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

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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,
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 isoosmotic 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 A₂ and a 5-lipoxygenase, (II) cell specific NADPH oxidases and generation of reactive oxygen species, (III) unidentified protein tyrosine kinases, (IV) phosphatidylinositol 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
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 PLA₂, 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. Hydrophathy 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 apoptosis.

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].

**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].

**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...
The 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 taurine-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’-Diisothiocyanato-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+/HCO3 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]).

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 A₂ (PLA₂) is the first known upstream transducer of cell swelling in the volume sensitive taurine release signalling pathway. PLA₂ subtypes vary between cell types, with the calcium-independent iPLA₂ and the secreted sPLA₂ subtypes involved in NIH3T3 [83], and the cytosolic cPLA₂ subtype in Ehrlich cells (see [46]). Following activation PLA₂ 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 A₄ (LTA₄) 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]). LTA₄ is successively converted to leukotriene C₄, D₄, and E₄, though the activity of glutathione S-transferases. LTD₂ 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 iPLA₂, and hence the swelling-induced mobilisation of AA in NIH3T3 cells [90]. CaM interacts with iPLA₂ in a catalytically inactive heterocomplex, and dissociation of calmodulin by reduced intracellular Ca²⁺ is necessary for iPLA₂ activity [91, 92]. From Fig. 2A it is seen that the volume sensitive taurine release is significantly inhibited by the iPLA₂ inhibitor Bromoeno 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 taurine release under hypoosmotic conditions depending on cell type and whether the taurine release is potentiated by increased intracellular Ca²⁺ mobilization [93, 94].

CK2 is known to phosphorylate and inhibit CaM, reducing its Ca²⁺-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 sPLA₂-activity in vitro as well as cPLA₂ 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 PLA₂-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 PLA₂.

Cell swelling results in an increased generation of reactive oxygen species (ROS) in e.g., NIH3T3, EAT and HTC cells [7, 83, 99, 100]. PLA₂ 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 H₂O₂ (see [102]). Increased availability of H₂O₂ 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 H₂O₂ including PTP1B [104], PP1, PP2A and PP2B [106, 107]. Exogenous addition of H₂O₂ or the tyrosine phosphatase inhibitor vanadate potentiates the taurine release significantly, whereas ROS-scavenging with the antioxidant butylated 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 H₂O₂ (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 b₅₅₅ 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^233^ [108], possibly preventing interaction of the regulatory complex p40-p67-p47 with flavocytochrome b₅₅₅ 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 H₂O₂ 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 H₂O₂ 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 H₂O₂ 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 H₂O₂ and in the presence of vanadate. However, inhibition of CK2 does not fully prevent H₂O₂ 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 H₂O₂-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 (Fe²⁺) [113], i.e., oxidation of Fe²⁺ to Fe³⁺ by H₂O₂ 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 (PIP₃, 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 wortmannin [18, 94, 115]. Generation of PIP₃ 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 glucocorticoid 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 PIP₃, 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 wortmannin (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-pyrole-2,5-dione (SB216763), has no effect on taurine release under isosomatic 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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