CHAPTER 1

INTRODUCTION – CREATINE: CHEAP ERGOGENIC SUPPLEMENT WITH GREAT POTENTIAL FOR HEALTH AND DISEASE

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1. THE BOOK

The appearance in print of the present volume of the "Subcellular Biochemistry" Series entitled "Creatine and Creatine Kinase in Health and Disease", edited by Gajja S. Salomons and Markus Wyss, seems entirely timely. The importance and physiological significance of creatine kinase (CK) as well as the pleiotropic effects of creatine (Cr) and phosphocreatine (PCr) in health and disease have been largely underappreciated historically. Based on new discoveries in recent years, however, they are currently attracting much interest and even experience center stage attention, for instance with the recently announced large clinical Cr study with Parkinson's patients in the USA (Couzin, 2007). The comprehensive earlier review on "Creatine and Creatinine Metabolism" by Markus Wyss and Kaddurah-Daouk (2000), as well as the most recent review on Cr by John and Margaret Brosnan (2007), give a pre-taste of this new and exciting era of CK- and Cr-related research to come.

The volume presented here, as well as a new book on "Molecular Systems Bioenergetics: Energy for Life, Basic Principles, Organization and Dynamics of Cellular Energetics", edited by Valdur A. Saks (2007), provide a comprehensive overview of the field, with emphasis on complementary facets of this broad topic. The Saks book focuses on new data and theories derived mainly from basic science, taking a holistic systems biology approach to explain CK and related phosphotransfer systems, including mathematical modeling of metabolic and signaling networks related to bioenergetics. Conversely, the present volume links the basics of the CK

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system and of Cr action to their practical relevance for health, sports, rehabilitation, neuromuscular and neurodegenerative diseases, as well as for patients suffering from Cr deficiency syndromes. The discovery of the latter syndromes, the clinical manifestation of which is described here in detail, is living proof that CK and Cr are indeed essential for normal body function, especially for brain development and mental performance. The bridge from basic science – unravelling the structure and physiological function(s) of the CK isoenzymes and their substrate Cr in cells with high and fluctuating energy turnover (chapters 1–3; this chapter; Ellington and Suzuki, 2007; Saks *et al.*, 2007) – to clinical applications – including the power of Cr supplementation to positively influence human health (chapters 10–12; Tarnopolsky, 2007; Klein and Ferrante, 2007; Hespel and Derave, 2007) and actually prevent clinical symptoms in patients with certain forms of inherited Cr deficiency syndromes if treated early in life (chapters 8 and 9; Stockler *et al.*, 2007; Schulze and Battini, 2007) – is extremely rewarding, not to speak of the potential socioeconomic and health benefits of such cheap intervention which, apparently, lacks any serious side effects (chapter 14; Persky and Rawson, 2007).

In the present book, after a short excursion into the basics of CK evolution (chapter 2; Ellington and Suzuki, 2007) and the thermodynamics and modeling aspects of the CK phosphotransfer network (chapter 3; Saks *et al.*, 2007), topics on Cr synthesis, trafficking and metabolism in brain and across the blood-brain and blood-retina barriers are discussed in detail in chapters 4 and 5 (Braissant *et al.*, 2007; Tachikawa *et al.*, 2007), followed by new insights into the function of the Cr transporter (CRT) that is responsible for specific uptake of Cr into target cells (chapter 6; Christie, 2007). Then, the physiological consequences of CK and guanidinoacetate methyltransferase (GAMT) gene knock-outs in transgenic mouse models (chapter 7; Heerschap *et al.*, 2007), the pathophysiology and treatment of human Cr deficiency syndromes (chapters 8 and 9; Stockler *et al.*, 2007; Schulze and Battini, 2007), as well as the clinical use of Cr in neuromuscular and neurometabolic disorders (chapter 10; Tarnopolsky, 2007) are discussed, followed by a general presentation of the neuroprotective role of Cr (chapter 11; Klein and Ferrante, 2007). After these chapters, more applied topics, such as the ergogenic effects of Cr in sports and rehabilitation (chapter 12; Hespel and Derave, 2007), the pharmacokinetics of Cr (chapter 13; McCall and Persky, 2007), the safety of Cr supplementation in general (chapter 14; Persky and Rawson, 2007), as well as aspects concerned with chemical synthesis, purity and regulatory status of Cr as a nutritional supplement (chapter 15; Pischel and Gastner, 2007) are presented. The book concludes with an outlook on some promising future avenues of Cr-related research (chapter 16; Wyss *et al.*, 2007).

This volume truly reflects the present state of the art on CK and Cr, written by international experts with experience and recognition in the field. The present set of chapters represents a balanced mix from basic science and applied research to sport, rehabilitation and clinical subjects and thus makes good reading for CK and Cr specialists, but also for educated laymen and persons with a strong interest in Cr-related topics. This volume will serve as a solid basis for a new and exciting

wave of innovative Cr research, which among others will encompass systems biology approaches, including proteomics and phospho-proteomics, to elucidate the pleiotropic effects of Cr supplementation on the cellular and whole-body level, with a great potential for unexpected novel findings concerning the multiple facets of the CK system, as well as of Cr supplementation. The fact that many decades of basic research on the functions of CK and Cr have finally led to practical applications for human health and disease, as well as to clinical treatments of patients, is rewarding both for basic scientists and clinicians. Thus, the present book makes good reading also from the perspective of science history and idea development.

2. HISTORICAL ASPECTS OF CREATINE KINASE AND CREATINE

2.1. Functions of Creatine Kinase and Phosphocreatine in Muscle Contraction

Historically, over the last century, the CK and Cr field went through alternating periods of excitement and depression, concerning new ideas and concepts of CK/PCr/Cr function, particularly in the area of muscle biochemistry. New discoveries related to the "true" function of PCr, the "energy-rich" version of Cr that had been discovered in 1927 simultaneously by Eggleton and Eggleton and by Fiske and Subbarow, met with great enthusiasm. For instance, the idea that PCr would represent the long sought-for "immediate" source of energy for muscle contraction, as suggested by the findings of Lundsgaard in 1930, led to the abolition of Otto Meyerhof's "lactate theory of muscle contraction". However, this excitement started to slowly wane after Lohmann and Lehmann discovered the CK reaction in the mid 1930ies, indicating that PCr could only be used as energy source for muscle contraction in the presence of adenine nucleotides, and in particular ADP. This view was supported in 1939 by the finding of Engelhardt and Lyubimova that myosin possesses ATPase activity, and in 1941 by the proof of the Nobel laureate Albert Szent-Györgyi that muscle contraction *in vitro* was inevitably associated with ATP utilization. However, this latter fact could never be confirmed in contracting intact muscle *in vivo*, but rather, as in the Lundsgaard experiment, contraction was always paralleled by a depletion in PCr. Thus, a long-lasting controversy was stirring up the muscle research community for many years to come, until Cain and Davies, who in 1962 used 2,4-dinitro-1-fluorobenzene (DNFB) to specifically inhibit the CK reaction in muscle, finally managed to prove that under these conditions, it was indeed MgATP, and not PCr, that was used as the immediate source of energy for muscle contraction *in vivo*. This data proved that PCr, the substrate of the CK reaction in conjunction with ADP, is used to regenerate the ATP hydrolyzed by the Ca^{2+}-regulated Mg^{2+}-dependent acto-myosin ATPase. This then concluded the "phosphocreatine theory" of muscle contraction, and the CK system got a rather dismal stamp for representing a trivial, simple, homogeneously distributed cellular ATP-buffering system. Accordingly, the muscle

research community placed its focus fully on questions on how exactly ATP was used for muscle contraction and cell motility, taking for granted its replenishment for these processes (for reviews on these historical events, including a list of original publications, see (Lipmann, 1977, 1979; Mommaerts, 1969; Rapoport, 1977).

2.2. Development of the PCr-shuttle or CK/PCr-circuit Concept

By contrast, the PCr-shuttle or CK/PCr circuit concept that was developed in the 1970ies (see below) initially faced rather vicious scepticism that persisted for a lengthy period of time (Wallimann, 1996; Wiseman and Kushmerick, 1995). Over the years, however, this opposition seemed to slowly fade away (Chance *et al.*, 2006). The physiological concept of the PCr-shuttle or CK/PCr-circuit (for reviews, see Bessman and Carpenter, 1985; Bessman and Geiger, 1981; Saks *et al.*, 1978; Wallimann and Hemmer, 1994; Wallimann *et al.*, 1992, 2007) has followed the general destiny of many discoveries in science: first, it must not be true and therefore cannot be true; second, it may be true, but it is not important; and third, it is generally accepted but mostly trivial knowledge already around for years. It seems, however, that time has come that this concept reaches the "accepted" if not "generally accepted" status, since nowadays, it is discussed and visualized as back-up support figures without the need for citation, as for example in a recent review on "The failing heart – an engine out of fuel" (Neubauer, 2007).

As already mentioned, CK was considered a strictly soluble metabolic enzyme just for trivial buffering of cellular ATP levels according to its equilibrium constant (Meyer *et al.*, 1984). The facts that (i) CK existed as several tissue-specific and developmentally regulated cytosolic isoforms (Eppenberger *et al.*, 1964, 1967), that (ii) a CK isoform was identified that is located within mitochondria (Jacobs *et al.*, 1964; Jacobus and Lehninger, 1973), and that (iii) a small but significant fraction of soluble muscle-type MM-CK was shown to bind specifically to the sarcomeric M-band of skeletal muscle (Turner *et al.*, 1973; Wallimann, 1975), to the sarcoplasmic reticulum (SR) (Rossi *et al.*, 1990), as well as to the plasma membrane (Saks and Kupriyanov, 1982; for a recent review, see Wallimann *et al.*, 2007), were met with some resistance by the muscle research community. It was said that a soluble enzyme cannot be compartmentalized in a cell, that neither mitochondria nor myofibrils would depend functionally on CK bound at these locations, and – above all – that myofibrillar CK would certainly not play a structural role in the M-line architecture as proposed by us (Wallimann, 1983). As a matter of fact, the first notion of the existence of a PCr-shuttle in muscle, put forward by Martin Klingenberg and colleagues in 1964 (Jacobs *et al.*, 1964), was based on the identification of an unique mitochondrial CK (mtCK) isoform, while the evidence for myofibrillar-bound CK was only incidental. These rather intuitive ideas concerning a PCr-shuttle were further developed by Naegle *et al.* (1964), Bessman and Fonyo (1966), Scholte (1973) and Jacobus and Lehninger (1973) into an "acceptor control concept". According to this concept, extra-mitochondrial creatine is able to stimulate mitochondrial respiration, with the

stimulation being mediated by mtCK. In other words, mitochondria are able to produce PCr as high-energy phosphate output by a process called "Cr-stimulated respiration" (Dolder *et al.*, 2003). With the unambiguous demonstration that a fraction of "cytosolic" muscle-type MM-CK is bound to the sarcomeric M-band, a more complete picture of the PCr-shuttle concept slowly emerged (Bessman and Geiger, 1981; Saks *et al.*, 1978; Schlattner *et al.*, 2006; Wallimann 1975; Wallimann *et al.*, 1977, 1992, 2007; Wegmann *et al.*, 1992; Wyss *et al.*, 1992; see also chapter 3; Saks *et al.*, 2007). Now, more than thirty years after these historical findings, many excellent publications from independent laboratories have corroborated (i) the concept of microcompartmentation of the CK isoenzymes, (ii) directional flux and transport of high-energy phosphates within a cell, (iii) functional coupling of mtCK to the adenine nucleotide translocase (ANT) of the inner and the voltage-gated anion carrier (VDAC = porin) of the outer mitochondrial membrane, thereby forming a mitochondrial energy channeling unit (Schlattner *et al.*, 2006; Wallimann *et al.*, 1998; see also chapter 3; Saks *et al.*, 2007), and (iv) the physiological function of the CK/PCr system in alleviating the diffusional limitations of adenine nucleotides, especially in polar cells such as spermatozoa (Kaldis *et al.*, 1997; Tombes and Shapiro, 1985), photoreceptor cells of the retina (Hemmer *et al.*, 1993) or inner ear sensory hair bundle cells (Shin *et al.*, 2007). The PCr-shuttle concept was also helped by integrating structural and functional aspects of the aesthetically rewarding three-dimensional structure of mitochondrial mtCK (Fritz-Wolf *et al.*, 1996; Schlattner *et al.*, 1998) and by experiments showing that muscle-type MM-CK was specifically anchored to the M-band by two lysine-charge clamps that are symmetrically exposed on the MM-CK dimer to make contact with the M-band proteins, myomesin and M-protein (Hornemann *et al.*, 2003; Wallimann *et al.*, 2007). It is important to note that the octameric structure of mtCK has been "invented" very early in evolution; that is, mtCK octamers are found already in certain species of sponges (Hoffman and Ellington, 2005; Hoffman *et al.*, 2006; see also chapter 2; Ellington and Suzuki, 2007), thus reinforcing the eminent importance of this octameric enzyme for cell and organ function.

2.3. Phenotypes of Creatine Kinase Knockout Mice

If CK, Cr and the PCr-shuttle were so important, how should we explain that the various CK isoenzyme knock-out mice are not lethal and show, at first glance, only relatively mild phenotypes (Steeghs *et al.*, 1995; see also chapter 7; Heerschap *et al.*, 2007)? As a matter of fact, these CK isoenzyme knock-out mice are around now for more than 10 years, and each year, by virtue of more in-depth studies and by using more sophisticated methods, some new severe and rather interesting impairments of normal cell and organ function are being discovered. The latest addition to this list is the finding that cytosolic brain-type BB-CK and ubiquitous mtCK double knock-out animals show significant impairment in inner ear physiology, exhibiting a great reduction in hearing threshold and abnormal tympanic function for body balance (Shin *et al.*, 2007). This is an interesting lesson for mouse geneticists, who

initially thought that the severity of phenotypes, after knocking out a given gene, would be proportional to the "importance" of this gene. Fact is, however, that the principle successfully used in the aviation industry, namely that the most important functionalities have to be backed-up by several independent alternative mechanisms, also seems to apply to biological systems. In other words, it became obvious that tampering with the CK system leads to a host of compensatory events that can be uncovered when analyzing these transgenic animals with appropriate diligence. For example, an amazing proliferation of mitochondria is seen in glycolytic fast-twitch fibers of CK knock-out animals that makes them look like insect flight muscle fibers (Novotova *et al.*, 2006; van Deursen *et al.*, 1993; Ventura-Clapier *et al.*, 2004). This compensation mechanism represents an effective means of decreasing diffusion distances for ATP from mitochondria to myofibrils, since it is no longer PCr and Cr, but ATP and ADP, that have to be shuttled along this line in these muscle fibers. Such compensatory measures of cells and organs are teaching a great lesson, in particular on the plasticity of biological organisms with their remarkable ability to adapt to challenging situations. In addition, they can provide direct hints at the deleted gene's functions. Despite the above-mentioned back-up systems and compensation mechanisms, the phenotypes of some CK knock-out mice are severe enough to make their survival in the wild seem unlikely, due to impaired voluntary running capacity (Momken *et al.*, 2005), reduced cardiac performance at high work-load (Crozatier *et al.*, 2002), as well as a number of neuro-behavioral deficits (Jost *et al.*, 2002; Streijger *et al.*, 2004, 2005). In addition, double knock-out mice with a deletion in both the cytosolic BB-CK and the ubiquitous mtCK isoenzymes displayed severe problems to hold body temperature and to breed (own unpublished observation), which can be deadly in a harsh natural environment.

2.4. Discovery of Creatine and First Trials with Creatine Supplementation

The French scientist, Michel Eugène Chevreul, discovered Cr in 1832 as a new organic constituent that could be extracted from meat ("kreas" in Greek). In 1847, the German scientist, Justus von Liebig, chemically identified Cr as methyl-guanidino-acetic acid, a relatively simple guanidino compound. Today, we know that Cr is found in fresh meat and fish in concentrations ranging from 3 to 10 grams per kg wet weight. Justus von Liebig supported his laboratory largely by producing and selling meat broth, the famous Liebig's meat extract or Fleischbrühe in German, which contained about 8% Cr (Sulser, 1968). This was obviously the first attempt to bring Cr supplementation into the public domain with a small spin-off company, as one would call it today, with more such to come in the 1990ies for selling Cr to athletes. In the 1880ies, creatinine (Crn) was discovered and it was realized that this compound was likely the natural breakdown product of Cr. In 1926, Chanutin surmised, based on what was probably one of the first Cr supplementation trials in the history of mankind, that creatine is absorbed by the intestine and, thus, can be taken up rather quantitatively from alimentary sources such as fresh fish and

meat (Chanutin, 1926). In 1927, about a century from the discovery of Cr, PCr was discovered (Eggleton and Eggleton, Fiske and Subbarow). For a review on the historic aspects of Cr, see Conway and Clark (1996).

Unfortunately, during the next decades, Cr supplementation was followed-up only with low-key research at most. Reminiscent of this time is that some body builders and weight-lifters resorted, as it was told, to "sweated beef", a method to extract Cr from meat by hot steam, resulting in a highly Cr-enriched meat juice that was anecdotally said among the members of the "scene" to be beneficial for muscle growth and performance. Not to forget is the so-called "Jewish medicine", a concentrated chicken soup from a fresh chicken boiled to perfection, used as traditionally inherited panacea within the Jewish community that served to "cure" almost everything. Finally, worth mentioning in the same context is the ritual consumption by the new mother of the after-birth placenta, which is also rich in Cr, a tradition followed by many ancestral civilizations. Could one of the most active ingredients in these concoctions have been Cr? Nobody knows, but in hindsight and in light of the present knowledge on the pleiotropic effects of Cr supplementation, it could have been the decisive active principle.

Serious, double-blinded and placebo-controlled research with Cr supplementation was started in the early 1990ies only, and the seminal papers on "Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation" (Harris *et al.*, 1992) and on the "Influence of oral creatine supplementation on muscle torque during repeated bouts of maximal voluntary exercise in man" (Greenhaff *et al.*, 1993) initiated a big boost in Cr supplementation studies, mostly with athletes (chapter 12; Hespel and Derave, 2007). Interestingly, Roger Harris published his study in the very same year as Linford Christie (100 m dash) and Sally Gunnell (400 m hurdles) celebrated their Olympic victories, thereafter mentioning that they both ingested Cr. It seems that, once again, top athletes with the help of sports doctors did out-run the basic scientists.

Many of the several hundreds of publications from leading sports physiology laboratories around the world are clearly proving that Cr, in contrast to many other sports nutrition supplements, is a true ergogenic aid (chapter 12; Hespel and Derave, 2007). Nowadays, hundreds of thousands, if not millions of athletes of all proveniences are consuming Cr worldwide to boost physical performance, without any serious side effects, it seems (chapter 14; Persky and Rawson, 2007).

2.5. Human Patients with Cr Deficiency Syndromes

The ultimate proof that the CK system and Cr as such are essential for normal brain development and brain function came actually from the discovery of Cr deficiency patients (see chapters 8 and 9; Stockler *et al.*, 2007; Schulze and Battini, 2007). The findings in man were subsequently supported by a transgenic mouse model, where one of the Cr biosynthesis enzymes, guanidinoacetate methyltransferase (GAMT), had been ablated (Renema *et al.*, 2003; Schmidt *et al.*, 2004; see also chapter 7; Heerschap *et al.*, 2007). Patients which lack Cr in the brain, either

due to a defect in Cr biosynthesis (Schulze, 2003; Stöckler-Ipsiroglu, 1997; Verhoeven *et al.*, 2005) or in the Cr transporter (deGrauw *et al.*, 2003; Kleefstra *et al.*, 2005; Rosenberg *et al.*, 2004), present with mental retardation, speech- and language delay, epilepsy and autistic-like behaviour (for details, see the relevant chapters 8 and 9 in this book; Stockler *et al.*, 2007; Schulze and Battini, 2007). Patients with a defect in Cr biosynthesis can be treated with Cr supplementation in combination with dietary restriction and/or additional supplements. These treatments, if started early, may prevent the development of clinical symptoms (Bianchi *et al.*, 2007; Schulze, 2003; Verbruggen *et al.*, 2007). These new and clinically relevant discoveries, supporting (i) the importance of the CK system and of Cr for normal physiological function of the human body, most importantly the brain, and (ii) justifying CK and Cr-related research in the realm of rare human diseases, gave a new wave of recognition to the CK/Cr system.

3. PLEIOTROPIC EFFECTS OF CREATINE

Common denominators for many muscular, neuromuscular and neurodegenerative diseases are (i) lowered energy status, that is decreased cellular energy reserves (PCr and ATP), (ii) accompanying chronic calcium overload, due to a misbalance in the energetics of calcium homeostasis, and (iii) concomitant formation of reactive oxygen species (ROS) (Rodriguez *et al.*, 2007; Tarnopolsky and Beal, 2001). Incidentally, the most obvious phenotype of knockout mice lacking CK in muscle were difficulties with calcium sequestration and muscle relaxation (Steeghs *et al.*, 1997). Accordingly, Cr supplementation was shown to improve calcium homeostasis in the *mdx* muscular dystrophy mouse model (Pulido *et al.*, 1998) and muscle relaxation in humans (Hespel *et al.*, 2002). These findings are easily explainable by the fact that the calcium pump ATPase of the SR is energetically very demanding and only works efficiently if a high local ATP/ADP ratio is maintained by the action of CK functionally coupled to this very SR calcium pump (Wallimann and Hemmer, 1994; Wallimann *et al.*, 2007). Cr supplementation in general increases the PCr pool of cells, provided that ATP synthesis is not impaired and that sufficient free ATP is available to reload the PCr buffer (Vandenberghe *et al.*, 1997). Thus indirectly, through an improved energy state, Cr would stabilize cellular calcium homeostasis and thereby prevent prolonged calcium overload which is thought to be responsible for the initiation of apoptosis, necrosis (Kroemer *et al.*, 2007) or cell destruction in general (Passaquin *et al.*, 2002), due to the initiation of mitochondrial permeability transition pore opening or due to the action of calcium-activated proteases, respectively.

As evidence for the above, *in vitro* treatment of cells as well as dietary supplementation of animals with Cr are highly beneficial with regard to protection from injury and enhancement of survival following noxious treatment (Brewer and Wallimann, 2000; Brustovetsky *et al.*, 2001; Sullivan *et al.*, 2000). Since Cr supplementation leads to an increase in intracellular PCr levels, a higher PCr/ATP ratio (Vandenberghe *et al.*, 1997) and thereby to a higher buffering

capacity for intracellular ATP and ADP concentrations, one could explain at least part of the Cr-dependent protection of cells from various stressors, especially the neuroprotective effects of Cr (see chapters 8–11; Stockler *et al.*, 2007; Schulze and Battini, 2007; Tarnopolsky, 2007; Klein and Ferrante, 2007), by the classical beneficial effects of Cr in improving the cellular energy state. Cr supplementation mediates remarkable neuroprotection in experimental animal models of amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), Parkinson's disease, and traumatic brain and spinal cord injury (see chapters 10 and 11 herein; Tarnopolsky, 2007; Klein and Ferrante, 2007). Prophylactic Cr administration also mediates neuroprotection in cerebral ischemia in mice (Adcock *et al.*, 2002; Prass *et al.*, 2007; Zhu *et al.*, 2004). Transgenic mice expressing high levels of BB-CK in liver cells, which normally express only very low levels of this enzyme, show a high degree of resistance to tumour necrosis factor-induced and hypoxia-induced apoptosis of liver cells when supplemented with dietary creatine (Hatano *et al.*, 2004). Furthermore, Cr exhibits a protective effect against ROS in cultured mammalian cells (Sestili *et al.*, 2006) and against UV-induced oxidative stress in intact human skin (Berneburg *et al.*, 2005; Lenz *et al.*, 2005).

It is thus believed that the presence of the CK system improves the stress resistance of cells, making them less susceptible to injury. Congruent with the above discussion, three non-exclusive mechanisms of action underlying the protective effect of creatine are presently discussed: (1) The energy buffer and storage function of the CK/PCr system is thought to keep the ATP/ADP ratio high during situations of stress, which otherwise might lead to cellular dysfunction and, eventually, cell death. A high cellular energy state and high local ATP/ADP ratios are particularly critical for efficient calcium pump function (Minajeva *et al.*, 1996; Wallimann and Hemmer, 1994). Thus, compromised cellular energetics lead to a unbalance in calcium homeostasis and, in the long run, to chronic calcium overload. A convincing proof for the utmost importance of the CK system for cellular calcium homeostasis is the phenotype of mice with ablated CK genes (de Groof *et al.*, 2002) that show problems with muscle relaxation and calcium re-uptake into the SR. (2) Cr is thought to act in concert with mtCK in inhibiting mitochondrial permeability transition pore opening, an early trigger of apoptosis (Dolder *et al.*, 2003; O'Gorman *et al.*, 1997). MtCK, a cubical-shaped octamer with a central channel, was shown to form a multienzyme complex with porin of the outer mitochondrial membrane and adenine nucleotide translocase (ANT) of the inner membrane (Brdiczka *et al.*, 1998; Schlattner *et al.*, 2006). This complex crosslinks the two mitochondrial membranes and forms a functionally coupled microcompartment for vectorial export of PCr into the cytosol. Disruption of the octameric structure of mtCK leads to an impairment of energy homeostasis and facilitated transition pore opening (O'Gorman *et al.*, 1997), which eventually results in activation of caspase cell-death pathways (Kroemer *et al.*, 2007). (3) A coordinated action of mtCK activity with oxidative phosphorylation is facilitated by tight functional coupling of mtCK with ANT (Dolder *et al.*, 2003), leading to the well-known phenomenon termed "creatine-stimulated respiration" that can also be demonstrated *in vivo*

(Kay *et al.*, 2000). Under physiological conditions, when mitochondrial respiration is directly stimulated by Cr, endogenous intramitochondrial adenine nucleotides are recycled inside mitochondria, and PCr is leaving the mitochondria as the energy-rich end product of respiration (Dolder *et al.*, 2003). The addition of Cr to respiring mitochondria also induces an optimal coupling of the respiratory chain to ATP generation by the F1-ATPase. This leads to a significantly reduced production by the respiratory chain of ROS in mitochondria, compared to mitochondria without Cr. Thus, the accompanying deleterious consequences of mitochondrial ROS for cell damage are minimized by Cr, as has recently been demonstrated convincingly with brain mitochondria (Meyer *et al.*, 2006).

In addition to the above, Cr is released from cells after hypo-osmotic swelling (Bothwell *et al.*, 2001) and, conversely, serves as an osmolyte that is taken up by cells under hypertonic stress (Alfieri *et al.*, 2006). Recent evidence indicates that Cr is not only synthesized in the brain where it is trafficking back and forth between various neuronal cell types and being taken up by neurons (chapters 4 and 5; Braissant *et al.*, 2007; Tachikawa *et al.*, 2007), but that it can also be released from neurons in an action-potential-dependent way, e.g. upon excitation of neurons (Almeida *et al.*, 2006). This exocytotic release of Cr, together with its action as a partial $GABA_A$ receptor agonist (De Deyn and Macdonald, 1990), indicates that Cr may act as a neuromodulator. The actions of Cr as an osmolyte and a neuromodulator, as well as its direct or indirect effects as an anti-oxidant (Berneburg *et al.*, 2005; Lenz *et al.*, 2005; Sestili *et al.*, 2006), represent non-energy related functions of Cr that may contribute to the pleiotropic effects observed with Cr.

Systems biology approaches involving the study of global gene expression of Cr-substituted versus Cr-depleted cells and organs or whole animals are likely to reveal novel and unexpected effects of Cr. A particularly interesting topic for study will be the connection of PCr and Cr to cellular signaling networks and, in particular, the AMPK signaling cascade. Recent breakthroughs in phosphoproteomics (Bodenmiller *et al.*, 2007) should facilitate investigation of whether Cr supplementation stimulates phosphorylation of AMPK and, thereby, triggers the AMPK signaling cascade.

4. CONCLUSIONS

After a continuous up-hill battle for most who have been active in the CK- and Cr-related research field, to obtain grants and funding over all those years, I would never have dreamed that, one day, some of the ideas and concepts elaborated by basic laboratory scientists would come to fruition with respect to human health and disease and that they could, potentially, have significant health and socio-economic benefits. This is certainly rewarding, and this spirit, together with a positive outlook for an interesting future for CK- and Cr-related research ahead, prevails throughout the book, including the concluding chapter (chapter 16; Wyss *et al.*, 2007). Again, this book is important, for it represents a timely synopsis of past achievements and new developments in the field. Significant advances have

been made in deciphering some important aspects of the molecular structure and function of CK, the involvement of this enzyme in cellular energy distribution networks, and in revealing new and unexpected facts on the pleiotropic effects of Cr. These effects are important for normal cell function and can be exploited for practical application in health and disease. The reader can expect to learn much about the multifaceted role of CK and Cr in the context of cellular bioenergetics and will appreciate the broad spectrum of multidisciplinary approaches from molecular to cellular and finally to clinical Cr-related research.

Again, overall, this book makes a highly recommended reading for everybody interested in bioenergetics, energy homeostasis, as well as in theoretical and applied aspects of CK and Cr research, including the potential benefits of Cr supplementation for human health and disease. I can endorse the outlook given in the final chapter (chapter 16; Wyss *et al.*, 2007), where some interesting new aspects and possibilities concerning the future directions of Cr-related research are given, more appropriate Cr supplementation dosages are recommended, a list of clinical applications of Cr is provided, and potentially valuable new applications of Cr are presented for healthy people, both young and old. Thus, it is obvious that Cr has evolved from a simple "energy precursor metabolite" to a "true" ergogenic dietary supplement and, finally, to a promising cheap therapeutic agent with a potentially huge health and socio-economic impact. An exciting new era for the Cr field is conveyed not only for the Cr research community but also for the general reader of this book.

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