also involve transient increase of $\Delta\Psi_m$. Diazoxide, a mitochondrial K_{ATP} channel opener, was found to prevent ATP depletion, loss of cytochrome *c*, and stabilized $\Delta\Psi_m$ after ischemia−reperfusion of cultured myocytes [63]. Recently, the decrease of ROS production in isolated mitochondria from heart, brain and liver through opening of the K(ATP) channel by

diazoxide (with consequent decrease of $\Delta\Psi_m$ [64]) was demonstrated [65]. In isolated mitochondria, cytochrome *c* oxidase is usually dephosphorylated [24], resulting in high $\Delta\Psi_m$ and increased ROS formation. Thus, the protective effect of diazoxide on ischemia–reperfusion injury could be partly explained by decreasing highly elevated $\Delta\Psi_m$ values through influx of K^+ into mitochondria via the K_{ATP} channel.

4.3. Thyroid hormones

Brain mitochondria from hypothyroid rats showed a lower mitochondrial membrane potential $\Delta\Psi_m$ than those of euthyroid rats [66], and hypothyroidism caused a decrease of H_2O_2 formation in rat heart mitochondria [67]. Thyroid hormones induced the formation of ROS and subsequent apoptosis in cultured human lymphocytes, and lymphocytes from patients with Graves' disease (hyperthyroidism) exhibited spontaneous apoptosis [68]. After heart ischemia–reperfusion, the rate and amount of H_2O_2 formation as well as the concentration of antioxidants were greater in rats of hyperthyroid compared to euthyroid rats [69]. Thyroid hormones increased the "oxidative stress" in mouse heart as observed by peroxidation of lipids and modification of mitochondrial DNA [70]. All these data could be explained by the abolishing effect of thyroid hormones on the allosteric ATP-inhibition of cytochrome *c* oxidase [25], inducing consequently an increase of $\Delta\Psi_m$, which is accompanied by formation of ROS [34,35].

4.4. Palmitate induces apoptosis

Accumulation of long-chain fatty acids in the heart has been proposed to play a role in the development of heart failure and diabetic cardiopathy. It is also believed that free fatty acids contribute to the pathogenesis of type 2 diabetes in humans. In particular palmitate was shown to induce apoptosis of cardiomyocytes [71], CHO cells [72], rat pancreatic islet cells [73], microvascular cells, and retinal pericytes [74]. Palmitate-induced programmed cell death could occur by two different pathways: one is associated with the production of ceramide and independent of ROS formation [75]. The second pathway involves the production of ROS in mitochondria [74], and could be prevented by radical scavengers [72]. In embryonic chicken cardiomyocytes, apoptosis was induced by palmitate but not by oleate [76]. From ROS production, induced by palmitate in microvascular cells and retinal pericytes from patients with diabetes, it was concluded that ROS are involved in the development of diabetic retinopathy [74]. The induction of apoptosis in beta-islet cells of rat pancreas by both, palmitate (+oleate) or by high glucose (17 mM), was related to oxidative stress [73]. These results are in accordance with the abolition of allosteric ATP-inhibition of cytochrome *c* oxidase by palmitate, resulting in increase of $\Delta\Psi_m$ and ROS formation.

4.5. High glucose-induced apoptosis in neurons

The association between oxidative stress and apoptosis is important in the pathogenesis of diabetes-specific microvascular diseases [77] as well as neurodegenerative diseases, such as Alzheimer's disease, Huntington's disease, muscular dystrophies, and in models of cerebral ischemia. Also diabetic neuropathy is characterized by loss of neurons. The process of apoptosis in primary dorsal root ganglion neurons by high concentrations of glucose (45 mM) was preceded by an intermediary hyperpolarization of $\Delta\Psi_m$, followed by depolarisation, ROS formation and activation of caspase-3 and -9; and these effects could be prevented by respiratory chain inhibitors (myxothiazol, thenoyltrifluoroacetone) [78]. Similarly, in human neuronal cell line SH-SY5Y, rat sensory neurons, and Schwann cells the induction of ROS production by 20 mM glucose was preceded by hyperpolarization of $\Delta\Psi_m$ [79].

In almost all regions of the brain, the presence of fatty acid synthetase was demonstrated histochemically, which, in contrast to liver, is not decreased during starvation [80]. The final product of fatty acid synthetase in animals is free palmitate, in contrast to palmitoyl-CoA, the end product of fatty acid synthesis in bacteria and plants. In addition to its main function as energy-rich fuel, palmitate could represent a second messenger of energy metabolism in animals.

We postulate that high glucose concentrations stimulate fatty acid synthesis as a consequence of increased acetyl-CoA and citrate levels in neurons, resulting in increased concentrations of free palmitate, which is not consumed as energy fuel in neurons. Free palmitate could turn off the allosteric ATP-inhibition of cytochrome *c* oxidase, leading to increased $\Delta\Psi_m$ values, the production of ROS, and induction of apoptosis. Increased ROS production as a consequence of elevated $\Delta\Psi_m$ values is generally accepted to be a cause of diabetes-specific microvascular diseases, but our view differs from that of Brownlee [77], who suggests overproduction of electron donors by the TCA cycle being the cause of increased $\Delta\Psi_m$.

4.6. p53-induced apoptosis

The tumor suppressor p53 stimulates the transcription of pro-apoptotic genes in certain cell types [81], and was shown to translocate to the mitochondria of apoptosing cells [82]. Overexpression of p53 in HeLa cells induced apoptotic cell death. The signal cascade involved first increased $O_2^{-\cdot}$ production, followed by hyperpolarization of $\Delta\Psi_m$ and increased H_2O_2 levels, and subsequent decrease of $\Delta\Psi_m$. p53-induced apoptosis was not associated with cytochrome *c* release, but involved caspase activation and up-regulation of Bax. Overexpression of Bcl-2 prevented the increase of $\Delta\Psi_m$ but not the increase of $O_2^{-\cdot}$ and H_2O_2 [83]. In addition, the induction of apoptosis by camptothecin or hypoxic stress in different cells was accompanied by translocation of p53 to mitochondria, and by an early increase of

$\Delta\Psi_m$ followed by its decrease [84]. The production of $O_2^{-\cdot}$ preceding the increase of $\Delta\Psi_m$ in HeLa cells [83] could indicate two different ROS signals during induction of apoptosis, one of which could originate outside of mitochondria (e.g. from plasma membrane NADH oxidase [33]) and initiates apoptosis, whereas the other originates from mitochondria as a consequence of $\Delta\Psi_m$ hyperpolarization.

4.7. Fas-induced apoptosis

The delivery of signals through the cell surface death receptor Fas or other receptors of the TNF (tumor necrosis factor) family has emerged as a major pathway in the elimination of unwanted cells under physiological and disease conditions [85]. In human T-cell leukemia cells (Jurkat cells) and peripheral blood lymphocytes, induction of apoptosis by anti-Fas antibody was preceded by $\Delta\Psi_m$ hyperpolarization and formation of ROS, which occurred before externalization of phosphatidylserine in the plasma membrane, activation of caspases, depolarisation of $\Delta\Psi_m$, and cell death [86]. In addition, anti-Fas antibodies caused a release of endogenous NO from Jurkat cells in sufficient amounts to inhibit cell respiration (cytochrome *c* oxidase activity). As shown in Ref. [87], the increase of $\Delta\Psi_m$ is generated from glycolytic ATP by reversal of the F_1F_0-ATPase reaction. Rat cortical neurons and astrocytes respond differently upon NO generated from its donor DETA-NO. Astrocytes show an inhibition of cell respiration, a subsequent increase in the rate of glycolysis as well as a persistent mitochondrial hyperpolarization without exhibiting apoptotic cell death, while neurons undergo mitochondrial depolarization with increased apoptosis. The increased $\Delta\Psi_m$ in astrocytes was prevented by inhibitors of the F_1F_0-ATPase and the adenine nucleotide carrier, or by glucose deprivation [88], indicating that hyperpolarization of $\Delta\Psi_m$ was based on hydrolysis of glycolytic ATP.

Why is $\Delta\Psi_m$ generated from glycolytic ATP higher than that produced by mitochondrial oxidative phosphorylation? After inhibition of cytochrome *c* oxidase by NO, subsequent generation of $\Delta\Psi_m$ is produced by the exchange of glycolytic ATP^{4-} for mitochondrial ADP^{3-} and reversal of the F_1F_0-ATPase reaction. We propose that $\Delta\Psi_m$, no longer controlled by allosteric ATP-inhibition of cytochrome *c* oxidase, is limited only by the proton permeability of the membrane, which increases exponentially above 140 mV [89]. Therefore, at high glycolytic ATP/ADP ratios elevated $\Delta\Psi_m$ values are produced, corresponding to the high values obtained in isolated mitochondria, where the allosteric ATP-inhibition is turned off [21].

4.8. Cancer cells

Cancer cells are generally more active than normal cells in metabolic ROS generation and are constantly under oxidative stress (for review see Ref. [90]). In addition, mitochondrial hyperpolarization is a shared feature of many tumor cell lines [91]. One explanation for the increased $\Delta\Psi_m$ in tumor cells could be the frequent occurrence of somatic mutations in mitochondrial DNA (for review see Ref. [92]). A defective synthesis of one of the thirteen mtDNA-encoded protein subunits of the four mitochondrial proton pumps of oxidative phosphorylation could impair mitochondrial ATP synthesis with consequently increased glycolytic ATP production. Similarly, in solid tumors the lack of oxygen supply could stimulate glycolytic ATP production. The glycolytic ATP would create $\Delta\Psi_m$ via reversal of F_1F_0-ATPase but without feedback control by the allosteric ATP-inhibition of cytochrome *c* oxidase, resulting in increased $\Delta\Psi_m$-values. This mechanism of $\Delta\Psi_m$ increase, however, depends on additional factors, such as sufficient glycolytic ATP production, and higher mitochondrial matrix pH to prevent binding of IF_1, the F_1F_0-ATP synthase inhibitor protein, which binds to F_1 at low pH thereby preventing ATP hydrolysis. It could be envisioned that the nuclear-encoded components of the respiratory chain complexes could still allow electron transfer from NADH via ubisemiquinone to molecular oxygen thus forming the superoxide radical anion $O_2^{-\cdot}$. An ‘‘injury’’ of the respiratory machinery, resulting in compensatory increases of glycolytic ATP production, was the basis of Warburg’s [93] hypothesis on cancerogenesis originally proposed 70 years ago.

Another explanation for increased $\Delta\Psi_m$ values in cancer cells could be turning off the allosteric ATP-inhibition by calcium-activated dephosphorylation of cytochrome *c* oxidase. Wojczak et al. [94] observed in Ehrlich ascites tumor and Zajdela hepatoma cells an increase of cytosolic and mitochondrial $[Ca^{2+}]$ by glucose and deoxyglucose, paralleled by hyperpolarization of $\Delta\Psi_m$ and increased reduction state of nicotine nucleotides. The authors suggested, however, that the inhibition of the F_1F_0-ATPase by calcium, which is more pronounced in tumor cells than in normal cells, caused the stimulation of $\Delta\Psi_m$ in tumor cells, in addition to stimulation of $\Delta\Psi_m$ by the activation of mitochondrial dehydrogenases by calcium [95].

5. Conclusion

Our review of the literature revealed no clear explanation for the hyperpolarization of $\Delta\Psi_m$ after induction of apoptosis by various parameters in different cell types, followed by ROS production. We hypothesize that preventing the allosteric ATP-inhibition of cytochrome *c* oxidase (by either calcium-activated dephosphorylation, 3,5-diiodo-L-thyronin or palmitate) may contribute to hyperpolarization of $\Delta\Psi_m$. Alternatively, the generation of high $\Delta\Psi_m$ values from glycolytic ATP when the respiratory chain is blocked could also explain some of the above observations. The reversible switching of the control of respiration either by the ATP/ADP ratio or by $\Delta\Psi_m$, resulting in $\Delta\Psi_m$ values

of 80–140 or 140–200 mV, respectively, could represent a general principle of the control of energy metabolism in animal cells. This hypothesis implies a reversible change from a resting to an excited state of energy metabolism with a higher rate of ATP synthesis (Fig. 1), but at the expense of oxidative stress [21,26–29]. However, the proposed mechanism for the control of $\Delta\Psi_m$ has not been tested in intact cells so far. Therefore, the aim of this paper is to encourage new investigations on the possible role of the allosteric ATP-inhibition of cytochrome *c* oxidase in the control of $\Delta\Psi_m$ in vivo, and thus in the progression of apoptosis.

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