Genetic Control of Neutralization of Angiotensin and Its Relationship to Essential Hypertension

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The tendency for the syndrome of essential hypertension to be familial has long been recognized.\(^1\)\(^2\) Early studies of such families suggest that the pattern of inheritance is one of a dominant character that somehow accounts for the presence of the elevated blood pressure. Some recent studies of population groups have added support to this concept.\(^3\)\(^4\) Contrariwise, other recent investigations have led to the conclusion that blood pressure, like individual height, is determined by a series of genes. Thus, a whole spectrum of blood pressures may be expected to appear in a population, and those observed in the upper extreme of this normal distribution are arbitrarily referred to as hypertension.\(^5\) Simply stated, is the syndrome of essential hypertension a distinct genetic and biochemical entity or merely an extreme of normal variation?\(^6\)

While the weight of evidence seems to favor the concept that hypertension is a distinct genetic entity, the biochemical abnormality that is brought about by this character and that leads to elevated blood pressure has not been suggested. A great deal of evidence is now available to indicate that the renin-angiotensin system is of fundamental importance in the causation of essential hypertension.\(^6\)\(^7\) When combined, these 2 general concepts imply that angiotensin is present in greater concentration in the circulation of the hypertensive subject as a result of gene action. The reason for the appearance of elevated blood pressure relatively late in life is obscure if it is conceived that the tendency to elaboration of excessive angiotensin is present from birth. Alternatively, perhaps the prehypertensive subject is characterized by an inability to destroy the vasoactive quality of angiotensin should it appear in the circulation. This character would have no associated infirmity and could be present from birth, yet would lead to elevated blood pressure if the concentration of angiotensin in the circulation gradually increased for nonspecific reasons in later years. It is the purpose of this paper to present evidence that this is the mode of genetic transmission of the syndrome of essential hypertension.

Methods

The method used was designed to test the ability of a subject to neutralize the vasopressor activity of angiotensin II with his peripheral blood in vitro. Angiotensin II was incubated with blood that was freshly drawn from a test subject; then the combination was reinfused to determine the effect on the subject's blood pressure.

One 0.5-mg. ampule of angiotensin II* was mixed in 20 ml. of saline the day of the experiment. The portion of this solution to be tested (15 \(\mu\)g. of angiotensin II in all experiments unless otherwise stated) was placed in a tuberculin syringe that was connected in turn to the side arm of a 3-way stopcock. The stopcock was placed on a 50-ml. siliconized syringe. The stopcock could then be connected to a needle that had been placed in the subject's antecubital vein. The syringe contained 3.0 mg. of heparin. About 5 ml. of blood were drawn into the syringe to assure good position of the needle. Then, the angiotensin II solution was introduced into the large syringe from the small one, via the stopcock. More blood was drawn into the mixture to a volume of 30 ml. Within 30 seconds from the time of introduction of the angiotensin II into the large syringe, the mixture was placed in a constant temperature water bath of 37 C. and agitated for exactly 10 minutes. The withdrawal and incubation procedure was arranged so that the blood was not exposed to air and was kept sterile throughout the procedure.

*Supplied as Hypertensin CIBA. All ampules were from a single lot.

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The blood-angiotensin mixture was infused into the donor from the same syringe, beginning exactly 15 seconds after the conclusion of the 10-minute incubation period. A constant speed infusion pump was used for this purpose. The syringe was connected to the previously established saline infusion at a point that was out of the subject’s sight. The plastic tube leading to the venous needle was covered with adhesive tape. This and other precautions made it impossible for the subject to observe the onset of the infusion of the blood-angiotensin mixture. The syringe was rotated at 1-minute intervals to prevent settling of the red cells.

Arterial blood pressure was measured by the auscultatory method. Brachial artery sounds were amplified as necessary in each subject. Generally, the disappearance of sounds rather than the change of sounds was taken as indicative of diastolic pressure level, except when the exact point of disappearance was not clear. In any event, the same end point was used throughout each experiment. The output of a strain gauge, connected to the blood pressure cuff, was displayed on an oscilloscopic screen to expand the scale of the diastolic pressure range.

All tests were performed with the subject resting in bed. The room was kept absolutely quiet, and many subjects dozed, once the experiment had begun. Although all of the subjects were told of the general plan to infuse the blood that had been withdrawn, the exact timing of the procedure was not explained. All of the details of the procedure were arranged so that the subject would not be alerted to a change of conditions.

The quantity of angiotensin II that was to be used in the experiments was selected by trial. Several concentrations were used to determine the largest amount of angiotensin II that usually would not elevate the subject’s blood pressure after incubation with 30 ml. of his blood when infused at a rate of 1.92 ml. per minute. Normotensive subjects (diastolic blood pressure less than 80 mm. Hg) who were over 40 years of age and had no family history suggestive of hypertension were used in these experiments. The blood-angiotensin mixtures that gave no rise in blood pressure were also infused at progressively faster rates. Thus, a small residuum of vasoactive angiotensin could be detected by a slight rise of blood pressure with very rapid infusion of the mixture.

The experiments described above led to the conclusion that 15 μg. of angiotensin II per 30 ml. of blood should be used for the definitive test procedure. This test was applied to patients with elevated blood pressure who were in the 40- to 60-year age range, had a strong family history of hypertension, and had no detectable cause of hypertension and, thus, were considered to be suffering from essential hypertension. Patients, aged 40 to 60 years, who were normotensive and who did not have a family history of hypertension were also studied. Finally, normotensive, healthy, first-year students at the Medical College of Georgia were subjected to this test.

The concentration of hemoglobin in the plasma of the blood-angiotensin mixture that remained in the intravenous tubing at the conclusion of the infusion was measured. The results of tests in which the concentration of hemoglobin exceeded 5 mg. per cent were discarded.

The mean of the diastolic blood pressures during the 10-minute period of incubation was subtracted from the mean of the diastolic blood pressures observed during minutes 3 through 9 of the infusion of the blood-angiotensin mixture. The value obtained was the rise in diastolic pressure that was induced by infusion of the blood-angiotensin mixture. The values reported were rounded off to the nearest preceding number.

Blood pressures of the parents of the first-year students were measured by fourth-year medical students. The fourth-year students, who did not know the results of the tests of the first-year students, visited these parents at their homes. They were preceded by a written explanation of the project. A detailed family history related to hypertension was obtained from each parent. Blood pressure was taken with the parent in the sitting position. It was taken repeatedly until it stabilized; then 5 successive readings were obtained. These 5 diastolic pressures were averaged. Data are reported in increments of 5 mm. Hg. Each level contains those values at that increment and those at the 4 succeeding 1 mm. Hg increments. Thus, the parents reported at the diastolic pressure level of 90 mm. Hg had a mean diastolic pressure of 90, 91, 92, 93, or 94 mm. Hg.

Results

Tests of the ability to neutralize the vasoconstrictor activity of angiotensin were performed on 94 of 100 members of the first-year class of the Medical College of Georgia. All of the subjects were normotensive. Their average (recumbent) resting diastolic blood pressures ranged from 60 to 78 mm. Hg. The age range of the group was 21 to 32 years. The blood pressures during the control periods in 2 of the subjects were too erratic to allow the test to be completed. The plasma hemoglobin concentration of the blood-angiotensin mixture exceeded 5 mg. per cent in 3 subjects.
Thus, there were 89 successfully completed tests, and the results are illustrated in figure 1. There was a bimodal distribution of response so that those students whose diastolic blood pressures increased by less than 10 mm. Hg during the infusion of the blood-angiotensin mixture are referred to hereafter as having had a negative test. Those students whose diastolic blood pressures rose 10 mm. Hg or more are referred to hereafter as having had a positive test.

The tests were repeated in 25 of the students, 16 of whom were classified as negative and 9 of whom were classified as positive after the first test. While there was some variation in blood pressure response between the 2 tests, the result as to positivity or negativity of the test was changed in only 1 instance.

Twenty-five of the students were subjected to infusions of 0.76 ml. per minute of 15 μg. angiotensin II in 30 ml. of saline. Twelve of these subjects had had positive tests, and 13 had had negative tests. The mean rise of diastolic blood pressure was 13 mm. Hg in the group with positive tests and 12 mm. Hg in the group with negative tests. The values were not significantly different.

The blood pressures of both parents of 72 of the students were obtained. Their ages ranged from 41 to 69 years. The studies of the remaining 17 families of the 89 first-year students that had been successfully tested were incomplete because of the death of 1 parent, 1 or both parents living out of the state, or illness of 1 parent. The average resting diastolic blood pressures of the parents in these 72 families are illustrated in figure 2. A bimodal distribution of results occurred. Those parents whose diastolic blood pressures were 90 mm. Hg or more are referred to as hypertensive; those whose diastolic blood pressures were less than 90 mm. Hg are referred to as normotensive. Some of the parents in the former group were receiving therapy for elevated blood pressure, but none of the parents in the latter group was receiving hypotensive agents.

The relationship between the results of the tests on the first-year medical students and the resting diastolic pressures of their parents are shown in table 1. If those parents in the entire group classified as hypertensive are considered to be the incidence of the phenotype in the parent population and if the gene frequency is calculated on the basis of a simple dominant gene (heterozygous or homozygous) resulting in a prehypertensive offspring, then it would be predicted that the 36 families consisting of 1 hypertensive and 1 normotensive parent would produce 20 prehypertensive offspring (positive tests) and 16 offspring who were not prehypertensive (negative tests).

The incidence of a family history suggestive of hypertension in either of the parents of the fathers or mothers of the first-year students with positive tests was 81 per cent (22 of 27). The incidence of such a history in the parents of fathers or mothers of the students with negative tests was 51 per cent (29 of 57). Fifteen students had normotensive parents and no history suggestive of hypertension in any of their grandparents. Fourteen of these students had negative tests, and 1 had a positive test.

Two families were studied in which both parents were clearly hypertensive (diastolic
blood pressure usually more than 95 mm. Hg). Reinfusion of the blood-angiotensin mixture resulted in rises of diastolic blood pressure of 11 and 13 mm. Hg in the parents of the first family. Diastolic blood pressure rose 12 mm. Hg in their son (21 years) but did not change during reinfusion of the blood-angiotensin mixture in either of their 2 daughters (18 and 23 years). Reinfusion of the blood-angiotensin mixture caused the diastolic blood pressure to rise 12 and 10 mm. Hg in the 2 parents of the second family. Their daughter (16 years) showed a rise of 12 mm. Hg with the test, while the son had an increase of diastolic pressure of only 4 mm. Hg with the test.

Fourteen patients who were considered to have essential hypertension were studied with the blood-angiotensin infusion test. Ten of these patients had a positive test, and 4 were negative by the criteria used for the student group. Eight of the 10 normotensive patients had negative tests.

Comment

Recent experiments of Tobian indicate that the renin-angiotensin system may have a role in the normal control of blood pressure that presumably is complementary in some way to sympathoadrenal control. The findings of Helmer and Judson are further evidence that an excess quantity of biologically active angiotensin is present in the circulation of patients with hypertension. These observations combine to imply that essential hypertension is somehow caused by an aberration of a system that ordinarily serves as a part of the homeostatic control of the level of blood pressure.

The results of several studies indicate that the syndrome of essential hypertension, and, thus, possibly the aberration of the renin-angiotensin system, is transmitted genetically. The question of the pattern of transmission has been somewhat controversial, especially recently. Results of older studies of familial patterns of hypertension led to the conclusion that it is transmitted as a dominant trait, but the more recent studies of Pickering seem to show that essential hypertension is not a distinct genetic entity. These studies have suggested that the level of blood pressure is controlled by a series of genes and that the individuals referred to as having essential hypertension are in reality in the upper portion of the normal range of blood pressures. Platt and his coworkers reached a different conclusion. They found that study of subjects in the 40- to 60-year age range (the approximate age range of parents reported here) does yield 2 populations with regard to blood pressure. The study of population groups which include subjects of all ages would tend to obscure the presence of the 2 groups of subjects in the middle age range since the prehypertensive individual would often appear to be normotensive until he reached the age of 40 and since hypertension not genetic in origin would appear in some subjects after the age of 60.

The available evidence implies then that essential hypertension is the result of an alteration in the normal function of the renin-angiotensin system and that this is brought
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about in some way by the action of a dominant gene. The question as to how this occurs is the next important consideration. The overall alternatives of too much angiotensin being produced versus too little being inactivated would seem to require investigation first. The studies reported here show that hypertensive subjects are not able to neutralize the vasoconstrictor activity of angiotensin with their peripheral blood in vitro so rapidly as normotensive subjects. The findings of Jablons indicate that this is also true of the cutaneous tissues in vivo. Finally, Mendelowitz et al. have found that hypertensives are not able to degrade radioactively tagged angiotensin with normal rapidity when it is introduced into the general circulation.

All these studies leave the general question as to whether or not this relative inability to inactivate angiotensin predated the onset of sustained hypertension or is merely a secondary result of the disease. This question becomes especially pertinent when one considers the additional finding of Jablons that intravenous infusion of angiotensin does impair the ability of a normotensive subject to neutralize the vasoconstrictor effects of angiotensin introduced into the skin.

The importance of studying the subject before he gets hypertension is clear. It is not possible to predict with accuracy whether or not a young individual will become hypertensive in later years. It is possible to predict, on the basis of past experience, that a proportion of the offspring of families with at least 1 hypertensive parent will be more prone to develop hypertension themselves. The results of the investigations here reported show that offspring in families with at least 1 parent who is hypertensive (as defined for purposes of this study) are more likely to have a relative inability to destroy angiotensin.

Bimodality of distribution of parents with regard to their resting diastolic blood pressures, bimodality of distribution of offspring with regard to their ability to neutralize angiotensin, and the clear relationship between these groups indicate a single factor genetic relationship between excessive diastolic blood pressure and the ability to neutralize angiotensin. The virtual absence of positive tests in the students from families with 2 normotensive parents, the virtual absence of positive tests in students with no evidence of hypertension in their parents or any of their grandparents, and the distribution of positive and negative tests in families with 1 hypertensive and 1 normotensive parent best fit the pattern of genetic transmission of a simple dominant gene. Application of the test procedure of reinfusion of blood-angiotensin mixtures into parents and offspring in families with 2 clinically hypertensive parents lent further support to this hypothesis. Even though both parents in these families were clearly hypertensive, their offspring were clearly positive or negative in their responses to the test. This finding seemed to support the concept that a dominant trait was or was not transmitted from these presumably heterozygous parents.

The question as to whether or not the homozygous state produces a more serious hypertension or is, in fact, the premalignant hypertensive state cannot be answered by these studies. If the homozygous state is more lethal, then there would have been fewer such subjects in the live parents studied here than would have been predicted. This would have the effect of reducing the number of students with positive tests in the offspring of families with 1 or with 2 hypertensive parents. In the series reported here, there were slightly fewer students with positive tests than would have been expected in these groups.

While it is possible that these tests are merely a reflection of differences in sensitivity to angiotensin, the failure of saline-angiotensin infusions to separate the 2 groups is against this possibility. The finding of Doyle that offspring of hypertensives are more reactive to small intra-arterial injections of angiotensin than normotensives may not be a sensitivity phenomenon but may be a reflection of the difference in the rate of neutralization of angiotensin by the blood in the prehypertensive subject.

These experiments shed no light on the
source of the neutralizing material or on the nature of this material. The finding of Fried-
man that plasma of ischemic kidneys will not inactivate angiotensin suggests the source.\(^{12}\)
While a peptidase is probably involved in the reaction, the modifications of concentrations
of the primary reactants, of their inhibitors, or of their activators possibly reflected by the
results of these tests remain to be studied.

The results of these and other experiments support the concept that so-called essential
hypertension is a distinct genetic entity, so that the designation hereditary hypertension
might be far more appropriate. While these results have no immediate clinical application,
with the possible exception of prognostic value, new approaches to the therapy of hyp-
ertension on the basis of this more fundamental concept of its etiology might well be
envisioned.

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