contained a water bottle but provided no access to alcohol. One hour after the animals were placed in them, all observation cages were placed on a large table and keys were shaken above the cages for 5 minutes. No unusual behavior, other than curiosity, was observed. No seizures nor partial seizures were observed.

Three hours after removal from the polydipsia regime, the same procedure was repeated. At the sound of the shaking keys, rats 4, 5, and 6 leaped out of their observation cages and ran about on the laboratory floor. However, no convulsions or tonic-clonic seizures occurred. After the key shaking, all rats were given the amount of pellets that would have been dispensed in the experimental situation during an equivalent time period.

Seven hours after removal, the same procedure was repeated once more. Rats 4, 5, and 6 duplicated their previous behavior; again, however, there were no convulsions or seizures, or any other indication of withdrawal symptoms.

Immediately following this phase of the experiment, all rats were placed in home cages with access to both water and 5 percent ethanol. With unlimited food supply and free access to water, the animals failed to maintain their previous level of ethanol consumption (Fig. 1). Average ethanol intake during this period was only 5.46 g/kg.

Replication of the Falk *et al.* procedure (*4*) did not replicate the previously reported results. Falk *et al.* interpreted their findings as being unequivocal evidence of ethanol dependence in rats by virtue of convulsions following withdrawal of the animals from alcohol. We found no indication of such behavior after identical subject treatment.

The shaking of keys after the removal of alcohol was reported by Falk *et al.* (*4*) to have triggered convulsive seizures that resulted in death for two animals; they exposed only three of the seven rats to the key-shaking stimulus. It seems questionable whether audiogenic seizures are indicative of alcohol dependence under these circumstances, and our failure to observe even these symptoms raises some question as to the reliability of this polydipsia procedure in the production of alcohol dependency in the rat. We believe that the convulsive behavior reported by Falk *et al.* may have been a result of the rats' inherent proneness to seizures. The animals' "natural" seizure susceptibility was not tested by Falk *et al.*; therefore some seizure-prone animals might have been included in the sample. We further believe, as do others (*5, 6*), that an appropriate criterion of an animal's dependence should include voluntary ethanol consumption maintained presumably to avoid withdrawal symptoms. This aspect of alcohol dependence was not examined by Falk *et al.*

In the present study, when rats were given free access to alcohol after a period of withdrawal, they failed to maintain their previous level of consumption. This can only suggest that these rats were not dependent on alcohol.

The incidence of death associated with seizures reported by Falk *et al.* (*4*) can possibly be explained in terms of the Selye general adaptation syndrome (*7*). In the last stage of this syndrome (exhaustion), the organism has theoretically consumed its internal resources for dealing with continued stress and further stress will often result in death. If we assume that Falk's sample of animals contained some rats naturally susceptible to audiogenic seizures, it is possible that the induction of seizures may have provided additional stress conditions which, when summed with physiological stress resulting from chronic high alcohol consumption, may have pressed the animals beyond the point of general adaptation exhaustion and, hence, resulted in death. It is possible, therefore, that the deaths observed by Falk *et al.* were not the result of withdrawal symptoms at all but were caused by a combination of stressors, chronic high-level ethanol consumption coupled with induced seizures.

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Adrenaline-Forming Enzyme in Brainstem: Elevation in Genetic and Experimental Hypertension

Abstract. *The adrenaline-forming enzyme (phenylethanolamine* N-*methyltransferase) was elevated in the* A_1 *and* A_2 *regions of the brainstem of 4-week-old spontaneously (genetic) hypertensive rats and in the* A_1 *region of adult experimentally (deoxycorticosterone acetate and sodium chloride) hypertensive rats. The administration of a phenylethanolamine* N-*methyltransferase inhibitor to experimentally hypertensive animals caused a reduction of the elevated blood pressure to normal values. These results implicate adrenaline-containing neurons in the brainstem in the development of hypertension.*

It has been recognized that peripheral and central noradrenergic nerves play a role in the regulation of blood pressure and in the expression of some forms of hypertension (*1*). Recent work has demonstrated the presence of other catecholamine-containing neurons (adrenaline) in the brain. The adrenaline-forming enzyme, phenylethanolamine *N*-methyltransferase (PNMT) (*2*), has been detected in certain areas of the brain by immunohistofluorescent techniques with the use of antibodies directed against bovine adrenal PNMT (*3*), as well as by the direct measurement of the enzyme (*4*). Adrenaline has also been detected in the same regions (*5*). Nerve tracts and cells containing PNMT were found to be highly localized in the A_1 and A_2 areas of the brainstem.

Table 1. Activity of phenylethanolamine *N*-methyltransferase (PNMT) in specific regions of the brainstem of spontaneously (genetic) hypertensive rats (SHR). Results are expressed as mean ± standard error of the mean (S.E.M.) of groups of 14 animals. Brain regions from individual rats were dissected and analyzed separately as described in the text.

Region	PNMT activity (picomoles per milligram of protein per hour)	
	Controls	SHR
A_1	23.2 ± 5.9	38.6 ± 3.5*
A_2	42.4 ± 6.0	65.8 ± 5.4**
Locus coeruleus	7.2 ± 1.3	6.4 ± 1.7

*Statistically significant $P < .05$ (Student's *t*-test) against control values. **Statistically significant $P < .01$ (Student's *t*-test) against control values.

The A_1 area of the rat brainstem contains the cell bodies of the catecholaminergic neurons that send their axons to the spinal cord (*6*). The A_2 area corresponds in part to the nucleus of the solitary tract in which the majority of the fibers of the carotid sinus nerve terminate (*6, 7*). It is densely supplied with noradren-

Table 2. Phenylethanolamine *N*-methyltransferase (PNMT) activity in discrete brain regions of Doca-salt hypertensive rats. Results are expressed as mean ± S.E.M. Numbers in parentheses indicate the number of animals analyzed for each group.

Region	PNMT activity (picomoles per milligram of protein per hour)			
	Control	Doca-salt	Control + SKF 7698	Doca-salt + SKF 7698
A_1	40.9 ± 4.5 (17)	89.9 ± 13.3 (18)*	30.6 ± 0.7 (5)**	21.1 ± 2.0 (5)*
A_2	118.9 ± 2.8 (18)	118.9 ± 10.3 (18)	80.2 ± 3.2 (5)**	72.8 ± 14.6 (5)*
Locus coeruleus	17.7 ± 0.9 (5)	25.2 ± 4.4 (5)	15.5 ± 0.3 (5)	26.4 ± 7.7 (5)

*Statistically significant $P < .01$ (Student's t-test) against the control group receiving no drugs. **Statistically significant $P < .02$ (Student's t-test) against the control group receiving no drugs.

ergic nerve terminals and also contains catecholaminergic cell bodies (*6*). These two areas contain the highest amounts of PNMT in the brain (*3, 4*) and are considered to be involved in blood pressure regulation (*7*).

This prompted us to investigate the changes in PNMT in specific nuclei of the brain in spontaneously (genetic) hypertensive rats (*8*) and unilaterally nephrectomized rats, made hypertensive by the administration of deoxycorticosterone acetate and sodium chloride (Doca-salt hypertensive rats) (*9, 10*). We wish to report that there is a marked elevation of PNMT activity in specific areas of the brainstem in both these forms of hypertension. In addition, we have found that the administration of a PNMT inhibitor reduces the blood pressure as well as brain PNMT activity in hypertensive animals.

Male spontaneously hypertensive rats (SHR) and normotensive rats of the Wistar-Kyoto substrain, from which SHR rats were derived, were raised under identical conditions for 1 week after being obtained from Taconic Farms, Germantown, New York. Doca-salt hypertension was produced as described earlier (*10*). Rats were killed by decapitation at 9:00 a.m. and brain areas were dissected by the punch technique as previously described (*11*). The PNMT was assayed as described previously (*4*). It has been shown that hypertension is not fully developed in the SHR rat at 3 to 4 weeks of age, although a marked elevation of sympathetic nerve activity can be demonstrated at this age by increased amounts of dopamine β-hydroxylase (an enzyme highly localized in sympathetic nerves) in blood. Blood levels of dopamine β-hydroxylase are reduced to normal values in adult animals when the hypertension is fully developed (*12*). The PNMT activity was therefore examined in the A_1 and A_2 regions, and the locus coeruleus (an area of the brain where the cell bodies of noradrenergic neurons are localized) (*6*) of SHR and Wistar-Kyoto rats at 4 weeks of age. There was a marked elevation (60 percent) of PNMT activity in the A_1 and A_2 regions, but not in the locus coeruleus of 4-week-old SHR rats as compared to their controls (Table 1). The biochemical mechanism for the increase in PNMT activity has not been examined.

The examination of PNMT activity in adult Doca-salt hypertensive rats showed more than a doubling of enzyme activity in the A_1 region of the brainstem (Table 2). There was no significant change in enzyme activity in the A_2 region or locus coeruleus. No significant changes in noradrenaline and dopamine concentrations and tyrosine hydroxylase activity were found in the A_1 and A_2 regions in the two forms of hypertension studied.

We have previously found that tranylcypromine (*trans*-D,L-2-phenylcyclopropylamine sulfate) and related compounds inhibit the in vitro activity of PNMT (*13*). Recently Pendleton *et al.* (*14*) have described a compound (SKF 7698) that also inhibits PNMT in vivo. In view of the marked elevation of PNMT activity in specific areas of the brainstem in hypertension, we examined the effects of an in vivo PNMT inhibitor (SKF 7698) (*14*) on PNMT activity in the brain and the blood pressure in Doca-salt hypertensive rats.

The administration of SKF 7698 reduced PNMT activity to below normal levels in the A_1 and A_2 regions but not in the locus coeruleus. Furthermore, the administration of the PNMT inhibitor reduced the blood pressure in Doca-salt hypertensive rats (150 to 180 mm-Hg) to normal levels (110 to 120 mm-Hg). The PNMT activity was also reduced in control animals but the blood pressure remained within the range of normal levels.

Our results are compatible with the earlier suggestions (*1*) of a possible involvement of central catecholaminergic neurons in hypertension. The elevation of PNMT activity indicates that adrenaline production in these neurons is increased during the development of hypertension. The enhanced PNMT activity of these neurons during the early phase of the hypertension may be a compensatory mechanism in response to the increased activity of peripheral sympathetic nerves (*12*) or a central mechanism for the initiation of the hypertensive disease.

The hypotensive effect of a PNMT inhibitor is of interest, although the site of action and specificity of its effects remain to be determined. The drug inhibits PNMT, not only in the brain, but also in the adrenal medulla, and possesses α-adrenergic blocking action (*14*). The use of PNMT inhibitors with selective central or peripheral action may clarify the site and mechanism of action and provide a new class of drugs for the study and treatment of some forms of hypertension.

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15. We thank H. Green (Smith Kline & French Laboratories, Philadelphia) for the supply of SKF 7698.

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