The Effects of Ethanol on Prolactin Signaling in Normal and Regenerating Rat Liver

E. Aksamitiene, A. N. Antony, A. Kiyatkin, J. B. Hoek
Department of Pathology, Anatomy & Cell Biology, Thomas Jefferson University
Philadelphia, Pennsylvania
19107

Abstract

Liver has a unique ability to regenerate itself after tissue damage or resection, while maintaining differentiated functions. The regeneration process is driven by a complex set of signals, involving multiple growth factors, cytokines, proteolytic enzymes, and cytokine receptors. A proliferation hormone, prolactin (PRL), has been shown to accelerate liver regeneration after partial hepatectomy and trigger tropic/originomic responses in isolated rat hepatocytes. By contrast, acute or chronic alcohol treatment impairs liver regeneration, which may contribute to alcoholic liver damage. However, it has been reported that ethanol consumption increases circulating levels of PRL. The objective of this study was to compare the effects of acute ethanol exposure on PRL-mediated downstream signaling and induction of key transcription factors in normal hepatocytes and in rat liver cells, isolated after partial hepatectomy, using quantitative Western blotting methods. Preincubation with a physiologically relevant dose of ethanol (50 mM) decreased PRL-induced tyrosine phosphorylation of the PRL receptor (PRL-R) and STAT proteins in freshly isolated rat hepatocytes, but augmented the activation of Ras/MAPK-activated protein kinase (MAPK) cascade. In addition, ethanol treatment inhibited the PRL-induced increase of c-Fos and c-Jun and decreased nuclear translocation levels. PRL signaling responses were significantly higher in regenerating hepatocytes than in normal hepatocytes, due to a 24 hours regenerating liver as compared to normal cells. These findings suggest that ethanol treatment may interfere with the pro-regenerative effects of PRL through a differential effect on signaling pathways downstream of the PRL receptor.

Introduction

The regeneration process after partial hepatectomy (PHx) or liver injury requires hepatocyte proliferation, which is facilitated by a simultaneous action of various growth factors and cytokines. In this study, we evaluated the effects of acute ethanol exposure on PRL-mediated signaling in normal rat liver and regenerating rat liver cells.

Methods

Materials and Methods

Isolation of hepatocytes. Adult male Sprague Dawley rats were anesthetized and subjected to two-thirds PHx by a ligature and resection of the liver median and left-lateral lobes, following the standard procedures. At 24 h post-PHx, the rats were sacrificed, and the livers were harvested by a standard procedure (2). Freshly isolated rat liver was perfused for 10 min with a warm (37ºC) carbogen-gassed (5% CO2, 95% O2) solution (-) or 50 mM DTT and subsequently washed twice. After removal of supernatant, the liver was minced to release the hepatocytes in collagenase buffer, filtered through a nylon mesh and centrifuged at 500×g for 3 min at 4°C. Detergent-insoluble materials were removed by centrifugation at 10,000×g for 10 min at 4°C. Equal amounts of solubilized proteins were dissolved in 4×NuPAGE LDS sample buffer (Invitrogen) supplemented with 50 mM DTT (Kupffer and stellate) cells and the cells from other organs as well as endocrine glands (Fig. 1). Serum levels of pituitary gland-derived hormone-protein (PLR), which reportedly stimulates cell cycle progression in liver, increase significantly at 10–15 min after PHx (1). PRL binding to its cognate class I cytokine family receptor (PRL-R) induces its homodimerization (2) and subsequent activation of non-receptor protein tyrosine kinases of the Src (p60c-Src) and Fc receptor family in 2D and 3D cultures of the plasma membrane (3). Briefly, phosphorylation of PRL-R by JAKs and activation of Src family kinases initiate the signal transduction of the JAK/STAT family (STAT1, 3 and 5) and the mitogen activated protein kinases (MAPK) cascade.

Results

Activation of transcription factors (STAT3, AP-1) Induction of immediate-early genes

Fig. 1. Steps in liver regeneration.

A. Control

E. NIH

B. ETOH

C. JUN

D. PRL

E. GAPDH

PRL induces its homodimerization (2) and subsequent activation of non-receptor protein tyrosine kinases of the Src (p60c-Src) and Fc receptor family in 2D and 3D cultures of the plasma membrane (3). Briefly, phosphorylation of PRL-R by JAKs and activation of Src family kinases initiate the signal transduction of the JAK/STAT family (STAT1, 3 and 5) and the mitogen activated protein kinases (MAPK) cascade.

Conclusion

1. Acute exposure to ethanol caused a suppression of prolactin-stimulated c-Fos, c-Jun and JunB protein expression in hepatocytes derived from both normal and regenerating liver.

2. PRL-receptor-stimulated gene expression is sensitive to acute ethanol due to the ethanol-mediated negative effects on JAK/STAT signaling pathway possibly through the upregulation of protein tyrosine phosphatases (e.g. PTP-1B activity), but not the expression levels of SOCS3.

Acknowledgements

This research was supported by NIH Grant #GM059570.

References