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Functional Aspects of Creatine Kinase in Brain

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Abstract
The distinct isoenzyme-specific localization of creatine kinase (CK) isoenzymes found recently in brain suggests an important function for CK in brain energetics and points to adaptation of the CK system to the special energy requirements of different neuronal and glial cell types. For example, the presence of brain-type B-CK in Bergmann glial cells and astrocytes is very likely related to the energy requirements for ion homeostasis (K^+-resorption) in the brain, as well as for metabolite and neurotransmitter trafficking between glial cells and neurons. In contrast, the presence of muscle-type M-CK, found exclusively in Purkinje neurons which also express other muscle-specific proteins, is very likely related to the unique calcium metabolism of these neurons. In addition, the developmentally late appearance of mitochondrial CK (Mi-CK) during brain development indicates an important function for Mi-CK in the oxidative energy metabolism of the brain. The physiological importance of the phosphocreatine circuit fully operating in adult brain has been corroborated by recent data from in vivo ^{31}P-NMR magnetization transfer measurements. Future investigations should concentrate on the possible involvement of CK in diseases of the CNS with altered energy metabolism, aspects of which are also discussed here.

The creatine kinase / phosphocreatine circuit for energy homeostasis

Creatine kinase (CK; ATP: Creatine N-phosphotransferase, EC 2.7.3.2), a key enzyme in the energy metabolism of cells with intermittently high and fluctuating energy requirements (e.g. skeletal and cardiac muscle, neural tissues like brain and retina, spermatozoa and electrocytes etc.) catalyzes the reversible transfer of the phosphoryl group from phosphocreatine (PCr) to ADP, to regenerate ATP. The enzyme is found in cytosolic (muscle-type CK, M-CK and brain-type CK, B-CK) [1] and mitochondrial [Mi_a-CK (ubiquitous) and Mi_b-CK (sarcomeric)] isoforms [2, 3]. The cytosolic and subcellularly associated CKs, together with the mitochondrial CK isoforms, constitute an intricate cellular energy buffering

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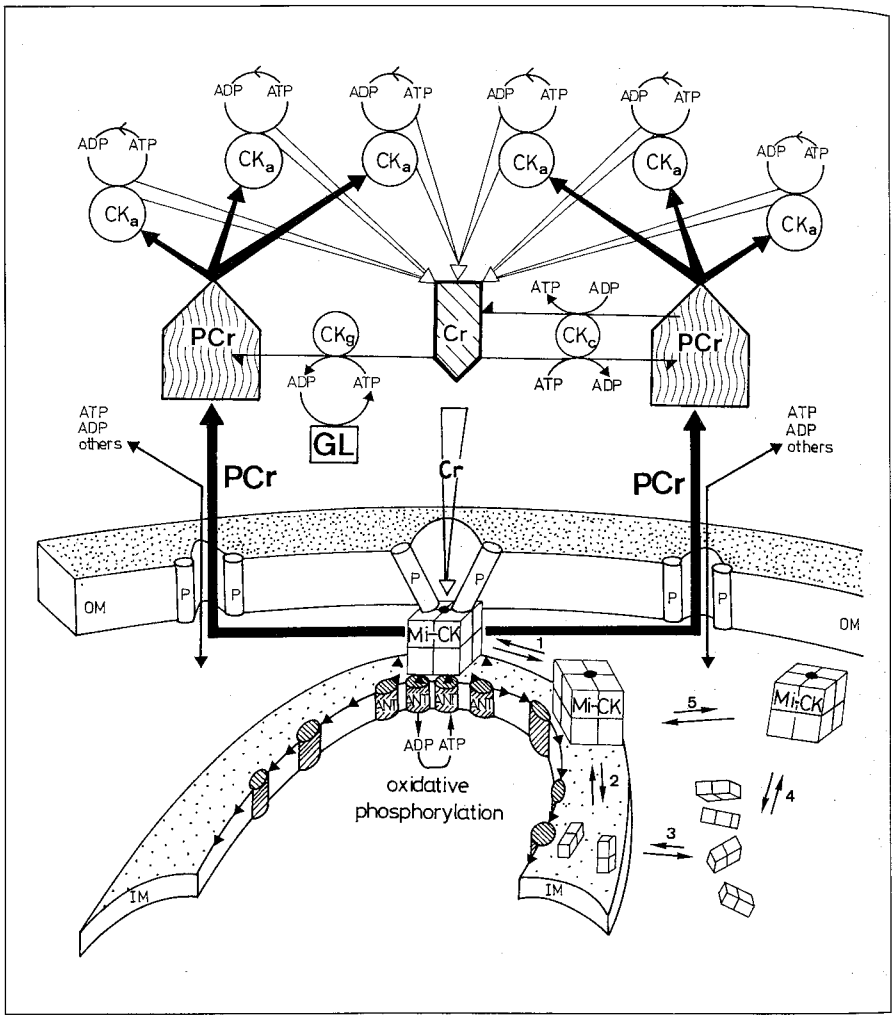

Fig. 1. The phospho-creatine circuit model for specialized cells with high and fluctuating energy metabolism. In a cell, ATP may be derived from two major synthetic pathways, that is, from oxidative phosphorylation and from glycogenolysis or glycolysis (GL). Four major compartments of CK are indicated: (1) strictly soluble CK (CK_c) freely equilibrating PCr/Cr and ATP/ADP ratios in the cytosol; (2) cytosolic CK functionally coupled to glycolysis (CK_g) [4, 23, 25–27] on the producing side of the PCr circuit; (3) Mi-CK being functionally coupled to oxidative phosphorylation [11–13]; (4) 'cytosolic' CK, specifically associated with subcellular structures (CK_a) at sites of high and fluctuating ATP requirements on the receiving end of the PCr circuit [for the different ATPases see 17, 19, 20, 42]. Note that in resting muscle for example, the relative pool sizes of [PCr] = approximately 20–40 m*M* and [Cr] = approximately 5–15 m*M* are much larger than those of [ATP] = approximately 3–5 m*M;* [ADP] = approximately 10–20 μ*M*. Also note that PCr and Cr are smaller and less charged molecules compared to the adenine nucleotides [for review see 4].

At the mitochondrial side, a cube-like mitochondrial creatine kinase Mi-CK octamer molecule with an internal channel [8], shown to interact with inner (IM) as well as outer mitochondrial membranes (OM), thus stabilizing contacts between IM and OM in vitro [10], is depicted in conjunction with the ATP/ADP translocator (ANT) of the IM, and with porin (P) of the OM, thus forming a multienzyme 'channel' [16] at the so-called 'mitochondrial energy transfer contact sites' [5]. The small black triangles (▲) in the IM and in association with ANTs represent cardiolipin molecules.

According to this model, ATP generated by oxidative phosphorylation, after transport through the IM by ANT in exchange for ADP, is transphosphorylated by Mi-CK to give PCr. A functional compartmentation between oxidative phosphorylation and CK, as well as between ANT and CK, has been demonstrated (see text). PCr, as a net product of oxidative phosphorylation, leaves the mitochondrion just beyond the contact sites through P of the OM in its high conductance, anion-selective state [for details see 5, 6]. Cr, on the other hand, is entering the contact sites through P of the OM within the contact sites, where the P channel is thought to be in its cation-selective state due to capacitive coupling of the IM and OM being in close apposition at these sites [5]. Possible regulatory aspects of Mi-CK in cellular energetics are depicted at the lower right: (1) the dynamic equilibrium of the enzyme, moving in and out of contact sites while bound to the IM (arrows, 1), the dynamic octamer/dimer equilibrium of the enzyme while bound to the IM (arrows, 2) or being in solution in the intermembrane space (arrows, 4), and (2) the differential pH-dependent association of the two oligomeric species of Mi-CK with the IM (3, dimers; 5, octamers; 2, octamerization of membrane-bound dimers on the IM), all observed in vitro [9], are indicated by numbers (1–5).

In this complex model, the CP/PCr system is proposed to be responsible for 'temporal' as well as 'spatial' energy buffering, with PCr not only representing an inert 'energy buffer' but also a 'transport form' of high-energy phosphates. Besides representing an ATP-buffering system and directing intracellular energy flux from sites of ATP generation to sites of ATP utilization by means of PCr and Cr, CK keeps intracellular [ADP] very low and thus prevents a net loss of adenine nucleotides. By speeding up 'communication' between sites of ATP production and ATP consumption, the PCr/CK system is also thought to accelerate and smoothen the transitions between different work states, that is, it may dampen oscillations in [ADP] and [ATP] and simultaneously reduce the transient times for reaching a new steady state at a given work load [6, 16]. In addition, the CK reaction (in the direction of ATP synthesis) removes protons produced by cellular ATPases and thus prevents intracellular acidification. Furthermore, inorganic phosphate (P_i) released as a consequence of PCr hydrolysis stimulates glycogenolysis, glycolysis and possibly also oxidative phosphorylation. Finally and most importantly, those fractions of CK which are functionally coupled to cellular ATPases (CK_a, at the figure top), such as the myofibrillar ATPase [23], to the sarcoplasmic

and transport system interconnecting intracellular sites of high-energy phosphate production, i.e. glycolysis and oxidative phosphorylation, with sites of energy consumption, e.g. ATPases and ion pumps via PCr and creatine (fig. 1) see below [for review see 4].

Mi-CK is located in the mitochondrial intermembrane space, where it is bound along the entire inner membrane, but also at peripheral sites where inner and outer membranes are in close proximity, that is at the contact sites [5–7]. There, Mi-CK can directly convert the intramitochondrially produced ATP to PCr, which then is exported into the cytosol where it serves at relatively high concentration (5–40 m*M*, depending on the tissue) as an easily diffusable energy storage and transport metabolite [for review see 4, 6]. Mi-CK, which in contrast to the dimeric cytosolic CK isoenzymes forms highly symmetrical, cube-like octameric structures [8, 9], has the specific ability to peripherally bind to lipid membranes and, most importantly, to mediate contact site formation between inner and outer mitochondrial membranes [10]. Moreover, Mi-CK is tightly functionally coupled to oxidative phosphorylation via the adenine nucleotide translocator (ANT) [11–13] which catalyses the antiport of ATP versus ADP through the inner membrane [14]. As a consequence, Mi-CK preferentially utilizes intramitochondrially produced ATP for PCr synthesis [12]. CK substrates and products also have to pass the outer mitochondrial membrane. Based on experiments showing that mitochondrial state 3 respiration can be effectively stimulated by creatine, leading to a net production of PCr by mitochondria [11–13], a functional coupling between Mi-CK and porin has also been postulated (see fig. 1) [5, for review see 4, 6]. Electron microscopy [8] and X-ray crystallography [15] have shown that the cube-like Mi-CK octamer contains a central channel running parallel to the 4-fold axis through the entire molecule. This structure/function relationship, together with the results discussed above, led to a hypothesis that Mi-CK could act as a connecting module between ANT and porin at the mitochondrial contact sites, thereby forming an efficient, tightly coupled multienzyme 'energy channel' that combines the export of mitochondrial energy equivalents with the interconversion of matrixgenerated ATP to PCr [16] (see fig. 1). Specific features of Mi-CK, e.g. a dynamic octamer/dimer equilibrium which is influenced by physiological parameters, as well as the differential pH-dependent interaction of Mi-CK octamers and dimers with mitochondrial membranes observed in vitro (fig. 1) [9, 10], may be important parameters for regulation of mitochondrial energetics.

The cytosolic CK isoforms, which are in part associated with subcellular structures, in turn utilize PCr to regenerate ATP at sites of high energy demand, e.g. at the sarcomeric M-band [17, 18], at the sarcoplasmic reticulum Ca^{2+} ATPase [19, 20] or in the outer segments of photoreceptor cells [21]. This presumably tightly regulated communication between mitochondrial and 'cytosolic' CK isoforms via creatine and PCr has been termed the 'phosphocreatine shuttle' or the 'PCr circuit (see fig. 1) [12, 13, 22, 23]. An important feature of this model is the 'spatial buffering', transport or shuttling function of the CK system [6]. The 'temporal' buffer function ascribed to the CK system serving mainly to keep [ATP] constant [24] is likely to be facilitated by the major portion of cytosolic CK. However, a significant portion of this cytosolic CK is also thought to be functionally coupled to glycolysis [4, 25–27].

Many of the above results have been obtained with muscle and muscle-type CKs as a model system, however, the brain isoenzyme B-CK [21, 28–31] as well as the brain-type mitochondrial CK [32] have also been extensively characterized. Thus, since CK isoenzymes, like in skeletal muscle and heart, are also abundant in brain, a similarly important function of this enzyme system for the energy homeostasis in the CNS can be inferred. Some

reticulum Ca^{2+} ATPase [19, 20] or to the Na^+/K^+ ATPase [42] increases the thermodynamic efficiency of these ATPases [4, 83, 84] by immediately rephosphorylating, via PCr, the ADP generated by the ATPases and thus maintaining very high local ATP/ADP ratios in the vicinity of these ATPases [for details see 4, 6].

According to such a model, in a cellular system performing work, only small pools of adenosine nucleotides (ATP and ADP) are turned over rapidly and in opposite direction at the producing and the receiving end of the PCr circuit. The latter sites are connected via CK isoenzymes specifically associated there. Accordingly, PCr and Cr, which are present at high concentrations, are acting as easily diffusible shuttle currencies. In most cells, ADP is normally not allowed to accumulate and therefore is kept at very low intracellular concentrations by CK and adenylate kinase. Thus, the problems of severe diffusion limitations of ADP can be overcome by the PCr circuit [4, 6, 22–24], since according to such a scheme, the 'transport of energy' is facilitated mostly via PCr. The model presented here stresses the functional coupling of ATP production with ATP utilization via CK and PCr, as well as the diffusional pathways of PCr and Cr. However, parallel pathways involving a direct transport of ATP may also operate at the same time. This model, originally developed for muscle, sperm and retina photoreceptor cells, as well as for electrocytes [for review and references see 4, 6] is very likely to be relevant also for brain energetics [40, 57, 88]. For complete reference list see recent reviews [4–6].

of the possible functions of the PCr/CK system are discussed with respect to new data concerning the localization of the different CK isoenzymes in brain, as well as recent in vivo ^{31}P-NMR measurements of brain function.

Energy Requirements in the Brain

ATP plays a fundamental role in brain function. Under normal conditions, it is synthesized almost exclusively by aerobic glycolysis involving mitochondrial oxidative phosphorylation, with only small contributions from anaerobic glycolysis [for review see 33]. The CK and adenylate kinase reactions operate efficiently in both neurons and glia to help maintain a constant ATP level. Even though the resting concentration of the high-energy phosphate metabolites in brain (2–2.5 m*M* in ATP; 4–6 m*M* in PCr [33]) do not appear to differ significantly between brain regions, the rates of ATP production and utilization vary widely throughout the brain. ATP consumption, is higher in grey versus white matter and is especially intense in regions rich in synaptic contacts such as the molecular layer of the cerebellum and the hippocampus; in parallel, rapid ATP synthesis is seen in these areas.

Such regional differences in energy metabolism have been observed in the brain by in vivo ^{31}P-NMR measurements, where the flux through the CK reaction in grey matter was higher by a factor of two compared to white matter [34]. Regional differences also exist between different cell types of the brain or even between different regions of a single cell, e.g. perikarion, dendrites, axonal endings etc. [35]. During stimulation of nerves, axon terminals rather than the neuronal cell bodies are the sites of enhanced metabolic activity [36]. In the brain, the maintenance of ionic gradients requires approximately 50–60% of the total O_2 consumed, i.e. some 12–16 µmol ATP/g wet weight min, of which the major fraction is used by the Na^+/K^+ ATPase [33]. The remaining 40–50% of energy consumption is used for activities such as protein and lipid biosynthesis, cell maintenance and repair, neurotransmitter metabolism including synthesis, packaging, transport and release, and for protein phosphorylation using up a sizable amount of energy in the brain [33]. A large part of the energy expenditure in the CNS occurs to restore ionic gradients altered during nerve excitation [37, 38]. The striking correlation between stimulation of energy metabolism and enhanced K^+ ion transport activity supports the concept that active regulation of the extracellular K^+ concentration has very high priority in the vertebrate brain [35]. It has been shown that astrocytes, representing the only major cell population in brain capable of utilizing fatty acids as a major energy substrate [39], display an intense oxidative metabolism; in contrast, isolated neurons express glycolytic activities. Therefore, since creatine kinase is thought to be crucial for the energy homeostasis in excitable tissues, such as the brain, and since the different CK isoenzymes, e.g. cytosolic as well as mitochondrial CK, have been shown to be connected to different energy producing pathways [4], it was of interest to investigate the in situ localization of these different CK isoenzymes in the CNS and to incorporate the results [40] (see below) into a functional concept with respect to the different energy requirements of the specialized cell types present in the brain. Many of the possible functions of CK in brain can be directly inferred by analogy, taking into account the experimentally well documented intimate functional coupling of CK to the myofibrillar actomyosin ATPase [17, 18, 23], to the Ca^{2+} ATPase of the sarcoplasmic reticulum [19, 20] or to the Na^+/K^+ ATPase in electrocytes of electric fish [41, 42].

Creatine Kinase in Brain

B-CK, the major 'cytosolic' CK isoenzyme present in brain [1], has been characterized extensively [21, 28–31, 43]. For chicken B-CK, a considerable heterogeneity was found, with two major B-type subunits and additional subspecies arising from alternative ribosomal initiation [44] and posttranslational modifications [21, 30, 43]. In the sixties, Jacobs et al. [45] and Swanson [46] reported on CK activity associated with brain mitochondria, which was later identified and characterized as genuine brain Mi-CK [32, 47, 48] representing the ubiquitous isoform Mi_a-CK [2, 3, 32]. Mi_a-CK, which was also characterized extensively [2, 3, 6, 32], was found to be localized preferentially as octamers in mitochondrial boundary membrane contact site fractions of brain mitochondria [49]. In addition, in several early studies the presence of muscle-type M-CK in brain was also postulated [for review see 40]. M-CK has indeed recently been demonstrated in postmortem human brain extracts by biochemical isolation and protein sequencing [50] and in chicken cerebellum by immunoprecipitation and immunoblotting [40].

Although the relative distribution of CK in different areas or cell types of the brain has been investigated in numerous studies, there is still no consistent and com-

plete overview of the in situ localization of CK isoenzymes in the brain. Regional variations in CK activity with comparably high levels in the cerebellum were reported in studies using native isoenzyme electrophoresis [51] or enzymatic CK activity measurements of either tissue extracts [52] or cultured brain cells [53]. In particular, the molecular layer of the cerebellar cortex contains high levels of CK activity [40, 52, 54]. In contrast, high levels of CK activity or messenger RNA levels of CK were shown in cultured oligodendrocytes [53, 55], representing a model for developing oligodendrocytes in vivo. In the adult, these typical glial cells are found predominantly in the white matter. Conflicting results have also been obtained using histochemical and immunohistochemical techniques; one report indicated that B-CK was present exclusively in astrocytes of human brain [56], whereas other research groups localized B-CK to both astrocytes and neurons, with a prominent CK content in large neurons of rat, human, gerbil and mouse brain [for review see 40]. Concerning the relatively small amounts of M-CK found in brain, the question arose of whether this atypical CK represented a contamination from vascular smooth muscle in the brain or whether this muscle M-CK isoform was indeed associated with neuronal cells. Having a carefully characterized set of highly specific antibodies against chicken CK isoenzymes at hand [40], we started to investigate the cellular distribution and localization of all chicken CK isoenzymes within the chicken brain. In addition, we investigated the localization, accumulation and developmental appearance of CK isoenzymes during maturation of the rat brain and correlated these data with in vivo ^{31}P-NMR CK reaction flux measurements [57].

Immunofluorescence Localization of CK Isoenzymes in Chicken Cerebellum

In this study, the cerebellar localization of CK isoenzymes, B-, M- and Mi_a-CK, was determined by conventional immunofluorescence microscopy, using different, highly isoenzyme-specific anti-CK antibodies (fig. 2). Anti-B-CK staining was found in all layers of the cerebellar cortex as well as in the deeper nuclei of the cerebellum, indicating that a high proportion of the cerebellar cell types contain B-CK. The labeling was most intense in Bergmann glial cells (BGC) (fig. 2a, b, small arrowheads in b point to BGC cell bodies). The processes of these cells, lying in the vicinity of Purkinje neurons (PN, large arrow), span radially through the entire molecular layer and finally form the membrana limitans [58] with their end-feet, which is also stained heavily (fig. 2a, arrowheads). Thus, the morphology of BGCs is perfectly matched by the intense anti-B-CK staining pattern (fig. 2a–c). Besides BGC, some other cell types in the molecular layer, such as basket cells and neurons in the deeper nuclei, contain B-CK [for details see 40]. Additionally, structures in the granule cell layer, likely to be glomeruli [59], as well as astrocytes in the granule cell layer contained significant anti-B-CK immunoreactivity (fig. 2a, b), whereas cerebellar white matter appears to contain rather low levels of B-CK. The latter finding is consistent with previous histochemical and ^{31}P-NMR data [34, 54].

Surprisingly, the 'cytosolic' muscle-type CK isoform, M-CK, was specifically and exclusively located in PNs (fig. 2d–f, see arrow in 1e); these cells were essentially unlabeled when brain sections were stained with anti-B-CK antibody (fig. 2b, large arrow). The distribution of M-CK within the PNs and their dendrites was nonuniform, with the proximal parts of Purkinje cell dendrites displaying the most anti-M-CK immunoreactivity. PNs were also slightly stained by the polyclonal anti-Mi_a-CK antibody (fig. 1h, large arrow), however, preliminary results using newly generated anti-Mi_b-CK peptide antibodies strongly indicate that Purkinje neurons express mainly 'sarcomeric' Mi_b-CK, the mitochondrial CK isoenzyme [Kaldis et al., unpubl. observation] which is generally expressed in muscle cells only. In contrast, newly generated anti-Mi_a-CK antibodies, raised against a synthetic peptide corresponding to the N-terminus of 'ubiquitous' Mi_a-CK, stained most of the other brain cells [Kaldis et al. unpubl.].

The exclusive presence of M-CK in PNs and the high amounts of B-CK found in BGCs may indicate that some characteristic properties of the different CK isoenzymes match the distinct energy requirements of these functionally specialized neuronal and glial cell types (see below). Similar differential localizations of specific isoenzymes in cerebellar Purkinje and BGC were reported for protein kinase C [60] and enolase [61] isoenzymes in the rat.

Proposed Functions of CK Isoenzymes in the Glomerular Structures

The granular layer of the cerebellum, especially the glomerular structures, contains high levels of Mi_a-CK as well as B-CK, as judged from the intensities of anti-CK antibody staining (fig. 2a, b, g, h). These structures, forming intimate synaptic as well as glial-neuron interac-

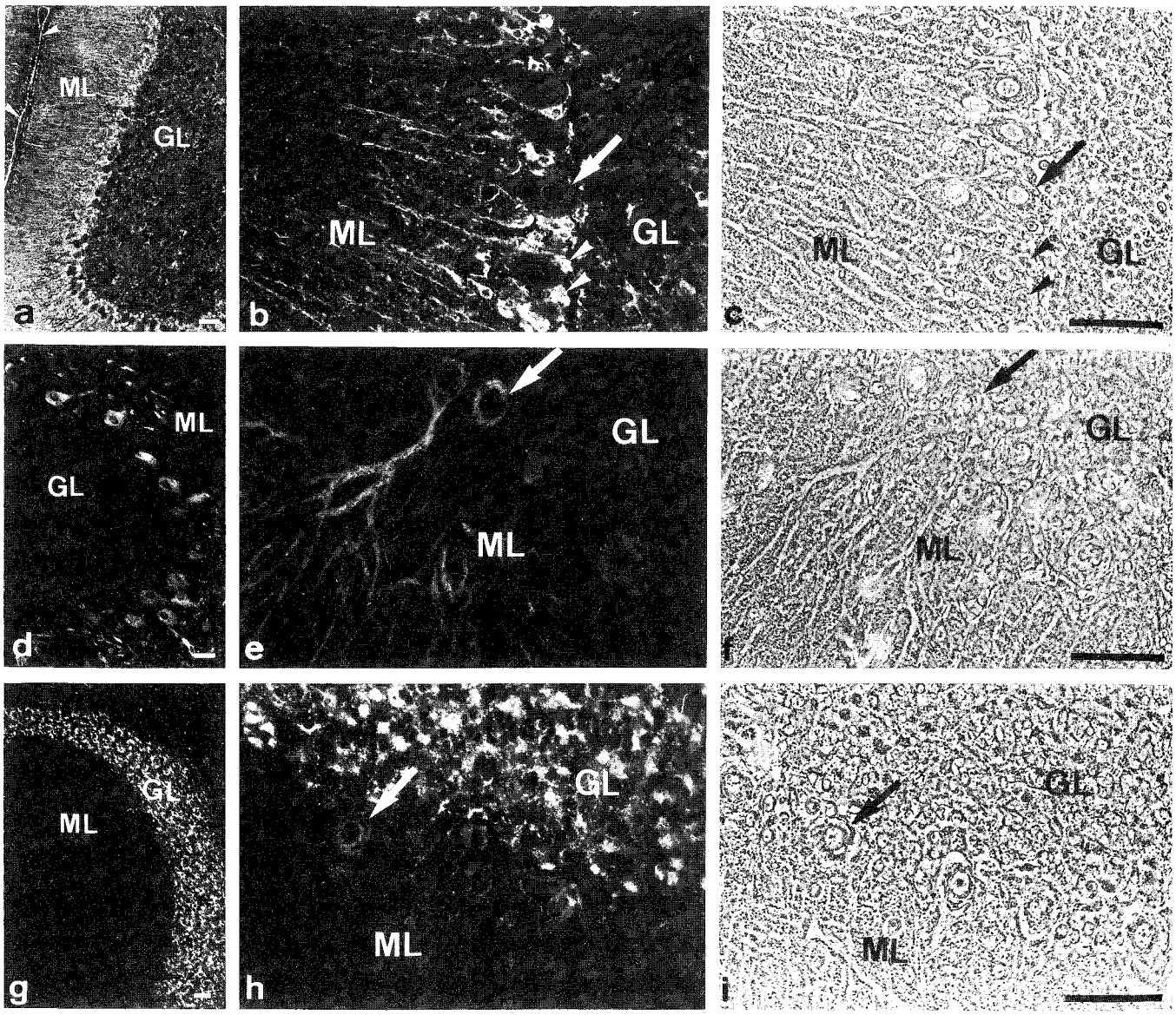

Fig. 2. Localization of brain-type B-CK, muscle-type M-CK and mitochondrial CK in chicken cerebellum. Chicken cerebellum or whole brains from 4-week-old chicken were fixed and embedded in paraffin by standard techniques [for details see 40]. Sections of 5-μm thickness were cut, deparaffinized and washed with tris-buffered saline (TBS: 150 m*M* NaCl, 50 m*M* Tris/HCl, pH 7.4). Nonspecific binding sites were blocked with TBG (1% BSA, 0.2% gelatine in TBS) for 30 min and incubated with primary antibodies, generally at dilutions between 1:100 and 1:300 in TBG, for 2 h in a moist chamber, followed by washing for 30 min in three changes of TBS. As second antibody, rhodamine-conjugated goat antirabbit IgG (Pierce), diluted 1:500 in TBG was used and the sections incubated again for 1 h. After 3 washes with TBS, specimens were mounted in buffered polyvinyl alcohol medium in the presence of *p*-phenylene diamine as anti-fading reagent. Low magnification overviews of immunostained cerebellar regions (**a, d, g**); and higher magnification immunofluorescence pictures in (**b, e, h**), with the corresponding phase contrast pictures (**c, f, i**), are shown after staining for brain-type B-CK (**a–c**); for muscle type M-CK (**d–f**); and for mitochondrial Mi_a-CK (**g–i**). Control sections, incubated with preimmunesera, followed by rhodamine-conjugated second antibody, displayed no significant unspecific staining [not shown here, see 4]. Small arrowheads indicate the cell bodies of BGCs (**b, c**) and the membrana limitans gliae (**a**) which are both strongly stained by anti-B-CK antibodies. Large arrows indicate the PNS which are strongly stained by anti-M-CK antibodies (**e, f**), weakly stained by anti-Mi_a-CK antibodies (**h, i**), but remain unstained by anti-B-CK antibodies (**b, c**). Note the anti-M-CK staining in the proximal processes of the PNs is not uniform, but vesicular (**e**). This would be consistent with a staining of the ER that is highly enriched in this region of PNs.

ML and GL refer to molecular and granule cell layers of the cerebellum, respectively. bar = 50 μm. An extensive study concerning the immunohistochemical localization of CK in chicken brain, including more details on the exact localization of the different CK isoenzymes, as well as on the characterization and specificity of the antibodies used was published elsewhere [4].

tions also called 'neuropils', are known to be rich in mitochondria and to display a very high energy metabolism. Large amounts of energy are needed in these structures for restoring of potassium ion gradients partially broken down during neuronal excitation, as well as for metabolite and neurotransmitter trafficking between glial cells and neurons [for review see 35]. Thus, the localization of both B- and Mi_a-CK isoforms within the same structures may be an indication that part of the energy consumed in these giant complexes of mossy fiber, Golgi cell and granule cell synapses (for more details concerning the localization of CK, see 40), might be provided by a 'phosphocreatine circuit', as it has been proposed for other excitable cells [4, 21].

Proposed Functions CK Isoenzymes in Bergmann Glial Cells, Astrocytes and Oligodendrocytes

The BGC is a specialyzed type of astroglial cell. It provides the migratory pathway of granule cell migration from the external granule cell layer to the internal granule cell layer during cerebellar development [58, 62]. Another main function of these cells is the proposed ATP-dependent spatial buffering of potassium ions [63, 64], released during the electrical activity of neurons. This function is also reflected by the morphology of BGC, which envelop the synaptic sites of Purkinje cell dendrites with the exception of those precise sites at which Purkinje spines make contact with parallel or climbing fibers [58]. Since BGC processes directly face the cerebrospinal fluid at the membrana limitans, these cells were suggested to be responsible for releasing the K^+ ions, taken up via ATP-driven Na^+/K^+-ion pumps from the extracellular space around the highly active PNs, into the subdural space, which acts as a K^+ sink [63]. It is therefore reasonable to assume that the high B-CK content of BGC (fig. 2a, b), which could easily be identified by their morphology and the staining pattern typical for these cells, reflects their high energy demands in relation to spatial K^+ buffering [35]. In this respect, it is interesting to note that Müller cells, representing a functionally and morphologically specialized astrocyte cell type found in the vertebrate retina, were also proposed to be involved in spatial K^+ buffering [64]. Like BGC, the Müller cells also contain significant amounts of B-CK [65].

The presence of B-CK in astrocytes [for details see 40, 53] is also compatible with the energy requirements of these cells which need energy for metabolic interaction with neurons, e.g. tricarboxylic acid cycle metabolite and neurotransmitter trafficking [35]. Since both BGC and astrocytes contain mitochondria and the latter cell type is known to display an intense oxidative metabolism [35], it is likely that both cell types also contain Mi-CK, although this cannot be shown unambiguously by our light microscopic study, but rather has to be demonstrated by immuno-gold labelling. An interesting finding in this context is the fact that astrocyte primary cell cultures from embryonic rat brain in contrast to neuron-rich cultures can accumulate creatine by a saturable Na^+-dependent creatine transport channel [66]. This may explain the relatively high creatine + PCr content of these cells. Very recently the creatine transporter, which is prominent in brain, muscle, heart and kindney, has been cloned and functionally expressed in transfected cell cultures [67]. Neurons do not import creatine via this transporter [66], but still contain CK and the corresponding substrates. This indicates that the capacity for the synthesis of creatine in brain found by Defalco and Davies [68], via amidino- and methyl-transferases [69], is most likely characteristic of neurons. This also would fit the observation that upon prolongued administration of the creatine analogue, beta-guanidino-propionic acid (GPA), which is known to block the creatine uptake mechanism, the creatine and PCr levels are decreased only about 20–40% in brain [70], whereas in skeletal muscle, the same treatment reduces these levels by 90–95% [71]. In brain, a GPA-inaccessible pool of PCr (60–80% of the total PCr), which could not be replaced by the analogue, was found by ^{31}P-NMR methods [70]. This residual fraction of PCr was hypothesized to represent the same compartmentalized pool, presumably in neurons (see above), which is stable in hypoxic or seizing animals [70]. This view is consistent with the observation that a cellular and subcellular compartmentation of energy metabolism exists in brain. This is very likely related to the presence of different cell types in this organ, e.g. astrocytes display higher rates of aerobic glycolysis and the capacity to increase this rate with increased energy demand is characteristic for these cells, [70, 72], whereas neurons rely more on the glycolytic pathway [35]. Finally, the high concentration of CK mRNA and CK activity found in oligodendrocytes [53, 55] may indicate a function of CK in oligodendrocyte metabolism, most likely related to the energy requirements needed for myelin synthesis, transport and assembly. This is supported by the fact that CK continue to rise during the period of most active myelination in rat brain and also in cell cultures [53].

Proposed Functions of CK Isoenzymes in PNs

PNs play a very important role in brain function. Receiving excitatory input from parallel fibers and climbing fibers, they represent the sole neuronal output structures of the cerebellar cortex. A remarkable feature of PNs is that a single PN makes hundreds of synaptic contacts to a single climbing fiber. Climbing fiber impulses evoke complex Ca^{2+} spikes and prolongued Ca^{2+}-mediated depolarizations in Purkinje cell dendrites [73–75], that is, in those processes which are thought to play a central role in the mechanism of cerebellar motoric learning [76].

The presence of the 'unusual' muscle-type M-CK (see fig. 2) [40], and most likely also the muscle-type Mi_b-CK [Kaldis, personal commun.] in PNs of chicken brain may reflect an adaptation of PNs to their very special energy requirements. It is known that PNs specifically express a whole variety of enzymes involved in Ca^{2+}-homeostasis [for ref. see 40]. Interestingly, several of these proteins are also muscle-type isoforms, e.g. the skeletal muscle-type ryanodine receptor is expressed in PNs [77]. Additionally, chicken PNs represent the only nonmuscle cell type in which calsequestrin, a typical protein of the sarcoplasmic reticulum, has been found [78, 79]. PNs contain the highest concentration of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) found in any nonmuscle cell [80] and also preferentially express a muscle-specific isoform of the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase, SERCA2b [81]. Recent in vivo ^{31}P saturation transfer experiments, showing that dihydropyridine calcium antagonists reduce the consumption of high-energy phosphates and concomitantly decrease the CK reaction flux in rat brain [82], corroborate the above conclusions that CK is directly or indirectly coupled to energetic processes needed for Ca^{2+} homeostasis or to cellular processes triggered by this second messenger.

Thus, the presence of muscle-type M-CK in PNs fits well into the general picture that PNs display some 'muscle-like' characteristics and may also reflect the better suitablility of M-CK, compared to other cytosolic CK isoenzymes, to associate with certain subcellular structures, e.g. with the endoplasmic reticulum (ER) membrane system. Chicken M-CK, in contrast to B-CK, was shown to be associated with both the myofibrillar M-line and the sarcoplasmic reticulum, where the enzyme is functionally coupled to the myosin ATPase [17] and the ATP-dependent Ca^{2+} pump [19], respectively. Very recently, the Ca^{2+} ATPase of rat skeletal muscle was shown to have preferential access to ATP generated by sarcoplasmic reticulum-bound CK [20]. The vesicular immunofluorescence staining seen in the proximal processes of PNs (fig. 1e) would be consistent with staining of ER vesicles which are prominent in this region of the cell. Thus, the role M-CK plays in muscle, that is, (1) supplying the Ca^{2+} pump of the sarcoplasmic reticulum with ATP, and (2) keeping local ATP/ADP ratios high in the vicinity of the Ca^{2+} pump, thereby increasing the thermodynamic efficiency of this ion pump [4, 83, 84], may be paralleled by M-CK bound to the ER in PNs. Our findings with chicken cerebellum are fully in line with the notion that PNs contain rather high concentrations of CK [104], but are at variance with the same study reporting on the exclusive presence of B-CK (at 2 pg B-CK/Purkinje cell), with no M-CK being present in PNs of rat cerebellum [104]. At the moment, we are unable to resolve this discrepancy. The fact that our anti-M and anti-B CK antibody staining is complementary for PNs and BGCs, however, makes us feel confident that our results with chicken are correct.

Proposed Functions of CK in Neurons

In brain, brain-type CK has also been found in association with synaptic vesicles [85], as well as with the plasma membrane [86]. Since a similar association between B-CK and synaptic vesicles as well as the plasma membrane has been demonstrated in electrocytes of Torpedo [41], the electrocyte system serves as a good analogy for the function of neuronal membrane-bound B-CK in brain. Convincing data concerning a direct functional coupling of CK with the Na^+/K^+ ATPase have been obtained by in vivo ^{31}P-NMR studies on electric fish, which showed that CK and the plasma membrane-bound Na^+/K^+ ATPase are tightly coupled in the resting as well as in the stimulated electric organ [42]. Additionally, CK bound to synaptic vesicles in electrocytes is involved in neurotransmitter release [87]. Thus, the fractions of CK, which are bound to synaptic vesicles and to the plasma membrane in neurons, are also likely to be involved in neurotransmitter release, as well as in the maintenance of membrane potentials and the restoration of ion gradients before and after electrical discharge, both in conjunction with the Na^+/K^+ ATPase [42, 88]. This is consistent with the fact that high energy turnover and, concomitantly, high CK concentrations have been found in those regions of the brain that are rich in synaptic connections, e.g. in the molecular layer of the cerebellum, in the

glomerular structures of the granule layer and also in the hippocampus [the latter finding is an unpubl. observation]. In neurons, the Na^+-extrusion activity facilitated via the neuron-specific Na^+/K^+ ATPase [89] is especially high in the synaptic regions [33]. This is also true for Ca^{2+} extrusion activity, mediated either via the plasma membrane Ca^{2+} pump responsible for net extrusion of calcium out of neurons or via the Na^+/Ca^{2+} exchange. The Na^+/Ca^{2+} exchange is driven by the Na^+ gradient which in turn is maintained by ATP indirectly through the operation of the Na^+/K^+ ATPase [for review see 90]. The observation that the rise in CK levels, observed in a fraction of brain containing nerve endings and synapses, parallels the neonatal increase in Na^+/K^+ ATPase is also suggestive that higher levels of PCr and CK are characteristic of regions in which energy expenditure for processes such as ion pumping are large [33]. In addition, protein phosphorylation which plays an important role in brain function, with some of the key proteins being phosphorylated and dephosphorylated at very high rates in neurons, is also thought to consume a sizable fraction of the total energy available to these cells [33].

Finally, CK, together with nerve-specific enolase, belongs to a group of proteins known as slow component-b (SC-b). These proteins are synthesized in neuronal cell bodies and are directed by axonal transport to the axonal extremities [91, 92]. The question of whether CK participates in the actual energetics of axonal transport remains to be answered. However, the association of a fraction of 'soluble' CK with SC-b proteins shows an intracellular compartmentation of the enzyme also in neurons. In addition, during preparation of neuron-specific enolase, brain CK is cochromatographed with the latter glycolytic enzyme [93], indicating a functional coupling of brain CK with glycolysis as was demonstrated in muscle [4, 25–27].

Developmental Changes in CK Activity and Phosphorus Metabolites in the Brain and in vivo Function of CK in Adult Brain

In the altricial neonate, including mouse, rat, rabbit, pig and human, marked quantitative and qualitative changes in the physiology of ATP metabolism occur postnatally [94]. Similarly, rather dramatic postnatal increases in total CK activity [47] as well as in PCr content were noted [95, 96]. For example, in the narrow time-window between days 12 and 15 of postnatal development of mouse and rat, (1) the in vivo rate of CK-catalysed ATP synthesis increased 4-fold, as measured by saturation transfer ^{31}P-NMR [94]; (2) the brain developed the capacity to increase ATP synthesis by oxidative phosphorylation in response to sudden changes in energy demand [94], and (3) a population of cerebral brain mitochondria appeared with tight contacts between inner and outer membranes [97]. Since Mi-CK has been identified in isolated contact site boundary membrane fractions of brain mitochondria [49] and since the octameric enzyme was shown to be able to induce contact formation between mitochondrial membranes [10], the appearance of the population of mitochondria described above may be related to the expression and accumulation of Mi-CK in these mitochondria. In rat brain, a 4- to 6-fold increase of Mi-CK activity has been measured to take place between days 12 and 20 of postnatal development concomitant with a corresponding 4-fold increase in the in vivo rate of CK-catalysed reaction flux [57]. These observations, showing that the developmental appearance of Mi-CK parallels the maturational changes in brain energy metabolism, suggest that Mi-CK, and CK in general, are critical in the control of cellular ATP metabolism in the adult brain [57]. It is interesting to note that in the developing rat cerebellum, creatine kinase activity was increased by vitamin D metabolites after vitamin D administration [98].

The interpretation that CK plays a key role in the energetics of the adult brain is supported by very recent in vivo ^{31}P-NMR magnetization transfer measurements showing that the pseudo first-order rate constant of the CK reaction (in the direction of ATP synthesis) as well as the CK flux correlate with brain activity, which was measured by EEG as well as by the amount of deoxy-glucose phosphate formed in the brain after administration of deoxy-glucose [88]. These data show that in vivo the CK/PCr system serves not merely as a temporal energy buffer, as suggested earlier [24], but also has a spatial energy buffer or transport function [4] with Mi-CK functioning as a key player in the intricate energy distribution system [6, 16] also in brain [57]. The topics discussed here illustrate that the control, coupling and kinetics of the PCr/CK/ATP system in brain are very complex. Nevertheless, CK and brain energetics may be relevant to clinical situations, such as the pathogenesis of hypoxia in the neonate, and of seizures, stroke and other pathologicald conditions. In the latter context it may be interesting to note that in multiple sklerosis (MS) plaques, a 35% decrease in the creatine concentration was observed [99], indicating an impairment of the cellular energy state in the MS lesions. Noninvasive ^{31}P-NMR methods, using

saturation transfer or inversion transfer techniques, are being applied now for functional measurements on the intact brain [34, 100], as well as for on-line functional imaging of high-energy phosphate metabolites within distinct regions of the brain [101, 102]. These approaches may provide important clues to identify certain pathological conditions associated with abnormal alterations of brain energetics. In fact, recent studies indicate that impaired energy metabolism can lead to neuronal cell death by a slow 'excitotoxic' mechanism representing one of the factors involved in Huntington's and Parkinson's disease [103].

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References

1 Eppenberger HM, Dawson DM, Kaplan NO: The comparative enzymology of CKs. I. Isolation and characterization from chicken and rabbit tissues. J Biol Chem 1967;242:204–209.

2 Hossle JP, Schlegel J, Wegmann G, Wyss M, Böhlen P, Eppenberger HM, Wallimann T, Perriard JC: Distinct tissue-specific mitochondrial creatine kinases from chicken brain and striated muscle with a conserved CK framework. Biochem Biophys Res Commun 1988;151:408–416.

3 Haas RC, Strauss AW: Separate nuclear genes encode sarcomer-specific and ubiquitous human mitochondrial creatine kinase isoenzymes. J Biol Chem 1990;265:6921–6927.

4 Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM: Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: The phosphocreatine circuit for cellular energy homeostasis. Biochem J 1992;281:21–40.

5 Brdiczka D: Contact sites between mitochondrial envelope membranes. Structure and function in energy- and protein-transport. Biochim Biophys Acta 1991;1071:291–321.

6 Wyss M, Smeitink J, Wevers RA, Wallimann T: Mitochondrial creatine kinase: A key enzyme of aerobic metabolism. Biochim Biophys Acta 1992;1102:119–166.

7 Jacob W, Biermans W, Bakker A: Mitochondrial contact sites: a dynamic compartment for creatine kinase activity; in De Deyn PP, Marescau B, Stalon V, Qureshi IA (eds): Guanidino Compounds in Biology and Medicine. London, Libbey, 1992, pp 165–174.

8 Schnyder T, Gross H, Winkler HP, Eppenberger HM, Wallimann T: Structure of the mitochondrial creatine kinase octamer: high resolution shadowing and image averaging of single molecules and formation of linear filaments under specific staining conditions. J Cell Biol 1991;112:95–101.

9 Schlegel J, Wyss M, Eppenberger HM, Wallimann T: Functional studies with the octameric and dimeric form of mitochondrial creatine kinase: Differential pH-dependent association of the two oligomeric forms with the inner mitochondrial membrane. J Biol Chem 1990;265:9221–9227.

10 Rojo M, Hovius R, Demel RA, Nicolay K, Wallimann T: Mitochondrial creatine kinase mediates contact formation between mitochondrial membranes. J Biol Chem 1991;266:20290–20295.

11 Bessman SP, Fonyo A: The possible role of the mitochondrial bound creatine kinase in regulation of mitochondrial respiration. Biochem Biophys Res Commun 1966;22:597–602.

12 Saks VA, Kuznetsov AV, Kupriyanov VV, Miceli MV, Jacobus WE: Creatine kinase of rat heart mitochondria: The demonstration of functional coupling to oxidative phosphorylation in an inner membrane preparation. J Biol Chem 1985;260:7757–7764.

13 Jacobus WE: Respiratory control and the integration of heart high-energy phosphate metabolism by mitochondrial creatine kinase. Annu Rev Physiol 1985;47:707–725.

14 Klingenberg M: The ATP/ADP translocation in mitochondria, a membrane potential controlled transport. J Membr Biol 1980;56:97–105.

15 Schnyder T, Sargent DF, Richmond TJ, Eppenberger HM, Wallimann T: Crystallisation and preliminary X-ray analysis of two different forms of mitochondrial creatine kinase from chicken cardiac muscle. J Mol Biol 1990;216:809–812.

16 Wyss M, Wallimann T: Metabolite channelling in aerobic metabolism. J Theor Biol 1992;158:129–132.

17 Wallimann T, Schlösser T, Eppenberger HM: Function of M-line-bound CK as intramyofibrillar ATP regenerator at the receiving end of the phosphocreatine shuttle in muscle. J Biol Chem 1984;259:5238–5246.

18 Krause SM, Jacobus WE: Specific enhancement of the cardiac myofibrillar ATPase by bound creatine kinase. J Biol Chem 1992;267:2480–2486.

19 Rossi AM, Eppenberger HM, Volpe P, Cotrufo R, Wallimann T: Muscle-type MM-creatine kinase is specifically bound to sarcoplasmic reticulum and can support Ca^{2+}-uptake and regulate local ATP/ADP ratios. J Biol Chem 1990;265:5258–5266.

20 Korge P, Byrd SK, Campbell KB: Functional coupling between sarcoplasmic-reticulum-bound creatine kinase and Ca^{2+}-ATPase. Eur J Biochem 1993;213:973–980.

21 Hemmer W, Riesinger I, Wallimann T, Eppenberger HM, Quest AFG: Brain-type creatine kinase in photoreceptor cell outer segments: role of a phosphocreatine circuit in outer segment energy metabolism and phototransduction. J Cell Sci 1993;106:671–684.

22 Bessman SP, Carpenter CL: The creatine-creatine phosphate energy shuttle. Annu Rev Biochem 1985;54:831–862.

23 Wallimann T, Eppenberger HM: Localization and function of M-line-bound creatine kinase: M-band model and creatine phosphate shuttle; in Shay JW (eds): Cell and Muscle Motility. New York, Plenum, 1985, vol 6, pp 239–285.

24 Meyer RA, Sweeney HL, Kushmerick MJ: A simple analysis of the 'phosphocreatine shuttle'. Am J Physiol 1984;246:C365–377.

25 VanWaarde A, Van den Thillart G, Erkelens C, Addink A, Lugtenburg J: Functional coupling of glycolysis and phosphocreatine utilization in anoxic fish muscle. J Biol Chem 1990;265:914–923.

26 Dillon PF, Clark JF: The theory of diazymes and functional coupling of pyruvate kinase and creatine kinase. J Theor Biol 1990;143:275–284.

27 Wegmann G, Zanolla E, Eppenberger HM, Wallimann T: In situ compartmentation of creatine kinase in intact sarcomeric muscle: the acto-myosin overlap zone as a molecular sieve. J Muscle Res Cell Motil 1992;13:420–435.
28 Quest AFG, Eppenberger HM, Wallimann T: Purification of brain-type creatine kinase (B-CK) from several tissues of the chicken: B-CK subspecies. Enzyme 1989;41:33–42.
29 Quest AFG, Eppenberger HM, Wallimann T: Two different B-type creatine kinase subunits dimerize in a tissue-specific manner. FEBS Lett 1990;262:299–304.
30 Quest AFG, Soldati T, Hemmer W, Perriard JC, Eppenberger HM, Wallimann T: Phosphorylation of chicken brain-type creatine kinase affects a physiologically important kinetic parameter and gives rise to protein microheterogeneity in vivo. FEBS Lett 1990; 269:457–464.
31 Hemmer W, Skarli M, Perriard JC, Wallimann T: Effect of okadaic acid on protein phosphorylation patterns of chicken myogenic cells with special reference to creatine inase. FEBS Lett 1993;327:35–40.
32 Wyss M, Schlegel J, James P, Eppenberger HM, Wallimann T: Mitochondrial creatine kinase from chicken brain. Purification, biophysical characterization and generation of heterodimeric and heterooctameric molecules with subunits of other creatine kinase isoenzymes. J Biol Chem 1990;265:15900–15908.
33 Erecinska M, Silver IA: ATP and brain function. J Cerebr Blood Flow Metabol 1989;9: 2–19.
34 Cadoux-Hudson TA, Blackledge MJ, Radda GK: Imaging of human brain creatine kinase activity in vivo. FASEB J 1989;3:2660–2666.
35 Hertz L, Peng L: Energy metabolism at the cellular level of the CNS. Can J Physiol Pharmacol 1992;70:S145–157.
36 Kadekaro M, Crane AM, Sokoloff L: Differential effects of electrical stimulation of sciatic nerve on metabolic activity in spinal cord and dorsal root ganglion in the rat. Proc Natl Acad Sci USA 1985;82:6010–6013.
37 Sokoloff L: The brain as a chemical machine. Prog Brain Res 1992;94:19–33.
38 Sokoloff L: Energy metabolism and effect of energy depletion or exposure to glutamate. Can J Physiol Pharmacol 1992;70(suppl): S107–112.
39 Edmond J, Robbins RA, Bergstrom JD, Cole RA, de Vellis J: Capacity for substrate utilization in oxidative metabolism by neurons, astrocytes and oligodendrocytes from developing brain in primary culture. J Neurosci Res 1987;18:551–561.
40 Hemmer W, Zanolla E, Furter-Graves EM, Eppenberger HM, Wallimann T: Creatine kinase isoenzymes in chicken cerebellum: specific localization of brain-type CK in Bergmann glial cells and muscle-type CK in Purkinje neurons. Eur J Neurosci, in press.
41 Wallimann T, Walzthöny D, Wegmann G, Moser H, Eppenberger HM, Barrantes FJ: Subcellular localization of creatine kinase in Torpedo electrocytes: Association with acetylcholine receptor-rich membranes. J Cell Biol 1985;100:1063–1072.
42 Blum H, Balschi JA, Johnson RG: Coupled in vivo activity of creatine phosphokinase and the membrane-bound (Na^+K^+)-ATPase in the resting and stimulated electric organ of the electric fish *Narcine brasiliensis*. J Biol Chem 1991;266:10254–10259.
43 Hemmer W, Glaser SJ, Hartmann GR, Eppenberger HM, Wallimann T: Covalent modification of creatine kinase by ATP: evidence for autophosphorylation; in Heilmeyer LMG (ed): Cellular Regulation by Protein Phosphorylation. NATO ASI Series vol H56. Berlin, Springer, 1991, pp 143–147.
44 Soldati T, Schäfer BW, Perriard JC: Alternative ribosomal initiation gives rise to chicken brain-type CK isoproteins with heterogenous amino termini. J Biol Chem 1990;265:4498–4506.
45 Jacobs M, Heldt HW, Klingenberg M: High activity of CK in mitochondria from muscle and brain. Evidence for a separate mitochondrial isoenzyme of CK. Biochem Biophys Res Commun 1964;16:516–521.
46 Swanson Ph: The particulate adenosine triphosphate creatine phosphotransferase from brain: Its distribution in subcellular fractions and its properties. J Neurochem 1967;14:343–356.
47 Booth RFG, Clark JB: Studies on the mitochondrially bound form of rat brain creatine kinase. Biochem J 1978;170:145–151.
48 Wevers RA, Reutlingsperger CPM, Dam B, Soons JBJ: Mitochondrial creatine kinase in the brain. Clin Chim Acta 1982;119:209–223.
49 Kottke M, Adams V, Wallimann T, Kumar-Nalam V, Brdiczka D: Location and regulation of octameric mitochondrial creatine kinase in the contact sites. Biochim Biophys Acta 1991;1061:215–225.
50 Hamburg RJ, Friedman DL, Olson EN, Ma TS, Cortez MD, Goodman C, Puleo PR, Perryman MB: Muscle creatine kinase isoenzyme expression in adult human brain. J Biol Chem 1990;265:6403–6409.
51 Chandler WL, Fine JS, Emery M, Weaver D, Reichenbach D, Clayson KJ: Regional creatine kinase, adenylate kinase and lactate dehydrogenase in normal canine brain. Stroke 1988;19:251–255.
52 Maker HS, Lehrer GM, Silides DJ, Weiss C: Regional changes in cerebellar creatine phosphate metabolism during late maturation. Exp Neurol 1973;38:295–300.
53 Manos P, Bryan GK, Edmond J: Creatine kinase activity in postnatal rat brain development and in cultured neurons, astrocytes and oligodendrocytes. J Neurochem 1991;56: 2101–2107.
54 Kahn MA: Effect of calcium on creatine kinase activity of cerebellum. Histochemistry 1976;48:29–32.
55 Molloy GR, Wilson CD, Benfield P, de Vellis J, Kumar S: Rat brain creatine kinase messenger RNA levels are high in primary cultures of brain astrocytes and oligodendrocytes and low in neurons. J Neurochem 1992;59:1925–1932.
56 Thompson RJ, Kynoch PAM, Sarjant J: Immunohistochemical localization of BB-CK to astrocytes in human brain. Brain Res 1980; 201:423–426.
57 Holtzman D, Tsuji M, Wallimann T, Hemmer W: Functional maturation of creatine kinase in rat brain. Dev Neurosci 1993; 15:261–270.
58 Rakic P: Neuron-glial relationship during granule cell migration in developing cerebellar cortex. A Golgi and electronmicroscopic study in Maccacus rhesus. J Comp Neurol 1971;141:283–312.
59 Palay S, Chan-Palay V: Cerebellar Cortex, Cytology and Organization. New York, Springer, 1974.
60 Mochly-Rosen D, Basbaum AI, Koshland DE Jr: Distinct cellular regional localization of immunoreactive protein kinase C in rat brain. Proc Natl Acad Sci USA 1987;84:4660–4664.
61 Schmechel D, Marangos PJ, Bright M, Goodwin FK: Brain enolases as specific markers of neuronal and glial cells. Science 1978;199: 313–315.
62 Hatten ME: Riding the glial monorail: a common mechanism for glial-guided neuronal migration in different regions of the developing mammalian brain. Trends Neurosci 1990;13: 179–184.
63 Newman EA: Regulation of potassium levels by glia cells in the retina. Trends Neurosci 1985;8:156–159.
64 Reichenbach A: Glial K^+ permeability and CNS K^+ clearance by diffusion and spatial buffering; in Abbott NJ (ed): Glial-Neuronal Interaction. New York, New York Academy of Sciences, 1991, pp 272–286.
65 Wallimann T, Wegmann G, Moser H, Huber R, Eppenberger HM: High content of creatine kinase in chicken retina: Compartmentalized localization of creatine kinase isoenzymes in photoreceptor cells. Proc Natl Acad Sci USA 1986;83:3816–3819.
66 Möller A, Hamprecht B: Creatine transport in cultured cells of rat and mouse brain. J Neurochem 1989;52:544–550.
67 Guimbal C, Kilimann MW: A Na^+-dependent creatine transporter in rabbit brain, muscle, heart and kidney. J Biol Chem 1993;268: 8418–8421.
68 Defalco AJ, Davies RE: The synthesis of creatine by the brain of intact rat. J Neurochem 1961;7:308–312.
69 Takeda M, Nakayama S, Tomino Y, Jung KY, Endou H, Koide H: Heterogeneity of transamidinase activity and creatine content along the rat nephron; in deDeyn PP, Marescau B, Stalon V, Qureshi IA (eds): Guanidino Compounds in Biology and Medicine. London, Libbey, 1992, pp 153–157.

70 Holtzman D, McFarland E, Moerland T, Koutcher J, Kushmerick MJ, Neuringer LJ: Brain creatine phosphate and creatine kinase in mice fed an analogue of creatine. Brain Res 1989;483:68–77.
71 Shoubridge EA, Radda GK: A ^{31}P-NMR study of skeletal muscle metabolism in rats depleted of creatine with the analogue GPA. Biochem Biophys Acta 1984;805:79–88.
72 Holtzman D, Olson JE: Developmental changes in brain cellular energy metabolism in relation to seizures and their sequelae; in Jaspers H, van Gelder W (eds): Basic Mechanisms of Neuronal Hyperexcitability. New York, Liss, 1983, pp 423–429.
73 Llinás R, Sugimori M: Electrophysiological properties of in vitro Purkinje cell dendrites in mammalian cerebellar slices. J Physiol 1980;305:197–213.
74 Eckerot CF, Oscarsson O: Prolonged depolarization elicited in Purkinje cell dendrites by climbing fiber impulses in the cat. J Physiol 1981;318:207–221.
75 Knöpfel T, Vranesic I, Staub C, Gähwiler BH: Climbing fiber responses in olivocerebellar slice cultures. II. Dynamics of cytosolic calcium in Purkinje cells. Eur J Neurosci 1991;3:343–348.
76 Ito M: The cellular basis of cerebellar plasticity. Curr Opin Neurobiol 1991;1:616–620.
77 Kuwajima G, Futatsugi A, Niinobe M, Nakanishi S, Mikoshiba K: Two types of ryanodine receptors in mouse brain: Skeletal muscle type exclusively in Purkinje cells and cardiac muscle type in various neurons. Neuron 1992;9:1133–1142.
78 Villa A, Podini P, Clegg DO, Pozzan T, Meldolesi J: Intracellular Ca^{2+}-stores in chicken Purkinje neurons: Differential distribution of low affinity-high capacity Ca^{2+} binding protein, calsequestrin, of Ca^{2+} ATPase and of ER lumenal protein, Bip. J Cell Biol 1991; 113:779–791.
79 Takei K, Stukenbrok H, Metcalf A, Mignery JA, Sudhof TC, Volpe P, DeCamilli P: Ca^{2+} stores in Purkinje neurons: Endoplasmic reticulum subcompartments demonstrated by the heterogeneous distribution of the $InsP_3$ receptor, Ca^{2+} ATPase and calsequestrin. J Neurosci 1992;12:489–505.
80 Michelangeli F, DiVirgilio F, Villa A, Podini P, Meldolesi J, Pozzan T: Identification, kinetics properties and intracellular localization of the (Ca^{2+}-Mg)-ATPase from the intracellular stores of chicken cerebellum. Biochem J 1991;275:555–561.
81 Campbell AM, Wuytack F, Fambrough DM: Differential distribution of the alternative forms of the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase, SERCA 2b and SERCA 2a, in the avian brain. Brain Res 1993;605:67–76.
82 Rudin M, Sauter A: Dihydropyridine calcium antagonists reduce the consumption of high-energy phosphates in the rat brain. A study using combined ^{31}P/^{1}H magnetic resonance spectroscopy and ^{31}P saturation transfer. J Pharmacol Exp Ther 1989;251:700–706.
83 Kammermeier H: Why do cells need phosphocreatine and a phosphocreatine shuttle. J Mol Cardiol 1987;19:115–118.
84 Läuger P: Ca^{2+}-pumps from sarcoplasmic reticulum; in Läuger P (ed): Electrogenic Ion Pumps. Mass., Sinderland, 1991, pp 227–251.
85 Friedhoff AJ, Lerner MH: CK isoenzyme association with synaptosomal membrane and synaptic vesicles. Life Sci 1977;20:867–872.
86 Lim L, Hall C, Leung T, Mahadevan L, Whatley S: Neuron-specific enolase and CK are protein components of rat brain synaptic plasma membrane. J Neurochem 1983;41: 1177–1182.
87 Dunant Y, Loctin F, Marsal J, Muller D, Parducz A, Rabasseda X: Energy metabolism and quantal acetylcholine release: effects of botulinum toxin, FDNB and diamide in the Torpedo electric organ. J Neurochem 1988; 50:431–439.
88 Sauter A, Rudin M: Determination of creatine kinase parameters in rat brain by NMR-magnetization transfer: Correlation with brain function. J Biol Chem 1993;268:13166–13171.
89 Sweadner KJ: Two molecular forms of Na^+/K^+-stimulated ATPase in brain. J Biol Chem 1979;254:6060–6067.
90 Carafoli E: Intracellular calcium homeostasis. Annu Rev Biochem 1987;56:395–433.
91 Brady ST, Lasek RJ: Nerve-specific enolase and creatine kinase in axonal transport: Soluble proteins and axoplasmic matrix. Cell 1981;23:515–523.
92 Oblinger MM, Brady ST, McQuarrie IG, Lasek RJ: Cytotypic differences in the protein composition of the axonally transported cytoskeleton in mammalian neurons. J Neurol 1987;7:433–462.
93 Gerbitz KD, Deufel T, Summer J, Thallemer J, Wieland OH: Brain specific proteins: Creatine kinase BB isoenzyme is cochromatographed during preparation of neuron-specific enolase from human brain. Clin Chim Acta 1983;133:233–239.
94 Holtzman D, McFarland EW, Jacobs D, Offutt MC, Neuringer LJ: Maturational increase in mouse brain creatine kinase reaction rates shown by phosphorus magnetic resonance. Dev Brain Res 1991;58:181–188.
95 Tofts P, Wray S: Changes in brain phosphorus metabolites during the post-natal development of the rat. J Physiol 1985;359:417–429.
96 Norwood WI, Ingwall JS, Norwood CR, Fossel ET: Developmental changes of creatine kinase metabolism in rat brain. Am J Physiol 1983;244:C205–210.
97 Holtzman D, Herman MM, Desantel M, Lewiston N: Effects of altered osmolality on respiration and morphology of mitochondria from the developing brain. J Neurochem 1979;33:453–460.
98 Binderman I, Harel S, Earon Y, Tomer A, Weisman Y, Kaye AM, Sömjen D: Acute stimulation of creatine kinase activity by vitamin D metabolites in the developing cerebellum. Biochim Biophys Acta 1988;972:9–16.
99 Davies SEC, Newcombe J, Williams SR, Clark JB: Decreased N-acetyl aspartate (and creatine) in postmortem multiple sklerosis in brain. J Neurochem 1993;61:S131.
100 Mora B, Narasimham PT, Ross BD, Allman J, Barker PB: ^{31}P-saturation transfer and phosphocreatine imaging in the monkey brain. Proc Natl Acad Sci USA 1991;88: 8372–8376.
101 Moonen CHT, VanZijl PCM, Frank JA, LeBihan D, Becker ED: Functional magnetic resonance imaging in medicine and physiology. Science 1990;250:53–61.
102 Kaupinnen RA, Williams SR, Busza AL, van-Bruggen N: Applications of magnetic resonance spectroscopy and diffusion weighted imaging to the study of brain biochemistry and pathology. Trends Neurosci 1993;16: 88–95.
103 Beal MF, Hyman BT, Koroshetz W: Do defects in mitochondrial energy metabolism underlie the pathology of neurodegenerative diseases? Trends Neurosci 1993;16:125–131.
104 Kato K, Suzuki F, Shimizu A, Shinohara H, Semba R: Highly sensitive immunoassay for rat brain-type creatine kinase: determination in isolated Purkinje cells. J Neurochem 1986; 46:1783–1788.