Insulin-like growth factor binding proteins in arterial hypertension: relationship to left ventricular hypertrophy

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Objective: It was reported previously that circulating insulin-like growth factor I levels are abnormally elevated in patients with essential hypertension and left ventricular hypertrophy. Tissue availability of the factor depends on the distribution of the circulating bound factor between its high- and low-molecular mass binding proteins, only the latter being able to cross the endothelium. The aim of this study was to investigate whether the presence of the different serum binding proteins is altered in patients with essential hypertension and left ventricular hypertrophy.

Design: The study was performed in 30 never-treated patients with essential hypertension and 30 age- and sex-matched normotensive subjects. Patients were separated into two groups according to the presence or the absence of echocardiographically determined left ventricular hypertrophy.

Methods: Plasma insulin-like growth factor I levels were determined by specific radioimmunoassay. The different molecular forms of its serum binding proteins were analysed by Western blotting using [^{125}I]-labelled insulin-like growth factor I. A densitometric scanning of the blots was performed to analyse the quantitative relationships between the different forms of binding proteins.

Results: Insulin-like growth factor I levels were significantly higher in the hypertensive patients with than in the hypertensive patients without left ventricular hypertrophy or in the normotensive subjects. Compared with the normotensive subjects, both hypertensive patients subgroups exhibited increased high-molecular mass binding protein type 3 and decreased low-molecular mass binding proteins types 1 and 2. However, changes in the binding proteins were more marked in the hypertensive patients without than in the hypertensive patients with left ventricular hypertrophy. Accordingly, the ratio of low- to high-molecular mass binding proteins (an index of insulin-like growth factor I bioavailability) was higher in the hypertensive patients with than in the hypertensive patients without left ventricular hypertrophy.

Conclusions: These results show that the distribution of the molecular forms of serum insulin-like growth factor binding proteins is altered in patients with essential hypertension, independently of insulin-like growth factor I levels. This suggests that regulation of the binding proteins is abnormal in essential hypertension. Whether the tissue availability of circulating insulin-like growth factor I is higher in hypertensive patients with than in hypertensive patients without left ventricular hypertrophy merits further investigation.

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Introduction

Recent studies [1,2] have shown that plasma levels of insulin-like growth factor I are increased in patients with essential hypertension compared with in normotensive subjects. Furthermore, an association has been found between high insulin-like growth factor I levels and left ventricular hypertrophy in hypertensive patients [1,2]. Interestingly, the ability of some antihypertensive drugs (angiotensin converting enzyme inhibitors) to induce regression of hypertensive cardiac hypertrophy might be related to their ability to normalize circulating insulin-like growth factor I levels [1,3].

Under physiological conditions 90% of all circulating insulin-like growth factor I is associated with several specific high-affinity binding proteins [4]. Accordingly, insulin-like growth factor I circulates by forming two complexes: the complex of relative molecular mass 150 000, which has been shown to contain the two major forms of binding proteins (relative molecular masses 41 500 and 38 500) and does not cross the capillary barrier, and the complex of relative molecular mass 40 000–50 000, which has been shown to contain two minor forms of binding proteins (relative molecular masses 34 000 and 30 000) and can cross the endothelium [4]. By controlling the distribution between the two complexes, insulin-like growth factor binding proteins may influence the bioavailability of the factor to their cellular receptors [4,5].

We hypothesized that an abnormal relationship between circulating insulin-like growth factor I and its binding proteins, leading to an increase in the availability of the factor to tissues, may be present in hypertensives with left ventricular hypertrophy. To test the hypothesis, we compared the presence of the various forms of binding proteins, obtained by Western blotting, in serum from essential hypertensive patients separated into two subgroups: those with left ventricular hypertrophy and those without left ventricular hypertrophy.

Subjects and methods

Subjects

The hypertensive group consisted of 30 never-treated Caucasian hypertensive patients. All had been classified as having mild-to-moderate hypertension in stage I or II of organ damage [6]. Causes of secondary hypertension were excluded after a complete medical work-up [7]. The control group consisted of 30 age- and sex-matched Caucasian normotensive subjects. None had any known family history of hypertension. Liver and endocrine functions were normal, and none of the subjects had a current illness. Dietary habits were similar in all subjects and patients.

Methods

Left ventricular structure was assessed by M-mode echocardiography (guided by two-dimensional echo). Measurements were obtained following the recommendations of the American Society of Echocardiography [8]. Left ventricular mass was calculated according to Devereux *et al.* [9] and corrected for body surface area (left ventricular mass index). The reproducibility of the method used for echocardiographic estimation of left ventricular mass was assessed by analysing the interobserver variability. Interobserver variability was determined by having a second observer recalculate left ventricular mass from the original trace made by the first observer in a series of 15 control subjects and 15 hypertensive patients. Estimates of left ventricular mass by the two observers were strongly correlated [$r=0.97$, $P<0.001$ (slope 45°), SD 8.9 g]. The presence of left ventricular hypertrophy was established when left ventricular mass index was >130 g/m^2 for males and >110 g/m^2 for females [10].

Plasma glucose and other serum metabolites were assayed by standard enzymatic methods. Plasma and urinary creatinine were determined by the picric acid method. The creatinine clearance was calculated from the standard equation. Urinary sodium was determined by flame photometry.

Blood samples for hormonal determinations were drawn from the antecubital vein at 0800 h after overnight fasting. Total plasma insulin-like growth factor I levels were quantitated as described previously, with some modifications [11]. The procedure included, first, a simple acid–ethanol extraction step in which insulin-like growth factor I is separated from its binding proteins and, secondly, a two-site immunoradiometric assay. Antiserum anti-insulin-like growth factor I was provided by Diagnostic Systems Laboratories Inc. (Webster, Texas, USA). Insulin and basal morning growth hormone levels were measured in plasma by routine radioimmunoassays. Plasma renin activity was determined by a radioimmunoassay for angiotensin I [12] in samples obtained after overnight resting (8–10 h). Plasma aldosterone was determined by routine radioimmunoassay.

Serum insulin-like growth factor binding proteins were analysed by Western blotting according to the methodology described previously by Hossenlopp *et al.* [13] and Hardouin *et al.* [14,15], with some modifications. To determine insulin-like growth factor binding proteins, all serum samples were taken between 0800 and 1000 h after overnight fasting, and stored at −20°C. The determination was made twice. Three reference serum pools were used: a pool of sera from 10 normotensive subjects; a pool of sera from 10 hypertensive patients without left ventricular hypertrophy; and a pool of sera from eight hypertensive patients with left ventricular hypertrophy. Briefly, 2-µl samples were diluted in 0.06 mol/l TRIS–HCl (pH 6.8) and 0.15 mol/l sodium chloride, then supplemented with 5% sodium dodecyl sulphate, 10% glycerol and 0.02% bromophenol blue (final volume

15 µl), heated for 20 min at 60°C and finally submitted to a sodium dodecyl sulphate–polyacrylamide gel electrophoresis gradient gel (10–15%) in the absence of reducing agent (except in the case of [^{14}C]-labelled reference proteins; Amersham International plc, Little Chalfont, Buckinghamshire, UK). The running conditions were constant voltage (60 V) for approximately 15 h, then constant current (30 mA/gel) until the marker dye exited. The proteins were then electroblotted on a nitrocellulose sheet for 2 h under constant current (0.8 A). After quenching at 4°C of the nitrocellulose with 5% Nonidet P-40 and 0.1% Tween-20, and overnight incubation with 200 000 c.p.m. [^{125}I]-insulin-like growth factor I at 4°C, the binding proteins were detected by autoradiography. The specificity of the binding was checked by incubating part of the nitrocellulose containing the binding proteins with excess unlabelled insulin-like growth factor I. Under these conditions the binding proteins were not visualized by autoradiography (data not shown).

After autoradiography of the gel, each line of the autoradiograph was scanned on a densitometer (Appraise; Beckman, Fullerton, California, USA) and the amount of each insulin-like growth factor binding protein in each sample was determined as the percentage of the total optical density per lane that occurred in bands representing the binding proteins (Fig. 1).

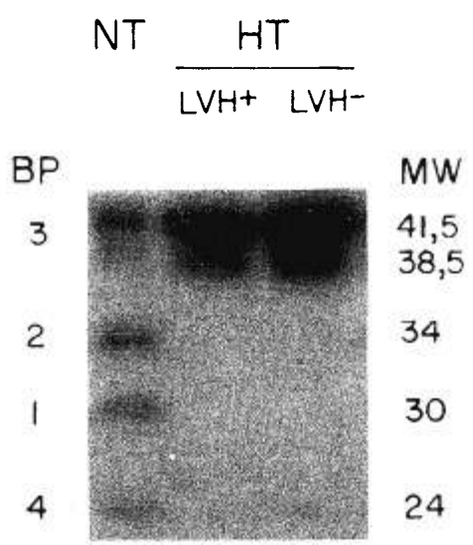

Fig. 1. Autoradiograph of Western blot analysis of serum insulin-like growth factor binding proteins (BP) in pools of sera from normotensive subjects (NT) and from hypertensive patients (HT) with (LVH+) and without (LVH–) left ventricular hypertrophy. The position of each BP is indicated on the left. The relative molecular mass (MW) of each (in thousands) is indicated on the right.

Statistical analysis

Values are expressed as means ± SEM. A multiple comparison test (Scheffé method) was used to compare values between control subjects and hypertensive patients both with and without left ventricular hypertrophy. The correlation between continuously distributed variables was tested by univariate regression analysis.

Results

Two subgroups of hypertensive patients were defined according to left ventricular mass index: those with left ventricular hypertrophy (n = 12, 40%) and those without left ventricular hypertrophy (n = 18, 60%). None of the normotensive subjects had left ventricular hypertrophy. The clinical and hormonal data for the two subgroups of hypertensive patients and the group of normotensive subjects are shown in Tables 1 and 2, respectively. It is observed that plasma insulin-like growth factor I levels were increased in the hypertensive patients with left ventricular hypertrophy compared with in the normotensive subjects and the other hypertensive patients. Insulin-like growth factor I level was positively correlated with left ventricular mass index in the combined subgroups of hypertensive patients ($r = 0.46$, $P < 0.05$; $y = 87 + 0.08x$). No correlations were found between insulin-like growth factor I level and other clinical and hormonal parameters measured in the present study.

Table 1. Clinical parameters in the normotensive control subjects and hypertensive patients.

		Hypertensives	
Parameter	Controls	With LVH	Without LVH
n	30	12	18
Age (years)	48 ± 8	48 ± 7	43 ± 5
Male : female	16 : 14	8 : 4	10 : 8
Duration of high blood pressure (months)	0	14 ± 8	16 ± 10
Body mass index (kg/m^2)	27.5 ± 0.5	29.4 ± 0.6*	28.8 ± 1.1*
Blood pressure (mmHg)			
Systolic	134 ± 2	165 ± 1*	154 ± 2*
Diastolic	80 ± 1	102 ± 1*	96 ± 1*
Mean arterial	98 ± 3	123 ± 1*	117 ± 2*
LVMI (g/m^2)	99 ± 11	112 ± 10†	101 ± 15
Glucose (mmol/l)	5.3 ± 0.3	5.3 ± 0.1	5.0 ± 0.2
Cholesterol (mmol/l)	5.67 ± 0.27	5.55 ± 0.20	5.80 ± 0.20
Triglycerides (mmol/l)	1.22 ± 0.16	1.54 ± 0.11*	1.50 ± 0.21*
Ccr (ml/min per 1.73 m^2)	98 ± 4	110 ± 15	107 ± 15
Urinary Na (mmol/day)	129 ± 14	133 ± 16	149 ± 21

Values are expressed as means ± SEM or as numbers of subjects. *$P < 0.05$, versus controls; †$P < 0.01$, versus controls and hypertensives without LVH. LVH, left ventricular hypertrophy; LVMI, left ventricular mass index; Ccr, creatinine clearance.

The Western blot analysis of serum insulin-like growth factor binding proteins is shown in Fig. 1. We refer to the various binding proteins in terms of the relative molecular masses deduced from sodium dodecyl sulphate–polyacrylamide gel electrophoresis and following the nomenclature in the literature [4]. In the normo-

Table 2. Hormonal parameters in the normotensive control subjects and hypertensive patients.

Parameter	Controls	Hypertensives	
		With LVH	Without LVH
IGF-I (ng/ml)	226±15	291±12*	222±20
Growth hormone (μg/l)	1.1±0.2	0.9±0.3	1.0±0.2
Insulin (μU/ml)	10±3	16±4	14±2
PRA (ng Ang I/ml per h)	1.1±0.5	1.4±0.6	1.4±0.4
Aldosterone (pg/ml)	229±66	253±43	233±70

Values are expressed as means±SEM. *$P<0.02$, versus controls and hypertensives without LVH. LVH, left ventricular hypertrophy; IGF-I, insulin-like growth factor I; PRA, plasma renin activity; Ang, angiotensin.

tensive control subjects there were two major forms of relative molecular masses 41 500 and 38 500 (binding protein type 3) and three minor forms of relative molecular masses 34 000, 30 000 and 24 000 (types 2, 1 and 4, respectively). Compared with in the normotensive subjects, binding protein type 3 level was increased in the two subgroups of hypertensive patients, particularly in those without left ventricular hypertrophy. The remaining binding protein levels were decreased in the two subgroups of hypertensive patients. Identical results were obtained in two separate determinations of binding proteins.

Figures 2 and 3 show the percentages of the total optical density corresponding to the different binding proteins for each lane. The percentages of the two bands corresponding to binding protein type 3 were higher ($P<0.05$) in the hypertensive patients without left ventricular hypertrophy (53.9±0.8 and 38.3±0.9%, respectively) than in the hypertensive patients with left ventricular hypertrophy (52.0±0.6 and 30.4±0.6%) and in the normotensive subjects (42.3±1.3 and 17.6±1.0%; Fig. 2). The percentages of binding proteins types 2 and 1 were lower ($P<0.001$) in the hypertensive patients without left ventricular hypertrophy (2.5±0.1 and 3.0±0.2%, respectively) than in the hypertensive patients with left ventricular hypertrophy (7.3±0.3 and 7.8±0.4%) and in the normotensive subjects (20.5±0.4 and 12.4±0.5%; Fig. 3). The percentages of binding protein type 4 were decreased ($P<0.001$) in the two subgroups of hypertensive patients (2.3±0.3 and 2.5±0.2%) compared with in the normotensive subjects (7.2±0.6%).

The ratio of the sum of the percentages of binding proteins types 1 and 2 divided by the percentage of type 3 was calculated as an index of insulin-like growth factor I bioavailability. The ratio was higher ($P<0.01$) in the hypertensive patients with left ventricular hypertrophy (0.184±0.022) than in the hypertensive patients without left ventricular hypertrophy (0.059±0.016). These ratios were lower ($P<0.001$) than the ratio calculated for the normotensive subjects (0.584±0.094).

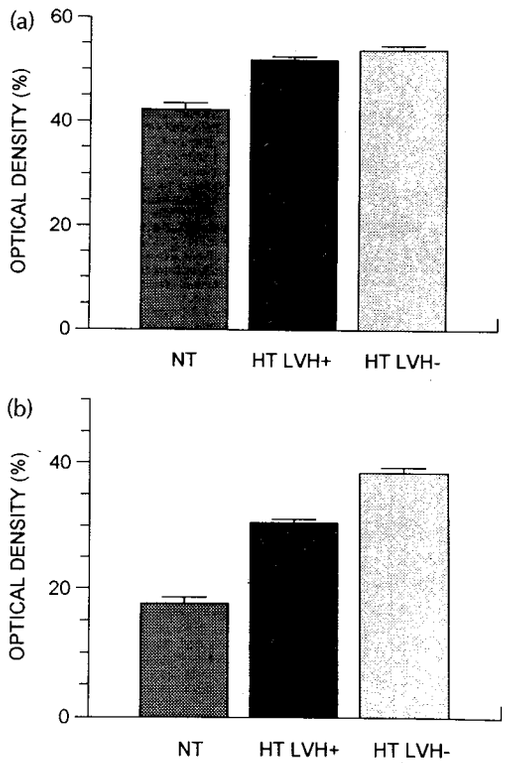

Fig. 2. Percentage of the total optical density corresponding to the two bands of insulin-like growth factor binding protein type 3 as shown in the autoradiograph of Fig. 1. (a) Component of relative molecular mass 41 500 and (b) component of relative molecular mass 38 500. NT, normotensive control subjects; HT, hypertensive patients with (LVH+) and without (LVH–) left ventricular hypertrophy. Values are expressed as means±SEM.

Discussion

The present study confirms previous data from our group [1,3] and others [2] showing that an association exists between exaggerated plasma insulin-like growth factor I levels and left ventricular hypertrophy in patients with essential hypertension. Thus, it appears that circulating insulin-like growth factor I might be related to the development of hypertensive cardiac hypertrophy. Two observations add further support to such a relationship: compared with control mice, transgenic mice expressing human insulin-like growth factor I exhibit increased serum levels of the factor in association with increased cardiac weight [16,17], and our group has shown previously [1,3] that the ability of antihypertensive drugs to induce regression of left ventricular hypertrophy in hypertensive patients appears to be related to their ability to normalize plasma insulin-like growth factor I levels.

However, the role of paracrine and autocrine regulation of insulin-like growth factor I at the cardiac level needs to be considered. Significant increases in left ventricular insulin-like growth factor I messenger RNA and protein have been shown to occur in different animal mod-

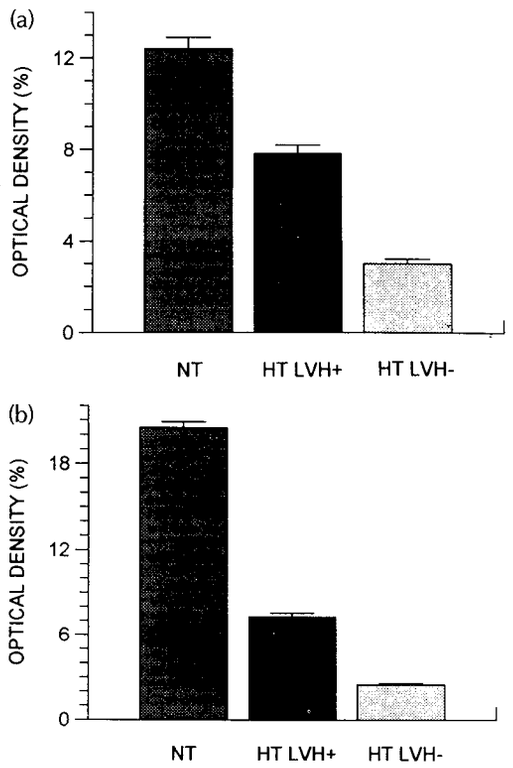

Fig. 3. Percentage of the total optical density corresponding to the bands of insulin-like growth factor binding proteins (a) type 2 and (b) type 1 as shown in the autoradiograph of Fig. 1. NT, normotensive control subjects; HT, hypertensive patients with (LVH+) and without (LVH–) left ventricular hypertrophy. Values are expressed as means ± SEM.

els of pressure-overload cardiac hypertrophy [18–20]. In contrast, neither insulin-like growth factor I messenger RNA nor the protein were increased in the hypertrophic hearts of mice expressing the factor [16]. Therefore, the data available do not allow it to be established whether an excess of autocrine or paracrine insulin-like growth factor I contributes to the development of left ventricular hypertrophy in essential hypertension.

The liver is the major source of circulating insulin-like growth factor I [5]. Its hepatic synthesis is under the control of growth hormone [5]. Although basal growth hormone levels were normal in the present group of hypertensive patients with left ventricular hypertrophy and with increased insulin-like growth factor I levels, we are aware that a dynamic determination of growth hormone levels is necessary to assess pituitary production of growth hormone. Nevertheless, the growth hormone levels of the hypertensive patients in the present study were below the critical value of 2 μg/l that has been found to exclude growth hormone hypersecretion [21]. Therefore, it appears that regulation of circulating insulin-like growth factor I levels is abnormal in hypertensives with left ventricular hypertrophy. Whether this reflects an altered interaction of growth hormone with its receptors in liver cells remains to be elucidated.

Alternatively, alterations in the growth hormone-independent extrahepatic synthesis of insulin-like growth factor I [22] could be considered. For instance, both endothelial cells and vascular smooth muscle cells are sites of insulin-like growth factor I synthesis and secretion [23–26]. Induction of a chronic increase in vascular load is associated with a marked increase in insulin-like growth factor I immunostaining of both endothelial cells and vascular smooth muscle cells in the rat femoral artery [27]. Accordingly, it has been proposed that circulating levels of insulin-like growth factor I in hypertensives with left ventricular hypertrophy could result from the pressure load stress exerted by exaggerated levels of blood pressure acting on the vascular system [2]. However, it is very unlikely that secretion of insulin-like growth factor I by vascular cells, reported to be in the range 0.006–0.018 ng/ml [23], should necessarily raise its plasma concentration, which is in the range 200–300 ng/ml.

The main finding of the present study is that the distribution of the different molecular forms of serum insulin-like growth factor binding proteins is shifted from the low-molecular mass forms (binding proteins types 1, 2 and 4) to the high-molecular mass forms (type 3) in hypertensives compared with in normotensives. However, the alteration was not uniform in all of the hypertensive patients studied. In fact, the intensity of the shift was less pronounced in the hypertensive patients with left ventricular hypertrophy than in the hypertensive patients without left ventricular hypertrophy.

The relationship that exists between circulating insulin-like growth factor I levels and the formation of the relative molecular mass 150 000 complexes with the type 3 binding protein [15], the long half-life of these complexes [28] and their near-inability to cross the capillary barrier [29] all support the hypothesis of their function as acting as an insulin-like growth factor I reserve and buffer. Unlike the relative molecular mass 150 000 complexes, the complexes of molecular mass approximately 40 000–50 000, composed of insulin-like growth factor I and its types 1 and 2 binding proteins, can cross the capillary barrier [29]. Thus, several observations have led to the suggestion that the role of these complexes would be to transport the insulin-like growth factor I to its target cells [15].

Therefore, although the index of insulin-like growth factor I bioavailability was abnormally diminished in the two subgroups of hypertensive patients, the present finding that the index is higher when cardiac hypertrophy is present than when it is not suggests that the availability of the circulating factor at its target cells, i.e. myocardial cells [30], is higher in hypertensives with left ventricular hypertrophy than in hypertensives without left ventricular hypertrophy.

Although this is the hypothesis suggested by the present findings, other possible interpretations are not excluded. For instance, it is important to know whether the degree of saturation of each particular form of the binding proteins with insulin-like growth factor I is altered in hypertensives and whether this influences the amount

of insulin-like growth factor I measured in those patients. Furthermore, there are other functions attributed to the binding proteins that also deserve consideration (i.e. modulation of the biological activity of insulin-like growth factor I).

Which are the causes of the abnormal patterns of serum insulin-like growth factor binding proteins found in patients with essential hypertension? The production of binding protein type 3 is increased in response to increases in growth hormone [15], insulin [31] and insulin-like growth factor I [32]. However, the levels of type 2 binding protein appear to be downregulated by growth hormone [15] and insulin [33]. Finally, the levels of type 1 binding protein in serum appear to be inversely related to the prevalent insulin levels [34].

Considering the values obtained for the different hormones measured in the present study (Table 2), it appears that the observed changes in binding proteins types 3 and 2 cannot be attributed to an excess of growth hormone. However, although an excess of insulin-like growth factor I might be responsible for the increase in binding protein type 3 present in hypertensives with left ventricular hypertrophy, this is not the case for hypertensives without left ventricular hypertrophy who also exhibit increased binding factor type 3 in the setting of normal levels of insulin-like growth factor I. A tendency to increased insulin levels was observed in the two present subgroups of hypertensive patients. Thus, it is tempting to speculate that this alteration might be involved in the abnormalities of insulin-like growth factor binding proteins present in the hypertensive patients studied here.

In summary, both the regulation of plasma insulin-like growth factor I levels and the regulation of its serum binding proteins are altered in patients with essential hypertension. In addition, the present findings suggest that the access of circulating insulin-like growth factor I to its target cells is altered in hypertensive patients. Further investigations are required to elucidate whether an excess of myocardial availability of circulating insulin-like growth factor I participates in the development of myocardial hypertrophy in patients with essential hypertension.

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