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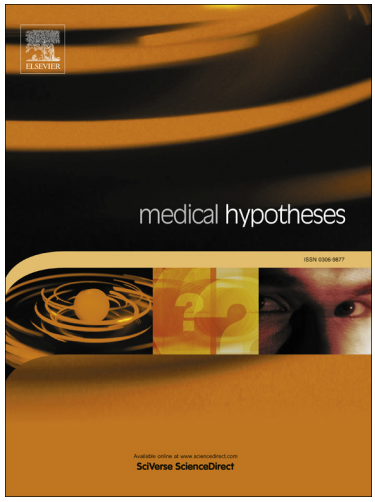

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Is the calcium transporter a potential candidate for heme transport?

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Abstract

Heme is of significant importance in iron nutrition and in systemic iron metabolism. The crux of the matter is that while much is known about non-heme metabolism, the vectorial import of exogenous porphyrin macromolecules into the enterocyte and possibly into blood circulation is still speculative. The inhibitory effect of calcium on heme iron absorption has been previously reported in the literature. This paper postulates that the gastrointestinal Ca transporter, TRPV6 might be a putative transporter of heme and that it is the mechanism of reduced heme absorption in the presence of Ca. The hypothesis needs to be investigated *in vitro* and *in vivo* with targeted TRPV6 deletion models to explore the nature of the competition of heme uptake by Ca. Studies are required to characterise fully this function in the gut and in systemic metabolism. If the hypothesis is proven, modulators of TRPV6 expression could have clinical implications in the management of heme-induced disorders.

Introduction

Transport of heme at both luminal and endogenous levels are quantitatively of vital importance in iron metabolism. Systemic transcellular heme transit is carried out by proteins or receptors such as LRP or HRG1 that are not expressed in the duodenum (1, 2). Consequently, the enterocyte vectorial apical heme transporter remains elusive and enigmatic in iron metabolism research since HCP1 transports folate with a higher affinity (3, 4). There is considerable evidence that the duodenal transporter of heme porphyrin proteins has higher transport kinetics than DMT1, the inorganic iron counterpart (5). It is presumably due, in part, to heme non-reactivity with luminal inhibitory dietary ligands. Consequently, heme iron sources from animal products are quantitatively of higher bioavailability than non-heme iron and can provide a relatively larger amount of iron to the body (6). The disparate haem and non-haem transport proteins are, however, paradoxically inhibited by calcium in models of both animal and human studies (7, 8). Inconsistencies or disparity of inhibition of Ca on iron absorption have in recent times been attributed to dose, time duration, and the Ca sources used in various experiments (8-10).

From previous studies towards a hypothesis

Heme and non-heme iron in luminal food matrix maintain distinct, discrete identities of which the former assumes supremacy of higher bioavailability only to culminate ultimately in a common pool of inorganic iron in the cytosol of the enterocytes. While there is evidence of a delayed systemic response to heme absorption when compared with non-heme iron (24), its existence as heme moieties after absorption seems to end in the cytosol. Evidence has shown that calcium exerts an inhibition of heme absorption at the initial mucosal uptake step rather than at the basolateral transfer into circulation (25). Although heme absorption, in a similar pattern to that of non-heme occurs in the duodenum, there is a decreasing

gradient along the lower gastrointestinal tract (19). In contrast, however, solubility of heme, unlike that of non-heme exhibits an increasing gradient with increasing pH of the lower gut. Consequently, higher heme absorption by transporters in the lower gut would benefit additionally from longer resident and transit time. Calcium has been shown to inhibit heme-induced cytotoxicity and carcinogenesis (20, 21) in the colon. TRPV6 is expressed in the duodenum and possibly in the lower section of the gastrointestinal tract.

The hypothesis

In light of the inhibitory effect of Ca on heme absorption, it is herein hypothesized that TRPV6, the Ca transporter, possibly transports heme and is inhibited by Ca. That, the putative function of TRPV6 as a heme transporter is competitively inhibited by heme. This hypothesis seems plausible because heme was demonstrated to bind and inhibit mammalian calcium-dependent Slo1 BK channels in the brain of the rat (26). These channels possess the conserved heme-binding sequence motif (CXXCH) (16, 27). The physiological consequences of this finding will have ramifications for other tissues and organs that express TRPV6 and possibly other calcium channels as well. What is particularly fascinating is the evidence that the G185R mutation turns DMT1, the non-heme membrane iron transporter into a calcium channel and this was ascribed possibly to a selective advantage of Ca flux to extend the lifespan of microcytic (mk) mice and Belgrade rats (28). Of significance importance, also, is the evidence that alludes to efficient heme uptake in the Belgrade rats when strain differences were eliminated in the control group (29, 30). Although the binding motif is not conserved in TRPV6, luminal competitive binding and interactions of calcium and heme might account for the mechanism of the inhibition on the transporter. While hemin carries a net positive charge, it did not induce electrochemical current in oocytes (4), the mechanism of inhibition could possibly be by coupling and binding to Ca in the lumen that consequently inactivates TRPV6. An alternative scenario is that heme-calcium complex induces conformational changes to the configuration of Ca transporter.

Testing the hypothesis

Studies are warranted using radio-labelled heme to investigate the kinetics and the nature of the inhibition of heme transport by the calcium channel. Studies will examine heme transport in cell cultures and investigate if amino-ethoxydiphenyl borate (2-APB), the inhibitor of Ca transporter, would diminish heme uptake in cells. *In vitro* binding characteristics and kinetics of binding of heme-calcium complexes will also be investigated. Calcium channel knockout mice, Belgrade rats, L-type Ca channel blockers, transgenic expression of TRPV6 and radio-labelled heme are valuable tools to employ in such studies. Heme absorption/uptake and competition of uptake by Ca will be carried out in these animal models. Techniques of immunohistochemistry and confocal microscopy will be employed to localise TRPV6 in different sections of the gastrointestinal tract. It is also important to decipher the intricacies and complexity of interactions involved in the

pleiotropic transporter functions of calcium channels which upon mutational transformation of DMT1 also assume a gain-of-function heme transporting role.

Implications of the hypothesis and conclusions

The hypothesis as proposed will be attested if heme absorption is inhibited by TRPV6 inhibitor, 2-APB and heme absorption is enhanced in calcium channel knockout mice and belgrade rats. The validation of the hypothesis will advance knowledge in the mechanism of heme absorption in the gastrointestinal tract. At the public nutrition level, the use of calcium supplements and rich sources of Ca in the diets will need to be moderated for specific iron nutrition disorders.

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