Demonstration of Endothelial-Activating Properties of Hypertensive Sera

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Sera from patients with hypertension were examined for their ability to influence endothelial cell function. Sera from 10 patients with hypertension and from 11 age-matched controls were incubated with endothelial cells and their effect on cell growth assessed by endothelial cell $^3$H-thymidine uptake. Sera from patients with hypertension had a more pronounced stimulatory effect on endothelial cell proliferation than control sera. In addition, hypertensive sera were also examined for their capacity to stimulate endothelial cell production of the vasoactive peptide endothelin. Confluent monolayers of endothelial cells were incubated with sera and the amount of endothelin generated in the supernatant measured by an immunoassay. Amounts of endothelin produced by the endothelial cells in response to hypertensive sera were significantly higher compared with the amounts produced in response to normal sera. The results of the studies suggest that a serum factor(s) in hypertensive patients may be of importance in modulating endothelial cell function. Such a factor(s) may have a significant role in the pathogenesis of hypertension. © 1996 American Journal of Hypertension, Ltd. Am J Hypertens 1996;9:1232–1235

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The endothelial cell is known to regulate the function and tone of vascular smooth muscle cells by the production of a variety of vasoactive substances. Examples of such substances are nitric oxide and prostacyclin both of which are vasodilatory, and the endothelins and thromboxane, both of which are vasoconstrictive. The roles of these substances in the pathogenesis of hypertension are unclear and are undergoing definition. Thus, several studies have shown a lack of response of hypertensive vessels to nitric oxide, indicating that insensitivity to this vasodilatory substance may be an important mechanism underlying the development of hypertension. The data on levels of circulating endothelin in the hypertensive state are not conclusive. Some studies have been able to show mildly elevated levels of this vasoconstrictive peptide in patients with hypertension, whereas others have been unable to demonstrate this finding. Another clue to the involvement of the endothelial cell in the genesis of hypertension is the finding of endothelial cell hyperplasia in the vessels of the hypertensive host, indicating that there is a stimulus for the proliferation of these cells in this disease state.

The present study has examined the sera of hypertensive patients for a factor(s) that can modulate endothelial cell function. We have been able to show that the sera of such patients stimulate endothelial cell proliferation, and endothelial cell production of endothelin.

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MATERIALS AND METHODS

Subjects
Sera were obtained from 10 male patients, aged 50 to 78, with hypertension. Hypertension was defined as a blood pressure greater than 140/90 mm Hg. Nine of the 10 patients had a diastolic blood pressure between 90 mm Hg and 100 mg Hg. None of the patients were on antihypertensive medication. Control sera were obtained from 11 age-matched healthy males.

Sera
Sera were obtained by letting blood stand for 60 min after which they were centrifuged at 1500 g × 15 min. Supernatants were stored in aliquots at -20°C until use.

Human Umbilical Arterial Endothelial Cells
These cells were obtained from Cell Systems (Kirkland, WA) and were identified by staining with anti-factor VIII. Cells were grown in special media containing endothelial cell growth supplement (Cell Systems, Kirkland, WA) until use.

Endothelial Cell ³H-Thymidine Assay
This is a modification of a previously described assay.² Aliquots of $1 \times 10^4$ cells were dispensed into the wells of flat-bottomed tissue culture plates (Microtest II, Falcon Labware) containing Dulbecco's Modified Eagle Medium (DMEM) and 1.5% human sera and incubated for 48 h in a 5% CO₂ humidified atmosphere at 37°C. Sixteen hours before the cells were harvested, they were pulsed with 1 µCi ³H-thymidine (20 µCi/mol) (New England Nuclear, Boston, MA) and incubation continued. At termination of the culture, medium was aspirated and cells washed twice with HBSS. An aliquot of 0.05% trypsin with 0.5 mmol/L EDTA (Gibco, Grand Island, NY) was added to each well; the cells were harvested onto glass fiber filters and washed with distilled water using a semiautomatic cell harvester (Skatron, Sterling, VA). Filter discs were punched out and transferred to scintillation vials containing Aquasol-2 (New England Nuclear, Boston, MA) and counted in a scintillation counter (LS-9800, Beckman Instruments Inc., Fullerton, CA). Assays were done in quadruplicate and are representative of three separate experiments.

Assay for Endothelial Cell Production of Endothelin
Confluent layers of endothelial cells were incubated with DMEM containing 5% human sera for 36 h. At the end of the incubation period, the supernatant was harvested and the cells counted for cell number. Endothelin was assayed in the supernatant by an immunoassay using a commercial kit (Amersham Life Sciences, Arlington Hts, IL). The antibody reacted equally with endothelin-1 and endothelin-2 but had 0.1% reactivity against endothelin-3 and the big endothelins. The lower limit of the assay was 14.8 pg/mL. All measurements were done on duplicate samples and are representative of two separate experiments. Results of assays of samples extracted by the Amersham Amprep C2 column were not significantly different from those assayed without extraction.

The concentrations of sera and times of incubation used for both assays were based on preliminary experiments testing various concentrations of sera at different time intervals on cell function.

RESULTS

The effect of sera on endothelial cell proliferation as assessed by ³H-thymidine uptake is shown in Figure 1. Sera from patients with hypertension stimulated the growth of endothelial cells more readily than that of control sera. The mean (± SE) ³H-thymidine uptake of cells cultured with hypertensive sera was 15,297 ± 744 disintegrations/min; this was significantly higher than the value (7,137 ± 381 dpm) obtained for cells incubated with control sera ($P < .0001$).

Figure 2 shows the amounts of endothelin generated by endothelial cells when they were incubated with hypertensive and control sera. The mean (± SE) concentration of endothelin produced by endothelial cells grown in hypertensive sera was 695 ± 21 pg/50,000 cells; this was significantly higher than the value obtained (612 ± 7 pg/50,000 cells) for cells incubated with control sera ($P < .0001$). Endothelin concentrations of sera from both patients and control were below the detectable limits of the assay.

DISCUSSION

The endothelial cell is a metabolically active cell that produces a variety of substances, including compounds that modulate vascular tone, such as nitric oxide, prostacyclin, thromboxane, and endothelin.
Endothelin is a peptide that has highly vasoconstrictive properties. It has a direct constrictor effect on vascular smooth muscle cells and when injected into the intact animal, produces a sustained increase in blood pressure.\(^6\) Its physiological properties make it a prime candidate molecule mediating the physiological and pathological changes found in the hypertensive state. To date, assays for circulating levels of endothelin in patients with mild to moderate hypertension have yielded inconclusive results. Although some studies have shown elevated levels of this peptide in this disease state, others have been unable to confirm the presence of this abnormality.\(^2\,^3\) It has been suggested, however, that circulating levels of the peptide may not accurately reflect what is occurring at vessel wall sites and that the more important effect of endothelin is its paracrine action on the vascular smooth muscle cell. Thus, it has been shown that endothelial cells in culture release twice as much endothelin in the basolateral direction as compared with the luminal one.\(^7\) Additionally, peripheral blood concentrations of endothelin may not reflect concentrations delivered to vascular smooth muscle cells, as the peptide may be degraded by enzymes present in those cells.\(^8\)

The current study of a fairly homogeneous group of mild hypertensive patients provides data that suggests that there is a factor(s) in the sera of these patients that acts on endothelial cells and stimulates them to proliferate more readily and to produce more endothelin than control normotensive sera. These data would explain the proliferation of endothelial cells that has been found in the hypertensive vessel wall\(^4\); more importantly, it would provide a basis for the increased vascular tone found in this disease state.

The limitations in the interpretation of the results are acknowledged. Thus the magnitude of the differences in endothelin production are small, and this may be consonant with the degree of hypertension found in the subjects. (It should be noted that in addition to its intrinsic ability to constrict blood vessels, endothelin also has a potentiating effect on the actions of other vasoconstrictive substances such as norepinephrine and serotonin.\(^9\) The tone of blood vessels depends on the balance between constrictive substances such as endothelin and thromboxane and dilatory substances such as prostacyclin and nitric oxide. The actions of this serum factor on the production of these vasoactive substances will also need to be defined. Finally, it has been pointed out that the alterations in endothelial function in hypertension may be the consequence rather than the cause of hypertension.\(^10\)

The identity of the serum factor(s) mediating both actions (growth promoting as well as endothelin stimulating) on the endothelial cell is undergoing clarification in continuing experiments. Two possibilities exist. The factor(s) responsible could be elevated levels of currently known growth factors that act on endothelial cells. Examples of these are basic fibroblast growth factor and vascular endothelial growth factor,\(^11\) both of which stimulate endothelial cell growth (their actions on endothelin production are unknown), and thrombin and transforming growth factor-\(\beta\) (together with a wide range of substances)\(^12\) both of which stimulate endothelin production. Alternatively, the factor could be a novel substance that may be elaborated only in the hypertensive host. Various factors have been characterized from the hypertensive host that have been demonstrated to elicit pressor responses in bioassay animals,\(^13\) to increase calcium uptake in platelets and in human neutrophils,\(^14,\,15\) and to inhibit Na, K ATPase; of special interest is the recent description of a factor of parathyroid gland origin, termed parathyroid hypertensive factor, which has been shown to stimulate vascular smooth muscle calcium uptake in parallel with its ability to raise systemic blood pressure\(^16\) and to raise cytosolic free calcium.\(^16\) Purification of the serum factor reported in this study will eventually enable us to determine whether it is different from these preceding factors.

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**REFERENCES**


