Calcium and Glutathione

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Extracellular glutathione (GSH) and oxidized glutathione (GSSG) can modulate the function of the extracellular calcium sensing receptor (CaSR). The CaSR has a binding pocket in the extracellular domain of CaSR large enough to bind either GSH or GSSG, as well as the derivatives L-cysteine glutathione disulfide (CySSG) and the compound cysteinyl glutathione (CysGSH). CySSG occurs naturally in the circulation and may be the preferred ligand for modulation of CaSR.

calcium-sensing receptor | L-cysteine-glutathione disulfide | glutathione | Ca

1. Introduction

Associations of calcium (Ca) and glutathione in various organs are not well understood. However, molecular modeling by Wang et al. [1] identified a glutathione binding site on the extracellular calcium sensing receptor (CaSR) and demonstrated glutathione-elicited changes in extracellular calcium responses mediated by CaSR transfected into a model cell system. With the perspective of more recent crystallographic studies of CaSR [2][3][4][5] and docking studies of various glutathionergic ligands with CaSR, this paper explores the potential actions that circulating glutathionergic compounds (reduced glutathione (GSH, PubChem CID 124886); oxidized glutathione (GSSG, PubChem CID 65359); and another circulating oxidized derivative of glutathione, L-cysteine-glutathione disulfide (CySSG, PubChem CID 10455148) may have that could be mediated via binding to the extracellular domain of CaSR.

1.1. GSH Synthesis and Relationship to GSH-Derivatives

GSH is a tripeptide (gamma-glutamyl-cysteinyl-glycine) made by non-ribosomal mechanisms. The rate-limiting enzyme in GSH synthesis is glutamate-cysteine ligase (GCL, EC 6.3.2.2), whose activity is limited by the amount of enzyme and the availability of cysteine. Glutathione synthase (GSS) couples glycine to the resultant gamma-glu-cys to make GSH. GSH reacts with oxidants via glutathione peroxidase (GPx) to form GSSG, form GS- adducts with various electrophiles (these are often toxicants that are thereby detoxified) via glustathione-S-transferases (GSTs), or glutathionylates proteins through reactions mediated by glutaredoxins and thioredoxins. Alternatively, GSH is transported out of cells where it can undergo further reactions. GSH can be resynthesized from GSSG by the actions of glutathione disulfide reductase (GR).

CySSG can be formed intracellularly or extracellularly by thiol-disulfide exchange with cystine [6], either spontaneously or via enzymatic catalysis by a thioltransferase [7][8]. CySSG is also produced spontaneously by the

reaction of GSSG and cysteine [6]; gamma-glutamyltransferase (GGT) produces CySSG and cystine from GSSG, a reaction that can be inhibited by AT-125 (Acivicin) [9]. GGT also degrades CySSG, which can also be inhibited by Acivicin [9]. All three forms of glutathione are found in mammalian circulation [10][11][12][13][14][15].

Plasma GSH and CySSG are usually in the 1–10 μM range, while GSSG is often 1 μM or less [10][15]. Many studies have used enzymatic methods to measure GSH; "oxidized glutathione" is often also measured after reduction of oxidized forms to GSH; however, this method does not distinguish between the two oxidized forms of glutathione. Measurement of both oxidized forms of glutathione requires HPLC, which can distinguish between CySSG and GSSG. In healthy people over a broad age range, plasma levels of GSH and CySSG are correlated (Pearson correlation coefficient = 0.622, $p < 0.001$ [10]). Plasma redox pairs have different Eh values (GSH/GSSG, −140 mV; Cys/CySS, −72 mV; and Cys-GSH/CySSG, −110 mV; [10]), indicating that relative concentrations of the reactants have different chemical potentials and are not at redox equilibrium in circulation. CySSG is also found in non-mammalian species, most notably in the polychaete *Nereis succinea* where it functions as a spawning pheromone [16][17][18][19].

1.2. CaSR Function in Parathyroid, Kidney, and Other Tissues

CaSR was first identified in parathyroid gland, where it solved the long-standing problem of what receptor mediated up- and down-regulation of parathyroid hormone (PTH) synthesis and release by Ca [20]. Unlike most secretory processes, PTH release is decreased by increases of extracellular Ca. CaSR was discovered to be a G-protein-coupled receptor that mediates responses in parathyroid cells by activating Gq, which regulates phospholipase C (PLC), and Gi, which inhibits cAMP synthesis (as reviewed by Ward [21]). CaSR activity is affected by small changes in Ca in the physiological extracellular range (1–10 mM) [20], and its sensitivity can be shifted by various agents, such as amino acids [22].

CaSR is found in many other tissues. In kidney, CaSR expression is particularly high in thick ascending limb (TAL) [21] but also occurs in many renal tissues, including proximal tubule, collecting duct, and juxtaglomerular apparatus [23]. Although approximately 65% of filtered Ca is reabsorbed in the proximal tubule, the proximal reabsorption is mostly not subject to regulatory control. Ca reabsorption in TAL and distal convoluted tubule is regulated in part by CaSR, coupled via G-protein mechanisms to cellular responses. The effect of these actions is to decrease cAMP, which would inhibit the luminal membrane cAMP-dependent Na-K-Cl cotransporter [24][25][26], thereby decreasing Na-reabsorption and inhibiting luminal (apical) K channels via phospholipase A2 and P-450 mediated synthesis of 20-hydroxyeicosatetraenoic acid (20-HETE) [27][28]. These multiple actions of the TAL CaSR cause changes in the transluminal voltage that ultimately cause a decrease in paracellular Ca reabsorption. Numerous other actions in kidney mediated by CaSR include increases in TAL PGE2 production [29], changes in aquaporin trafficking and water transport regulation in renal collecting duct [30], decreases in renin secretion by juxtaglomerular cells [31] and stimulation of claudin-14 expression in TAL mediated by a microRNA-signaling pathway downstream from CaSR activation [32].

CaSR protein is also expressed in the gastrointestinal system, bone cells, the nervous system, etc., where these receptors may mediate other Ca-sensitive responses [33]. Among other tissues expressing CaSR are liver cells that stimulate bile flow [34], endothelial cells and vascular smooth muscle cells in many tissues [35] notably in pulmonary arteries [36], pancreatic beta cells [37][38], and taste buds [39][40][41].

1.3. Amino Acid and Peptide Modulation of CaSR Activity

The sensitivity of the CaSR to Ca is enhanced by a variety of naturally occurring organic molecules of which amino acids were among the earliest to be described; tryptophan, phenylalanine, tyrosine, and histidine are among the most effective modulators of CaSR activity, generally having EC50 concentrations in the range of 1–10 mM [42][43][44]. Zhang et al. [5] discovered a novel derivative of tryptophan, L-1,2,3,4-tetrahydronorharman-3-carboxylic acid, bound to CaSR and having EC50 of approximately 2 µM. In studies of CaSR in taste buds, modulatory effects of small gamma-glutamyl peptides have been the focus, among which the most effective dipeptides were gamma-glutamyl-alanine, gamma-glutamyl valine, and gamma-glutamyl-cysteine [39][40].

Larger peptides have also been shown to modulate the activity of CaSR, including GSH and GSSG at micromolar and lower concentrations. The study of gamma-glutamyl peptides found that CaSR activity was enhanced by GSH as well as other gamma-glutamyl tripeptides, including gamma-glutamyl-S-methylcysteinylglycine and gamma-glutamylvalylglycine [39][40], generally exhibiting EC50 values in the micromolar range. Wang et al. [1], studying HEK-293 cells transfected with CaSR and tested in a calcium release assay, showed that the Ca response was enhanced in the presence of either GSH or GSSG. EC50 values were <1 µM for both, compared to an EC50 for phenylalanine of 300 µM in the same assays [1]. Neither CySSG nor CysG were tested.

Of particular note is that both GSH and GSSG produced similar physiological responses, supporting the idea that these responses are mediated by binding to a receptor and are not due to interactions with intracellular redox mechanisms, which might have been an alternative explanation if only one of them had been active. A similar conclusion that the action of glutathione is associated with binding, not redox regulation, has been drawn regarding the male spawning response of the polychaete *Nereis succinea*, which is also equally well activated by GSH and GSSG [19]. In the case of *N. succinea*, however, CySSG has also been tested and is effective at eliciting the response at about ten times lower concentration than either GSH or GSSG [19]. Unfortunately, little is known about the structure of the glutathionergic receptor in *N. succinea* and whether it may be part of the C-family of G-protein coupled receptors from which CaSR evolved in vertebrates. Nevertheless, the binding sites of all three of these glutathionergic compounds to potential receptors is of interest.

Homology modeling to the C class of G-protein coupled receptors [1] and recent crystallographic and cryo-EM studies of CaSR [2][3][4][5] have revealed that the modulator binding pocket in the extracellular domain of CaSR is large enough to bind either GSH or GSSG, as well as the natural derivative CySSG) and a related synthetic compound, cysteinyl glutathione (CysGSH). Molecular modeling of the docking of each of these compounds in the binding pocket of CaSR indicate that CySSG and CysGSH may actually bind with up to ten times greater affinity

than either GSH or GSSG [1]. CySSG occurs naturally in the circulation and, hypothetically, may be the preferred ligand for modulation of CaSR.

2. Associations of Ca with Glutathionergic Metabolism

Several experiments have examined the association of Ca with GSH metabolism. The rise in GSH synthesis in RAW264.7 macrophage tumor cells in response to gamma rays is Ca dependent [45]: GCLC mRNA increased with a similar time course to GSH, a response that was inhibited in cells cultured with 1 mM EGTA (Ca chelator) or BAPTA/AM (intracellular Ca chelator). Coordinate regulation of gene transcription for GCLC, GSS, and other glutathione-regulating genes is mediated by Nrf2, an activator of antioxidant response elements in their 5′-flanking promoter regions [46][47]. In human keratinocytes, activation of Nrf2 by arsenite was reduced by depleting cells of Ca in Ca-free media [48]. Ca-calmodulin inhibition of CK2 kinase activity mediates the response, i.e., low Ca results in higher CK2 activity, which phosphorylates Nrf2, making it more vulnerable to degradation. A treatment that reduced UV-radiation damage in lens tissue decreased the expression of CaSR at the same time that markers of oxidative stress (SOD and “T-AOC,” said to measure total antioxidant content, but is not a direct measurement of glutathione) were increased [49].

Changes in glutathione metabolism in liver cells are particularly significant as the liver is the major source of circulating glutathione [50]. GSH in hepatocytes was increased by 3.5 mM extracellular Ca compared to 0 mM extracellular Ca [51]. In a recent study, ionizing radiation increased liver Ca, accompanied by large decreases in total glutathione and glutathione-regulating enzymes, interpreted as a large relative increase in oxidant status, as compared to antioxidant status [52].

These previous studies relating Ca and glutathione have generally interpreted their findings in terms of redox status of cells and, except for the study UV-radiation damage in lens tissue [49], have not considered possible roles of CaSR in the mechanisms that might be involved. Nevertheless, evidence exists to indicate that glutathione metabolism may be interactive with calcium signaling and possibly related to extracellular calcium concentration via CaSR.

3. Proposed Role of Glutathionergics in Regulating CaSR Function

The above information can be summarized as follows: First, GSH and its oxidized derivatives CySSG and GSSG are found in mammalian circulation at micromolar concentrations and exhibit changes correlated with age and health. Second, CaSR is found in many tissues, including parathyroid gland, kidney, and bone, where it participates in regulation and utilization of extracellular Ca. Third, amino acids and peptides, including GSH and its derivatives, can sensitize CaSR responses to Ca; in taste buds GSH and related compounds can activate CaSR under ambient Ca conditions. Fourth, consistent with the functional effects of glutathionergics on CaSR, the binding pocket at which amino acids exert their effects on CaSR is large enough to accommodate peptides, including glutathione and

its oxidized derivatives. Fifth, extracellular Ca in the same concentration range as is regulated by CaSR in parathyroid, kidney, and bone tissues can modify glutathione synthesis, particularly in hepatocytes, the major source of circulating glutathione.

Given the above observations, researchers therefore propose that circulating glutathionergics (GSH, GSSG, and/or CySSG) bind to and sensitize CaSR to extracellular Ca and thereby participate in the homeostatic regulation of extracellular Ca. A subsidiary hypothesis, based on the physiological principle that homeostatic systems usually have feedback to the source of the regulatory signal, is that extracellular Ca effects on glutathione metabolism in the liver, the major source of circulating glutathionergics, may constitute a feedback mechanism for this hypothesized glutathionergic Ca regulatory mechanism. The association of CySSG changes with age and health and the greater affinity of CySSG for CaSR in model docking simulations may indicate an importance for this derivative of CySSG that has heretofore been overlooked.

This proposed role broadens current views about the functions of glutathione, emphasizing an extracellular receptor-mediated role for glutathionergics, complementary to their well-known intracellular actions regulating the intracellular redox state of cells. This paper highlights the potential biological actions of plasma CySSG and further emphasizes the peptide binding site on CaSR as a potential target the development of drugs that can be used in treating kidney, Parkinson’s and other diseases.

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