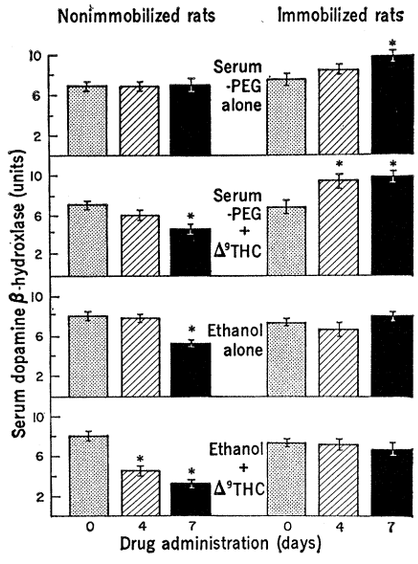

Fig. 1. Effects of Δ^9THC and ethanol on serum dopamine β-hydroxylase of naive (nonimmobilized) and immobilized rats. Animals were injected daily with Δ^9THC in an inert vehicle [a mixture of serum and polyethylene glycol (*PEG*)], Δ^9THC in ethanol, ethanol alone, or serum–polyethylene glycol alone. Blood samples for determining serum enzyme activity were drawn before injection of drugs and also 20 hours after the fourth and seventh injections. Immobilization was started 45 minutes after each injection and lasted 2 hours each day. Results are expressed as units of enzyme activity per milliliter of serum and are mean values (± standard error of mean) for six to eight rats. The asterisk indicates $P < .01$ compared with control.

ized rats were bled in identical fashion. Serum DBH activity was determined by a sensitive enzymatic assay (*5*), modified as described (*1*).

Results are summarized in Fig. 1. Serum DBH activity decreased significantly (by 36 to 40 percent) after the seventh treatment with Δ^9THC (in serum–polyethylene glycol) or with ethanol alone. Combined treatment for 4 days with Δ^9THC and ethanol potentiated the effects obtained when each drug was given alone, although after 7 days the effects of the drugs appeared to be additive. Serum–polyethylene glycol alone had no effect on serum DBH activity and thus served as an inert vehicle. Repeated immobilization for 7 days produced a significant elevation (30 percent) of serum DBH activity in animals treated with vehicle alone. The Δ^9THC (in vehicle) facilitated the immobilization-induced increase in serum DBH activity after 4 days of drug treatment. Ethanol, on the other hand, whether given alone or in combination with Δ^9THC, completely blocked the rise in serum DBH activity induced by repeated immobilization.

These studies show that Δ^9THC and ethanol significantly affect sympathetic nervous activity, as reflected by changes in serum DBH activity. The observed changes in serum DBH activity cannot be attributed to interference of the drugs with measurement of enzyme activity, since the environmental setting in which these drugs are given appears to influence not only the magnitude but also the direction of the response to the drugs. In naive animals, Δ^9THC appears to have a sympatholytic effect, whereas in animals subjected to immobilization stress, Δ^9THC appears to potentiate the sympathetic response to 4 days of repeated stress. The sympatholytic effect of Δ^9THC in naive rats appears also to be enhanced by the simultaneous administration of ethanol. This apparent synergistic interaction is of interest in view of the reported enhancement by alcohol of marijuana effects in humans (*6*). On the other hand, in rats subjected to immobilization stress, ethanol not only blocks the sympathetic response to the stress, but also effectively abolishes the effect of Δ^9THC in this setting. These observed differences in drug effects with differing environmental conditions suggest that Δ^9THC and ethanol exert their effects on the sympathetic nervous system through different mechanisms. The interaction of marijuana (and Δ^9THC) with other drugs is a subject of increasing social concern, and it is hoped that these studies will stimulate investigation in this area.

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Angiotensin-Sodium Interaction in Blood Pressure Maintenance of Renal Hypertensive and Normotensive Rats

Abstract. *A specific inhibitor of angiotensin II was used in rats to investigate whether angiotensin is involved in the maintenance of blood pressure in one-kidney Goldblatt hypertension, in which plasma renin levels are not usually increased. The inhibitor produced marked falls in blood pressure, often down to normal levels in the hypertensive animals only when they were depleted in sodium and not after sodium repletion. Much lesser but still significant falls in blood pressure were also produced in normotensive sodium-depleted rats but not in repleted rats. We conclude that the importance of angiotensin for maintaining blood pressure is largely determined by its relation to available sodium or fluid volume, since the renin component in maintenance of either the hypertensive or the normotensive state could be exposed only by sodium deprivation. Therefore, volume expansion per se or other pressor factors may be involved in maintaining blood pressure of these sodium-replete normotensive or hypertensive animals.*

Renin, an enzyme secreted by the kidneys, acts on a plasma globulin to release a decapeptide which is then converted by pulmonary and plasma enzymes to the octapeptide angiotensin II, the most powerful pressor substance known. In experimental renovascular hypertension, produced by clipping one renal artery with the contralateral kidney left untouched (two-kidney Goldblatt hypertension), increased renin levels have been demonstrated in both the acute and the established phases (*1–3*). In this model, administration of angiotensin II antibodies or a peptide inhibitor of angiotensin II, or of an inhibitor of the conversion of angiotensin I to angiotensin II, all have produced marked falls of blood pressure, which indicates a causal role for renin (*4, 5*).

In contrast, in chronic renal hyper-

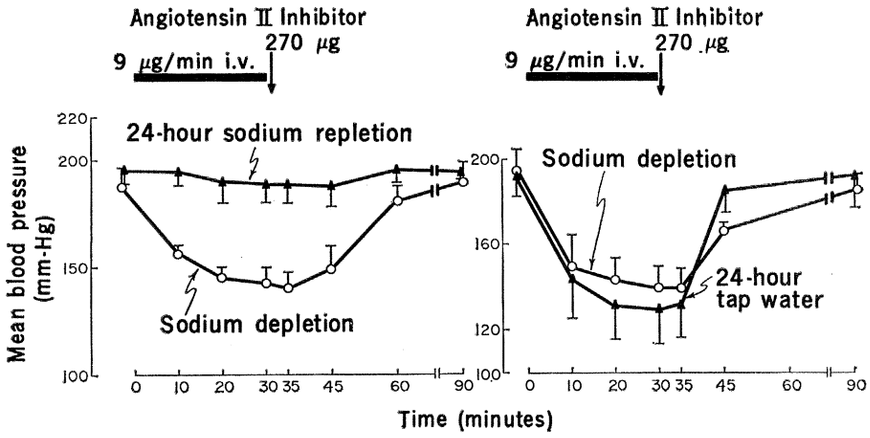

Fig. 1. (Left) In sodium-depleted, one-kidney hypertensive animals (group 1), angiotensin inhibitor produced striking falls in blood pressure; whereas 24 hours after sodium repletion decrements in blood pressure were insignificant. (Right) In similar animals (group 2) the inhibitor produced a marked fall in blood pressure during the first phase of the experiment, and again 24 hours later without sodium repletion.

tension produced by clipping one renal artery and removing the contralateral kidney (one-kidney Goldblatt hypertension), plasma renin and angiotensin II levels are usually normal (*2, 6, 7*). Moreover, administration of angiotensin II antibodies or inhibitor produced no significant fall of blood pressure in the established form (*4, 5*), although it did in its early phase (*8*). Since relatively higher total body sodium has been described in the one-kidney model (*9*), it would appear that hypervolemia is involved in this hypertension with angiotensin having no demonstrable role.

In order to investigate further the possible role of angiotensin in one-kidney Goldblatt hypertension, the present experiment was designed to evaluate the effects of an angiotensin II inhibitor in one-kidney Goldblatt hypertension under different conditions of sodium balance.

Male Wistar rats weighing 140 to 150 g, group 1 ($N = 7$) and group 2 ($N = 5$), had a silver clip placed on the left renal artery, and a right nephrectomy was performed. Group 3 ($N = 6$) underwent a right nephrectomy only and group 4 ($N = 6$) were normal unoperated animals. All four groups were fed a low-sodium diet (*10*) for 4 weeks prior to the study, with free access to food and water.

On the day of the study, the animals were anesthetized with ether; the femoral vein was cannulated with a PE 10 catheter and the external iliac artery with a PE 50 catheter. Arterial pressure was monitored with a Sanborn pressure transducer. Upon awakening, the animals were maintained in a semirestrained position. After a 30-minute control period, the pressure response to 100 ng of angiotensin II given intravenously was determined. Subsequently, [sarcosine^1-Ala^8]angiotensin II, a highly specific competitive inhibitor of angiotensin II (*11*) was infused at a rate of 9 μg/min for 30 minutes. Thereafter, 270 μg of the inhibitor were infused rapidly in 3 minutes. Immediately afterward, the blocking effect of the inhibitor was evaluated by a rapid injection of 200 ng of angiotensin II. When the first phase of the study was completed all animals were maintained in the semirestrained position for 24 hours, during which they had free access to fluid and food. Groups 1, 3, and 4 received food containing salt and 0.9 percent saline to drink. Animals of group 2 were maintained on the low-sodium diet and tap water. During the 24-hour interim period, venous and arterial catheters were kept patent with slow infusion of 2 ml of isotonic fluid through each catheter (saline for groups 1, 3, and 4; dextrose for group 2). After this 24-hour period, the same procedure was reapplied to all animals.

In the sodium-depleted, one-kidney hypertensive animals (group 1), the angiotensin II inhibitor produced an immediate and progressive fall in blood pressure of 32.4 ± 4.1 (mean ± standard error) mm-Hg after 10 minutes and a maximum fall of 47.6 ± 4.8 mm-Hg after 30 minutes ($P < .01$). The additional bolus of inhibitor did not further decrease the blood pressure. However, after 24 hours of salt repletion, during which the animals drank 148 ± 33.2 ml of saline and their weight increased by 18.2 ± 5.3 g, infusion of the inhibitor produced no significant change in blood pressure. The maximum observed decrement in blood pressure was 9.3 ± 3.5 mm-Hg after 35 minutes (Fig. 1, left).

In group 2, also sodium-depleted one-kidney hypertensive animals, the angiotensin inhibitor produced a similar fall in pressure of 53.0 ± 5.2 mm-Hg after 30 minutes ($P < .01$). Then, dur-

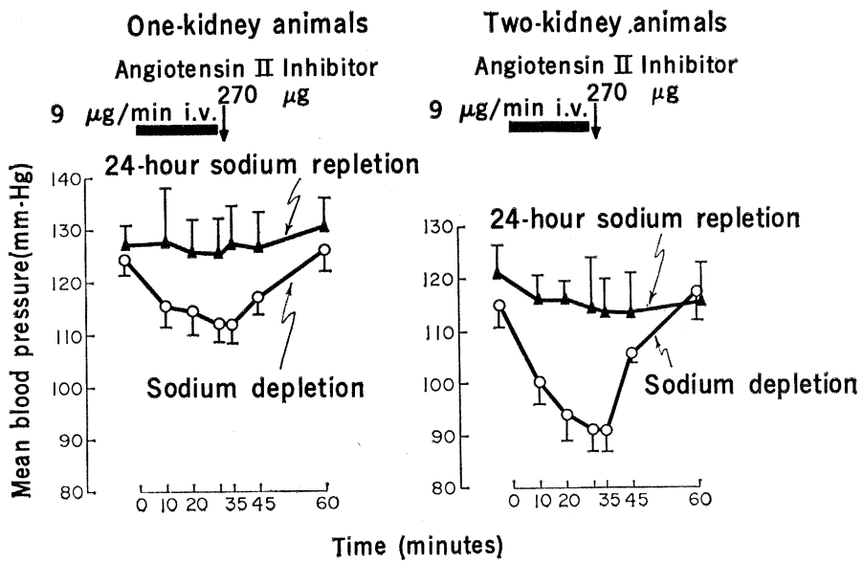

Fig. 2. (Left) Infusion of angiotensin inhibitor significantly lowered blood pressure in uninephrectomized, normotensive sodium-depleted animals (group 3). After 24 hours of sodium repletion this hypotensive response was abolished. (Right) Normal sodium-depleted animals (group 4) also exhibited significant hypotensive responses to the infusion of the inhibitor, which again were abolished by sodium repletion for 24 hours.

ing 24 hours on low-salt diet and tap water, the animals drank 17.6 ± 8.8 ml of tap water and their weight increased by 2.0 ± 4.9 g. In contrast to the pressure response observed in group 1 animals, after sodium depletion in the group 2 rats the repeat infusion of angiotensin inhibitor still lowered the blood pressure—this time by 63.8 ± 7.1 mm-Hg at 30 minutes ($P < .01$) (Fig. 1, right). In both groups arterial pressure returned within 90 minutes to preinfusion levels.

In group 3, uninephrectomized normotensive rats (Fig. 2, left), the initial infusion of angiotensin inhibitor produced a maximum fall in blood pressure of 13.3 ± 1.6 mm-Hg after 30 minutes ($P < .01$). Twenty-four hours later, after ingesting 96.0 ± 20.1 ml of saline, their body weight had increased by 8.6 ± 4.6 g. During the second phase the maximum fall in blood pressure was only 1.0 ± 1.9 mm-Hg ($N = 4$). In group 4, sodium-depleted normal animals (Fig. 2, right), the first infusion reduced the blood pressure by 25.0 ± 2.6 mm-Hg at 35 minutes ($P < .01$). Twenty-four hours later, after ingesting 136.0 ± 7.0 ml of saline and with an increase in body weight of 10.7 ± 8.4 g, the blood pressure fell insignificantly after infusion ($N = 4$). In all groups the test dose of angiotensin II given immediately after infusion of the inhibitor in both experiments had no pressor effect.

Brunner and associates (*4*) have presented evidence for two different mechanisms in experimental renovascular hypertension. In animals allowed free access to dietary sodium, the infusion of either a peptide inhibitor or antibodies to angiotensin II induced a marked fall of blood pressure in two-kidney animals with Goldblatt hypertension, while neither procedure affected the blood pressure in the one-kidney Goldblatt model. These observations are in keeping with a number of other reports implicating renin only in the two-kidney form of renovascular hypertension. Collectively, these reports have tended to exclude a role for abnormal renin secretion in the maintenance of hypertension in the one-kidney animals (*2, 5, 12*).

In contrast, other investigators (*13–15*) using active immunization have suggested that angiotensin II is not involved in pathogenesis of either one- or two-kidney animals with Goldblatt hypertension. However, the failure to lower blood pressure in these experiments could be due to an overriding of the antibody by endogenous release of renin induced by angiotensin feedback inhibition.

In our present experiments high blood pressure was induced in one-kidney animals and sustained despite a low-sodium diet for 4 weeks. Infusion of the angiotensin inhibitor in these animals induced marked falls of blood pressure to normal or near-normal levels. After sodium repletion, the renin dependency of the hypertension was abolished. This suggests that sodium depletion or associated volume shrinkage has unmasked abnormal or inappropriate renin secretion as a key factor in maintaining the hypertension in the one-kidney model during sodium depletion, even though the levels of renin measured under random or non-depleted conditions have been reported to be normal in this model (*2, 6, 7*).

Accordingly, under appropriate circumstances Goldblatt hypertension in both types of animals (one-kidney and two-kidney) can be shown to be renin-dependent. In this context, the seemingly normal renin levels reported in the one-kidney model could in fact be inappropriately high for the state of volume expansion. Viewed in this way, both types of renal hypertension can be explained in terms of an abnormal interaction of sodium balance with renin levels, with the two types exhibiting only quantitative differences in their deranged relationship of sodium to renin. The insignificant changes in blood pressure produced by the inhibitor in the salt-repleted hypertensive animals are not against this concept, and they may be considered to represent the other extreme of a two-component blood pressure maintaining system in which, after sodium repletion, the hypertensive state is converted from a largely renin-dependent to a largely or entirely volume-dependent condition. It is likely that between these two extremes of sodium balance, variable influences of the renin and sodium components together summate to sustain the hypertension. According to this concept, one might a priori expect a fall in blood pressure with angiotensin blockade even in sodium-repleted one-kidney animals with Goldblatt hypertension. The fact that no significant effect of the angiotensin inhibitor in lowering blood pressure was observed in that situation may be attributed to several factors, that is, over-expansion of volume by the maximum sodium loading, or inability of the inhibitor to reveal renin dependency unless relatively extreme conditions of such dependency are present.

In the normotensive uninephrectomized animals of group 3 and the normotensive intact animals of group 4, the angiotensin inhibitor produced significant but less striking falls in blood pressure after sodium depletion, while again, sodium repletion abolished any hypotensive effect. These observations complement those recently described in dogs, in which it was found that not only the blood pressure but also the aldosterone secretion was reduced (*16*). Taken in conjunction with studies of the hypertensive animals, these findings provide considerable additional support for the concept that the blood pressure of both renal hypertensive and normal subjects is determined by the summation of the interaction between angiotensin levels and the state of sodium balance (*17, 18*).

These results may provide a better basis for understanding high blood pressure in a whole variety of human disorders and particularly those due to chronic renal diseases. In such patients who exhibit either normal or low levels of plasma resin, the hypertension is probably largely on a sodium and volume basis and may be corrected by sodium depletion. Other patients in this category with high levels of plasma renin have a large renin component and in extreme instances may require bilateral nephrectomy for correction. In between these two extremes, hypertension results from an abnormal interaction between the renin and volume factors.

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Alcohol-Induced Hyperlipidemia and Beta Lipoproteins

Abstract. *Alcohol addicts with a primary type IV hyperlipoproteinemia show a striking elevation of triglycerides in the serum during long periods of alcohol consumption as compared with controls, without an accompanying significant increase in free fatty acids in the serum. These data suggest that this genetically related lipid abnormality may be a significant factor in the pathogenesis of alcohol hyperlipemia and the alcohol-induced fatty liver.*

Although there is a high correlation between chronic alcohol abuse and cirrhosis of the liver, not all alcoholics develop this form of hepatic disease. The specific factors that predispose certain individuals to the development of the alcohol-induced fatty liver, acute hepatic necrosis, or Lannec's cirrhosis, associated with chronic drinking, are as yet undetermined and have puzzled biomedical scientists for more than a century (*1, 2*). This report describes for the first time the role of a genetically related derangement in lipid metabolism which may be a significant factor in the pathogenesis of hyperlipemia in alcohol addicts. The impetus for our studies was provided by the comprehensive investigations of fat transport and lipoproteins carried out by Frederickson and his associates (*3*). A possible relationship between the expression of a type IV hyperlipoproteinemia and excessive alcohol intake, as well as emotional stress and carbohydrate metabolism alterations, was also suggested by the studies of Frederickson and his associates (*3*).

Although the exact incidence of type IV primary hyperlipoproteinemia is unknown, the presence of this disorder in the general population is quite common. Genetically determined primary type IV hyperlipoproteinemia is expressed as a dominant trait and is often observed in 50 percent of adult relatives of those who have the genetic abnormality.

Primary type IV hyperlipoproteinemia has also been implicated in the pathophysiology of pancreatitis and premature atheromatosis.

The mechanisms by which alterations in lipid transport and metabolism produce derangements of hepatic function are unknown. However, chronic alcohol intake in alcoholics has been associated with reciprocal elevations in serum triglycerides and free fatty acids (*4*). During experimentally induced intoxication, there was an initial fall in free fatty acids and an elevation in serum triglycerides. As drinking continued and blood alcohol concentrations increased, serum triglycerides fell with a concomitant elevation in free fatty acids (*4*). However, two limitations affect the interpretation of these several studies: (i) lipoprotein profiles of subjects were not determined and (ii) alcohol was administered on a programmed dosage basis along with an adequate diet. Although programmed alcohol administration represents a good clinical research design, it does not simulate the patterns of real life drinking accompanied by the marginal dietary intake observed in chronic alcohol addicts and therefore may limit the generality of associated biological changes (*5*).

The major purpose of our study was to determine whether there is any relation between primary lipoprotein abnormalities and ethanol-induced alterations in serum lipids. Concentrations of lipids and lipoprotein profiles in serum were determined in alcoholic males prior to, during, and after free-choice alcohol consumption (a pattern of drinking analogous to the manner in which such individuals drink in real life). Although these subjects had access to an adequate diet, on the basis of previous clinical findings, it was anticipated that they would tend to decrease caloric intake during prolonged drinking periods (*5*).

Thirteen male alcohol addicts between the ages of 28 and 51 were admitted to the clinical research ward of the National Institute of Alcohol Abuse and Alcoholism and informed consent was obtained from each subject. All subjects were in good health and showed no evidence of any medical (including hepatic and pancreatic) or major psychiatric disorder as determined by appropriate clinical and laboratory examinations. Subjects had a 3- to 33-year history of alcoholism (as defined by tolerance and physical dependence). The history of recent alcohol intake and the duration of abstinence was comparable for all subjects.

After the subjects completed a period of acclimation to the research ward, a minimum of three consecutive daily blood samples were obtained and used for assay of serum lipids and lipoproteins. All samples in our study were collected from fasted (8 hours) subjects. During the remainder of the study, blood samples were obtained each morning for serum lipid and lipoprotein determinations (*6–8*). Triglycerides were determined by the method of Noble and Campbell (*6*). Cholesterol was determined by the procedure of Zondag and Van Boelzeler (*7*). Free fatty acids were determined by a method described by Dole (*8*). Profiles of pre-beta lipoprotein (these proteins migrate in front of beta lipoproteins on paper electrophoresis and lag behind beta lipoproteins on acrylamide gel electrophoreses) were determined with a disc-gel technique described by Frings and his associates (*9*). Reagent kits (Q.D.L., Canalco Diagnostic Products, Rockville, Maryland) were used for these determinations. In any instance where disc gel electrophoresis data were not clear, confirmation of serum lipoprotein phenotyping was carried out by analytical ultracentrifugation.

After a baseline phase of 7 to 9 days during which the subjects did not consume any alcohol, an 11- or 12-day period of spontaneous alcohol intake was instituted; this period was followed by a 7- to 10-day withdrawal period. Subjects could consume up to 32 ounces of 100-proof beverage alcohol daily. Each morning subjects were given 32 tokens to buy alcohol from an automatic dispensing apparatus; 1 ounce of alcohol was dispensed for each token spent. Blood alcohol concentrations were determined every 4 hours with a breathalyzer device. Subjects could drink at any time, but were encouraged to sleep during the hours of 12:30 and 7:30 a.m. to permit electroencephalogram recording of sleep patterns. Although all subjects reduced caloric intake from food when drinking, there