though Defendi and Roosa do not specifically address the issue of incomplete neonatal thymectomy, their table 1 (p. 126) includes data showing polyoma virus tumors were successfully transplanted in 1 of 18 control mice, 2 of 25 mice with sham thymectomies, 1 of 5 mice with incomplete thymectomies, and 8 of 8 mice with complete thymectomies. The results for the incompletely thymectomized mice are different from those for the sham-operated mice at $P < .25$ (chi-square test).
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High Angiotensin-Converting Enzyme Activity in the Neurohypophysis of Brattleboro Rats

Abstract. *The activity of angiotensin-converting enzyme is significantly higher in the intermediate and posterior pituitary lobes of Brattleboro rats than in Long-Evans control rats. The high activity level was reversed by vasopressin treatment. Conversely, angiotensin-converting enzyme activity was significantly lower in the anterior pituitary of Brattleboro rats than in Long-Evans rats, and this activity level was not affected by vasopressin. These findings suggest an inverse relation between vasopressin and angiotensin systems in the posterior and intermediate lobes of the pituitary gland.*

Two hormones, angiotensin II and vasopressin, regulate water balance in mammals (*1*). Angiotensin II increases fluid intake, whereas vasopressin is antidiuretic. An interaction between the renin-angiotensin and vasopressin systems has been established (*2*). After its synthesis and transport in the hypothalamo-neurohypophyseal system and its release into the general circulation, vasopressin is able to control the release of renin from the kidney (*3*). Angiotensin and vasopressin have also been postulated to interact at the neurohypophyseal level. Administration of angiotensin II increases the release of vasopressin from the neurohypophysis (*4*). The localization of the precursor of angiotensin II, angiotensin I (*5*), and the existence of a highly active angiotensin-converting enzyme (ACE) (E.C. 3.4.15.1) in the neurohypophysis (*6*) suggest an interaction between renin-angiotensin and vasopressin systems in this organ.

The interaction can be advantageously studied in homozygous Brattleboro rats (*7*). In these animals, diabetes insipidus is associated with high plasma renin activity and angiotensin II concentration (*8*) without changes in renin substrate (*9*). The high plasma renin activity can be corrected by treatment with vasopressin (*10*), but vasopressin is ineffective against the high isorenin activity in the adrenal gland and hypothalamus of these rats (*10*).

Homozygous Brattleboro rats lack angiotensin II binding sites in the neurohypophysis (*11*). To determine whether there are other abnormalities in the renin-angiotensin system in the neurohypophysis of Brattleboro rats and whether these changes can be influenced by vasopressin, we studied ACE activity in the posterior, intermediate, and anterior pituitary lobes of Long-Evans rats and age-matched, heterozygous and homozygous male Brattleboro rats (*12*).

The animals were decapitated between 9:00 and 11:00 a.m. and blood samples were taken from the trunks, poured into ice-chilled tubes containing heparin, and centrifuged. A piece of lung was removed and the pituitary glands were separated into anterior, posterior, and intermediate lobes under a dissecting microscope. These tissues were homogenized in cold 0.1*M* tris buffer (*p*H 7.4) containing 1 m*M* parachloromercuriphenylsulfonic acid, and portions of the homogenate were analyzed for protein content (*13*) and ACE activity (*14*). ACE activity was also measured in duplicate 10-μl samples of plasma.

There were significant differences between the Brattleboro and Long-Evans rats in ACE activity in the three lobes of the pituitary gland. ACE activity in the

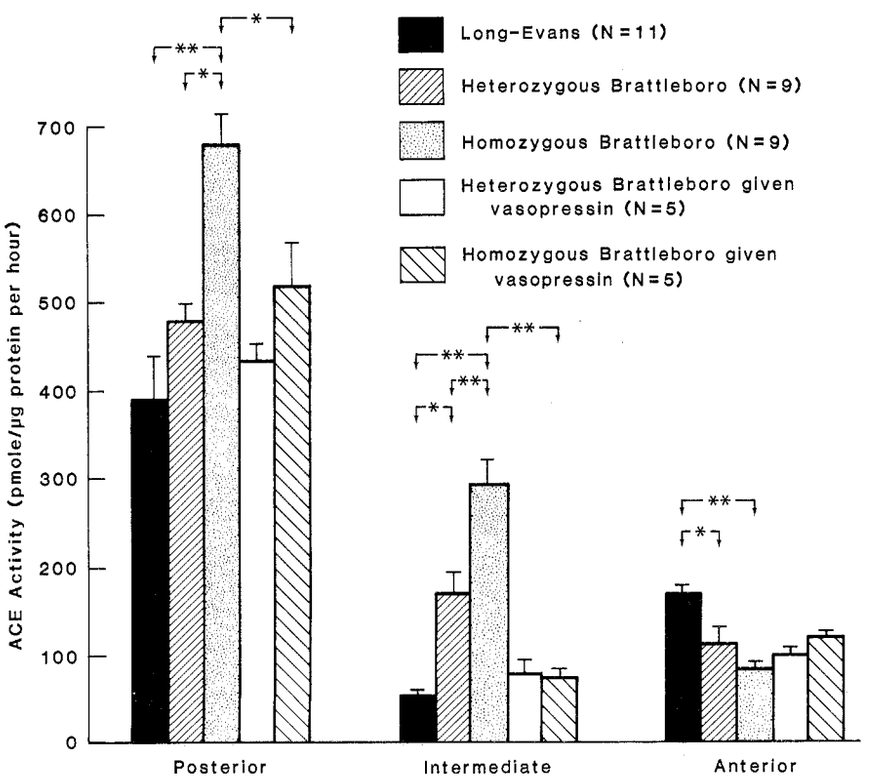

Fig. 1. Angiotensin-converting enzyme activity in the three lobes of pituitary glands from Long-Evans, heterozygous Brattleboro, and homozygous Brattleboro rats and the effect of vasopressin. The data are means ± standard errors. Single asterisks indicate significant differences at $P < .05$; double asterisks indicate $P < .01$ (analysis of variance followed by multiple comparisons among individual means).

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posterior lobe was higher in heterozygous Brattleboro rats than in Long-Evans rats and even higher in homozygous Brattleboro rats. The same differences in ACE activity were observed for the intermediate lobe but were even more pronounced. These high activities were reversed by vasopressin treatment (Fig. 1). Conversely, ACE activity in the anterior pituitary was lower in homozygous Brattleboro rats than in Long-Evans rats, with the heterozygous rats having intermediate activity; and the low activities were not reversed by vasopressin (Fig. 1). While homozygous Brattleboro rats lack vasopressin, the heterozygous rats show levels of vasopressin in their pituitary glands and plasma which are intermediate between those of homozygous Brattleboro rats and Long-Evans rats (*15*).

Our findings suggest that the high ACE activity in the posterior and intermediate lobes of the Brattleboro rat pituitary is related to the altered vasopressin metabolism (*16*) and indicate control of ACE activity by vasopressin. The vasopressin-induced reversal of the high ACE activity could be mediated by a mechanism at the neurohypophyseal level or indirectly by changes in the formation of angiotensin II. Thus, an interaction between the vasopressin and renin-angiotensin systems can be postulated at the neurohypophyseal level. However, ACE activity is not restricted to cleavage of angiotensin I; this enzyme also participates in the metabolism of bradykinin and enkephalins (*17*). In particular, the intermediate lobe of the pituitary contains large quantities of opioid peptides (*18*), and low levels of enkephalin have been described in the intermediate pituitary lobe of homozygous Brattleboro rats (*19*). For these reasons it is also possible that the effects of vasopressin on ACE activity in this structure reflect an interaction between vasopressin and the opioid peptide system (*19, 20*).

The cause of low ACE activity in the anterior pituitary of homozygous Brattleboro rats is not known. A direct role of vasopressin in the anterior pituitary can be postulated, since this peptide is present in hypophyseal portal blood (*21*). Adrenocorticotropic hormone, whose release is stimulated by vasopressin and angiotensin II (*22*), is present in abnormally low concentrations in the pituitary gland of homozygous Brattleboro rats (*23*). Adrenocorticotropic hormone is metabolically related to the common precursor of opiate peptides (*24*). High concentrations of β-endorphin and related peptides are present in the anterior pituitary of the rat, and the β-endorphin concentration is even higher in the anterior pituitary of homozygous Brattleboro rats (*20*). Opioid peptides competitively inhibit ACE activity in vitro, suggesting that these peptides function as ACE substrates (*25*). The low level of ACE activity in the anterior pituitary of homozygous Brattleboro rats indicates a relation between this enzyme and the metabolism of anterior pituitary peptides and may be responsible for the altered anterior pituitary function in these rats (*26*).

Table 1. Angiotensin-converting enzyme activity in plasma and lung tissue of Long-Evans (N = 11), heterozygous Brattleboro (N = 9), and homozygous Brattleboro (N = 9) rats. Values are means ± standard errors.

Strain	Activity in plasma (pmole/μl per hour)	Activity in lung (pmole/μg protein per hour)
Long-Evans	948 ± 83	507 ± 32
Heterozygous Brattleboro	868 ± 89	552 ± 27
Homozygous Brattleboro	967 ± 67	538 ± 30

There was no difference in ACE activity in plasma and lung tissue between the Brattleboro and Long-Evans rats (Table 1), indicating that low ACE activity is not a generalized phenomenon in Brattleboro rats. On the contrary, plasma renin activity is reported to be high in Brattleboro rats and is reversible with vasopressin administration (*11*).

These results indicate that vasopressin regulates the renin-angiotensin system to different degrees in different organs. Peripherally, vasopressin may modulate renin activity; in the pituitary gland, vasopressin may affect the renin-angiotensin system through a modulation of ACE activity.

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12. The rats were maintained on a 12-hour light-dark cycle (lights on at 6:00 a.m.) and controlled temperature for 2 weeks before being killed. They received unlimited food and water. Mean daily water intake and urine volume for Long-Evans rats was 24 + 1 and 10 + 2 ml, respectively; for heterozygous Brattleboro rats, 24 ± 5 and 8 ± 2 ml; and for homozygous Brattleboros, 153 ± 2 and 144 ± 18 ml. Five homozygous and five heterozygous Brattleboros received vasopressin (Pitressin tannate in peanut oil; two 1.5-U intramuscular injections daily at 8:00 a.m. and 6:00 p.m. for 1 week). This treatment resulted in a decrease in the water intake and urinary volume of homozygous Brattleboros to 32 ± 6 and 14 ± 13.5 ml, respectively.
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