

## Quantitative analysis of EGF-induced signaling responses in hepatocytes from control and ethanol-fed rats.

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Chronic ethanol intake interferes with growth factor signaling pathways and affects growth and repair responses, including liver regeneration (1). Building on our previous computational modeling studies of EGF induced signaling responses in rat hepatocytes (2-4), we are using a combination of experimental and computational modeling studies to identify the nature of the cellular state changes following chronic ethanol feeding of rats that results in an altered EGF response. Experiments on isolated hepatocytes demonstrate that chronic ethanol has a differential effect on tyrosine phosphorylation and activation of the EGF receptor (EGFR) and its downstream signaling proteins ranging from ultra low to very high, sensitivities in response patterns of different proteins with different EGF concentrations (Fig.1). To account for these differential response patterns, we built on our kinetic model of the EGFR signaling network, consisting of about 130 ordinary differential equations. Changes in the kinetics of activation of the EGFR signaling system at 1 and 20 nM EGF in hepatocytes from pair-fed control rats were first described quantitatively using a numerical fitting procedure to develop a predictive mathematical model and determine values of its kinetic parameters and protein concentrations. The results of this analysis can be summarized as follows:

1. Shc phosphorylation patterns (Fig.1B) respond effectively to low EGF concentration, presumably because Shc can be phosphorylated not only by EGFR tyrosine kinase, but also by tyrosine kinases of the src family, markedly left-shifting its dose-dependence.
2. Ultrasensitivity to changes in EGF concentration in PLC $\gamma$  and PI3K phosphorylation patterns (Figs.1C and D) could be explained by: a) a well established positive feedback loop (Gab1-pY $\rightarrow$ PI3K $\rightarrow$ PIP3 $\rightarrow$ Gab1-pY) in the PI3K-Gab1 pathway, b) multi-site phosphorylation of PLC $\gamma$  by EGFR tyrosine kinase, which has *positive feedback loop features* and can result in ultrasensitivity and bistability.
3. Maximal Ras and ERK activation (Figs.1F,G) were the same with EGF concentrations of 1 and 20 nM despite considerable decrease in the Ras-activating Sos signal (Fig.1E) suggesting a mechanism to generate a proportional decrease in the deactivating RasGAP signal.

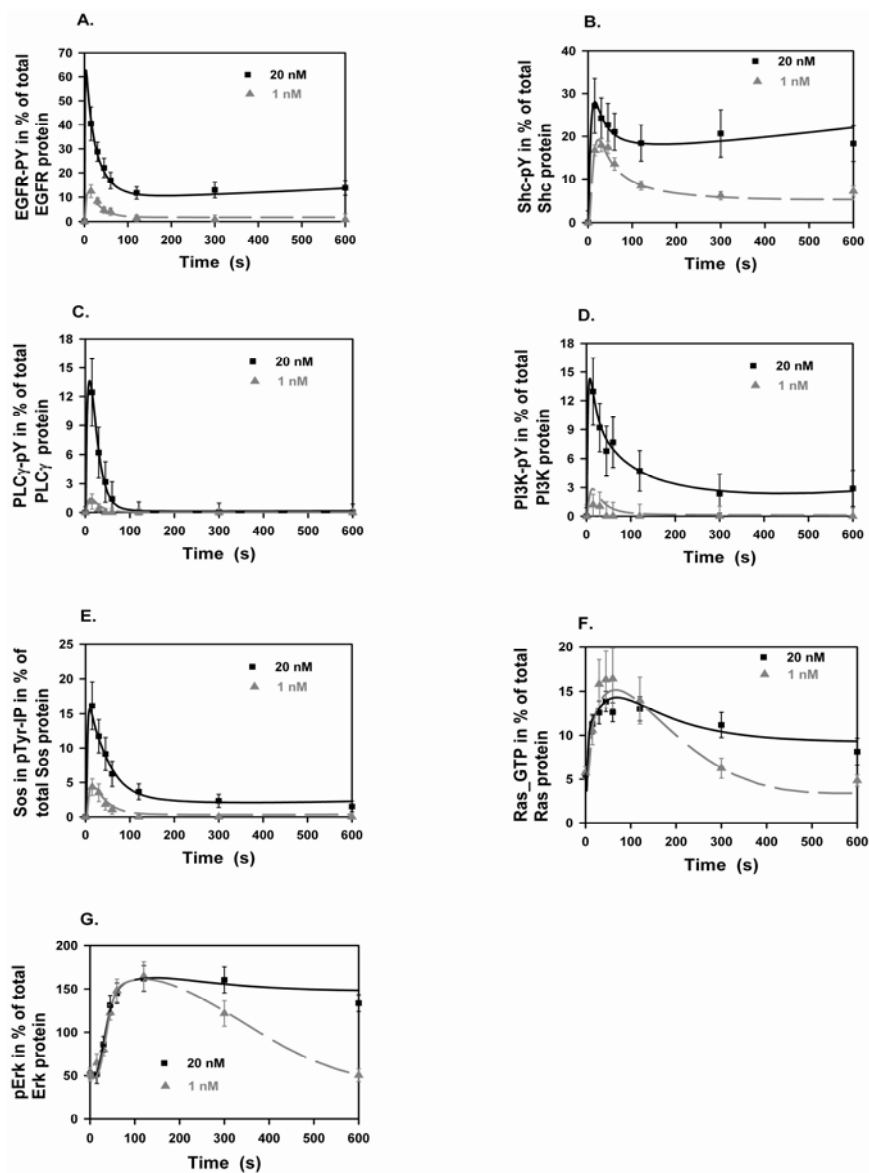
A quantitative analysis of the effects of chronic ethanol feeding suggests possible mechanisms for ethanol-induced alterations in EGFR signaling machinery. The conclusions of this part of the work are:

1. Prominent ethanol-dependent changes in EGF-induced signaling pathways occur at EGFR: a). A 2-fold irreversible inactivation of EGFR with chronic ethanol consumption;. b).A 5-fold decrease in dimerization of EGFR; c). EGFR phosphatases are drastically inhibited. Together, these alterations result in a considerable decrease in the peak phosphorylation of EGFR. However, the sustained EGFR phosphorylation level after stimulation with EGF is higher than in the control animals (Fig.2A).
2. Ethanol-induced decrease in maximal EGFR activation has differential effects on peak phosphorylation levels of downstream target proteins. The most marked effects of ethanol occur in the phosphorylation of PLC $\gamma$  and PI3K (Figs. 2C,D), but Shc phosphorylation (Fig.2B) and maximal Ras and ERK activation were unaffected (Figs.2F,G). Explanations

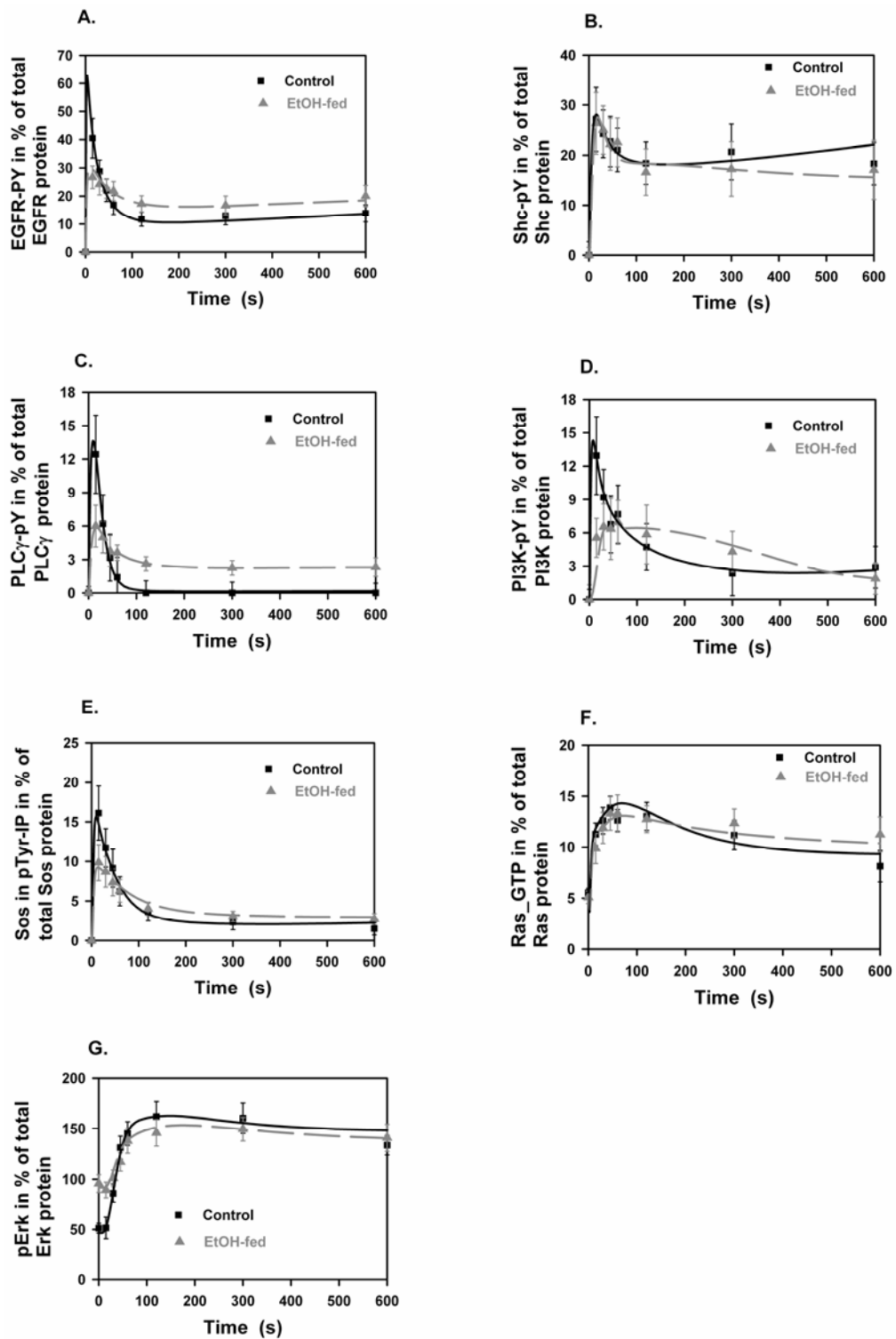
could be similar to those used to account for the differential effects of EGF concentration. However, the ethanol-induced changes in activation of downstream target proteins have ethanol specific features, such as a slower rate of decay of the initial phosphorylation pulse and the increased sustained levels compared to the control cells, which can be explained by decrease in phosphatase activity in ethanol-fed rats. We suggest that such changes may reflect increased oxidative stress in cells from ethanol-fed animals that may selectively alter activity levels of tyrosine phosphatases and related signaling proteins.

#### References

1. Diehl A.M. Am J Physiol 288:G1-G6, 2005.
2. Kholodenko, BN. et J Biol Chem 274:30169-30181, 1999.
3. Moehren, G. et al, Biochemistry 41:306-320, 2002.
4. Markevich, N. et al. Syst. Biol. (Stevenage) 1:104-13, 2004.



**Fig.1. Computer simulation (solid and dashed curves) of experimentally observed EGF-induced responses in control rats at 1 (triangles) and 20 (squares) nM EGF.**



**Fig.2. Computer simulation (solid and dashed curves) in EGF-induced responses in control (squares) and ethanol-fed (triangles) rats at 20 nM EGF.**