natively, this response could have been due to residual adenohypophysial tissue not completely removed by the hypophysectomy or to other unknown factors (*21*).

We believe that ACTH acting within the central nervous system mediates the increased grooming observed in the novel environment. The presence of antiserum to ACTH in the ventricular fluid attenuates this response. Our results provide good evidence that the secretion of a peptide hormone into the brain can mediate a complex behavioral response. This response is observed physiologically under conditions known to cause release of ACTH. These findings support those of others suggesting that the cerebrospinal fluid functions to transport intracerebrally secreted hormones to their sites of physiological and behavioral action (*23*).

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13. Antiserum to human ACTH (batch 2) was donated by C. D. West, National Pituitary Agency (University of Maryland School of Medicine), National Institute of Arthritis, Metabolism, and Digestive Diseases. This antiserum has full activity against ACTH 1-39 or ACTH 11-24 but only partial activity against ACTH 1-24. There was no cross-reactivity with ACTH 1-10, ACTH 4-10, ACTH 1-16, ACTH 17-39, ACTH 25-39, β-lipotropin, or β-endorphin (D. Krieger, personal communication). The freeze-dried antiserum was redissolved at full strength in water and diluted 1:1 (by volume) with isotonic saline for injection. Control serum was obtained from nonimmunized rabbits.
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Accumulation of a Tetrahydroisoquinoline in Phenylketonuria

Abstract. *3′,4′-Deoxynorlaudanosolinecarboxylic acid (DNLCA), a tetrahydroisoquinoline derived from dopamine and phenylpyruvic acid, has been detected by computerized mass fragmentography in urine of phenylketonuric children and in urine and brain of rats with experimentally induced hyperphenylalaninemia. Levels of DNLCA in brain of treated animals were more than tenfold higher than controls, and the excess tetrahydroisoquinoline appeared to accumulate in the cerebellum and cortex. DNLCA is a noncompetitive inhibitor of dopamine β-hydroxylase (inhibition constant, K_{i_s} = 0.42 mM) and is taken up by the brain.*

Recently we demonstrated the presence of a tetrahydroisoquinoline alkaloid, 3′-*O*-methylnorlaudanosolinecarboxylic acid (MNLCA) (*1*) in parkinsonian patients treated with L-dopa (*2*). We now report on the occurrence of a related catecholamine derivative in phenylketonuric children and in rats with an experimentally induced hyperphenylalaninemia.

3′,4′-Deoxynorlaudanosolinecarboxylic acid (DNLCA) was prepared by Pictet-Spengler condensation of dopamine and phenylpyruvic acid, a reaction that can occur in low yields spontaneously under physiological conditions (*1, 2*). DNLCA was characterized as its hydrochloride: melting point, 239° to 241°C (decomposes); ultraviolet wavelength of maximum absorption (λ_{max}; 1 *N* HCl) 285 nm (log molar absorptivity 3.55); nuclear magnetic resonance spectrum [$(CD_3)_2SO$-CF_3COOH] chemical shift, 6.6 (singlet H-5), 7.1 to 7.6 (multiplet remaining aromatic H atoms), 2.6 to 4.0 (multiplet αH, H-3, H-4); mass spectra *m/e* 299 (M^+ 1.7 percent), 281 (11 percent), 255 (39 percent), 254 (91 percent), 253 (30 percent), 208 (100 percent), 164 (67 percent), 162 (65 percent), 91 (50 percent). Using a computerized gas-liquid chromatography–mass spectrometric method (GC-MS) (*2–4*), we have found DNLCA in the urine of four phenylketonuric children ranging in age from 5 to 15 years (45, 100, 69, and 60 ng/ml, respectively). Although all of the children were on a normal diet, the average of their DNLCA concentrations was twice that of three age-matched controls (35, 34, and 34 ng/ml). Urinary metabolite patterns were obtained by paired-ion, reversed phase, high-pressure liquid chromatography (*5*). A direct correlation was observed between the concentration of urinary phenylpyruvate and DNLCA.

Additional data were obtained by injecting rats with *p*-chlorophenylalanine

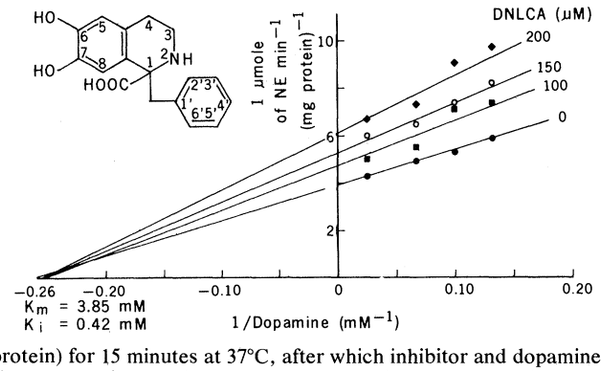

Fig. 1. Incubation mixtures contained 200 μl of 1*M* potassium phosphate buffer (*p*H 6.2), 50 μl of 20 m*M* pargyline, 50 μl of 0.2*M* ascorbate, 200 μl of 0.25 m*M* *p* - chloromercuribenzoate, 50 μl of 0.2*M* sodium fumarate, and 80 μl of a catalase solution (1 mg protein/ml, 27,000 units) which was added last. This solution was incubated with 40 μl of enzyme (bovine adrenal dopamine β-hydroxylase, 5 to 10 units per milligram of protein) for 15 minutes at 37°C, after which inhibitor and dopamine were added to give the final concentrations as indicated. After 40 minutes the reaction was terminated by addition of 200 μl of 25 percent aqueous trichloroacetic acid. Samples were then taken to dryness, dissolved in a mixture of methanol and 1 percent aqueous acetic acid (3:7 by volume), and subjected to paired-ion, reversed phase, high-pressure liquid chromatography as described (*5*). Norepinephrine (NE) concentrations were estimated from peak height analysis with an ultraviolet spectrophotometric detector (*5*). Kinetic constants were obtained by the method of least squares.

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Table 1. Regional distribution of DNLCA in brain of hyperphenylalaninemic (PKU) rats. Male Sprague-Dawley rats (250 g) were injected intraperitoneally with 500 μmole of phenylalanine daily and 250 μmole of *p*-chlorophenylalanine every other day. After 2 weeks on the regimen, the animals were anesthetized with ether and their brains were removed and dissected over ice (*14*). Regions were immediately homogenized in cold 1*N* HCl. The values expressed represent the mean of determinations made on two groups of animals; each group was obtained by pooling tissue from three brains and subjecting them to repetitive scan GC-MS (*2–4*). The DNLCA concentration in whole brain was determined to be 10 ng/g with a range of 3 to 21 ng/g for normal rats ($N = 6$) and 169 ng/g with a range of 30 to 371 ng/g for treated animals ($N = 7$). To ascertain that the experimental rats were hyperphenylalaninemic, we excised and homogenized their livers. Homogenates were centrifuged at 20,000*g*, and the supernatants were treated with trichloroacetic acid to precipitate protein. Analyses of the supernatant by automated amino acid analyzer revealed that the livers from experimental rats contained 71 ng of *p*-chlorophenylalanine per gram of tissue. In addition, the ratio of free hepatic phenylalanine to tyrosine changed from 1.14 in controls to 4.2 in treated animals.

Region	Control group ($N = 6$)		Experimental animals			
			Group 1 ($N = 6$)		Group 2 ($N = 6$)	
	Concentration (ng/g)	Total amount (ng)	Concentration (ng/g)	Total amount (ng)	Concentration (ng/g)	Total amount (ng)
Medulla Oblongata and pons	35	8.2	43	7.6	14.0	3.8
Midbrain	12	8.3	12	7.9	9.5	5.8
Cortex	10	6.6	105	74.6	379.0	249.0
Cerebellum	71	20.1	725	198.0	479.0	151.0

and phenylalanine to induce hyperphenylalaninemia in rats (Table 1) (*6*). Hepatic phenylalanine and tyrosine were monitored in treated and control animals. Brain tissue of rats maintained on the regimen described in Table 1 contained concentrations of DNLCA that were more than tenfold higher than in controls, and the excess of the tetrahydroisoquinoline in the treated animals appeared to accumulate in the cerebellum and cortex (*7*). The white matter of these two brain regions sustain the most severe neurological damage in phenylketonuria, that is, amyelination, status spongiosus, and gliosis (*8*). Compared to other brain regions, cerebellum and cortex also undergo a more extensive development in the postnatal period, thus they would be most susceptible to marked changes in metabolism. Furthermore, when rat pups were given *p*-chlorophenylalanine and phenylalanine, their cerebellums were not only reduced in weight as compared to controls but they also contained less myelin as measured by histological staining (*9*).

To determine whether DNLCA can be taken up by brain, we injected [3,4-^{3}H]-DNLCA (212,000 count/min, 600 μg) into the tail vein of male Swiss albino mice (25 g). The animals were killed 20 minutes later and their brains were immediately frozen on Dry Ice. Each brain was added to a mixture of perchloric acid and 30 percent hydrogen peroxide (1 : 2 by volume) and heated at 75°C for 3 hours. The radioactivity in the solubilized material was then counted. Under these conditions 0.48 percent of the total radioactivity injected was recovered in brain ($N = 4$).

DNLCA proved to be an inhibitor of dopamine β-hydroxylase in vitro. Lineweaver-Burk plots of dopamine hydroxylation in the presence of various concentrations of the tetrahydroisoquinoline indicated a kinetic pattern of noncompetitive inhibition (Fig. 1). The inhibition constant (K_i) of $10^{-4}M$ suggests a possible physiological significance, if compartmentalization of DNLCA occurs (see Table 1). Plasma serotonin, norepinephrine, and epinephrine concentrations are depressed in phenylketonuria (*10*). Decreased concentrations of dopamine, norepinephrine, epinephrine, and serotonin, and their catabolites, in brain as well as urine of phenylketonurics (*10*) have been attributed to inhibition of tyrosine hydroxylase or L-aromatic amino acid decarboxylase, but recent evidence (*11*) argues against inhibition of these enzymes. Phenylalanine proved to be an excellent substrate for tyrosine hydroxylase in the presence of the naturally occurring cofactor, tetrahydrobiopterin. Earlier studies in vitro (*12*) on L-aromatic amino acid decarboxylase indicate that phenylpyruvate is only a weak inhibitor of this enzyme (*13*). Our findings suggest that DNLCA could contribute by inhibiting dopamine β-hydroxylase. Furthermore, the presence of excessive concentrations of phenylpyruvate in plasma (up to 0.1 m*M* in phenylketonurics) would establish conditions conducive to condensation of this α-keto acid not only with dopamine and norepinephrine but also serotonin (*1*), thereby diminishing these biogenic amines.

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