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Secretion of endothelin and related peptides from renal epithelial cell lines

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Using specific radioimmunoassays (RIAs) for endothelin (ET) and big ET, we have studied whether ET and related peptides are secreted from renal epithelial cell lines ($LLCPK_1$ and MDCK) of non-endothelial origin. Dilution curves of extracts of conditioned media from both $LLCPK_1$ and MDCK cell lines were parallel to those of standard porcine (p) ET and big pET in each RIA. Both cell lines incubated in serum-free medium secreted ET- and C-terminal fragment (CTF)-like immunoreactivity (LI) of big ET as a function of time. Reverse-phase HPLC coupled with both RIAs of the extracted media from both cell lines revealed a single component with ET-LI coeluting with pET(1–21) and several components with CTF-LI, one corresponding to the elution position of big pET(1–39), one to its CTF(22–39), and the others eluting earlier than CTF. These data indicate that endothelin and related peptides are synthesized by and secreted from cells other than endothelial cells.

Endothelin; HPLC, reverse-phase; RIA; (Renal cell line)

1. INTRODUCTION

Endothelin (ET) is a potent vasoconstrictor peptide recently isolated and sequenced from the supernatant of cultured porcine aortic endothelial cells [1]. It has been suggested that the 21-residue porcine (p) ET is derived from the 203-residue precursor molecule, prepro-ET, through an unusual proteolytic process in endothelial cells; big pET(1–39), a presumptive intermediate form, is processed to mature pET(1–21) by a putative ET-converting enzyme. We have recently demonstrated that bovine and human endothelial cells in culture actually synthesize and secrete, in addition to ET, big ET and its C-terminal fragment (CTF) into medium [2].

In this communication we have studied whether renal epithelial cell lines derived from non-endothelial origin produce and secrete ET and related peptides.

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2. MATERIALS AND METHODS

2.1. *Cell culture*

MDCK (ATCC CCL34) and $LLCPK_1$ (ATCC CRL1392) cell lines were cultured in Dulbecco's modified Eagles' medium (DMEM) containing 10% fetal calf serum (FCS) and antibiotics. After reaching confluency they were fed with serum-free DMEM. Conditioned media were collected at 2–3 day intervals, pooled, and stored at −40°C until processed for extraction. Cell numbers were counted using a Coulter counter, model ZM (Coulter Electronics, Hialeah, FL).

2.2. *Extraction and chromatographic procedures*

The conditioned media (300 ml) from both cells were acidified with trifluoroacetic acid (TFA) and applied to Sep-Pak C-18 cartridges (Waters Associates, Milford, MA) and eluted with 70% acetonitrile/0.1% TFA, as reported previously [3]. The extract was subjected to a reverse-phase HPLC using a 0.45 × 25 cm octadecyl-silica column (JASCO, Tokyo) eluted with a linear gradient (15–60%) of acetonitrile in 0.09% TFA for 60 min at a flow rate of 1 ml/min, as described [2]. Each eluate was subjected to subsequent RIAs. The recoveries of standard pET, big ET, and its CTF were 96%, 89% and 90%, respectively.

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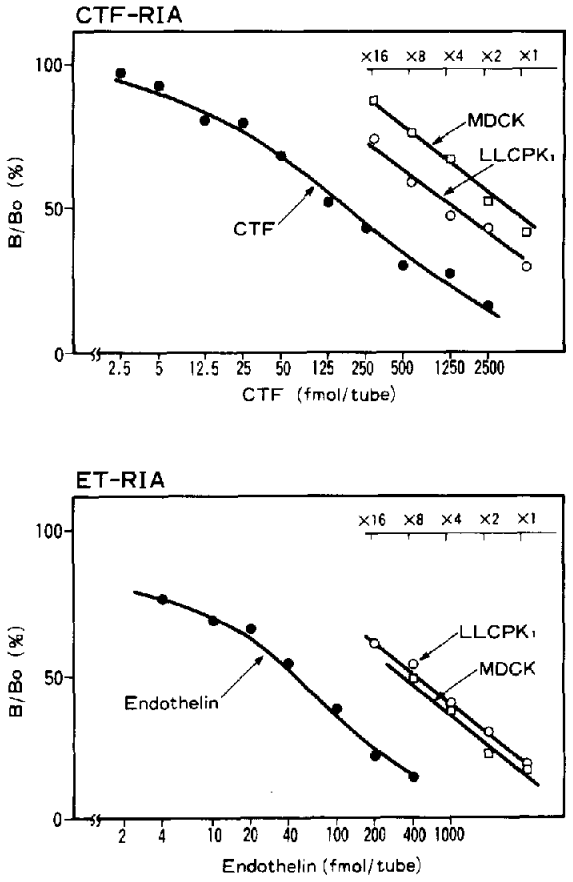

Fig.1. Radioimmunoassays for the C-terminal fragment (CTF) of big ET and ET. Dilution curves of the same extracts of conditioned media from $LLCPK_1$ cells (○) and MDCK cells (□) are compared with standard curves in CTF RIA (upper panel) and ET RIA (lower panel).

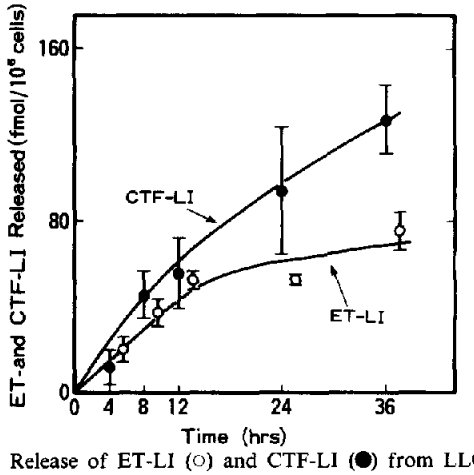

Fig.2. Release of ET-LI (○) and CTF-LI (●) from $LLCPK_1$ cells as a function of time. Each point is the mean of 6 measurements; bars indicate SEM.

2.3. *RIAs for ET and big ET*

RIAs for ET and big ET were performed as recently described [2,3]; the antibody used for ET RIA mainly recognizes the C-terminal region of pET, but not big pET, whereas the antibody used for big ET RIA recognizes the C-terminal fragment(22–39) of big pET, but not pET.

3. RESULTS

As shown in fig.1, dilution curves generated by the extracts of the conditioned media from $LLCPK_1$ and MDCK cell lines were parallel to those of standard CTF of big pET (upper panel) and pET (lower panel) in each RIA. Both ET-like immunoreactivity (LI) and CTF-LI of big ET were concomitantly secreted linearly from the $LLCPK_1$ cells under a serum-free condition, reaching a plateau after 12 h (fig.2), while the amounts of ET-LI and CTF-LI released from MDCK cells were far less than those from $LLCPK_1$ cells (data not shown). Reverse-phase HPLC profiles of ET-LI and CTF-LI in the extracts of the conditioned media from $LLCPK_1$ and MDCK cell lines are shown in fig.3. The elution profiles of the media from $LLCPK_1$ cells (fig.3, upper panel) revealed a single minor component with ET-LI coeluting with standard pET(1–21), and two components with CTF-LI: a major peak coeluting with standard CTF(22–39), and another minor peak eluting earlier than CTF. In the media from MDCK cells (fig.3, lower panel), a single ET-LI peak corresponding to pET(1–21) and three minor components with CTF-LI were observed; one coeluting with big pET(1–39), one with CTF(22–39), and the other eluting prior to CTF(22–39).

4. DISCUSSION

The present study demonstrates for the first time that ET and related peptides are synthesized by and secreted from established renal epithelial cell lines ($LLCPK_1$ and MDCK) of non-endothelial origin. The apparent parallelism between the dilution curves of the extracts of conditioned media from both cells strongly suggests that ET and related peptides immunologically similar to ET and big ET are released from these cell lines. Both ET-LI and CTF-LI from the $LLCPK_1$ cell line accumulated in the medium time-dependently under serum-free condition, although the amounts of ET

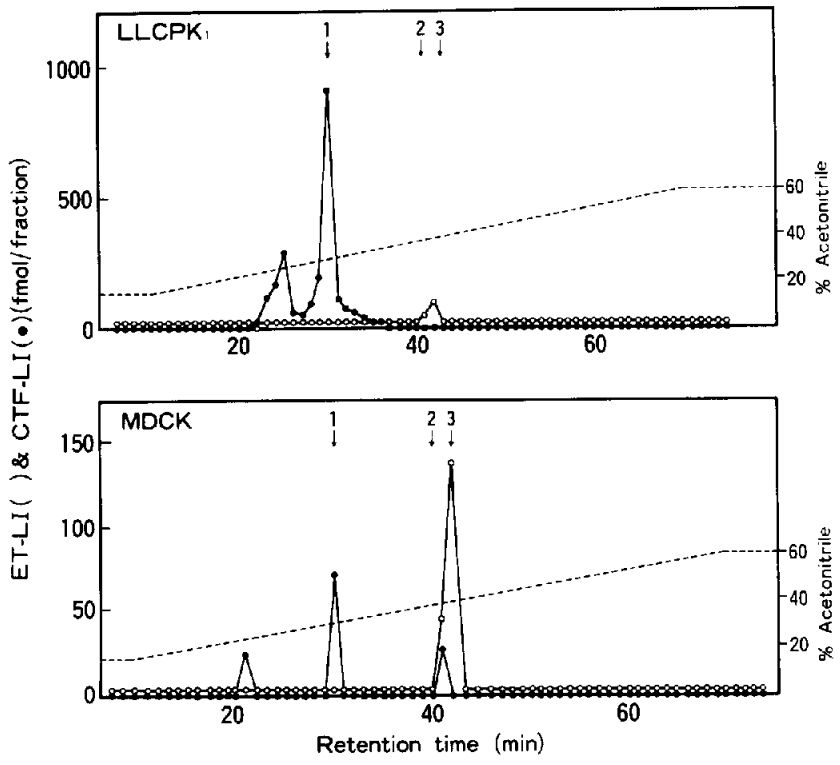

Fig.3. Reverse-phase HPLC profiles of ET- and CTF-like immunoreactivity (LI) in extracts of conditioned media from $LLCPK_1$ cells (upper panel) and MDCK cells (lower panel). Arrows indicate the elution positions of (1) CTF(22–39), (2) big pET(1–39), and (3) synthetic pET(1–21), respectively.

and related peptide in the medium from $LLCPK_1$ cells were far greater than those from MDCK cells. These data strongly suggest that these renal cell lines actually synthesize and secrete ET and related peptides. Reverse-phase HPLC coupled with RIAs for ET and CTF of big ET of the extracts of the conditioned media from both renal cell lines revealed one major peak with ET-LI coeluting with pET(1–21) and several components with CTF-LI. $LLCPK_1$ cells secreted two major CTF-LI components, one coeluted with CTF(22–39) and the other eluted earlier than CTF. They may represent proteolytic products of CTF(22–39) and shorter fragment(s) generated from big ET. In contrast, MDCK cells secrete three minor CTF-LI components: one coeluting with big ET, one with CTF(22–39), and the other eluting earlier than CTF(22–39). The present results from non-endothelial cells are in sharp contrast to those obtained from cultured human and bovine endothelial cells, which produced and secreted equimolar amounts of ET(1–21) and CTF(22–39) in addition to big ET [2], indicating the existence of an ET-converting enzyme responsible for processing big ET. Taken together, these data suggest that renal epithelial cell lines have an ET processing mechanism different from that of endothelial cells, possibly by other proteolytic enzymes responsible for further processing of big ET, other than ET-converting enzyme.

The MDCK cell line retains many of the differentiated properties typical of renal tubular cells in the loop of Henle or in the distal tubule [4–6], whereas the $LLCPK_1$ cell line exhibits some of the properties of proximal tubular cells [7–9]. We observed dome formations in all confluent cultures of the two cell lines, suggesting functional plasma membrane polarization with the apical (microvillus) cell surface in contact with the medium and the formation of occluding junctions. The presence of ET and related peptides in the conditioned media may suggest that they are released

from the apical (microvillus) membrane of these cells. If ET or related peptides are secreted from renal tubular cells into the lumen in vivo, physiologic effects on more distal tubular cells can be anticipated. Further studies should be performed to elucidate the effect of ET on transepithelial transport in renal tubular cells.

REFERENCES

[1] Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K. and Masaki, T. (1988) Nature 332, 411–415.

[2] Emori, T., Hirata, Y., Ohta, K., Shichiri, M., Shimokado, K. and Marumo, F. (1989) Biochem. Biophys. Res. Commun., in press.

[3] Emori, T., Hirata, Y., Ohta, K., Shichiri, M. and Marumo, F. (1989) Biochem. Biophys. Res. Commun. 160, 93–100.

[4] Cereijido, M., Robbins, E.S., Dolan, W.J., Rotunno, C.A. and Sabatini, D.D. (1978) J. Cell Biol. 77, 853–880.

[5] Misfeldt, D.S., Hamamoto, S.T. and Pitelka, D.K. (1976) Proc. Natl. Acad. Sci. USA 73, 1212–1216.

[6] Gansch, C.R., Hard, W.L. and Smith, T.F. (1966) Proc. Soc. Exp. Biol. Med. 122, 931–935.

[7] Mullin, J.W., Weibel, J., Diamond, L. and Kleinzellar, A. (1980) J. Cell Physiol. 104, 375–379.

[8] Hull, R.N., Cherry, W.R. and Weaver, W. (1976) In Vitro 12, 670–677.

[9] Perantoni, A. and Berman, J.J. (1979) In Vitro 15, 446–454.