LOCALIZATION OF ENDOTHELIN-1 IN GUT TISSUE: IMMUNOCYTOCHEMICAL STUDY

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Endothelins are newly discovered bioactive peptides isolated from supernatant of cultured endothelial cells (EC) (5). The discovery of endothelins (ET) has generated major interest because of their vasoactive potency and possible involvement in diseases (3). There are reports in vitro that ET release is enhanced during hypoxia. The present study was undertaken to locate endothelin-1 (ET-1) in EC and monitor possible changes brought about by chronic hypoxia in the microvasculature of the intestinal mucosa. Adult male Wistar rats were maintained in a flexible film hypoxic chamber for 10 days. The fractional inspired oxygen (FIO2) was maintained at 10%, with carbon dioxide and excess humidity removed by means of filters (4). Normoxic control animals were maintained on the same diet and exposed to the same light/dark cycle. After Sagatel anaesthesia animals were perfusion-fixed with 4% paraformaldehyde, 1,8% DL-lysine HCL and 0,2% sodium m-periodite. Pieces from the large intestine were processed for electron microscopy without osmification. Material was embedded in LR White resin (London Resin Co, U.K). Post-embedding immunogold staining was performed (1) using antibody to ET-1 (Cambridge Research Biochemicals, U.K) with all appropriate controls.

ET-1 immunoreactive sites in the large intestine in normal control tissue were confined to the vascular endothelium and the brush-border epithelium of the mucosa. The immunolabelling signal, however, was low. Not all EC profiles were labelled. Gold particles were found mainly at the endothelial matrix. In epithelial cells, small clusters of gold particles were found at the basal portion and few at the brush-border region. Hypoxic gut tissue showed a marked increase in the intensity of labelling. In EC, gold particles were distributed in large groups in the cell periphery as well as in the subendothelium. There was no association of gold label with pinocytotic vesicles. In epithelial cells clusters of immunogold label were concentrated in all regions of the cytoplasmic matrix. Gold particles were encountered also in vacuole-like spaces facing the apical portion of the cell. The brush border itself was most heavily labelled (Fig. 1). Immunoreactive product was also concentrated at the periphery.
between two neighbouring cells.

Our results demonstrate that immunolabelling with ET-1 of the endothelial and epithelial cells of the intestinal mucosa is increased during chronic hypoxia. It is recently shown that ET-1 is released from mesenteric arteries in response to hypoxia. Hypoxia-related states like asthma, may trigger the synthesis and release of ET-1. Hypoxia-induced increase of ET-1 immunolabelling in epithelial cells is, however, unexpected, moreover the heaviest labelling is seen at the brush border. Previous investigations have reported the presence of ET in gut tissue but its exact localization is not clarified. Recently, a novel peptide-vasoactive intestinal contractor (VIC) (2) was found to belong to the endothelin family. It is expressed in the intestine but not in EC. It remains to be clarified whether ET-1 and/or VIC are synthetized in epithelial cells. VIC differs from ET-1 in only 3 aminoacid residues. Our ultrastructural findings are also suggestive of luminal and paracrine secretion of these products from the epithelium. Further studies are needed to ascertain the synthesis and secretion of ET-1 in gut epithelial cells and their physiological role in the gut.