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ANGIOTENSIN II STIMULATION OF THE RAT PITUITARY TUMORAL CELL PROLIFERATION *IN VITRO*

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SUMMARY: The effect of angiotensin II (AT II) on proliferation of rat pituitary tumoral cells was investigated *in vitro*. The tumoral cells were isolated from the prolactin-secreting pituitary tumors induced by stilboestrol implantation. The incorporation of [^{3}H]-thymidine into DNA was used as an index of cell proliferation. It was found that AT II significantly enhanced the [^{3}H]-thymidine incorporation into pituitary tumoral cells in the concentrations of 10^{-10} and 10^{-8}M. The stimulatory effect disappeared at the concentration of 10^{-6}M. The possible involvement of pituitary renin-angiotensin system in pituitary tumorigenesis was discussed.

Angiotensin II circulating in blood is known to be generated by a cascade of proteolytic cleavages, where renin produced by juxtaglomerular cells of the kidney cleaves angiotensinogen, a precursor originating from the liver, to from a decapetide angiotensin I (AT I). The latter is transformed under the influence of the so-called converting enzyme into the active octapeptide angiotensin II (AT II). However, now it is well known that the kidney is not a unique source of renin and angiotensins can be produced also in other tissues and organs. One of them is the anterior pituitary. Renin has been detected in the anterior pituitaries of many mammalian species including rats and men [1]. In the rat pituitary both renin and AT II immunoreactivity was found in gonadotrophs [2,3]. In human pituitaries, renin, angiotensinogen and converting enzyme were localized in lactotrophs [4]. The presence of renin RNA in the rat pituitary was also shown [5]. Although AT II receptors in the anterior pituitary were characterized [6], the role of the adenohypophysial renin-angiotensin

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system (RAS) remains unclear. Several data indicate that AT II can effect the pituitary secretions in the paracrine manner [7,8]. So far, the effects of AT II on the pituitary cell growth have not been reported. Thus, it seemed reasonable to study the effect of AT II on the proliferation of the rat pituitary tumoral cells.

METHODS

Induction of pituitary tumors in rats. Female, four-weeks-old Wistar rats were used in the experiment. Pituitary tumors were induced by subcutaneous implantation of a single Silastic capsule containing 8-10 mg of diethylstilboestrol (DES; Stilboestrol, Sigma) in the lumbar region. Such capsule was estimated to release 18-45 μg/day of stilboestrol.
Pituitary tumoral cell cultures. Forty nine days following DES capsules implantation the animals were killed by decapitation and pituitary tumors were aseptically removed. The suspension of tumor cells was obtained by enzymatic digestion with 0.4% collagenase (537 U/mg, clostridium histolyticum, type, I, Sigma). This procedure yielded to a population containing more than 95% of viable cells. Following 30 min. of preincubation the cells were counted and resuspensed in RPMI-1640 medium (Gibco), with 20 mM HEPES buffer, penicillin (100 U/ml), streptomycin (100 μg/ml) and 15% fetal calf serum (Gibco). One ml aliquots of the cell suspension, each containing 0.5 x 10^6 cells, were distributed into plastic tubes (12 x 75 mm, Kimble Products, Houston, Texas). The tubes were incubated at 37°C in the humidified atmosphere of 95% air and 5% CO_2. The angiotensin II - (Angiotensin-Germed) dissolved at the desired concentrations were added to the appropriate tubes. After 1h of incubation 2 μCi/ml of [^{3}H]-thymidine, sp. act. 28.9 Ci/mM (Amersham Center, England) was added in 50 μl of the medium. The incubation was terminated 24h later and cells were washed with 2 ml cold 0.9% NaCl. After the precipitation of DNA the radioactivity was measured and expressed as the mean counts per minute ([^{3}H] cpm/0.5x10^6 cells). The values represent mean of two experiments (each in triplicate) $\pm$SD. Student t-test or Aspin-Welsh test were used to determine the statistical significance.

RESULTS

The data are presented in Fig. 1. As can be seen there, the exposure of pituitary tumoral cells to AT II in the concentrations of 10^{-10} and 10^{-8} M resulted in an enhancement of the [^{3}H]-thymidine incorporation. The stimulatory effect disappeared at the AT II concentration of 10^{-6} M.

DISCUSSION

The observation reported above indicates that AT II my stimulate rat prolactinoma cells proliferation. It is not surprising since AT II is a potent mitogenic factor for

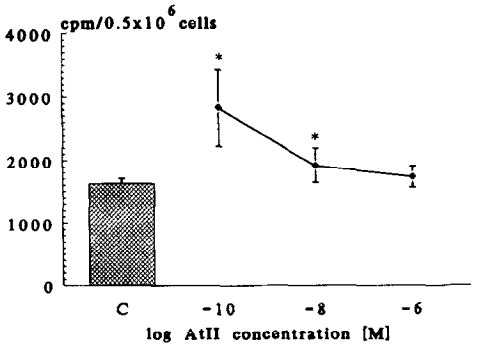

Fig. 1. Effect of angiotensin II (AT II) on the incorporation of [^{3}H]-thymidine into DNA of rat pituitary tumor cells. *$p < 0.05$ vs control.

adrenocortical [9,10] and arterial smooth muscle cells [11]. Moreover, an inhibitor of converting enzyme captopril as well as AT II antagonist saralasin were found to inhibit the neuroblastoma cell growth *in vitro* [12]. It has been also suggested that the mas oncogene encodes the AT II receptor [13]. Our finding taken together with the fact that AT II is generated within the anterior pituitary, suggests that this peptide hormone may act as a local factor controlling the pituitary growth. However, the suggestion needs further studies to be proven, especially using the inhibitors of AT II synthesis and/or action. The proliferative response of tumoral lactotrophs to AT II suggests that lactotrophs are target cells for the paracrine action of this peptide. This suggestion is compatible with the recent data of Kubota and Aso [8] who have shown that AT II is responsible for gonadoliberin-induced release of prolactin. Another question is whether the pituitary RAS might be involved in the pituitary tumorigenesis. Such a presumption seems to be attractive since estrogens are well known to enhance the angiotensinogen production in the liver as well as to stimulate the pituitary growth and tumorigenesis [14,15].

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