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 10000 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, NJ, 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 respectively. 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 phospholipase C to generate inositol triphosphate and diacylglycerol. 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 adrenocorticotropin (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
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