SHORT COMMUNICATION

The stimulatory effect of endothelin-1 on regenerating adrenal cortex is reversed by nifedipine

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Abstract

Endothelin-1 (ET-1), a potent vasoconstrictor, was found to act in non-vascular tissues, for example it enhanced aldosterone output from adrenal zona glomerulosa. As the adrenal cortex is capable of regeneration after enucleation, it seemed of interest to study the effects of ET-1 on adrenocortical regeneration. The study was performed on adult rats subjected to left adrenal enucleation combined with contralateral adrenalectomy. Mitotic index was employed to assess the proliferation of regenerating adrenal cortex cells. Plasma corticosterone was measured by a standard RIA kit. ET-1 significantly raised the mitotic index of regenerating rat adrenal cortex by six days after surgery. On the other hand, nifedipine reduced the proliferation ratio and abolished the stimulatory influence of ET-1. Similarly, ET-1 enhanced corticosterone output from the regenerating adrenal cortex, and this could be prevented by the addition of nifedipine.

This study has shown that ET-1 might act as a regulatory factor on the regenerating adrenal cortex via calcium channels.

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Introduction

The rat adrenal cortex is capable of regeneration after removal of most of the cortex and medulla by enucleation. This process starts from the subcapsular zone and is most intensive 3–7 days after surgery (1).

Endothelin-1 (ET-1), a potent vasoconstrictor, can also affect non-vascular tissues. For example, it was found that ET-1 can stimulate aldosterone secretion (2).

The mechanism of ET-1 action is unclear, although calcium seems to play an important role (3). Therefore, it seemed of interest to study the effect of ET-1 and nifedipine, a calcium channel blocker, on the regenerating adrenal cortex.

Materials and methods

The investigation was performed on 40 male Wistar rats weighing 160–180 g. The animals were kept under controlled photoperiods (12 h light : 12 h darkness) and temperature (22–24 °C) with constant access to tap water and rat chow. All rats were subjected to the enucleation of the left adrenal gland combined with the removal of the opposite gland. For enucleation, the adrenal was gently raised with forceps and, after an incision was made in the capsule, the contents were extruded with curved forceps. Surgery was carried out under ether anesthesia.

The animals were divided into 4 groups, each comprising 10 rats. Ten rats received 5 ng/kg ET-1 (Peninsula Lab. Inc., Belmont, CA, USA), 10 animals received 5 mg/kg nifedipine (Nifedipine-guttae, Ratiopharm GmbH, Hamburg, Germany), 10 rats were given ET-1 (5 ng/kg) and nifedipine (5 mg/kg), and the remaining animals served as controls and were given 0.9% NaCl. All agents were given as subcutaneous injections, 12 h and 1 h before decapitation.

On the 6th day after operation, the animals were decapitated at 1000 h. 2 h after an intraperitoneal injection of colchicine (Fluka AG, Buchs, Switzerland, 1 mg/kg). The mitotic index was employed to evaluate adrenocortical cell proliferation. To establish this parameter, the regenerating adrenal cortex after a routine histological treatment was examined microscopically and metaphases were counted in 10 000 cells. The mitotic index was determined as the number of mitoses/1000 cells. Trunk blood was collected from each animal. The plasma corticosterone concentration was measured by a commercially available RIA kit specific for rat corticosterone (DRG Inc., Mountainside, NY, USA).

The results were expressed as means ± s.e.m. and were analyzed by Student’s t-test.
Results
Six days after operation, the mean mitotic index was
10.82 ± 0.64 in the control group. ET-1 stimulated the
proliferation ratio (19.15 ± 0.74; P < 0.01), and this
effect could be prevented by nifedipine (12.04 ± 0.46;
P < 0.01 when compared with the ET-1-treated group).
Nifedipine alone caused a well-marked inhibition of cell
growth ratio (6.84 ± 0.34; P < 0.01 compared with the
control group).
The plasma corticosterone concentration was higher
in ET-1-treated animals than in the control group.
125.89 ± 14.29 vs 60.1 ± 2.15 ng/ml, P < 0.001 respec-
tively. On the other hand, nifedipine alone decreased
corticosterone secretion (42.31 ± 3.74, P < 0.01) and
reversed the stimulatory effect of ET-1 (48.51 ± 5.43;
P < 0.01 compared with ET-1-treated animals).

Discussion
ET-1 and ET-1 binding sites are widely distributed in
non-vascular tissues including brain, kidney, liver,
spleen and adrenal glands (2, 4, 5).
ET-1 has been shown to stimulate aldosterone
secretion in dispersed zona glomerulosa cells and, in
addition, some investigators have reported a similar
effect of pharmacological concentrations of ET-1 in vivo
(4, 6, 7). There is also some evidence that ET-1 might
enhance cortisol and corticosterone release from
dispersed human and rat zona fasciculata cells (8).
Moreover, ET-1 could stimulate mitotic activity in the
zona glomerulosa of the intact adrenal cortex (9).
It is noteworthy that the growth-promoting effect of
ET-1 seems to be more universal. ET-1 was shown to
stimulate DNA synthesis and proliferation of Swiss 3T3
cells, fibroblasts, melanocytes, cardiocytes, epithelial
carcinoma cell lines and vascular smooth muscle
cells (10).
The exact mechanism of ET-1 action remains unclear.
After binding to its receptor ET-1 activates phospho-
lipase C to generate inositol triphosphate and diacyl-
glycerol. This promotes release of calcium ions from
intracellular stores and influx of calcium through the
voltage-dependent calcium channels (2, 3).
On the other hand, it is believed that cells require
calcium ions during both the S phase and mitosis (11).
The presence in the adrenal cortex of voltage-dependent
calcium channels sensitive to dihydropyridines has been
clearly demonstrated (12, 13).
Some authors believe that the mitogenic action of
ET-1 on vascular smooth muscle cells and rat glial
cells depends, in part, on its ability to mobilize extracellular calcium, and this could be reversed by
nifedipine (10).
Although ET-1 has been reported to stimulate adreno-
corticotropin (ACTH) secretion (14), such indirect action
of ET-1 is rather doubtful, as the adrenal regeneration is
independent of ACTH (15).

In conclusion, this study reveals that ET-1 can
stimulate adrenocortical regeneration and this effect
can be abolished by a calcium channel antagonist.

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References
1 Taki MT & Nickerson PA. Differentiation and proliferation of
adrenocortical cells during the early stage of regeneration.
Laboratory Investigation 1985 53 91–100.
2 Masaki T. Endothelins: homeostatic and compensatory actions in
the circulatory and endocrine systems. Endocrine Reviews 1993
14 256–268.
3 Pollock DM, Keith TL & Highsmith RE. Endothelin receptors and
4 Lerman A. Hildebrand FL, Margules KB, O'Murchu B, Perrella MA.
Heublein DM et al. Endothelin, a new cardiovascular regulatory
5 Sakurai T & Goto K. Endothelins. Vascular action and clinical
6 Cozza EN, Gomez-Sanchez CE, Foecking MF & Chiov S. Endothelin
binding to cultured calf adrenal zona glomerulosa and stimula-
tion of aldosterone secretion. Journal of Clinical Investigation 1989
84 1032–1035.
7 Gomez-Sanchez CE, Cozza EN, Foecking MF, Chiov S & Ferris MW.
Endothelin receptor subtypes and stimulation of aldosterone
8 Hinson JP, Vinson GP, Kapas S & Teja R. The role of endothelin in
the control of adrenocortical function: stimulation of endothelin
release by ACTH and the effects of endothelin-1 and endothelin-3
on steroidogenesis in rat and human adrenocortical cells. Journal of
9 Mazzocchi G, Malendowicz LK, Meneghelli V & Nussdorfer GG.
Endothelin-1 stimulates mitotic activity in zona glomerulosa of
10 Battistini B, Chaillet P, D’Orleans-Juste P, Briere N & Siros P.
Growth regulatory properties of endothelins. Peptides 1993 14
385–399.
11 Fujita T & Nakao Y. Cellular calcium: cell growth and dif-
ferentiation. In Calcium in Human Biology pp 421–46. Ed BRC
12 Durroux T, Galloc-Payet N & Payet MD. Three components of the
calcium current in cultured glomerulosa cells from rat adrenal
13 Zienlewiski W. Effect of nifedipine on adrenocortical cell
proliferation and steroidogenesis in early stage of adrenal
14 Vierhapper H, Holstein U, Roden M & Nowotny P. Effect of
endothelin-1 in man—impact on basal and stimulated concen-
trations of luteinizing hormone, follicle stimulating hormone,
thryotropin, growth hormone, corticotropin and prolactin.
Metabolism 1993 42 902–906.
15 Estivariz FE, Morano MJ, Carino M, Jackson S & Lowry PJ. Adrenal
regeneration in the rat is mediated by mitogenic N-terminal
proopiomelanocortin peptides generated by changes in precursor
processing in the anterior pituitary. Journal of Endocrinology 1988
116 207–216.

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