Hypertension Reduces the Number of Beta-adrenergic Receptors in Rat Brain Microvessels

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SUMMARY Beta-adrenergic receptor function was measured in cerebral microvessels of spontaneously and DOCA-salt hypertensive rats using 125I-iodohydroxybenzylpindolol (IYP). Both in genetic and in experimental hypertension, a significant decrease in the number of β-receptor sites was observed, without receptor affinity changes. These results suggest that alterations of central adrenergic regulation of small vessels may participate in the pathogenetic mechanisms leading to the development of the central hypertensive disease.

SEVERAL studies indicate that abnormalities in the functioning of the cerebral and peripheral adrenergic system may participate in the development and in the expression of hypertension. In particular, changes in catecholamine synthesis, content and turnover have been observed in various brain regions, sympathetic ganglia and peripheral tissues of hypertensive rats.1-10 Recently, changes of central catecholamine metabolism were shown to be associated with alterations of adrenergic receptor function and norepinephrine uptake in several brain areas of hypertensive rats.11, 12

The altered patterns of adrenergic transmission in hypertension may influence the cerebral microvasculature. In fact, various data suggest that brain microvessel function is altered in the presence of hypertension. In particular, morphologic studies in experimental hypertensive monkeys and in humans with essential hypertension13 have revealed the presence of cerebral capillary injury, such as increased diameter, endothelial degeneration and deposition of collagen.

Biochemical and physiologic evidence indicates that hypertension causes an increase in the permeability of the blood-brain barrier (BBB).14, 15 It has been reported that during acute elevation of systolic blood pressure there is a breakdown of the BBB, characterized by increased cerebral blood flow, extravasation of markers (which normally do not penetrate the BBB) and cerebral edema.16-18 Furthermore, in patients with severe hypertension (e.g., hypertensive encephalopathy), an impaired capacity of cerebral blood flow autoregulation has been reported.19, 20

Recently, the presence of β-adrenergic receptors in cerebral capillaries was demonstrated, and these receptors may have a role in the regulation of microvascular function.21-23 On the basis of these observations, β-adrenergic receptor function has been measured in brain capillaries of spontaneously and DOCA-salt hypertensive rats to investigate its involvement in cerebral microvascular alterations in hypertension.

Methods

Animals and Deoxycorticosterone Acetate Treatment

Adult male spontaneously hypertensive rats (SHR) and Wistar normotensive controls (250 g) were purchased from Charles River, Japan.

Adult male uninephrectomized Sprague-Dawley rats (250–280 g) were purchased from Charles River, Italy. In the latter animals, hypertension was induced by administering a biweekly subcutaneous injection of 15 mg/kg deoxycorticosterone acetate (DOCA, Cortinon) and 1% saline, as drinking water, for 4 weeks. Normotensive control rats were given tap water.

Blood Pressure Recording

Systolic blood pressure was measured by a tail-cuff method. In SHR and in DOCA-salt hypertensive rats, blood pressure values were significantly higher than those of the normotensive controls: 194 ± 7 mm Hg for SHR and 120 ± 8 mm Hg for Wistar controls, and 210 ± 9 mm Hg for DOCA-salt treated rats and 122 ± 6 mm Hg for their controls (mean ± SD).

Isolation of Cerebral Capillaries

Brain microvessels were isolated according to a method previously reported.21 Each experiment was performed using 10–12 rats per group. Rats were killed by decapitation. Gray matter of cerebral cortices was rapidly cleaned from pia membrane and white matter. Cortices of each experimental group were pooled, minced and homogenized by hand in a Teflon-glass homogenizer in gassed (95% O₂, 5% CO₂) Ringer’s solution that contained 1.2-mM MgCl₂, 15-mM Hepes, 5-mM glucose and 1% fraction V bovine serum albumin (BSA) (pH 7.4, 5 ml for each cortex). The homogenate was passed through 700-μm, 200-μm and 100-μm nylon meshes and centrifuged at 1000 g for 10 minutes. The resulting pellet was suspended in 20 ml of the same buffer now containing 30% BSA (pH 7.4 adjusted by 1N NaOH) and centrifuged at 1000 g for 15 minutes. The resulting small pellet was suspended in 5 ml of 1% BSA buffer. The floated thick layer was suspended in the supernatant and recentrifuged at 1000 g for 15 minutes. The pellet was suspended in 5 ml of 1% BSA buffer and combined with the first suspension. The suspension was passed through a column (1.2 cm for 1.2 cm) of glass beads (0.2–0.5 mm in diameter) and washed with 1% BSA buffer. The glass beads, with attached capillary segments, were taken from the...
column, suspended in buffer and shaken to release the capillaries. Practically pure capillaries were obtained by passing the suspension through another glass bead column. The purity was controlled by phase-contrast microscopy.21

The microvessels, free of neuronal and glial elements, were collected and washed twice in 154 mM NaCl-20 mM Tris HCl buffer (pH 7.5) by centrifugation and suspended in the same buffer for the binding assay. The ratio of milligrams of protein of microvessels to milligrams of cortex tissue from hypertensive rats is similar to that from normotensive animals.

125I-iodohydroxybenzylpindolol Binding Assay

Beta-adrenergic receptor density was measured using the specific radioligand [125I]-iodohydroxybenzylpindolol (IHYP, 2 Ci/μmol, Amersham).21 Aliquots of microvessel suspension were incubated at 37°C for 30 minutes. The incubation medium contained IHYP at various concentrations (25–170 pM), 100-μM GTP and 100-μM phenolamine to reduce nonspecific binding. The incubation was stopped by addition of 4.5 ml of 154 mM NaCl-20 mM Tris-HCl buffer (37°C, pH 7.5). Each sample was rapidly filtered through glass fiber filters (Whatman GF/B) and washed three times with 4.5 ml of buffer. Radioactivity was counted with efficiency of 55% (Packard 5110).

In a previous report, we have shown that IHYP binding to cerebral capillaries is characterized by saturation, high affinity, stereospecificity and reversibility.21 Specific binding of IHYP was defined as the difference of IHYP bound in the absence and in the presence of 20-μM (-)isoproterenol. The concentration of (-)isoproterenol is 100 times higher than the Kd value.

Specific binding was about 70% of the total binding at the Kd concentration of IHYP in both hypertensive and control groups. Protein content was measured by the method of Lowry et al.24

Results

Beta-adrenergic receptor function in cerebral microvessels was measured by the method of Scatchard.25 Figure 1 shows the saturation binding isotherm of IHYP in cerebral microvessels of SHR and normotensive Wistar controls. The maximum number of binding sites (Bmax) for SHR is 31% lower than that of normotensive rats (107 ± 6 fmol/mg protein and 154 ± 8 fmol/mg protein, respectively); no significant changes in Kd values were observed (74 ± 5 pM and 82 ± 9 pM, respectively).

A reduction of the number of β receptors in brain capillaries was also found in DOCA-salt treated rats. Figure 2 shows the Scatchard analysis of IHYP binding to cerebral microvessels of DOCA-salt hypertensive rats and normotensive controls.

The results are comparable with those from SHRs. The Bmax value for experimental hypertensive rats is 28% lower than that of normotensive controls (96 ± 8 fmol/mg protein and 132 ± 9 fmol/mg protein, respectively); no significant changes of Kd values were detected (87 ± 7 pM and 95 ± 8 pM, respectively).

Discussion

Various experimental observations suggest that cerebral microvasculature is under neuronal influence, mostly exerted by adrenergic neurons. In fact, ultrastructural studies have revealed the existence of adrenergic fibers originating from the locus coeruleus and innervating small brain vessels,26,27 whereas physio-
logic experiments suggest that the destruction or stimulation of the central noradrenergic system may influence BBB permeability and cerebral blood flow.28-30

Biochemical studies have demonstrated the presence of norepinephrine in brain capillaries, its synthesizing and catabolizing enzymes, and $\beta$-stimulated adenylate cyclase.31-33 Recently, the existence and the characterization of $\beta$-adrenergic receptors in rat and human cerebral microvessels has been reported.21,23 Two populations of $\beta$-adrenergic receptors have been demonstrated, with a large predominance of the $\beta_2$ type.22 The population of $\beta$ receptors measured in our experimental conditions is predominantly of the $\beta_2$ type.

The fact that the intraventricular administration of norepinephrine increases oxygen consumption and cerebral glucose uptake, and that these effects are prevented by the $\beta$-antagonist propranolol,34,35 may indicate a potential physiologic function of $\beta$-adrenergic receptors located in cerebral microvessels.

Our data suggest that functional alterations that occur with hypertension, such as increased BBB permeability and impaired cerebral blood flow autoregulation, may be partially related to the diminished $\beta$-adrenergic receptor density in brain capillaries. The data reported in the paper are consistent with previous observations of decreased catecholamine responsiveness of SHR brain capillary adenylate cyclase.36 In both genetic and DOCA-salt induced hypertension, the activity of the sympathetic nervous system is enhanced and plasma catecholamine levels are elevated.8,9,37-39 The observed reduction of $\beta$-adrenergic receptors may therefore be the consequence of an altered pattern of central adrenergic neurons controlling brain microvasculature as well as of increased plasma catecholamine levels. Brain aging in rats induces a similar reduction of $\beta$-receptor function.40 Although the blood pressure was not measured in those rats, we assumed that hypertension may at least partially responsible for the changes.

The concomitant events of altered neuronal and humoral regulation of $\beta$-adrenergic receptors may be responsible for microvessel dysfunction in hypertension. This disorder may be involved in the pathogenesis of hypertensive encephalopathy and in other neurologic syndromes complicating malignant hypertension.

References
31. Lai FM, Udenfriend S, Spector S: Presence of norepinephrine and...
Hemodynamic, Hormonal and Electrolyte Responses to Prenalterol Infusion in Heart Failure

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SUMMARY  The hemodynamic, hormonal and electrolyte effects of prenalterol, a synthetic selective \( \beta_1 \) agonist, were studied in six patients with New York Heart Association functional class II and III heart failure. Prenalterol was infused incrementally at 60, 120 and 240 nmol/min, each rate for 24 hours, producing steady-state plasma prenalterol levels of 52 ± 3, 121 ± 6 and 194 ± 9 nmol/l, respectively (mean ± SEM). Hemodynamic and hormonal measurements were performed before, during and after prenalterol administration under conditions of constant body posture and a regulated intake of dietary sodium and potassium.

Prenalterol induced a statistically significant increase in cardiac index (from 2.6 ± 0.2 to 3.1 ± 0.3 l/min/m²), with parallel increases in stroke index (from 28 ± 2 to 34 ± 2 ml/beat/m²). Forearm blood flow measurements increased (from 2.9 ± 0.5 to 4.1 ± 0.6 ml/min/100 g), while calculated systemic vascular resistance fell, as did pulmonary capillary wedge pressure (from 13.7 ± 0.6 to 10.5 ± 1.7 mm Hg). The drug did not alter heart rate, arterial pressure, right heart pressures or the frequency of ventricular premature beats. Prenalterol increased plasma renin activity (from 2.9 ± 0.8 to 6.6 ± 1.8 nmol/l/hour), angiotensin II (from 59 ± 12 to 89 ± 22 pmol/l), urinary aldosterone excretion (from 41 ± 10 to 78 ± 34 nmol/day) and plasma insulin (from 10.6 ± 2.2 to 19.8 ± 3.9 mU/l). Circulating catecholamines, cortisol, glucose, glucagon or pancreatic polypeptide did not change. Dose-response studies in five patients showed dose-dependent increments in hemodynamic variables, while hormonal changes plateaued at the second dose level. We conclude that prenalterol infusion augments myocardial contractility, reduces systemic vascular resistance, and stimulates insulin release and the renin-angiotensin-aldosterone system.

NO MAJOR ADVANCE in chronic inotropic treatment for heart failure has taken place since the introduction of digitals, and the place of this drug for patients in sinus rhythm is still uncertain.1 Several drugs have been investigated.2-4 Reports that a selective \( \beta_1 \) agonist, prenalterol, improves myocardial function in patients with heart failure are encouraging, especially because the drug may be effective when given by mouth.3 However, available data relate largely to its administration over a period of minutes only and dose-response information is scarce. Further, the effects of the drug on neurohormonal systems and electrolytes have received scant attention. The present study documents hemodynamic, hormonal and electrolyte changes during 3 days of an incremental prenalterol infusion in six patients with cardiac failure.

Methods

The protocol was approved by the hospital’s ethical committee. All patients gave informed written consent.

Clinical details of the six patients are summarized in table 1. All had suffered at least one episode of pulmonary edema but had responded to routine treatment. At the time of entrance to the study, their therapy had not changed for at least 3 months. Patient 1 was on perhexilene and disopyramide, which were discontinued 1 week before the study.
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