Studies of the Mechanisms Underlying Impairment of Beta-Adrenoceptor-Mediated Effects in Human Hypertension

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SUMMARY To investigate the impairment of beta-adrenoceptor responsiveness in human hypertension, we evaluated the effect of an oral salt load (400 mEq/day of NaCl for 7 days) on plasma catecholamine concentrations and beta-adrenoceptor-mediated effects in 11 young patients with mild essential hypertension. Responses of heart rate and plasma cAMP to isoproterenol administration were used as indices of beta-adrenoceptor responsiveness. Salt loading induced a significant reduction in the dose of isoproterenol required to raise the heart rate by 25 bpm (CD 25) (from 7.6 ± 1.5 to 5.3 ± 0.9 µg, p < 0.05) and an increase in the slopes of the regression lines for heart rate changes and isoproterenol doses (ΔHR/IS) (from 3.3 ± 0.6 to 4.7 ± 0.7, p < 0.05) and for plasma cyclic AMP (cAMP) level changes and isoproterenol doses (ΔcAMP/IS) (from 0.3 ± 0.06 to 1.4 ± 0.3, p < 0.05). After salt loading there was a significant reduction in plasma catecholamine concentrations with a significant relationship between changes in upright plasma epinephrine levels and changes in CD 25 (r = 0.904, p < 0.01) and in the slopes for ΔHR/IS (r = 0.983, p < 0.001) and ΔcAMP/IS (r = 0.922, p < 0.001). These results support the hypothesis that the impairment of beta-adrenoceptor sensitivity observed in human hypertension is associated with a beta-adrenoceptor overstimulation due to chronically elevated adrenergic tone. (Hypertension 5: 584-590, 1983)

KEY WORDS • salt load • catecholamines • cAMP

ALTHOUGH a reduction in beta-adrenoceptor responsiveness in hypertensive patients is well documented,1-3 the nature of this phenomenon and the mechanisms underlying it have not yet been fully elucidated. Bertel et al.2 found an inverse relationship between plasma catecholamines and beta-adrenoceptor responsiveness in hypertensive subjects and hypothesized that the marked decrease could be a consequence of beta-adrenoceptor overstimulation due to chronically elevated adrenergic tone. Such a hypothesis is based chiefly on three observations: 1) desensitization to catecholamines of beta-adrenoceptors has been described in aging men and in patients with congestive heart failure,4-5 who have elevated plasma catecholamine concentrations and attenuated cyclic adenosine monophosphate (cAMP) responses of peripheral blood lymphocytes to isoproterenol; 2) it has been suggested that plasma catecholamine levels regulate beta-adrenoceptor density in human leukocytes;6 and 3) improved beta-adrenoceptor responsiveness has been demonstrated in hypertensive patients after an increase in dietary sodium intake,3 which is known to reduce catecholamine release.7 However, a causal relation between plasma catecholamine levels, which reflect catecholamine release from the peripheral sympathetic system, and beta-adrenoceptor responsiveness in hypertensive patients has yet to be demonstrated. We therefore designed the present study to obtain new insights into the relationship between changes in plasma catecholamine levels and changes in adrenergic beta-adrenoceptor mediated effects in hypertension. For this purpose we chose to use a week of oral salt loading, which is known to alter catecholamine levels6,7 within the physiological range. As indices of beta-adrenoceptor sensitivity we used the changes in heart rate and plasma cAMP induced by isoproterenol administration.

Patients and Methods

The study was carried out in 11 male volunteers who gave their written consent prior to participation in the study. The subjects, who had mild essential hypertension, were aged from 15 to 35 years (mean age, 25 ± 2
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years). All subjects had average supine diastolic pressure, measured on four occasions at least 1 week apart, between 90 and 110 mm Hg.

As recommended by the American Heart Association, blood pressure measurements were made with a standard sphygmomanometer on subjects lying on a comfortable bed after a 10-minute rest in a darkened room. Secondary forms of hypertension were ruled out on the basis of the history and physical examination, complete blood count, urinalysis, urine culture, serum and urinary electrolytes, blood urea nitrogen, serum creatinine, plasma renin activity, and plasma aldosterone (supine and after 1 hour upright), urinary vanillylmandelic acid and free catecholamine concentrations, thyroid function test, rapid-sequence intravenous pyelogram, and renal scintigram. Patients with major disease other than hypertension were excluded from the study.

Procedures

Each patient had taken no drugs at least 2 weeks before the studies were begun. In the week preceding the study, each patient was given a daily diet containing 80 mEq sodium, 120 mEq potassium, 65 g protein, 50 g fat, 270 g carbohydrate, 100 mg phosphorus, as meat, eggs, bread, vegetables, fruit. Personal food preferences were followed as much as possible.

Testing of Beta-Adrenoceptor Sensitivity to Isoproterenol

The isoproterenol study was undertaken at 9 a.m. with the patient supine, not sedated, and after an overnight fast. Bolus injections of isoproterenol in 0.9% NaCl were given i.v., in a maximum injection volume of 1 ml, through an indwelling cannula previously placed in an antecubital vein, starting with a dose of 0.1 μg/m² body surface area. The heart rate was continuously monitored by ECG before and after each dose; the blood pressure was measured, always by the same person, before the injection and at 30-second intervals after isoproterenol administration. The heart rate (HR) was allowed to return to control values before subsequent doses of isoproterenol were given. The dose of isoproterenol was gradually increased to define the dose-HR response curve. From this curve, the dose that increased a stable HR by 25 bpm, i.e., the chronotropic dose 25 (CD₂₅), was derived. The doses of isoproterenol administered were also plotted against the corresponding increase in HR for each subject and analyzed by linear regression. The slopes of the regression lines so obtained were used as an additional index of beta-adrenoceptor responsiveness. A bolus of saline was randomly interposed as a control and did not induce any changes in blood pressure (BP) or HR. Full stop tachyphylaxis does not occur in this isoproterenol bolus test, nor does the test interfere with neurotransmitter reuptake. The subjects were asked to breathe regularly during the study. The resting HR (preinjection) was calculated as the mean of 10 R-R intervals. The postinjection peak HR was calculated from the shortest 3 R-R intervals after each injection. To evaluate the response of plasma cAMP to isoproterenol, a constant infusion of the drug was given by means of a pump, at doses of 1, 1.8, 2.4, 3.5, and 4.8 μg/min, each dose level being maintained for 4 minutes. The ECG was monitored continuously during the infusion. Blood samples for determination of plasma cAMP were taken at 5 and 1 minutes before the beginning of the infusion and then every 5 minutes thereafter.

Salt Loading

The day after the first isoproterenol test, all subjects were given an oral salt load by adding to the diet described above 400 mEq/day sodium chloride, divided into 8 doses of 50 mEq each, wrapped up in wafers. This regimen was maintained for 7 days. Water intake was allowed as desired. The subjects were weighed after voiding the bladder at the beginning and the end of the salt-loading period.

Twenty-four-hour urine specimens were obtained on the day before the first isoproterenol test and on the last day of the loading period, to determine diuresis, natriuresis, and kaliuresis. Blood samples were taken from supine patients before each isoproterenol test to determine serum electrolytes, creatinine, hematocrit, plasma epinephrine, and norepinephrine. Additional blood samples for determination of plasma catecholamines were also taken after 3 minutes of orthostasis. Comparison of the plasma norepinephrine responses after 3 minutes of standing can give valuable information, since at this time, according to our previous time course experiments (unpublished data), over 80% of the norepinephrine response is already developed (81.2% ± 8.0%, in respect to the norepinephrine increment at the plateau, n = 10). Similar time courses have been reported by Christensen and Tohmeh et al.

After 7 days of salt loading, the isoproterenol tests were repeated.

Analysis

Urinary and serum electrolytes were measured with a flame photometer. Creatinine was measured by an automated technique. Changes in plasma volume were estimated from the changes in peripheral microhaematocrits, using the formula:

\[ P = \frac{100(100-H_1)}{100(H_2-H_1)} \frac{H_2}{H_1} \]

in which P is the percent change in plasma volume and H₁ and H₂ are the initial and the final packed cell volumes. Epinephrine and norepinephrine were measured in 50 mcl of plasma simultaneously by a sensitive and specific radioenzymatic method, as previously described.

Plasma cAMP levels were determined by the competitive binding assay of Brown et al. Radioactivity was measured by liquid scintillation counting. Incubating the cAMP samples for 1 hour at 30°C with beef heart PDE (0.5 U/ml) destroyed more than 98% of measurable cAMP; recovery of known quantities of cAMP exceeded 80%.
Plasma renin activity was measured according to Menard and Catt\textsuperscript{18} (sensitivity: 5 ± 0.4 pg/tube angiotensin I, i.e., PRA = 0.06 ng·ml·hr\textsuperscript{-1}) and plasma aldosterone concentration according to McKenzie and Clements\textsuperscript{19} (sensitivity 6 ± 1 pg/ml).

Statistical Methods

Slopes of the regression lines obtained by plotting the doses of isoproterenol vs the corresponding values of increases in HR (ΔHR/IS) or of the increase in cAMP concentrations (ΔcAMP/IS) were always significant and therefore used for further analysis. The slopes obtained for the values before and after salt loading were then plotted against CD\textsubscript{25} and against the corresponding values of upright concentrations of epi-nephrine and norepinephrine.

Student's t test was used to compare pre- and post-salt loading mean values of the slopes of the regression lines found for 1) isoproterenol doses vs changes in heart rate (ΔHR/IS); and 2) isoproterenol doses vs changes in cAMP concentrations (ΔcAMP/IS). Values are given as mean ± 1 SEM.

Results

Patient Characteristics

Tables 1 and 2 show the characteristics of the patients and changes induced by the salt loading. No significant correlation was found between the values of plasma renin activity and those of both catecholamines, either supine or upright. However, the upright position induced significant increases in both norepinephrine and epinephrine plasma concentrations (table 2). The percent changes in plasma epinephrine concentration were correlated (r = 0.89, p < 0.001, n = 9) with those in PRA induced by upright position. Data from nine patients were analyzed since for the remaining two patients plasma catecholamines and cAMP were not assayed.

Testing of Beta-Adrenoceptor Sensitivity to Isoproterenol before Salt Loading

As expected, isoproterenol administration induced an increase in heart rate and plasma cAMP in all patients. The mean values of CD\textsubscript{25} were 7.59 ± 1.5 μg (fig. 1). A significant inverse correlation was found between CD\textsubscript{25} and upright PRA (r = 0.9452, p < 0.001, n = 11). Moreover, CD\textsubscript{25} was also correlated with basal heart rate (r = 0.7029, p < 0.05, n = 11) but not with age. There was also a significant correlation between the percent increase in PRA induced by orthostasis and the corresponding percent increases in...
epinephrine \( (r = 0.89, p < 0.001, n = 9) \) and norepinephrine \( (r = 0.83, p < 0.01, n = 9) \) plasma concentrations.

There were significant relationships between the changes in heart rate and plasma cAMP, and the increasing doses of isoproterenol. In particular, the mean slopes of the regression lines between heart rate and isoproterenol and cAMP and isoproterenol were \( 3.34 \pm 0.56 \) and \( 0.34 \pm 0.06 \), respectively. If the individual slopes between the changes in heart rate and the dose of isoproterenol administered were plotted against the CD25 values, a significant inverse correlation was found \( (r = 0.838, p < 0.01, n = 9) \).

Salt Loading

The effects of high sodium intake on blood pressure, heart rate, hematocrit, body weight, plasma catecholamines, plasma cAMP, plasma and urinary sodium and potassium levels, plasma renin activity and aldosterone, are shown in tables 1 and 2. Significant increases in diuresis, natriuresis, and body weight and decreases in heart rate and hematocrit were found. No significant changes in kaliuresis, plasma sodium and potassium levels, systolic and diastolic blood pressure, supine plasma catecholamines or cAMP concentrations were induced by the salt load. The mean plasma volume increased by \( 14.8\% \pm 5.3\% \). After salt load, neither epinephrine nor norepinephrine plasma concentrations were significantly modified by the upright position (table 2). However, salt loading potentiated the heart rate responses to standing. The mean percent increase in heart rate \( (29.7\% \pm 5.5\%) \) was significantly greater than that observed before salt loading \( (9.7\% \pm 3.2\%) \) \( (p < 0.01) \).

Testing of Beta-Adrenoceptor Sensitivity to Isoproterenol after Salt Loading

As shown in figure 1, salt loading induced a significant reduction in CD25 \( (\text{from } 7.6 \pm 1.5 \text{ to } 5.3 \pm 0.95, \mu g, p < 0.05, n = 11) \) and a significant increase in the mean slope of the regression lines obtained by plotting the isoproterenol doses vs the corresponding increase in heart rate \( (\Delta \text{cAMP/IS}) \) \( (\text{from } 0.3 \pm 0.06 \text{ to } 1.4 \pm 0.3, p < 0.05, n = 9) \). After salt loading, the relationship between the CD25 and the slope of \( \Delta \text{HR/IS} \) regression line was still statistically significant \( (r = 0.827, p < 0.01, n = 9) \). Moreover, a significant inverse relationship between the percent changes in CD25 and the corresponding changes in the slopes of the \( \Delta \text{HR/IS} \) regression lines was observed.

Finally, the percent changes in plasma epinephrine concentrations in the upright position induced by the salt loading were significantly correlated with the percent changes in CD25 \( (r = 0.904, p < 0.01, n = 9) \) (fig. 2) and with the percent changes in the slopes of \( \Delta \text{HR/IS} \) \( (r = 0.983, p < 0.001, n = 9) \) (fig. 3) and \( \Delta \text{cAMP/IS} \) \( (r = 0.922, p < 0.001, n = 9) \) (fig. 4) regression lines induced by salt loading. No such correlations were found with respect to norepinephrine.

**Discussion**

In a previous study we reported that the addition of 400 mEq/day of sodium chloride to a diet containing 80 mEq/day of sodium induced a significant enhancement of adrenergic beta-adrenoceptor responsiveness in hypertensive patients but not in normal subjects. Furthermore, the increased salt intake was able to abolish the difference in beta-adrenoceptor responsiveness between normal and hypertensive subjects present before the salt loading. Therefore, we decided to use this experimental approach to investigate the nature of the pressure-related impairment in beta-adrenoceptor sensitivity in essential hypertension.
Available evidence suggests that beta-adrenergic agonists elicit a physiological response from target tissues by a sequence of events that includes hormone binding to a cell surface receptor that is coupled to adenylate-cyclase. Then there is an increase in intra-cellular cAMP concentration, which, in turn, activates a protein kinase that phosphorylates regulatory proteins that ultimately regulate the physiological activity of the cell. Consequently, isoproterenol infusion induces an increase in cAMP release, which may be expected to be reduced in patients with depressed beta-adrenoceptor responsiveness. It should be noted that only a fraction of the cyclic nucleotides generated is released from the cell. Thus, their concentration in the extracellular fluids may be used to detect changes in their release from various tissues. Although this method provides only an indirect evaluation of cell metabolism and does not allow precise identification of the organ where cAMP is released, it permits the observation of rapid changes in vivo and is suitable to investigate physiological and pharmacological modulations (diet, posture and drugs) or correlations with other humoral factors.

In addition, to obtain a more integrated view of adrenoceptor-mediated cardiovascular function, we also assessed the chronotropic effect of isoproterenol administration and evaluated the relationship between cAMP production and HR response induced by beta-adrenoceptor stimulation. CD25 may be considered a reliable index of beta-adrenoceptor density since Fraser et al. have reported a direct correlation between these two parameters in humans. Measurement of CD25 does not take into account the basal HR, however, which is different before and after salt load. Therefore, we also performed a dose-response curve by plotting the increase in HR against the dose of isoproterenol administered. We believe that this kind of analysis gives a more complete evaluation of the changes in beta-adrenoceptor sensitivity induced by salt load.

In agreement with previous observations, the increase in dietary salt intake induced a significant improvement in beta-adrenoceptor responsiveness in our patients. In particular, there was a reduction in CD25 and an increase in the slopes of ΔHR/IS and ΔcAMP/IS regression lines. Furthermore, after salt loading there was also a sharper increase in HR induced by orthostasis. However, this phenomenon cannot be safely considered as a further evidence of improved beta-adrenoceptor responsiveness, since the high HR...
values present in our patients before salt load may account for the small percent increase in HR induced by orthostasis in this condition. The high HR values could be explained by the very young age of some patients of our hypertensive group. On the other hand, the possibility that our patients were "hyperdynamic" hypertensives can be ruled out by the fact that mean cardiac index, as assessed by echocardiography in the basal state, was 2.97 ± 0.11 liter/min/m²; this value may be considered similar to those previously reported in "nonhyperdynamic" hypertensives.25 26

On the other hand, the restriction of the study population to a young age group strengthens further the conclusion of the study. In fact, an age-relating change in beta-adrenoceptor sensitivity has been reported by previous authors.2-27 Therefore, to investigate the nature of the pressure related decrease in beta-adrenoceptor sensitivity, young patients are needed in order to minimize the possibility of an age-related decrease in beta-adrenoceptor sensitivity. Furthermore, the high values of HR, which probably reflect an elevated sympathetic tone, may account for the high values of CD25 observed in our patients. A significant relationship between CD25 and HR was found in our study group, while we failed to observe any age-related trend as to isoproterenol sensitivity. The close relationship between beta-adrenoceptor responsiveness, as evaluated by CD25, and sympathetic activity is confirmed by the existence of statistically significant correlation between CD25 and the percent increase in plasma renin activity induced by orthostasis. While different factors regulate the basal value of PRA, sympathetic tone is the major determinant of postural increase in PRA as also demonstrated by the significant correlation between PRA and plasma epinephrine and norepinephrine responses to orthostasis.

The reason for the impairment of beta-adrenoceptor responsiveness in hypertension is not clear. It has long been suspected11-13 that this phenomenon is due to a tachyphylaxis to endogenous catecholamines. Our results seem to confirm this view. After salt loading, decreased upright plasma epinephrine and norepinephrine levels were found, suggesting that the amount of circulating catecholamines in our salt-loaded patients was also reduced during the various stimuli (postural or of other nature) of normal everyday life. Thus, it appears possible that the observed improvement in beta-adrenoceptor responsiveness was causally related to a diminished release of epinephrine and norepinephrine from the peripheral sympathetic system. It should be also noted that this protocol does not allow us to rule out a direct effect of sodium per se on beta-adrenoceptor-mediated effects. However, the inverse relationship between the changes in beta-adrenoceptor sensitivity and those in the sympathetic tone reported by previous authors using different techniques2 seems to corroborate the hypothesis that the improvement in beta-adrenoceptor-mediated effects induced by salt loading in our patients was mediated through the reduction in sympathetic tone.

In addition, the state of sodium balance is known to influence the responsiveness to vasoactive substances such as angiotensin II and norepinephrine in experimental animals.28 29 However, an increase in the number, affinity, or type of sympathetic receptor sites, induced by the level of sodium balance, has not been clearly demonstrated.

Interestingly enough, we found a significant correlation between the reduction in upright plasma epinephrine levels and the changes in all the indices of beta-adrenoceptor responsiveness. The finding that only the changes in upright epinephrine concentration were correlated with the changes in beta-adrenoceptor sensitivity is in keeping with the observation that, before salt loading, only the upright values of plasma epinephrine were found to be abnormally elevated, while norepinephrine concentration did not differ from that of normotensive subjects. When the plasma catecholamine values of our hypertensives were compared with those of 10 normotensive men (mean age 25 ± 2 years, range, 16 to 32 years) previously studied for another purpose under similar experimental conditions (epinephrine: supine = 50 ± 6 pg/ml; 3-minute upright = 50 ± 7 pg/ml; norepinephrine: supine = 252 ± 25 pg/ml; 3-minute upright = 555 ± 88 pg/ml), only the upright epinephrine concentration was found to be significantly elevated (p < 0.05) in our hypertensive patients. Furthermore, other authors have previously found elevated plasma epinephrine2, 20-23 and normal norepinephrine concentrations in hypertensive patients.34-37

Elevated plasma epinephrine levels seem to express increased sympahtoadrenal activity (reactivity) in hypertension, and conceivably the excess of epinephrine may with time densensitize beta-adrenoceptors. In normal subjects, Fraser et al.5 found that the relationship between plasma epinephrine and leukocyte beta-receptor density was stronger than that based on norepinephrine concentrations, consistent with the greater affinity of epinephrine than norepinephrine for the beta-receptors. The observation that changes in plasma epinephrine concentrations correlate with changes in cardiac responsiveness to isoproterenol could indicate that in hypertensive patients, as in normotensive subjects, beta-adrenoceptor sensitivity is mainly controlled by circulating epinephrine. This observation lends further support to the hypothesis that the reduced beta-adrenoceptor responsiveness observed in hypertensive patients is the consequence of the sympathoadrenal over-activity present in these patients. There is biochemical evidence for desensitization at various sites in the beta-adrenergic receptor-adenylate cyclase system. In most tissues that have been investigated, tachyphylaxis to the effect of an adrenergic agonist has been accompanied by an unchanged affinity for the agonist, but with a decrease in the apparent number of beta-adrenergic receptors as assessed by the agonist binding.38 39 This desensitization to chronically elevated catecholamine levels might be attributed to structural changes, including endocytosis of the receptor complex, as suggested by Chang and Costa40 and as documented in other effector-receptor interaction.41
References


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Hypertension. 1983;5:584-590
doi: 10.1161/01.HYP.5.4.584

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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