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Regulation of Taurine Transport Systems by Protein Kinase CK2 in Mammalian Cells

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Key Words

Volume regulation • Reactive oxygen species • GSK3β • CK2 • PTEN • PI3K

Abstract

Maintaining cell volume is critical for cellular function yet shift in cell volume is a prerequisite for mitosis and apoptosis. The ubiquitously and evolutionary conserved serine/threonine kinase CK2 promotes cell survival and suppresses apoptosis. The present review describes how mammalian cells regulate the cellular content of the major cellular organic osmolyte, taurine with emphasis on CK2 mediated regulation of active taurine uptake and volume-sensitive taurine release. Furthermore, we discuss how CK2-mediated regulation of taurine homeostasis is potentially involved in cellular functions such as proliferation and survival.

Introduction

Imbalance in cell volume control affects intracellular signaling, cellular metabolism, cell cycle regulation, cell migration and cell fate. On the other hand cells use a shift in cell volume to trigger essential cellular processes e.g., an increase in cell volume is as a prerequisite for mitosis and migration [1, 2], whereas cell shrinkage proceeds apoptosis [3, 4], see [5, 6] for reviews). Water is osmotically obliged to follow net movement of osmolytes, hence preservation of cell volume involves active uptake and passive release of ions and organic osmolytes. Rapid changes in the extracellular osmolarity offset membrane potential or change the intracellular ionic environment, and to avoid cellular or tissue dysfunction cells accumulate ions and organic osmolytes following cell shrinkage in a process termed regulatory volume increase (RVI), whereas they release the osmolytes following cell swelling in a process designated regulatory volume decrease (RVD) (see [7, 8]). Taurine is a semi-essential, highly abundant β-amino sulfonic acid, and one of the primary organic osmolytes involved in the maintenance of mammalian cell volume. The cellular taurine content is a balance between active uptake, synthesis and passive release. Taurine is actively accumulated in the cells via the taurine transporter TauT, and passively released by an as yet unidentified swelling induced release pathway. Human adults has the capability to synthesize taurine from the non-essential amino acid cysteine or alternatively from the essential amino acid methionine, mainly in the liver and to a minor extend in the brain [9]. Human neonates,

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on the other hand, have to rely on dietary uptake as the primary taurine source (see [10]). The bulk taurine is intracellular and the normal taurine plasma concentration is estimated at 10-100 µM. Taurine constitutes more than 50% of the free amino acid pool in tissues such as heart and retina with concentrations of 6 and 40 mM, respectively [11]. Besides being involved in osmotic regulation taurine is proposed to be involved in antioxidative defence, detoxification, neuromodulation, insulin signalling, calcium signalling, as well as cholesterol regulation [12-17]. Studies on taurine deficient cats reveal severe retinal degeneration, growth retardation and dysfunction of the central nervous system, as well as serious disturbances in the CNS development, causing anencephaly or hydrocephaly (see [14]).

Previous reports have introduced the serine/threonine kinase CK2 as a regulator of the active taurine uptake and the passive taurine release [18, 19]. CK2 is constitutively active and upregulated in most types of cancers as well as growth stimulated cells where it stimulates proliferation and suppresses apoptosis [20] (see [21]). In addition, inhibition of CK2 sensitizes cancerous cells to induction of apoptosis [22, 23] (see [24, 25]). Using mouse NIH3T3 fibroblasts and Ehrlich Lettré ascites tumor cells (ELA), which express low and high levels of CK2, respectively, it has been demonstrated that pharmacological inhibition of CK2 potentiates taurine uptake and reduces taurine release under isotonic conditions (Fig. 1). Furthermore, inhibition of CK2 also results in decreased activity of the swelling induced taurine release pathway (Fig. 2) [18]. As the effect of CK2 inhibition on taurine transport was similar in the two cell lines it was suggested that CK2 suppresses taurine accumulation regardless to the CK2 expression level.

CK2 consists of two regulatory and two catalytic subunits that form a hetero tetramer. Even though the crystal structures of CK2 / CK2 subunits are available [26] and CK2 has been shown to target a broad range of substrates involved in intracellular homeostatic processes, e.g., cell cycle control, cell dynamics (morphology/ cytoskeleton), gene expression, proliferation, transformation , and apoptosis [27, 28], knowledge to the regulation of CK2 activity is more limited, i.e., regulation through phosphorylation/dephosphorylation, recruitment by protein-protein interaction [29, 30], spatial-temporal organization of subunits [31, 32], aggregation / dissociation of subunits following shift in ionic strength [30, 33, 34] are considered. Drug resistance is accompanied by CK2 overexpression / increased CK2 activity, and treatment with CK2 inhibitors induces cell death even in drug resistant cells [35]. The effect of CK2 inhibition on active taurine uptake involves modulation of taurine transport kinetics, whereas the effect on taurine release involves reduction in the activity of the efflux pathway. Taurine release is significantly increased following cell swelling and involves (I) cell specific subtypes of phospholipase A_2 and a 5-lipoxygenase, (II) cell specific NADPH oxidases and generation of reactive oxygen species, (III) unidentified protein tyrosine kinases, (IV) phosphatidyl inositol 3-kinase (PI3K), normally recognized for its involvement in cell growth, proliferation and cell survival, and (V) glycogen synthase kinase (GSK3β), associated with glucose homeostasis. We will in this review discuss

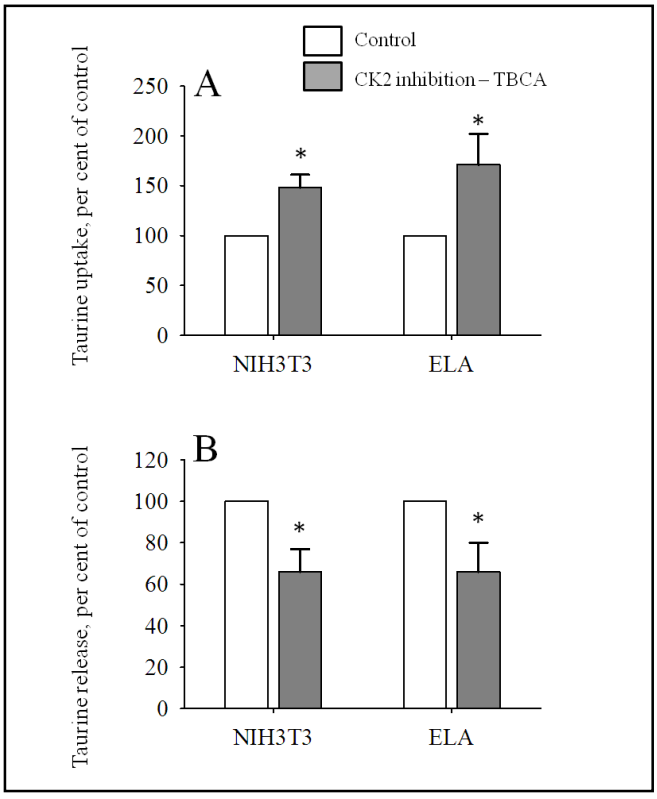

Fig. 1. CK2 inhibition favors taurine accumulation in NIH3T3 and ELA cells under isoosmotic conditions. A: CK2 inhibition potentiates taurine uptake. Taurine influx was estimated under isoosmotic conditions from the initial ^{3}H-taurine uptake in control cells (open bar) and cells treated with the CK2 inhibitor (E)-3-(2,3,4,5-tetrabromophenyl) acrylic acid (TBCA, grey bar). B: CK2 inhibition reduces taurine release. Taurine efflux was estimated under isoosmotic conditions using tracer technique. The fractional rate constant for the release of labeled taurine was used to indicate the activity of the taurine leak pathway in the absence (open bar) or presence of TBCA (grey bar). Values are given relative to control ± SEM. Values are from [18]. * Significant different from control values.

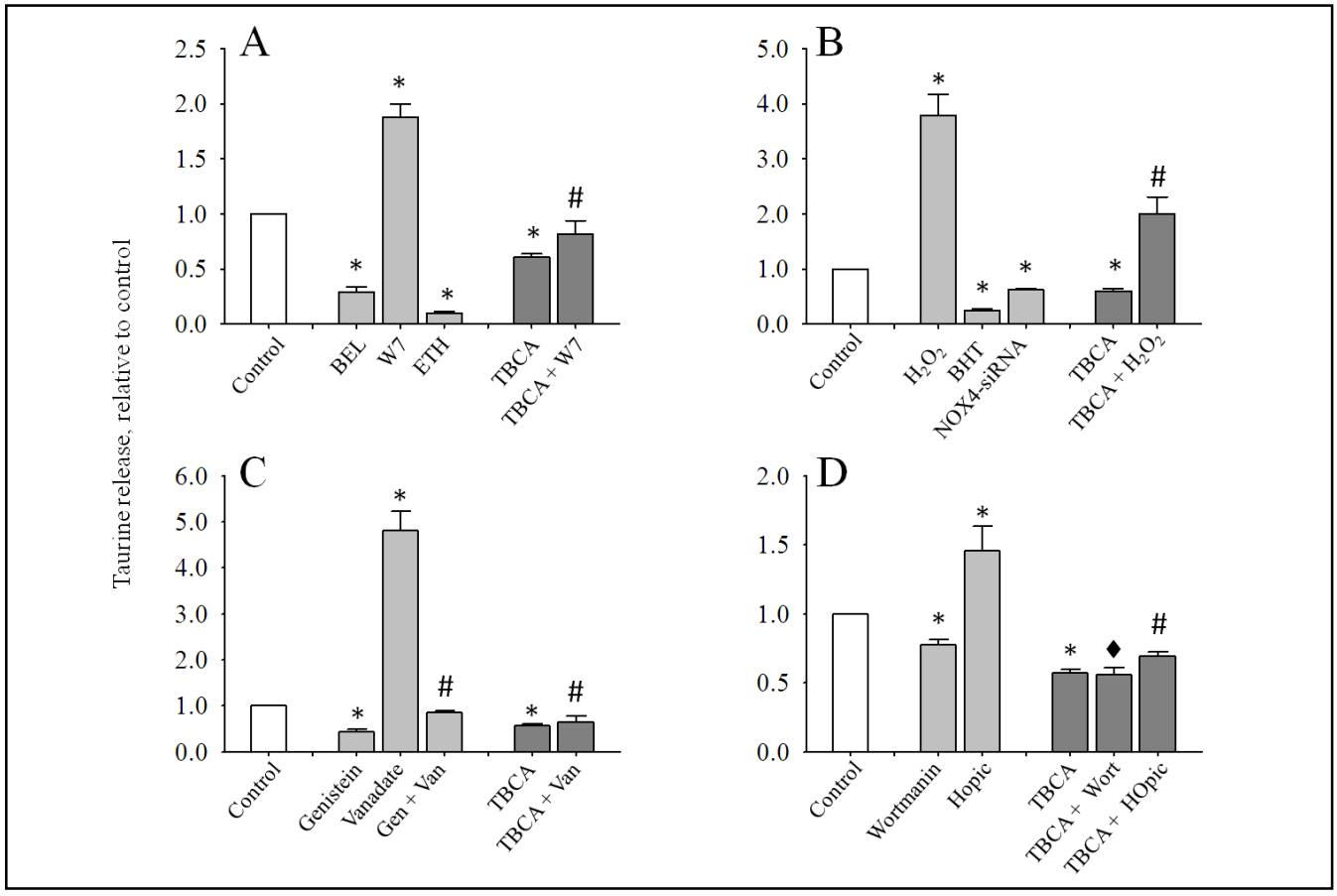

Fig. 2. Activation and modulation of volume sensitive taurine release in NIH3T3 cells. Taurine efflux from NIH3T3 cells was estimated under hypoosmotic conditions using tracer technique. The maximal rate constant obtained after reduction in the extracellular osmolarity from 335 mOsm to 200 mOsm was taken to represent the activity of the volume-sensitive taurine leak pathway. A: CK2 modulates the volume-sensitive taurine efflux pathway downstream to the Ca^{2+}-independent phospholipase A_2 ($iPLA_2$) and the 5-lipoxygenase. Taurine release was estimated in the absence (control) or presence of the $iPLA_2$ inhibitor bromoenol lactone (BEL), the calmodulin antagonist N-(6-aminohexyl)-5-chloro-1-naphathalene sulphonamide (W7), the 5-lipoxygenase inhibitor ETH 615-139 (ETH) or TBCA in combination with W7. Values from [18, 90]. B: CK2 modulates the volume-sensitive taurine efflux pathway downstream to the ROS sensitive step. Taurine release was estimated in the absence (control) or the presence of H_2O_2, the water-soluble antioxidant butulated hydroxytoluene (BHT), following NOX4 silencing (siRNA, NOX4-si) or TBCA in combination with H_2O_2. Values from [90, 99]. C: CK2 modulates the volume-sensitive taurine efflux pathway downstream to the step modulated by protein tyrosine phosphorylation. Taurine release was estimated in control cells and cells exposed to the protein tyrosine kinase inhibitor genistein (Gen), the protein tyrosine phosphatase inhibitor vanadate (Van), genistein plus vanadate or TBCA in combination with vanadate. Values from [90]. D: CK2 modulates the volume-sensitive taurine efflux pathway downstream to PI3K. Taurine release was estimated in control cells (open bar) and cells exposed to the PI3K inhibitor Wortmanin (Wort), the PTEN inhibitor HOpic, the CK2 inhibitor TBCA or TBCA in combination with Wortmanin and HOpic. Values from [18]. *, # and ♦Significant different from control values, equivalent with no TBCA, and equivalent with no genistein, respectively.

multiple potential targets of CK2 in regulation taurine release, and present data that indicate that CK2 interferes with a step downstream to the known swelling induced signal transducers including PLA_2, NADPH oxidase and PI3K. Figure 4 presents a tentative model for CK2-mediated regulation of taurine accumulation and release with emphasis on the latter.

Taurine uptake - TauT

Taurine uptake is mediated by the taurine transporter TauT (SLC6A6), a member of the SLC6 (solute carrier 6) family of sodium- and chloride-dependent transporters, which consists of at least 16 highly homologous members [36] including transporters for neurotransmitters

(GABA, glycine, dopamine, noradrenaline, serotonin) as well as transporters for creatine. Cloning of the *TauT* gene from mouse [37], rat [38], dog [39], human [40] and fish [41] revealed that it codes for an about 620 amino acid protein with an estimated molecular weight at 70 kDa. Mammalian TauT show a 90% sequence identity and it appears that several TauT isoforms occur in e.g. mouse fibroblasts, which can be distinguished by their molecular weight in Western blotting analysis [19, 42, 43] as well as their affinity towards taurine [42] and their Na^+:taurine stoichiometry [18] in kinetic analysis. Hydropathy plots indicate that TauT contains 12 transmembrane domains and drawing an analogy with the structure of other members of the Na^+-Cl^--coupled SLC6 transporter family and the leucine transporter for leucine from Aquifex aeolicus (LeuTAa) [44, 45] it is revealed that the binding pockets for Na^+ and taurine in TauT are coordinated by residues on TM1, TM3, TM6 and TM8 and that variation in Na^+:taurine stoichiometry reflect variation in Na^+ binding sites [44]. Taurine uptake is initiated by the binding of sodium to intra-membrane domains of TauT, changing the tertiary structure and hence facilitating binding and transport of taurine (see [46]). Chloride is in addition to sodium, needed to reach maximal transport rates. The Na^+ Cl^- taurine stoichiometry is generally 2.5:1:1 [47] although this ratio is susceptible to modulation and varies between cell types (see [46]), potentially as a result of TauT-subtypes or differential regulation.

Knock-out of the taurine transporter TauT, and the concomitant failure to accumulate cellular taurine causes programmed cell death of photoreceptor cells in mice, either due to decreased antioxidative capacity or due to impairment of retinoid transport between pigment epithelium and retina [14, 48, 49]. Furthermore, TauT knock-out mice has a 25% lower than normal birth weight, chronic liver disease, reduced exercise capacity and destruction of nerve cells in the CNS as well as in the olfactory epithelia and the auditory nerve [14, 48-51]. The immediate effects caused by taurine deficiency as observed in cats, could possibly be indirectly caused by osmotic perturbations and cellular stress due to reduced anti-oxidative capacity, although the mechanisms are probably diverse. In this context it is noted that treatment with platinum drugs, e.g. cisplatin induces activation of p53 and repression of *TauT* and that overexpression of TauT prevented the cisplatin-induced apoptosis and renal dysfunction [52]. Similarly it has been demonstrated that knock down of TauT in ELA cells resulted in a significant increase in cisplatin-induced apoptosis [53]. It is speculated that increased TauT activity could limit cell shrinkage during the apoptotic process and hence limit initiation of apoptose.

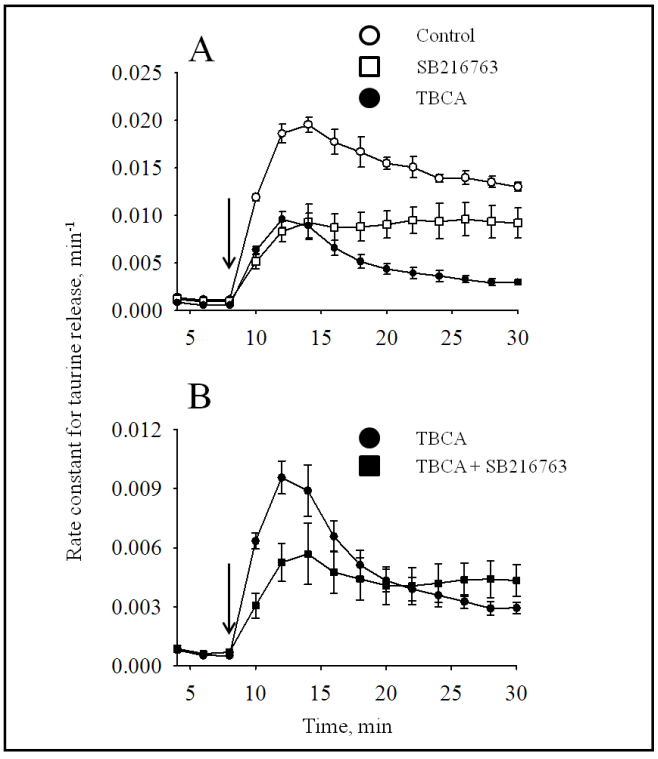

Fig. 3. GSK3 and CK2 inhibit the volume-sensitive taurine efflux pathway via separate signaling pathways. Taurine efflux from NIH3T3 cells was estimated under isoosmotic (335 mOsm) and hypoosmotic (200 mOsm) conditions using tracer technique. The rate constant for taurine efflux was estimated every 2 min and plotted as a function of time. Arrow indicates shift to hypotonicity. A: Experiments were performed in the absence (control) or the presence of the GSK3 inhibitor (3-(2,4-dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione (SB216763), or TBCA. B: Experiments were performed in the presence of TBCA and TBCA in combination with SB216763. Values are from [18].

Taurine uptake is in addition to uptake via TauT also mediated by the transporter PAT1, which belongs to the SLC36 family [54]. However, PAT1 is a pH depend, Na^+- / Cl^--independent transporter with a low affinity towards taurine, i.e., is PAT1 is assumed to be responsible for taurine uptake in the intestine during a taurine rich meal [54].

TauT regulation, long-term

Long term regulation of the Na^+-coupled taurine uptake via TauT involves modulation of the transcription of the *TauT* gene coding for TauT via its promoter, changes in translation rates of mRNA to protein, and

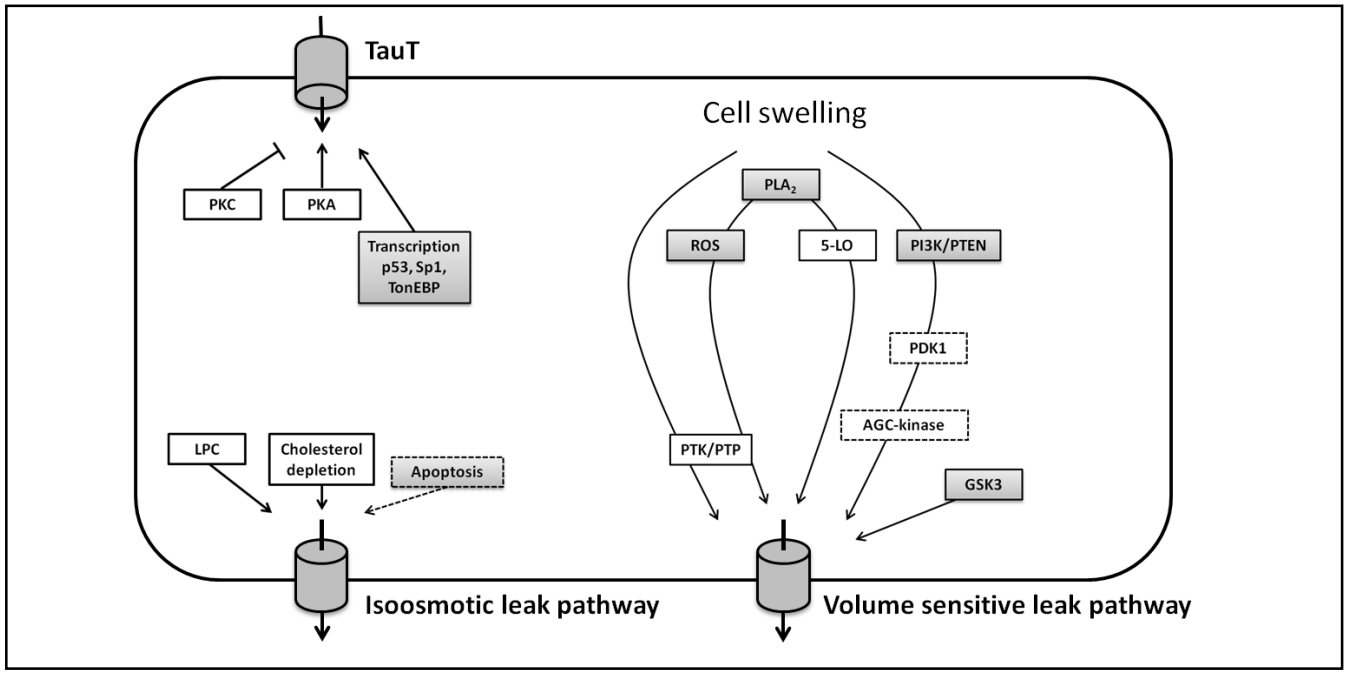

Fig. 4. Regulation of taurine transport systems by protein kinase CK2 in mammalian cells. Taurine is accumulated via the Na^+-dependent transporter TauT and released via a Isoosmotic leak pathway and a Volume sensitive leak pathway. TauT is regulated through phosphorylation by the AGC-kinases PKC and PKA, as well as through transcriptional control by p53, Sp1 and TonEBP. The isoosmotic leak pathway is activated by apoptosis and modulated via membrane content of lysophospholipids (LPC) and cholesterol. Swelling induced taurine release involves (I) activation of cell specific PLA_2 subtypes and followed by ROS-generation (NADPH oxidases) and fatty acid oxidation by a 5-lipoxygenase; (II) increased tyrosine phosphorylation due to tyrosine kinase activation and ROS/vanadate-sensitive protein tyrosine phosphatase inactivation; (III) activation of PI3K and potentially AGC-kinases (PDK-1, PKC, Akt/PKB, PKA and SGK); (IV) GSK3 activity. Dashed boxes indicate putative actors in swelling induced taurine release signaling. Grey boxes and transporters indicate CK2 modulation. See text for details.

translocation of transporter between intracellular stores and the plasma membrane. The *TauT* promoter contains DNA consensus binding sites for the zinc finger-containing transcription factors Sp1 (required for basal promoter activation), TG repeats (critical for full expression of TauT), binding site for the Wilms tumor suppressor (WT1, required for enhanced TauT transport activity), a tonicity-enhancer element (TonE, required for hypertonicity-induced upregulation of *TauT* transcription), a consensus binding site for the transcriptional regulator p53 (p53 binding suppresses TauT transcription) as well as a putative taurine response element (involved in taurin-induced downregulation of *TauT* transcription) [55, 56]. CK2 targets Sp1, p53, topoisomerase, RNA polymerase as well as the TonE binding protein (see [57, 58]) and it has been demonstrated that inhibition of CK2 reduce TonEBP activity [58] and prevents hypertonicity-induced upregulation of *TauT* transcription in NIH3T3 cells [19]. At the same time alters the subcellular TauT localization, i.e., from being evenly distributed in control cells TauT localizes at the endoplasmatic reticulum following CK2 inhibition [19]. Taurine concentration is high in non-adherent Ehrlich ascites tumor cells [59] but becomes reduced as cells adhere to a surface [53] or develop a multidrug resistant phenotype [42]. Drug resistant phenotype is accompanied by an overexpression of catalytic CK2 subunits and increased CK2 catalytic activity [35]. At the same time it has been demonstrated that daunorubicin resistance in Ehrlich ascites tumor cells is accompanied by a significant down regulation of the taurine uptake at the TauT mRNA and protein level and as the down regulation is unrelated to p-gp overexpression [42] Hence, it seems plausible that CK2 affects TauT expression at the transcriptional level.

TauT regulation, acute

Acute regulation of the TauT activity involves shift in pH and the membrane potential (uptake is reduced following acidification of the extracellular medium and depolarisation of the plasma membrane) and phosphorylation / dephosphorylation of TauT or a putative regulator of TauT (see [46]). TauT contains several putative

consensus sites within its intracellular domains for phosphorylation by the serine/threonine kinases PKA, PKC and CK2 and it has been demonstrated that activation of PKA stimulates taurine uptake, whereas activation of PKC inhibits taurine uptake in Ehrlich ascites tumour cells and renders the cells insensitive towards PKA stimulation [60]. Inhibition of protein phosphatases by calyculin A, i.e., retaining proteins in a more phosphorylated state retain TauT in a state with low transport activity where its apparent affinity towards Na^+ and taurine, its transport capacity as well as the Na^+:taurine stoichiometry are reduced compared to unpertubated cells [61]. The PKC-mediated down-regulation of taurine uptake involves phosphorylation of TauT at a highly conserved serine-322 [62] and decreased maximal velocity [19, 43, 60, 63, 64], which in some cases reflect a reduction in the number of transporters in the plasma membrane [65]. Kinetic analysis of taurine uptake in the NIH3T3 and ELA cells revealed that the increase in taurine uptake following CK2 inhibition reflected an increase in TauT´s affinity towards taurine and its transport capacity as well as a reduction in the Na^+:taurine stoichiometry [18]. In this context it is noted that motif scanning reveals a threonine-28, located at the cytosolic site and hence in close contact with TM1, which is a putative CK2 target. It is assumed that TauT activity under steady state is constrained by CK2, i.e., CK2 inhibition elicits a conformational change in TauT and hence substrate binding and the Na^+:taurine stoichiometry. As CK2 inhibition reduces the Na^+:taurine stoichiometry one would expect that Na^+:taurine stoichiometry in NIH3T3 cells, that express low levels of the CK2 regulatory/catalytic subunits should be lower than the Na^+:taurine stoichiometry in ELA cells, which express high levels of CK2 units [18]. This is not the case, i.e., the Na^+:taurine stoichiometry is 2,6 in NIH3T3 and 1.8 in ELA [18], indicating that normal TauT function indeed depends on CK2 activity but is unaltered by the change in the CK2 expression level.

Taurine release, pathways

The swelling-induced taurine release is triggered by acute swelling and increases with the degree of swelling (see [7, 10, 66]). It has been estimated that the fractional release of taurine and other amino acids accounts for 10% of the osmolyte release within the first minutes of RVD, increasing to as much as 30% if hypotonicity prevails (see [46]). The taurine release, activated within seconds to minutes following exposure to hypotonic medium [66], is sodium independent, bi-directional, and non saturable up to 50 mM taurine (see [46]). Activity of the swelling induced taurine release pathway is significantly reduced following cell cycle arrest in the G0/G1 stage, and it has been demonstrated that inhibition of the swelling induced taurine release pathway by tamoxifen and 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) arrests proliferating cervical cancer SiHa cells in the G0/G1 phase [67].

The swelling induced release pathway has yet to be identified. However, electrophysiological studies has demonstrated that swelling induced taurine release is mediated by anion channels (see [66]), and that the swelling-induced taurine release is inhibited by the use of anion channel blockers such as NPPB or 4,4'-Diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS) in Ehrlich Ascites Tumor cells (EAT), NIH3T3, MDA-MB-231 and MCF-7 [46, 68, 69]. However, taurine has an isoelectric point of 5.15 (pKas values: 1.5 and 8.8 (see [46]) making 96% of the taurine pool zwitterionic and the remaining 4% anionic at physiological pH of 7.4, and as the electrophysiological measurements were usually made under non-physiological conditions e.g. high taurine concentrations or high pH which increases the fraction of taurine on anion form, anionic taurine release would have been favoured. Furthermore, the anion blockers commonly used are non-selective, i.e., NPPB uncouples mitochondrial ATP synthesis, resulting in partial depletion of cellular ATP [70], DIDS blocks the Na^+/HCO_3 exchanger (NBCe1-A, [71]) and the Monocarboxylate Transporter 1 (MCT1, [72]) whereas other blockers reduce the intracellular concentration of ATP via chloride channels, thereby affecting the activation of the taurine release (see [46]). Although it has been proposed that volume sensitive chloride and taurine release is mediated by some of the same channels, it has been shown that the sensitivity towards DIDS and oleic acid [68], time course for activation/inactivation of taurine and chloride release differs in e.g. HeLa [73] and MDA-MB-231 human breast cancer cells where chloride release was transient, and the taurine release sustained [69]. Other examples of difference in the release includes volume sensitive taurine release in the absence of chloride channel activity in Xenopus oocytes, and that volume sensitive release of chloride and taurine has been demonstrated to happen independently from the other (see [7]). The great majority of chloride and taurine release is probably mediated by separate systems (see [7]), with the taurine efflux mediated by an anion channel, although this notion is mostly based on the sensitivity of the taurine release towards anion

channel blockers [68]. The volume and DIDS sensitive taurine release pathway has in addition to taurine, been shown to be permeable to other non-essential amino acids such as alanine and glycine [59] as well as sorbitol, choline and thymidine [74]. A common pathway for taurine, glutamate and aspartate has also been proposed, although this release might be mediated by a chloride channel (see [66]).

TauT is possibly involved in isoosmotic taurine release, but not significantly involved in the swelling induced release (see [7]). Taurine release under isotonic conditions is increased following exposure to lysophosphatidylcholine (LPC) [75-77] and following acute cholesterol depletion [78]. Furthermore, release of taurine [79] and amino acids in general [3] has also been correlated with apoptosis. LPC is produced after cell swelling [80] and during ischemia [81], but is has been demonstrated the taurine release, induced by LPC exposure and cholesterol depletion under isotonic conditions, is unaffected by anion channel blockers, which all block the volume sensitive taurine [75, 78]. Similarly, taurine release during apoptosis seems to be insensitive to anion channel blockers [79]. As changes in the physical state of the membrane occur during LPC-treatment, cholesterol depletion [78, 82] and as part of the apoptotic process it is plausible that potentiation of the isoosmotic taurine release by these treatments does not involve activation of the volume sensitive pathway.

Taurine release, regulation of the swelling induced signalling pathway

Swelling induced taurine release is activated by an unknown sensor of cell swelling (see [46]). Activation of phospholipase A_2 (PLA_2) is the first known upstream transducer of cell swelling in the volume sensitive taurine release signalling pathway. PLA_2 subtypes varies between cell types, with the calcium-independent $iPLA_2$ and the secreted $sPLA_2$ subtypes involved in NIH3T3 [83], and the cytosolic $cPLA_2$ subtype in Ehrlich cells (see [46]). Following activation PLA_2 localizes to the perinuclear membrane and catalyses hydrolyses of membrane phospholipids into arachidonic acid (AA) and lysophospholipids (LPL) [80, 84, 85]. The 5-lipoxygenase (5-LO) co-localizes to the nuclear membrane where it associates with the membrane-bound 5-lipoxygenase-activating protein (FLAP) and catalyzes the conversion of AA to leukotriene A_4 (LTA_4) through 5-hydroperoxyeicosatetraenoic acid (5-HPETE) [86-89]. Inhibition of 5-LO or the 5-LO/FLAP interaction blocks the swelling induced taurine release, e.g., with the 5-lipoxygenase inhibitor ETH (see Fig. 2A, [46]). LTA_4 is successively converted to leukotriene C_4, D_4 and E_4 though the activity of glutathione S-transferases. LTD_4 in nM concentration is demonstrated to elicit K^+ as well as taurine loss and accelerate the RVD response in Ehrlich ascites tumor cells (see [46]). Calcium activated calmodulin (CaM) is an inhibitor of $iPLA_2\beta$ and hence the swelling-induced mobilisation of AA in NIH3T3 cells [90]. CaM interacts with $iPLA_2$ in an catalytically inactive heterocomplex, and dissociation of calmodulin by reduced intracellular Ca^{2+} is necessary for $iPLA_2$ activity [91, 92]. From Fig. 2A it is seen that the volume sensitive taurine release is significantly inhibited by the $iPLA_2$ inhibitor Bromoenol lactone (BEL) and stimulated following exposure to the calmodulin antagonist *N*-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride (W7). It is noted that inhibition of CaM can lead to a reduced taurin release under hypoosmotic conditions depending on cell type and whether the taurine release is potentiated by increased intracellular Ca^{2+} mobilization [93, 94].

CK2 is known to phosphorylate and inhibit CaM, reducing its Ca^{2+}-sensitivity and interaction with other proteins [95, 96], making CaM the potential target in the CK2-mediated effect on swelling induced taurine release. On the other hand, Inhibition of CK2 stimulates $sPLA_2$-activity *in vitro* as well as $cPLA_2$ activity in lysed synaptosomes [97, 98], which would reduce swelling induced taurine release. From Fig. 2A it is seen that inhibition of CK2 results in decreased taurine release rates when PLA_2-activity is stimulated by the calmodulin antagonist W7 under hypoosmotic conditions. The taurine efflux with TBCA alone is not significantly different from the efflux when TBCA and W7 are used in combination, indicating that CK2 regulate taurine downstream to PLA_2.

Cell swelling results in an increased generation of reactive oxygen species (ROS) in e.g., NIH3T3, EAT and HTC cells [7, 83, 99, 100]. PLA_2 activity is required for the swelling induced ROS generation in NIH3T3 [90]. Addition of LPL, e.g., lysophosphatidic acid (LPA) and the PKC activator phorbol myristate acetate (PMA) potentiates swelling-induced ROS production as well as the concomitant taurine release. The effect of LPA and PMA on taurine transport is impaired in the presence of an NADPH oxidase inhibitor [99, 101], i.e., PKC and LPA interferes at a step upstream to generation of ROS. NADPH oxidases generates superoxides which are converted by dismutation to the lipid permeable H_2O_2 se (see [102]). Increased availability of H_2O_2 leads to activation

of tyrosine kinases and reduced phosphatase activity caused by oxidation of the thiol residue at an essential cysteine in the active site [103]. Several protein phosphatases have been shown to be oxidized by the otherwise weak oxidant H_2O_2 including PTP1B [104], PP1, PP2A and PP2B [106, 107]. Exogenous addition of H_2O_2 or the tyrosine phosphatase inhibitor vanadate potentiates the taurine release significantly, whereas ROS-scavenging with the antioxidant butulated hydroxytoluene (BHT), knock-down of the NADPH oxidase with siRNA or protein tyrosine kinase inhibition with genistein block the swelling induced taurine release even in the presence of vanadate or H_2O_2 (see Figs. 2B and C) [90]. This indicates that tyrosine phosphorylation is downstream in the swelling-induced taurine release signalling cascade and that ROS inhibit a protein tyrosine phosphatase, hence amplifying the effect of the protein tyrosine kinase. Several tyrosine kinases have been assigned a role in the regulation of the swelling-induced taurine release as judged from the effect of pharmacological inhibition (see [90, 107]).

NADPH oxidase (NOX) is a multimeric heterocomplex composed of flavocytochrome b_{558} consisting of a Nox enzyme, $p22^{phox}$, plus the regulatory subunits $p40^{phox}$, $p47^{phox}$, $p67^{phox}$, Rac1/2. CK2 has been indicated to phosphorylate $p47^{phox}$ at Ser^{208} and Ser^{283} [108], possibly preventing interaction of the regulatory complex p40-p67-$p47^{phox}$ with flavocytochrome b_{558}, and thereby generation of ROS [108]. Activation of NOX can be induced by arachidonic acid which induces a conformational change in $p47^{phox}$, thereby activating the oxidase [109]. However, this conformational change also significantly increases CK2 phosphorylation *in vitro* [110]. Furthermore, inhibition of CK2 results in increased translocation of $p47^{phox}$ to the cell membrane [108] and in increased production of ROS [108, 111], making CK2 a potential negative regulator of NOX activity [108]. The NADPH oxidase subunits; $p67^{phox}$, $p47^{phox}$, NOXO1, $p22^{phox}$, NOX4 and DUOX2 are expressed in NIH3T3 cells [99]. However, only NOX4 and $p22^{phox}$ have been proven sufficient for generation of ROS [99, 102], and are therefore proposed to constitute the volume-sensitive NADPH oxidase in NIH3T3 cells [99], reducing but not excluding a potential role of CK2 regulation of NOX4 though $p47^{phox}$ in these cells. On the other hand, inhibition of CK2 was demonstrated to increase the production of H_2O_2 as a result of induced apoptosis [111]. CK2 inhibition has been demonstrated to result in reduced superoxide concentration at the same time as the increased H_2O_2 production, indicating that increase in ROS is not necessarily caused by increased NADPH-activity, but more likely a result of accelerated superoxide dismutation [112]. These previous studies place CK2 as a negative regulator of ROS-production, reducing general availability of H_2O_2 either directly through phosphorylation of $p47^{phox}$ in some cell types, or indirectly by suppression of apoptosis [22, 23] (see [24, 25]). From Figs. 2B and C it is seen that taurine release rates are reduced by CK2-inhibition in the presence of 0.5 mM exogenous H_2O_2 and in the presence of vanadate. However, inhibition of CK2 does not fully prevent H_2O_2 induced stimulation of the swelling induced taurine release whereas the vanadate-induced potentiation is fully prevented, indicating CK2-mediated regulation of the taurine efflux pathway parallel to ROS-stimulation and downstream to vanadate-stimulation. It is possible that CK2 regulates the taurine release on the level of tyrosine kinase-activity as the effect of TBCA on vanadate-induced potentiation of swelling induced taurine release is similar to the effect of the tyrosine kinase inhibitor genistein (Fig. 2C) (see [46]). This interpretation of data indicates that H_2O_2-induced stimulation of the swelling induced taurine release does not only function through modulation of tyrosine-phosphorylation state. Other ROS targets for modulation of the taurine-release signalling cascade could be the activity of 5-LO and phosphatidylinositol (3,4,5)-trisphosphate-kinase (PI3K) signalling. 5-LO is a non-heme iron containing enzyme which is inactive when the iron is in the ferrous state (Fe^{2+}) [113], i.e., oxidation of Fe^{2+} to Fe^{3+} by H_2O_2 could potentiate 5-LO activity.

PI3K is activated by cell swelling [114] possibly through activation of the epidermal growth factor receptor (EGFR) [115], G protein-coupled receptors and tyrosine kinase receptors, resulting in generation of phosphatidylinositol (3,4,5)-trisphosphate (PIP_3, see [116]). Taurine release, activated by either cell swelling (Fig. 2D) or by cell swelling in combination with thrombin-induced PAR-receptor activation, is significantly decreased by PI3K inhibition with wortmanin [18, 94, 115]. Generation of PIP_3, by PI3K results in activation of the so-called AGC-kinases including notably members such as PDK-1, PKC, Akt/PKB, PKA and serum and glycocorticoide kinase (SGK), several of which are involved in control of proliferation, programmed cell death (apoptosis) and ion transport (see [117]). The phosphatase and tensin homolog (PTEN) dephosphorylates PIP_3, opposing the action of PI3K. PTEN is reversibly inactivated by ROS through oxidation [118-121], and by CK2 through phosphorylation [118]. From Fig. 2D it is seen that inhibition of PTEN by HOpic potentiate the

swelling induced taurine release. Inhibition of CK2 annuls potentiation by HOpic and is not additive with wortmanin (Fig. 2D), indicating that CK2 interferes a step downstream to PI3K-signalling. The Glycogen synthase kinase 3 (GSK3) is inactivated downstream to PI3K-activation through Akt/PKB, and is implicated in various cellular functions including glycogen metabolism, wnt signalling and cell migration (see [122-124]). GSK3 has an uncommon prerequisite for priming phosphorylation, i.e., GSK3 substrates must be phosphorylated at the position n+4 where n is the GSK3 target residue, and CK2 has been demonstrated to prime the phosphorylation of glycose synthase by GSK3 [125] (see [123]). Inhibition of GSK3 by (3-(2,4-dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione (SB216763), has no effect on taurine release under isoosmotic conditions [18], whereas it reduces the swelling-induced taurine release significantly (Fig. 3A). The effect of GSK3-inhibition is additive to the inhibition of CK2 by TBCA (Fig. 3B). Examining the traces in Fig. 3 reveals that inhibition of GSK3 results not only in reduced maximum taurine release rates, but also in sustained taurine release, i.e., the taurine release is not inactivated with time (Fig. 3A). Furthermore, inhibiting CK2 in addition to GSK3 leads to overall reduced, but sustained taurine release, i.e., the release characteristics are dominated by GSK3-inhibition although CK2-inhibition reduces overall release (Fig. 3B). We suggest that GSK3 is a novel regulator of swelling induced taurine release. However, CK2 and GSK3 are most likely not operating in concert, that is, CK2-priming followed by GSK3-phosphorylation, as swelling induced taurine release characteristics following CK2-inhibition would resemble that of GSK3-inhibition. Furthermore, as Akt/PKB inactivates GSK3 following PI3K-activation it would seem unlikely that PI3K regulates taurine release through Akt/PKB-GSK3. A more direct role of AGC-kinase members on regulation of the swelling induced taurine release is not excluded.

The constitutively active CK2 is upregulated in various forms of cancer and the present data indicate that CK2 indeed limits taurine uptake and stimulates taurine release in mouse fibroblasts independently of the CK2 expression level. Using CK2 inhibitors in cancer treatment would favour retention of taurine in cells through reuptake of taurine and presumably limit net loss of taurine and hence apoptotic cell shrinkage. Provided CK2 inhibitors increase cytotoxic drug accumulation through inhibition of drug exit via the P-glycoprotein in drug resistant tumor cells [35], this would mean that CK2 inhibition could limit net loss of taurine and other amino acids (preservation of energy supply) in healthy dividing cells and hence increase their survival during the chemical drug treatment.

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References

1 Habela CW, Ernest NJ, Swindall AF, Sontheimer H: Chloride accumulation drives volume dynamics underlying cell proliferation and migration. J Neurophysiol 2009;101:750-757.

2 Habela CW, Sontheimer H: Cytoplasmic volume condensation is an integral part of mitosis. Cell Cycle 2007;6:1613-1620.

3 Poulsen KA, Andersen EC, Hansen CF, Klausen TK, Hougaard C, Lambert IH, Hoffmann EK: Deregulation of apoptotic volume decrease and ionic movements in multidrug-resistant tumor cells: role of chloride channels. Am J Physiol Cell Physiol 2010;298:C14-C25.

4 Ernest NJ, Habela CW, Sontheimer H: Cytoplasmic condensation is both necessary and sufficient to induce apoptotic cell death. J Cell Sci 2008;121:290-297.

5 Lang F, Foller M, Lang K, Lang P, Ritter M, Vereninov A, Szabo I, Huber SM, Gulbins E: Cell volume regulatory ion channels in cell proliferation and cell death. Methods Enzymol 2007;428:209-225.

6 Lang F, Ritter M, Gamper N, Huber S, Fillon S, Tanneur V, Lepple-Wienhues A, Szabo I, Gulbins E: Cell volume in the regulation of cell proliferation and apoptotic cell death. Cell Physiol Biochem 2000;10:417-428.

7 Hoffmann EK, Lambert IH, Pedersen SF: Physiology of cell volume regulation in vertebrates. Physiol Rev 2009;89:193-277.

8 Lambert IH, Hoffmann EK, Pedersen SF: Cell volume regulation: physiology and pathophysiology. Acta Physiol (Oxf) 2008;194:255-282.

9 Huxtable RJ: Taurine in the Central Nervous-System and the Mammalian Actions of Taurine. Prog Neurobiol 1989;32:471-533.

10 Huxtable RJ: Physiological actions of taurine. Physiol Rev 1992;72:101-163.

11 Jacobsen JG, Smith LH: Biochemistry and physiology of taurine and taurine derivatives. Physiol Rev 1968;48:424-511.

12 Lourenço R, Camilo ME: Taurine: a conditionally essential amino acid in humans? An overview in health and disease. Nutr Hosp 2002;17:262-270.

13 Aerts L, Van Assche FA: Taurine and taurine-deficiency in the perinatal period. J Perinat Med 2002;30:281-286.

14 Warskulat U, Heller-Stilb B, Oermann E, Zilles K, Haas H, Lang F, Haussinger D: Phenotype of the taurine transporter knockout mouse. Methods Enzymol 2007;428:439-58.
15 Kearns S, Dawson R Jr: Cytoprotective effect of taurine against hypochlorous acid toxicity to PC12 cells. Taurine 4: Taurine and Excitable Tissues Adv Exp Med Biol 2000;483:563-570.
16 Atmaca G: Antioxidant effects of sulfur-containing amino acids. Yonsei Med J 2004;45:776-788.
17 Schaffer SW, Azuma J, Mozaffari M: Role of antioxidant activity of taurine in diabetes. Can J Physiol Pharmacol 2009;87:91-99.
18 Hansen DB, Guerra B, Jacobsen JH, Lambert IH: Regulation of taurine homeostasis by protein kinase CK2 in mouse fibroblasts. Amino Acids 2011;40:1091-1106.
19 Jacobsen JH, Clement CA, Friis MB, Lambert IH: Casein kinase 2 regulates the active uptake of the organic osmolyte taurine in NIH3T3 mouse fibroblasts. Pflugers Arch 2008;457:327-337.
20 Sarno S, Pinna LA: Protein kinase CK2 as a druggable target. Mol Biosyst 2008;4:889-894.
21 Guerra B, Issinger OG: Protein kinase CK2 in human diseases. Curr Med Chem 2008;15:1870-1886.
22 Zhu D, Hensel J, Hilgraf R, Abbasian M, Pornillos O, Deyanat-Yazdi G, Hua XH, Cox S: Inhibition of protein kinase CK2 expression and activity blocks tumor cell growth. Mol Cell Biochem 2010;333:159-167.
23 Schneider CC, Hessenauer A, Montenarh M, Gotz C: p53 is dispensable for the induction of apoptosis after inhibition of protein kinase CK2. Prostate 2010;70:126-134.
24 St-Denis NA, Litchfield DW: Protein kinase CK2 in health and disease: From birth to death: the role of protein kinase CK2 in the regulation of cell proliferation and survival. Cell Mol Life Sci 2009;66:1817-1829.
25 Trembley JH, Wang G, Unger G, Slaton J, Ahmed K: Protein kinase CK2 in health and disease: CK2: a key player in cancer biology. Cell Mol Life Sci 2009;66:1858-1867.
26 Niefind K, Raaf J, Issinger OG: Protein kinase CK2 in health and disease: Protein kinase CK2: from structures to insights. Cell Mol Life Sci 2009;66:1800-1816.
27 Cozza G, Bortolato A, Moro S: How druggable is protein kinase CK2? Med Res Rev 2010;30:419-462.
28 Canton DA, Litchfield DW: The shape of things to come: An emerging role for protein kinase CK2 in the regulation of cell morphology and the cytoskeleton. Cell Signal 2006;18:267-275.
29 Montenarh M: Cellular regulators of protein kinase CK2. Cell Tissue Res 2010;342:139-146.
30 Olsten MEK, Weber JE, Litchfield DW: CK2 interacting proteins: Emerging paradigms for CK2 regulation? Mol Cell Biochem 2005;274:115-124.
31 Faust M, Montenarh M: Subcellular localization of protein kinase CK2. A key to its function? Cell Tissue Res 2000;301:329-340.
32 Theis-Febvre N, Martel V, Laudet B, Souchier C, Grunwald D, Cochet C, Filhol O: Highlighting protein kinase CK2 movement in living cells. Mol Cell Biochem 2005;274:15-22.
33 Olsen BB, Guerra B, Niefind K, Issinger OG: Structural basis of the constitutive activity of protein kinase CK2. Methods Enzymol 2010;484:515-529.
34 Litchfield DW: Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. Biochem J 2003;369:1-15.
35 Di MG, Brustolon F, Tosoni K, Belli S, Kramer SD, Pinna LA, Ruzzene M: Comparative analysis of CK2 expression and function in tumor cell lines displaying sensitivity vs. resistance to chemical induced apoptosis. Mol Cell Biochem 2008;316:155-161.
36 Chen NH, Reith MEA, Quick MW: Synaptic uptake and beyond: the sodium- and chloride-dependent neurotransmitter transporter family SLC6. Pflugers Arch 2004;447:519-531.
37 Liu QR, Lopez-Corcuera B, Nelson H, Mandiyan S, Nelson N: Cloning and expression of a cDNA encoding the transporter of taurine and beta-alanine in mouse brain. Proc Natl Acad Sci USA 1992;89:12145-12149.
38 Smith KE, Borden LA, Wang CH, Hartig PR, Branchek TA, Weinshank RL: Cloning and expression of a high affinity taurine transporter from rat brain. Mol Pharmacol 1992;42:563-569.
39 Uchida S, Kwon HM, Yamauchi A, Preston AS, Marumo F, Handler JS: Molecular cloning of the cDNA for an MDCK cell Na^+- and Cl^-- dependent taurine transporter that is regulated by hypertonicity. Proc Natl Acad Sci USA 1992;89:8230-8234.
40 Ramamoorthy S, Del Monte MA, Leibach FH, Ganapathy V: Molecular identity and calmodulin-mediated regulation of the taurine transporter in a human retinal pigment epithelial cell line. Curr Eye Res 1994;13:523-529.
41 Pinto W, Ronnestad I, Jordal AE, Gomes AS, Dinis MT, Aragao C: Cloning, tissue and ontogenetic expression of the taurine transporter in the flatfish Senegalese sole (Solea senegalensis). Amino Acids 2011, in press.
42 Poulsen KA, Litman T, Eriksen J, Mollerup J, Lambert IH: Downregulation of taurine uptake in multidrug resistant Ehrlich ascites tumor cells. Amino Acids 2002;22:333-350.
43 Voss JW, Pedersen SF, Christensen ST, Lambert IH: Regulation of the expression and subcellular localisation of the taurine transporter TauT in mouse NIH3T3 fibroblasts. Eur J Biochem 2004;271:4646-4658.
44 Yamashita A, Singh SK, Kawate T, Jin Y, Gouaux E: Crystal structure of a bacterial homologue of Na^+/Cl^--dependent neurotransmitter transporters. Nature 2005;437:215-223.
45 Gether U, Andersen PH, Larsson OM, Schousboe A: Neurotransmitter transporters: molecular function of important drug targets. Trends Pharmacol Sci 2006;27:375-383.
46 Lambert IH: Regulation of the cellular content of the organic osmolyte taurine in mammalian cells. Neurochem Res 2004;29:27-63.
47 Voss JW, Pedersen SF, Christensen ST, Lambert IH: Regulation of the expression and subcellular localization of the taurine transporter TauT in mouse NIH3T3 fibroblasts. Eur J Biochem 2004;271:4646-4658.
48 Heller-Stilb B, van Roeyen C, Rascher K, Hartwig HG, Huth A, Seeliger MW, Warskulat U, Haussinger D: Disruption of the taurine transporter gene (taut) leads to retinal degeneration in mice. Faseb J 2002;16:231-3.
49 Warskulat U, Borsch E, Reinehr R, Heller-Stilb B, Roth C, Witt M, Haussinger D: Taurine deficiency and apoptosis: findings from the taurine transporter knockout mouse. Arch Biochem Biophys 2007;462:202-209.
50 Warskulat U, Flogel U, Jacoby C, Hartwig HG, Thewissen M, Merx MW, Molojavyi A, Heller-Stilb B, Schrader J, Haussinger D: Taurine transporter knockout depletes muscle taurine levels and results in severe skeletal muscle impairment but leaves cardiac function uncompromised. Faseb J 2004;18:577-579.
51 Warskulat U, Borsch E, Reinehr R, Heller-Stilb B, Monnighoff I, Buchczyk D, Donner M, Flogel U, Kappert G, Soboll S, Beer S, Pfeffer K, Marschall HU, Gabrielsen M, Amiry-Moghaddam M, Ottersen OP, Dienes HP, Haussinger DH: Chronic liver disease is triggered by taurine transporter knockout in the mouse. Faseb J 2006;20:574-6.
52 Han X, Yue J, Chesney RW: Functional TauT protects against acute kidney injury. J Am Soc Nephrol 2009;20:1323-1332.
53 Tastesen HS, Holm JB, Moller J, Poulsen KA, Moller C, Sturup S, Hoffmann EK, Lambert IH: Pinpointing differences in cisplatin-induced apoptosis in adherent and non-adherent cancer cells. Cell Physiol Biochem 2010;26:809-820.

54 Anderson CM, Howard A, Walters JR, Ganapathy V, Thwaites DT: Taurine uptake across the human intestinal brush-border membrane is via two transporters: H^+-coupled PAT1 (SLC36A1) and Na^+- and Cl^--dependent TauT (SLC6A6). J Physiol 2009;587:731-744.

55 Han X, Budreau AM, Chesney RW: Identification of promoter elements involved in adaptive regulation of the taurine transporter gene: role of cytosolic Ca^{2+} signaling. Adv Exp Med Biol 2000;483:535-544.

56 Han X, Patters AB, Jones DP, Zelikovic I, Chesney RW: The taurine transporter: mechanisms of regulation. Acta Physiol 2006;187:61-73.

57 Meggio F, Pinna LA: One-thousand-and-one substrates of protein kinase CK2? Faseb J 2003;17:349-368.

58 Ferraris JD, Williams CK, Persaud P, Zhang Z, Chen Y, Burg MB: Activity of the TonEBP/OREBP transactivation domain varies directly with extracellular NaCl concentration. Proc Natl Acad Sci USA 2002;99:739-744.

59 Hoffmann EK, Lambert IH: Amino acid transport and cell volume regulation in Ehrlich ascites tumour cells. J Physiol 1983;338:613-625.

60 Mollerup J, Lambert IH: Phosphorylation is involved in the regulation of the taurine influx via the beta-system in Ehrlich ascites tumor cells. J Membr Biol 1996;150:73-82.

61 Mollerup J, Lambert IH: Calyculin A modulates the kinetic constants for the Na^+-coupled taurine transport in Ehrlich ascites tumour cells. Biochim Biophys Acta 1998;1371:335-344.

62 Han X, Budreau AM, Chesney RW: Ser-322 is a critical site for PKC regulation of the MDCK cell taurine transporter (pNCT). J Am Soc Nephrol 1999;10:1874-1879.

63 Han X, Budreau AM, Chesney RW: Role of conserved peptide in taurine transporter inactivation modulated by protein kinase C. J Am Soc Nephrol 1996;7:2088-2096.

64 Qian X, Vinnakota S, Edwards C, Sarkar HK: Molecular characterization of taurine transport in bovine aortic endothelial cells. Biochim Biophys Acta 2000;1509:324-334.

65 Loo DD, Hirsch JR, Sarkar HK, Wright EM: Regulation of the mouse retinal taurine transporter (TAUT) by protein kinases in Xenopus oocytes. FEBS Lett 1996;392:250-254.

66 Shennan DB: Swelling-induced taurine transport: Relationship with chloride channels, anion-exchangers and other swelling-activated transport pathways. Cell Physiol Biochem 2008;21:15-28.

67 Shen MR, Chou CY, Ellory JC: Swelling-activated taurine and K^+ transport in human cervical cancer cells: association with cell cycle progression. Pflugers Arch 2001;441:787-795.

68 Lambert IH, Hoffmann EK: Cell swelling activates separate taurine and chloride channels in Ehrlich mouse ascites tumor cells. J Membr Biol 1994;142:289-298.

69 Shennan DB, Thomson J, Gow IF: Osmoregulation of taurine efflux from cultured human breast cancer cells: Comparison with volume activated Cl^- efflux and regulation by extracellular ATP. Cell Physiol Biochem 2006;18:113-122.

70 Lukacs GL, Nanda A, Rotstein OD, Grinstein S: The chloride channel blocker 5-nitro-2-(3-phenylpropyl-amino) benzoic acid (NPPB) uncouples mitochondria and increases the proton permeability of the plasma membrane in phagocytic cells. FEBS Lett 1991;288:17-20.

71 Lu J, Boron WF: Reversible and irreversible interactions of DIDS with the human electrogenic Na/HCO_3 cotransporter NBCe1-A: role of lysines in the KKMIK motif of TM5. Am J Physiol Cell Physiol 2007;292:C1787-C1798.

72 Wilson MC, Meredith D, Bunnun C, Sessions RB, Halestrap AP: Studies on the DIDS-binding site of monocarboxylate transporter 1 suggest a homology model of the open conformation and a plausible translocation cycle. J Biol Chem 2009;284:20011-20021.

73 Stutzin A, Torres R, Oporto M, Pacheco P, Eguiguren AL, Cid LP, Sepulveda FV: Separate taurine and chloride efflux pathways activated during regulatory volume decrease. Am J Physiol 1999;277:C392-C402.

74 Hall JA, Kirk J, Potts JR, Rae C, Kirk K: Anion channel blockers inhibit swelling-activated anion, cation, and nonelectrolyte transport in HeLa cells. Am J Physiol 1996;271:C579-C588.

75 Lambert IH, Falktoft B: Lysophosphatidylcholine induces taurine release from HeLa cells. J Membr Biol 2000;176:175-185.

76 Lambert IH, Falktoft B: Lysophosphatidylcholine-induced taurine release in HeLa cells involves protein kinase activity. Comp Biochem Physiol A Mol Integr Physiol 2001;130:577-584.

77 Lambert IH, Nielsen JH, Andersen HJ, Ortenblad N: Cellular model for induction of drip loss in meat. J Agric Food Chem 2001;49:4876-4883.

78 Villumsen KR, Duelund L, Lambert IH: Acute cholesterol depletion leads to net loss of the organic osmolyte Taurine in Ehrlich Lettré tumor cells. Amino Acids 2010;39:1521-36.

79 Moran J, Hernandez-Pech X, Merchant-Larios H, Pasantes-Morales H: Release of taurine in apoptotic cerebellar granule neurons in culture. Pflugers Arch 2000;439:271-277.

80 Thoroed SM, Lauritzen L, Lambert IH, Hansen HS, Hoffmann EK: Cell swelling activates phospholipase A2 in Ehrlich ascites tumor cells. J Membr Biol 1997;160:47-58.

81 Kinnaird AA, Choy PC, Man RY: Lysophosphatidylcholine accumulation in the ischemic canine heart. Lipids 1988;23:32-35.

82 Lundbaek JA, Andersen OS: Lysophospholipids modulate channel function by altering the mechanical properties of lipid bilayers. J Gen Physiol 1994;104:645-673.

83 Lambert IH: Activation and inactivation of the volume-sensitive taurine leak pathway in NIH3T3 fibroblasts and Ehrlich Lettre ascites cells. Am J Physiol Cell Physiol 2007;293:C390-C400.

84 Pedersen S, Lambert IH, Thoroed SM, Hoffmann EK: Hypotonic cell swelling induces translocation of the alpha isoform of cytosolic phospholipase A2 but not the gamma isoform in Ehrlich ascites tumor cells. Eur J Biochem 2000;267:5531-5539.

85 Pedersen SF, Poulsen KA, Lambert IH: Roles of phospholipase A(2) isoforms in swelling- and melittin-induced arachidonic acid release and taurine efflux in NIH3T3 fibroblasts. Am J Physiol Cell Physiol 2006;291:C1286-C1296.

86 Rådmark OP: The molecular biology and regulation of 5-lipoxygenase. Am J Respir Crit Care Med 2000;161:S11-S15.

87 Radmark O, Samuelsson B: 5-Lipoxygenase: mechanisms of regulation. J Lipid Res 2009;50:S40-S45.

88 Radmark O, Samuelsson B: 5-lipoxygenase: regulation and possible involvement in atherosclerosis. Prostaglandins Other Lipid Mediat 2007;83:162-174.

89 Newcomer ME, Gilbert NC: Location, location, location: compartmentalization of early events in leukotriene biosynthesis. J Biol Chem 2010;285:25109-25114.

90 Lambert IH: Reactive oxygen species regulate swelling-induced taurine efflux in NIH3T3 mouse fibroblasts. J Membr Biol 2003;192:19-32.

91 Wolf MJ, Wang J, Turk J, Gross RW: Depletion of intracellular calcium stores activates smooth muscle cell calcium-independent phospholipase A2. A novel mechanism underlying arachidonic acid mobilization. J Biol Chem 1997;272:1522-1526.

92 Jenkins CM, Wolf MJ, Mancuso DJ, Gross RW: Identification of the calmodulin-binding domain of recombinant calcium-independent phospholipase $A_{2\beta}$, implications for structure and function. J Biol Chem 2001;276:7129-7135.
93 Lambert IH, Hoffmann EK: Regulation of taurine transport in Ehrlich ascites tumor cells. J Membr Biol 1993;131:67-79.
94 Cruz-Rangel S, Hernandez-Benitez R, Vazquez-Juarez E, Lopez-Dominguez A, Pasantes-Morales H: Potentiation by thrombin of hyposmotic glutamate and taurine efflux from cultured astrocytes: signalling chains. Neurochem Res 2008;33:1518-1524.
95 Arrigoni G, Marin O, Pagano MA, Settimo L, Paolin B, Meggio F, Pinna LA: Phosphorylation of calmodulin fragments by protein kinase CK2. Mechanistic aspects and structural consequences. Biochemistry 2004;43:12788-12798.
96 Greif DM, Sacks DB, Michel T: Calmodulin phosphorylation and modulation of endothelial nitric oxide synthase catalysis. Proc Natl Acad Sci USA 2004;101:1165-1170.
97 Shimoyama Y, Sakamoto R, Akaboshi T, Tanaka M, Ohtsuki K: Characterization of secretory type IIA phospholipase A2 (sPLA2-IIA) as a glycyrrhizin (GL)-binding protein and the GL-induced inhibition of the CK-II-mediated stimulation of sPLA2-IIA activity in vitro. Biol Pharm Bull 2001;24:1004-1008.
98 Piomelli D, Greengard P: Bidirectional control of phospholipase A2 activity by Ca^{2+}/calmodulin-dependent protein kinase II, cAMP-dependent protein kinase, and casein kinase II. Proc Natl Acad Sci USA 1991;88:6770-6774.
99 Friis MB, Vorum KG, Lambert IH: Volume-sensitive NADPH oxidase activity and taurine efflux in NIH3T3 mouse fibroblasts. Am J Physiol Cell Physiol 2008;294:C1552-C1565.
100 Varela D, Simon F, Riveros A, Jorgensen F, Stutzin A: NAD(P)H oxidase-derived H2O2 signals chloride channel activation in cell volume regulation and cell proliferation. J Biol Chem 2004;279:13301-13304.
101 Ørtenblad N, Young JF, Oksbjerg N, Nielsen JH, Lambert IH: Reactive oxygen species are important mediators of taurine release from skeletal muscle cells. Am J Physiol 2003;284:C1362-C1373.
102 Katsuyama M: NOX/NADPH oxidase, the superoxide-generating enzyme: its transcriptional regulation and physiological roles. J Pharmacol Sci 2010;114:134-146.
103 Barford D, Das AK, Egloff MP: The structure and mechanism of protein phosphatases: insights into catalysis and regulation. Annu Rev Biophys Biomol Struct 1998;27:133-164.
104 Lee SR, Kwon KS, Kim SR, Rhee SG: Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. J Biol Chem 1998;273:15366-15372.
105 Meinhard M, Grill E: Hydrogen peroxide is a regulator of ABI1, a protein phosphatase 2C from Arabidopsis. Febs Letters 2001;508:443-446.
106 Lambert IH, Klausen TK, Bergdahl A, Hougaard C, Hoffmann EK: ROS activate KCl cotransport in nonadherent Ehrlich ascites cells but K^+ and Cl^- channels in adherent Ehrlich Lettre and NIH3T3 cells. Am J Physiol Cell Physiol 2009;297:C198-C206.
107 Pasantes-Morales H, Lezama RA, Ramos-Mandujano G: Tyrosine kinases and osmolyte fluxes during hyposmotic swelling. Acta Physiol (Oxf) 2006;187:93-102.
108 Park HS, Lee SM, Lee JH, Kim YS, Bae YS, Park JW: Phosphorylation of the leucocyte NADPH oxidase subunit p47(phox) by casein kinase 2: conformation-dependent phosphorylation and modulation of oxidase activity. Biochem J 2001;358:783-790.
109 Shiose A, Sumimoto H: Arachidonic acid and phosphorylation synergistically induce a conformational change of p47(phox) to activate the phagocyte NADPH oxidase. J Biol Chem 2000;275:13793-13801.
110 Park HS, Lee SM, Lee JH, Kim YS, Bae YS, Park JW: Phosphorylation of the leucocyte NADPH oxidase subunit p47(phox) by casein kinase 2: conformation-dependent phosphorylation and modulation of oxidase activity. Biochem J 2001;358:783-790.
111 Ahmad KA, Wang GX, Ahmed K: Intracellular hydrogen peroxide production is an upstream event in apoptosis induced by down-regulation of casein kinase 2 in prostate cancer cells. Mol Cancer Res 2006;4:331-338.
112 Hanif IM, Ahmad KA, Ahmed K, Pervaiz S: Involvement of Reactive Oxygen Species in Apoptosis Induced by Pharmacological Inhibition of Protein Kinase CK2. Natural Compounds and Their Role in Apoptotic Cell Signaling Pathways. Ann NY Acad Sci 2009;1171:591-599.
113 Pergola C, Werz O: 5-Lipoxygenase inhibitors: a review of recent developments and patents. Expert Opin Ther Pat 2010;20:355-375.
114 Wang GX, McCrudden C, Dai YP, Horowitz B, Hume JR, Yamboliev IA: Hypotonic activation of volume-sensitive outwardly rectifying chloride channels in cultured PASMCs is modulated by SGK. Am J Physiol Heart Circ Physiol 2004;287:H533-H544.
115 Franco R, Lezama R, Ordaz B, Pasantes-Morales H: Epidermal growth factor receptor is activated by hyposmolarity and is an early signal modulating osmolyte efflux pathways in Swiss 3T3 fibroblasts. Pflugers Arch 2004;447:830-839.
116 Oudit GY, Penninger JM: Cardiac regulation by phosphoinositide 3-kinases and PTEN. Cardiovasc Res 2009;82:250-260.
117 Bayascas JR: PDK1: the major transducer of PI 3-kinase actions. Curr Top Microbiol Immunol 2010;346:9-29.
118 Silva A, Yunes JA, Cardoso BA, Martins LR, Jotta PY, Abecasis M, Nowill AE, Leslie NR, Cardoso AA, Barata JT: PTEN posttranslational inactivation and hyperactivation of the PI3K/Akt pathway sustain primary T cell leukemia viability. J Clin Invest 2008;118:3762-3774.
119 Lee SR, Yang KS, Kwon J, Lee C, Jeong W, Rhee SG: Reversible inactivation of the tumor suppressor PTEN by H_2O_2. J Biol Chem 2002;277:20336-20342.
120 Leslie NR, Bennett D, Lindsay YE, Stewart H, Gray A, Downes CP: Redox regulation of PI 3-kinase signalling via inactivation of PTEN. EMBO J 2003;22:5501-5510.
121 Cao J, Schulte J, Knight A, Leslie NR, Zagozdzon A, Bronson R, Manevich Y, Beeson C, Neumann CA: Prdx1 inhibits tumorigenesis via regulating PTEN/AKT activity. EMBO J 2009;28:1505-1517.
122 Wu D, Pan W: GSK3: a multifaceted kinase in Wnt signaling. Trends Biochem Sci 2010;35:161-168.
123 Frame S, Cohen P: GSK3 takes centre stage more than 20 years after its discovery. Biochem J 2001;359:1-16.
124 Sun T, Rodriguez M, Kim L: Glycogen synthase kinase 3 in the world of cell migration. Dev Growth Differ 2009;51:735-742.
125 Fiol CJ, Mahrenholz AM, Wang Y, Roeske RW, Roach PJ: Formation of protein kinase recognition sites by covalent modification of the substrate. Molecular mechanism for the synergistic action of casein kinase II and glycogen synthase kinase 3. J Biol Chem 1987;262:14042-14048.