ECGF and Heparin Determine Differentiation of Cloned Cerebral Endothelial Cells In Vitro

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ABSTRACT

Protein expression patterns of morphologically different cloned capillary endothelial cells from porcine and murine brain cortices were examined. Type I cells, grown in medium containing heparin and endothelial cell growth factor (ECGF), exhibited a polygonal, cobblestone appearance and appeared to replicate the cells of the blood-brain barrier endothelium. Type II cells, grown in medium without heparin and ECGF, were elongated and appeared to replicate capillaries in central nervous system tissue.

Cells of both phenotypes stained positive by the specific endothelial cell marker Bandeiraea simplicifolia lectin. The expression of α smooth-muscle actin (mRNA and protein) was taken as a marker for type II cells. By use of 2-D gel images and the GELLAB II system, a data base was created revealing that two proteins (90 kDa, pI 5.1, and 35 kDa, pI 5.7) were exclusively expressed in type I cells. Furthermore, the synergistic action of ECGF and heparin in respect to the phenotypic determination of cerebral endothelial cells was demonstrated.

Index Entries: Cerebral endothelial cells; endothelial cell growth factor; heparin; blood-brain barrier; phenotypic switch.

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INTRODUCTION

The blood–brain barrier (BBB) is created by a continuous layer of endothelial cells that forms the capillary walls in the central nervous system. Specific properties of the cerebral endothelial cells (cEC), such as tight junctions, less transcytosis, and a selective membrane transport system, are the basis for proper BBB function, i.e., the maintenance of a stable chemical environment in the CNS (Bradbury, 1979; Pardridge, 1983). Breakdown or "leakiness" of the BBB has been implicated in several brain diseases, including tumors and strokes as well as degenerative dementia (Müller-Hill and Beyreuther, 1989) and multiple sclerosis (Lossinsky et al., 1989).

A few years ago, Stewart and Wiley (1981) showed that the neural environment of the cEC plays an important role in the induction and maintenance of the BBB. Since then, there have been many findings (Janzer and Raff, 1987; Joó, 1992). To this end, morphological characteristics, as well as biochemical and functional properties, have been used as parameters for BBB function (Rubin et al., 1991).

We have recently shown that the induction of BBB-related γ-glutamyltranspeptidase and Na+,K+-ATPase activities in cloned cEC depends entirely on whether endothelial cells growth factor (ECGF) and heparin have been added to the culture medium (Bauer et al., 1990; Tontsch and Bauer, 1991). cEC grown in ECGF/heparin-supplemented medium show a cobblestone-like morphology and proliferate at a high rate. On contact with glial and neuronal plasma membranes, cells of this phenotype (type I cEC) respond with an increase in BBB-associated enzyme activities. Removal of growth factor from the culture medium results in a spindle-formed phenotype (type II cEC), a low proliferation rate and a loss of responsiveness to neural stimulation (Bauer et al., 1990).

In this article we demonstrate that cells of the two phenotypes differ also in their protein expression pattern, as revealed by 2-D gel analysis. Moreover, our data indicate that ECGF and heparin act synergistically with respect to the phenotypic determination of cloned cEC.

MATERIALS AND METHODS

Cell Culture

Microvessels from murine and porcine brain cortices were isolated as has been described earlier (Tontsch and Bauer, 1989). Capillaries were digested with 0.075% collagenase type I (Sigma, Deisenhofen, Germany) at room temperature for 10 min and plated onto culture dishes (Nunc, Roskilde, Denmark) in medium M199 (Seromed, Berlin, Germany) sup-