FREE COMMUNICATIONS III

Factor VIII: Synthesis and Clinical Aspects

IN VITRO STUDIES OF SYNTHESIS AND RELEASE OF FACTOR VIII RELATED PROTEIN BY ENDOTHELIAL CELLS.
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In vivo studies have shown that many stimuli such as epinephrine, exercise and pregnancy lead to a rise in factor VIII levels. However, the physiologic mechanisms controlling factor VIII levels are poorly, if at all, understood. Since endothelial cells synthesize factor VIII related protein (FVIII:RP) and can be grown in tissue culture, they provide a suitable in vitro model to study synthesis and release of FVIII:RP. Endothelial cells were harvested by collagenase digestion from human umbilical cords and grown in medium 199 supplemented with 20 to 30% pooled human serum. Confluent cultures were washed and then maintained in medium 199 supplemented with 20% fetal calf serum. Release of FVIII:RP into the medium was measured by immunoradiometric assay. Labeled amino acids were added to the medium for studies of FVIII:RP synthesis. Incorporation of radioactive label into FVIII:RP was measured in binding studies using a specific immunoabsorbent. Epinephrine in concentrations from 1 ng to 10 µg per ml had no effect on rate of release of FVIII:RP from cultured endothelial cells, suggesting that the in vivo effect of epinephrine is not due to a direct action on endothelial cells. In a separate experiment, exogenous FVIII:RP was added to the culture medium at a high concentration (2 units FVIII:RP per ml) along with ^3H Leucine. A control without exogenous FVIII:RP incorporated as much radioactivity into FVIII:RP as did the culture with added FVIII:RP. This result suggests that there is no end product inhibition of FVIII:RP synthesis which operates on the endothelial cell.

THE MODIFICATION OF FACTOR VIII PROCOAGULANT ACTIVITY IN TISSUE CULTURE MEDIA SUPPLEMENTED WITH FETAL CALF SERUM. Nancy W. Stead and P.A. McKee. Duke University Medical Center, Durham NC, U.S.A.

Cultured human umbilical vein endothelial cells produce a protein that reacts with rabbit antibody to purified plasma factor VIII (FVIII)/von Willebrand factor (vWF) and has vWF activity, but not FVIII procoagulant activity (PCA) (Jaffe *et al.* JCI 52:2757, 1973). In our laboratory endothelial cell cultures produce vWF activity that increases to 3 µg/culture by 72 hrs; partially purified protein from culture media forms an immunoprecipitin line of identity with human, but not bovine, plasma FVIII/vWF. Of the 3 functions of plasma FVIII/vWF protein, PCA, but not vWF activity or antigenicity, is modified by trace proteases. An assay that measures hydrolysis of p-nitroaniline from the tripeptide Bz-phe-val-arg-p-nitroanilide detects trace proteases in growth media; 0.001 NIH u thrombin releases measurable quantities of p-nitroaniline. Culture media without fetal calf serum (FCS) did not hydrolyze the tripeptide. Media 20% in: FCS, FCS heated to 56° for 30 min, FCS made 1.7x10⁻³M diisopropylfluorophosphate (DFP), FCS and 4.4x10⁻⁸ moles soybean trypsin inhibitor, or FCS and 4.4x10⁻⁸ moles Trasylol releases 3.5x10⁻⁶, 3.6x10⁻⁷, 3.6x10⁻⁷, 3.5x10⁻⁶ and 2.8x10⁻⁶ mmole of p-nitroaniline per ml of media per min. PCA of FVIII/vWF molecule is measured by one stage (PCAl) and two stage (PCA2) assays. Although >85% PCAl or PCA2 of purified plasma FVIII/vWF added to media without FCS remains at 6 hours, varying amounts of PCAl and only 10% PCA2 of the FVIII/vWF added to media 20% in FCS remained in 6 hrs. Hence endothelial cells make a protein with vWF activity and FVIII/vWF antigenicity; but were the protein to have PCA at the time of release, trace proteases in the growth media could quickly destroy it.