Angiotensin in Human Leukocytes of Patients with Insect Venom Anaphylaxis and Healthy Volunteers

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Key Words

Human leukocytes
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Angiotensin I
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Introduction

Angiotensin I (ANG I) and angiotensin II (ANG II) are part of the renin angiotensin system (RAS) which is a biologically active regulatory system involved in the regulation of blood pressure and fluid and electrolyte balance. The active component of the RAS is the peptide ANG II. It has been shown that ANG II, apart from its role in cardiovascular functions, stimulates protein, DNA and RNA synthesis as well as cell proliferation [1, 2]. Likewise, ANG II has some effects on the regulation and modulation of inflammation and immune functions, e.g. chemotaxis, migration, proliferation and differentiation of mononuclear leukocytes [3-5]. Although these findings indicate a possible role for ANG II in the regulation and modulation of immune functions, little is known about the presence or origin of ANG II in leukocytes. The aim of this study was to determine whether angiotensin peptides are present in human leukocytes and whether these cells possess the pathway for the biological synthesis of ANG II. Since ANG II concentrations were decreased in plasma of patients with Hymenoptera venom anaphylaxis [6], we were interested to investigate ANG II in leukocytes of patients with Hymenoptera venom anaphylaxis before and after hyposensitization.

Material and Methods

Blood samples were collected from healthy nonallergic volunteers and patients with Hymenoptera venom anaphylaxis. Leukocytes were prepared by sedimentation in 6% dextran. Angiotensin peptides were extracted from leukocytes with a mixture of acetone, 1 N HCl and water. ANG I and ANG II were measured radioimmunologically in the reconstituted extracts and HPLC was used to characterize the ANG I and ANG II immunoreactive material. Leukocytes were incubated in vitro with the ^3H -labeled amino acid isoleucine and the extracted radioactive material was characterized on an HPLC gel filtration column and a reverse-phase C18 column. The extracted radioactivity was further characterized by its ability to bind to anti-ANG I or anti-ANG II antibodies in the presence of excessive unlabeled Ile5-ANG I or Ile5-ANG II.

Results

Immunoreactive ANG I and ANG II were identified in leukocyte extracts. The concentration of ANG I and ANG II in healthy volunteers was 32.0 ± 3.6 and 13.1 ± 1.3 fmol/mg protein ($n = 24$). The peptides were characterized on HPLC as Ile5-ANG I, He5-ANG II and ANG II metabolites.

Patients with Hymenoptera venom anaphylaxis ($n = 22$) showed significantly reduced ANG II levels when compared to healthy nonallergic volunteers: 6.8 ± 0.8 versus 13.1 ± 1.3 fmol/mg protein, respectively. A significant positive correlation between the severity of the clinical symptoms of anaphylaxis and the ANG II concentration was found.

Human leukocytes isolated from the blood of healthy volunteers were incubated in vitro with ^3H -labeled amino acid isoleucine to investigate whether these cells were capable of synthesizing angiotensin. The cells showed a time-dependent uptake of radioactivity. The radioactive material extracted from the cells eluted from an HPLC gel filtration

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column with the same retention time as synthetic Ile5-ANG I and He 5-ANG II (fig. 1). The radioactive material also bound to anti-ANG I or anti-ANG II antibodies. However, rechromatography of the radioactive material which eluted from the gel filtration column on a reverse-phase C18 column eluted in the void volume of the column and could be identified as ^3H -isoleucine.

25,000

Discussion

ANG I and ANG II were identified in human leukocytes. The ANG I and ANG II immunoreactivity was characterized on HPLC as small amounts of Ile5-ANG I, Ile5-ANG II and ANG II metabolites confirming earlier results from our laboratory and reports in the literature [7, 8].

Patients with a history of Hymenoptera venom anaphylaxis showed significantly lower ANG II concentrations in their leukocytes as compared to healthy nonallergic controls. Successful hyposensitization induced a significant increase in the ANG II concentrations indicating a possible relationship between the endocrine RAS and the immune system. The appearance of ANG II in leukocytes indicated that an endogenous RAS capable of synthesizing ANG II is present. However, no radioactive-labeled ANG I or ANG II were identified after in vitro incubation of the cells with the ^3H -labeled amino acid isoleucine. This shows that renin and angiotensinogen, the precursor for ANG I and ANG II, are not present in human leukocytes. Further evidence for the absence of an endogenous RAS in leukocytes as obtained previously, where we were unable to identify renin and angiotensinogen or their mRNAs [unpubl. data]. Although these findings clearly demonstrate the presence of ANG II in human leukocytes, its source remains unclear. These findings also suggest a possible role for ANG II in Hymenoptera

venom anaphylaxis with a putative interaction between the immune and the cardiovascular system.

Time (h)

Thyroglobulin

Bovine serum albumin Carbonic anhydrase Cytochrome C

Cyanocobalamin U V

Fraction No.

Fig. 1. a Incorporation of the ^3H -labeled amino acid isoleucine in human leukocytes in vitro. A time-dependent uptake of radioactivity was found with a maximum between 2 and 4 h. Results are expressed as means $\pm$ SE ($n = 7$). b HPLC characterization of the radioactivity extracted from leukocytes on a Bio Sil TSK 125 gel filtration column. The radioactive material eluted in the low-molecular-weight range of the column with the same retention time as Ile5-ANG I or Ile5-ANG II. Arrows indicate the retention times of several molecular-weight markers. — = 2 h incubation; - - - = 8 h incubation.

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