Table 2. Concentrations of certain free amino acids in tobacco leaves. Concentrations are in nanomoles per gram, fresh weight. Fully expanded young leaves with veins removed were homogenized at room temperature. An equal volume of 10 percent trichloroacetic acid was added to the homogenate and then centrifuged. The acid-soluble supernatant was run on an amino acid analyzer. Each value was calculated from three replicates. Level of glycine, alanine, and proline are included to demonstrate that the increases in methionine in mutants 2 and 3 are specific to that amino acid.

Tobacco	Methionine	Glycine	Alanine	Proline
Havana Wisconsin 38	0.4 ± 0.2	1.3 ± 0.3	1.8 ± 0.3	0.3 ± 0.1
Mutant 1	.3 ± .2	1.4 ± .3	1.7 ± .5	.4 ± .2
Mutant 2	1.9 ± .5	1.7 ± .5	2.0 ± .4	.5 ± .2
Mutant 3	2.4 ± .6	1.2 ± .2	1.5 ± .3	.4 ± .2

wild-type variety from which they were selected. The naturally occurring resistance of Burley 21 is superior to that of the mutants. The failure to observe chlorosis in the mutant plants is due primarily to resistance to the action of the toxin, for neither bacterial multiplication nor toxin appearance is inhibited in the mutants.

The level of free amino acids in young, fully expanded leaves of wild-type and mutant tobacco is presented in Table 2. Mutants 2 and 3 both show significant specific increases in the level of free methionine. Free methionine levels are also increased in callus cultures and stem and root tissue in these mutants. The methionine content of the total proteins of these tissues is not significantly increased.

Even though MSO and the toxin produced by *P. tabaci* resemble methionine structurally, current evidence suggests that they may interfere with the enzyme glutamine synthetase rather than directly with methionine metabolism (*7*). However, levels of glutamine are not significantly altered in the leaf tissue of the mutant plants. Elevated intracellular levels of methionine may possibly protect against MSO uptake (*8*).

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Δ^9-Tetrahydrocannabinol and Ethanol: Differential Effects on Sympathetic Activity in Differing Environmental Setting

Abstract. *Serum dopamine β-hydroxylase activity, a useful biochemical index of peripheral sympathetic nervous activity, was measured in rats treated with Δ^9-tetrahydrocannabinol or ethanol or both substances. After 7 days of treatment with either substance, serum dopamine β-hydroxylase activity decreased significantly. Combined treatment with both agents enhanced the effects of each given alone. In rats subjected to immobilization stress, treatment with Δ^9-tetrahydrocannabinol appeared to potentiate the stress-induced increase in serum enzyme activity. Treatment with ethanol, with or without Δ^9-tetrahydrocannabinol, effectively blocked this increase in enzyme activity. These results show that both substances have significant effects on the sympathetic nervous system which are critically influenced by environmental setting.*

The social use of marijuana has been guided more by folklore than by scientific knowledge. Street lore suggests that the effects of marijuana can be enhanced by the simultaneous use of alcohol (especially in the form of sweet wines). While this practice may seem well based from what we know of the pharmacological effects of these agents, little biochemical evidence for their interaction is available. The synthesis of Δ^9-tetrahydrocannabinol (Δ^9THC), believed to be the major active substance in marijuana, has permitted a more systematic study of the interactions of Δ^9THC with other drugs. We report the results of one such study. Using a simple biochemical marker, dopamine β-hydroxylase (DBH) in serum, we found that Δ^9THC and ethanol have profound effects on the sympathetic nervous system and that these effects (and their interactions) are critically influenced by the environmental setting in which the drugs are given.

Dopamine β-hydroxylase catalyzes the formation of the neurotransmitter noradrenaline from dopamine. The enzyme is present in the serum of man and other mammalian species (*1*). Serum DBH arises mainly from sympathetic nerve terminals (*2*) and is elevated by stress of various types (*3*). The activity of this enzyme is therefore a useful biochemical index of the activity of the sympathetic nervous system and provides a convenient means to study the effects of drugs and their interactions on sympathetic nervous activity. We investigated the effects of Δ^9THC and ethanol on sympathetic nervous activity of normal rats and rats subjected to repeated immobilization, a procedure used as an experimental model of stress in animals (*4*).

Sprague-Dawley rats weighing about 200 g were divided into four groups. One group (12 rats) received Δ^9THC (20 mg per kilogram of body weight) in combination with ethanol (400 mg/kg); another group (16 rats) received Δ^9THC (20 mg/kg) in a mixture of homologous serum and polyethylene glycol (3 : 1, by volume); and two groups (12 rats each) received either ethanol (400 mg/kg) or serum–polyethylene glycol mixture alone. Half the rats in each group were immobilized with a specially designed restraining device (*4*) for 2 hours daily for seven consecutive days. The remaining rats in each group served as nonimmobilized controls. The Δ^9THC was either dissolved in ethanol or suspended in serum–polyethylene glycol. All animals were injected daily (100 μl of drug or vehicle subcutaneously) at the same time, and immobilization was started 45 minutes after the injection. Baseline blood samples were drawn from the tail vein in all animals before the experiment was begun, and samples were also taken 20 hours after the fourth and seventh immobilization periods. Immobilized and nonimmobil-

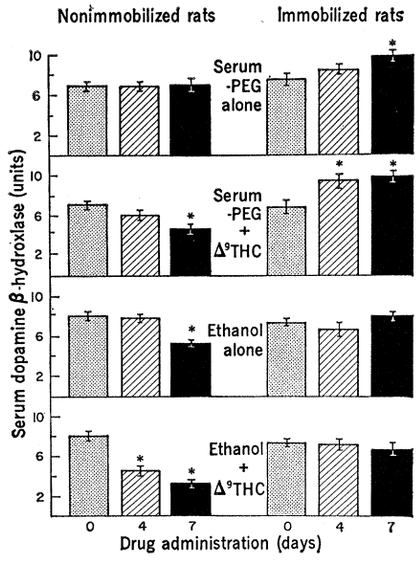

Fig. 1. Effects of Δ^9THC and ethanol on serum dopamine β-hydroxylase of naive (nonimmobilized) and immobilized rats. Animals were injected daily with Δ^9THC in an inert vehicle [a mixture of serum and polyethylene glycol (*PEG*)], Δ^9THC in ethanol, ethanol alone, or serum–polyethylene glycol alone. 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-