THROMBIN RECEPTORS:
MOLECULAR CLONING AND REGULATION OF mRNA EXPRESSION

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Submitted to the faculty of the University Graduate School

in partial fulfillment of the requirements

for the degree

Doctor of Philosophy

in the Department of Physiology and Biophysics

Indiana University School of Medicine

August 1996

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July 29, 1996

ABSTRACT

Vascular endothelial and smooth muscle cells, are endowed with two thrombin receptor systems with opposite functions. The proteolytically-activated thrombin receptor (PATR) system is procoagulant and proinflammatory; the thrombomodulin (TM) receptor system is anticoagulant and anti-inflammatory and converts thrombin from a procoagulant to an anticoagulant enzyme. The goal of our studies was to examine the biochemical and molecular regulation of these two thrombin receptor systems. Our data indicate that thrombin stimulation of cultured bovine aortic smooth muscle cells results in substantial increases in TM mRNA levels (probably due to an increase in the mRNA stability) and an increase in PATR mRNA. To further study receptor interactions, we successfully identified a cell line, Daudi, which lacks TM as well as PATR mRNA and a control cell line, K-562, which is strongly PATR mRNA positive and weakly TM mRNA positive. In experiments measuring phosphoinositide hydrolysis, we found substantial responsiveness to thrombin in the K-562 cells but not in the Daudi cells. The thrombin effect was reproduced by a synthetic hexameric thrombin receptor activating peptide ($TRAP_6$) and was augmented in experiments using a TM-specific antibody. These studies taken together suggest a "one-way" traffic arrangement whereby thrombin increases TM mRNA via the activation of PATR.

In the course of our studies, we have noticed substantial species-specific differences between bovine and human endothelial cell monolayer responses to human and bovine thrombins. These observations suggested that sequence specificity of either PATR or thrombin may be important in determining species responsiveness. Whereas the structure of the human PATR is known, this information is not available for the bovine PATR. We therefore cloned bovine PATR by PCR and standard cloning strategy. Structural considerations of human and bovine thrombins and human and bovine PATRs suggested

that a critical amino acid switch in thrombin (human Lys 149E to bovine Glu 149E) might account for species-specific differences in endothelial cells exposed to thrombin. This was confirmed in experiments where a human thrombin mutant (149E Lys to Glu) was found to assume the characteristics of bovine thrombin. Together these data may help provide a better understanding of the structure-function relationships between thrombins and PATRs.

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