A NEW TARGET FOR FUTURE ANTIEMETICS: INHIBITION OF GLYCOGEN SYNTHASE KINASE (GSK-3)

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Introduction

- Cisplatin-like chemotherapeutics cause vomiting via release of multiple neurotransmitters [e.g. dopamine, serotonin (5-HT), or substance P (SP)] from the gastrointestinal enterochromaffin (EC) cells and/or the brainstem via a calcium-dependent process (Darmani et al., 2014).
- Specific emetogens such as agonists of 5-HT₃R (e.g. 5-HT or 2-Me-5-HT), neurokinin NK₁R (GR73632), dopamine D₂R (apomorphine or quinirole), cholinergic M₁R (McN-A343 or pilocarpine), as well as the L-type Ca²⁺ channel agonist FPL64176 (Darmani et al., 2014), and the SERCA inhibitor thapsigargin (Zhong et al., 2016) evoke vomiting in vomit-competent species.
- The emetic loci involved in vomiting include the EC cells in the gastrointestinal tract (GIT) and the brainstem dorsal vagal complex [area postrema (AP), nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus (DMNX)]. The AP and NTS have an incomplete blood-brain barrier and are thus sensitive to emetogens present in the blood.

- We currently investigate post-receptor intracellular emetic signaling pathways.
- This study focuses on involvement of the serine/threonine kinase, glycogen synthase kinase-3 (GSK3), in vomiting. GSK-3 is encoded by two known genes, GSK3 α and GSK3 β . GSK3 is a downstream target protein for the phosphatidylinositol (3,4,5)-trisphosphate (PIP3)/protein kinase B (Akt) signaling pathway. Activation of Akt signaling can be followed by phosphorylation GSK3 α/β at Ser 21/9 and its subsequent inactivation (Kim et al., 2014).

Experimental strategies

- Effect of diverse emetogens on phosphorylated GSK3 (pGSK3) levels in the brainstem.
- Immunohistochemistry of the brainstem sections containing the AP/NTS/DMNX emetic nuclei to determine pGSK3 expression in the brainstem.
- The role of GSK3 in emesis: evaluation of emesis induced by the specific emetogens in the presence of a GSK3 inhibitor.

References

- Darmani et al. Broad-spectrum antiemetic potential of the L-type calcium channel antagonist nifedipine and evidence for its additive antiemetic interaction with the 5-HT₃ receptor antagonist palonosetron in the least shrew (*Cryptotis parva*). Eur J Pharmacol. 2014; 722: 2-12.
- Kim et al. The protective effect of hispidin against hydrogen peroxide-induced apoptosis in H9c2 cardiomyoblast cells through Akt/GSK-3 β and ERK1/2 signaling pathway. Exp Cell Res. 2014 Oct 1;327(2):264-75.
- Zhong et al. Thapsigargin-induced activation of Ca(2+)-CaMKII-ERK1/2 in brainstem contributes to substance P release and induction of emesis in the least shrew. Neuropharmacology. 2016; 103: 195-210.

Results

1. Specific emetogens increase phospho-GSK3 α/β at Ser21/9 in the least shrew brainstem

GSK3 α/β phosphorylation at Ser21/9 was examined via performing Western blots on brainstem protein extracts collected at various times from least shrews treated intraperitoneally with fully effective emetic doses of diverse emetogens such as : i) selective and/or nonselective agonists of serotonergic 5-HT₃ (e.g. 5-HT or 2-Me-5-HT, 5 mg/kg)-, tneurokinin NK₁ (e.g. GR73632, 5 mg/kg)-, dopamine D₂ (e.g. apomorphine or quinirole, 2 mg/kg)-, cholinergic M₁ (e.g. pilocarpine or McN-A343, 2 mg/kg)-receptors; and ii) calcium mobilizers including the L-type calcium channel agonist FPL 64176 (10 mg/kg), and the SERCA inhibitor thapsigargin (0.5 mg/kg).

2. Expression of phospho-GSK3 α/β in the least shrew brainstem emetic nuclei

Mice

Least shrew

Brainstem emetic nuclei

- AP: area postrema
- NTS: nucleus tractus solitarius
- DMNX: dorsal motor nucleus of the vagus

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3. The broad-spectrum antiemetic efficacy of the GSK3 inhibitor SB216763

A 30 minute pretreatment with a low-dose (0.25 mg/kg, i.p.) of the specific GSK3 inhibitor SB216763 significantly reduced both vomit frequency and percentage of shrews vomiting in response to fully efficacious doses of specific emetogens, except pilocarpine and quinirole.

Conclusions

- Time-dependent increase in GSK3 α/β phosphorylation at Ser21/9 in the brainstem occurs following the peripheral administration of specific emetogens in least shrews.
- A low dose of the GSK3 inhibitor SB216763 exerted potent antiemetic effects against vomiting caused by diverse emetogens.
- Phospho-GSK3 at Ser21/9 expresses throughout the brainstem emetic nuclei, including the AP, NTS, and DMNX.
- This study implies a pivotal role for GSK3 in vomiting.