EPINEPHRIN AND NOREPINEPHRIN LEVELS IN DILUTE-LETHAL MICE

Charles H. Doolittle and Harold Rauch

Department of Zoology
University of Massachusetts
Amherst, Massachusetts

Received October 16, 1964

Dilute-lethal ($\underline{d}^1$) is a juvenile lethal mutation which acts as a recessive allele both to full color ($\underline{D}$) and to Maltese dilution ($\underline{d}$) (Searle 1952). In addition to dilution of coat color, mutant mice ($\underline{d}^1\underline{d}^1$) are characterized by abnormal behavior: during the second week after birth they exhibit locomotor difficulties in the form of pronounced overbalancing accompanied by convulsive limb movements of the clonic-tonic type. At about two weeks of age opisthotonic seizures appear, recurring with increasing frequency and severity; death ensues at about three weeks of age. Examination of the central nervous system has revealed a pattern of myelin degeneration in various elements of the brain (Kelton and Rauch, 1962). Recently Rauch and Yost (1963) showed reduction in activity of the phenylalanine-pyruvate transaminase and phenylalanine hydroxylase enzyme systems in mutants as compared to normal littermates. The combination of symptoms (lightened hair color, tremors, epileptiform seizures, myelin degeneration) with abnormal phenylalanine metabolism suggests a similarity to the human hereditary condition called phenylketonuria.

Weil-Malherbe (1955) and Nadler and Hsia (1961) have shown that phenylketonurics have markedly lower levels of both epinephrin and norepinephrin in plasma and urine than do control patients. Reported here are the results of a study to determine whether similar decreases occur in dilute-lethal mice.

Materials and Methods

The animals used were from an inbred strain (DL) developed in our

laboratory by repeated brother-sister matings for 18 to 20 generations. Matings are heterozygote by heterozygote ($\underline{Dd^1}$ x $\underline{Dd^1}$) so that normals and mutants appear in each litter; mutant mice can be distinguished from their normal littermates on the basis of coat color as early as four to five days of age. Catecholamine levels in adrenal glands and in urine were determined. The pairs of adrenal glands from three mice were pooled for each assay. Six determinations were performed at each of the ages 5, 7, 10, 14, 18, and 21 days for both normal and mutant animals. The catecholamines were extracted from the glands after a modification of Crout et al. (1961) and were assayed by the fluorometric micromethod of differential estimation of Von Euler and Floding (1955); the fluorescence was read in a Coleman electrophotofluorometer.

Urine was collected from the mouse colony and pooled according to phenotype and age (14, 18 and 21 days). The urine was acidified with HCl and kept frozen during collection until the 3.0 ml needed for the determination was obtained. The procedure used for sample isolation and fluorescence development was essentially that described in the G. K. Turner Associates Manual of Fluorometric Clinical Procedures, (see also Von Euler and Lishajko 1959, Bertler et al. 1958, Jacobs et al. 1961) modified only by reducing volumes because of the small amounts of urine available. For the same reason only one or two samples at each age could be tested. Fluorescence was read in the Turner Model 111 Fluorometer. The catecholamines for standards were obtained from Nutritional Biochemicals Corporation.

Results and Discussion

The results of the assays of catecholamines in the adrenals of normals and dilute-lethals are given in Table 1. By 14 days of age epinephrin and norepinephrin levels are significantly higher in mutants than in normal littermates; these differences become more pronounced with age until the mutants die.

Table 1. Adrenal Catecholamine Levels

AGE	EPINEPHRIN			NOREPINEPHRIN		
	D-	d^1d^1	t	D-	d^1d^1	t
5	230±87	260±76	.63	169±64	153±61	.44
7	266±49	334±49	2.40*	131±76	149±50	.48
10	335±87	393±97	1.10	109±72	111±53	.05
14	433±15	543±62	4.20**	110±38	170±40	2.60*
18	487±72	625±73	3.20*	148±27	211±53	4.20**
21	605±130	756±114	2.20*	119±82	256±125	2.20*

Mean ± standard deviation expressed in micrograms of catecholamine per gram of adrenal glands. n=6; t=relative deviate; age in days.
*Significant at .05 level. **Significant at .01 level.

The results of the assays in urine are shown in Table 2. Although the number of determinations is small, it appears that epinephrin levels are considerably higher in dilute-lethals than in normals while norepinephrin levels are about the same.

Table 2. Urinary Catecholamine Levels

AGE	EPINEPHRINE		NOREPINEPHRINE	
	D-	d^1d^1	D-	d^1d^1
14	4.2	12.9	148	156
18	12.5	24.8	254	238
21	15.5	37.8	276	248
Adult	26.1	-	279	-

Average expressed in micrograms of catecholamine per liter of urine. n=2 except for 21 days where n=1; age in days.

Phenylketonuria results from the failure to hydroxylate phenylalanine to tyrosine (Jervis, 1953) and more specifically from a defect in the phenylalanine hydroxylase enzyme system (Kaufmann, 1958). The excess phenylalanine is presumably transaminated to phenylpyruvic acid

(Meister et al. 1956) which in turn may be decarboxylated to phenylacetic acid (Moldave and Meister, 1957). Fellman (1956) and Davison and Sandler (1958) have attributed reduced catecholamines in phenylketonurics to the inhibition of DOPA decarboxylase in the metabolic chain to norepinephrin and epinephrin by phenylalanine derivatives such as phenylpyruvic and phenylacetic acids. Boylen and Quastel (1961) showed that epinephrin-C^{14} formation from tyrosine-C^{14} in guinea pig medulla slices was inhibited by sodium phenylpyruvate at levels comparable to those found in phenylketonurics, while high concentrations of phenylalanine had no such effect. In dilute lethal mice reduced transaminase activity along with reduced hydroxylase activity results in increased phenylalanine levels without concomitant increases in phenylpyruvate and phenylacetate. The absence of these derivatives in excess may explain the lack of reduction in catecholamine formation.

It has been shown that stress causes increased synthesis and secretion of epinephrin and norepinephrin (Danowski 1962). The elevations observed in dilute-lethals may be a reflection of the stress imposed on the animals by their continuous convulsive behavior, for increasing catecholamine levels correspond in time with the appearance and increasing frequency and severity of the seizures. We have stressed mutants and normal littermates by exposure to cold and have found rapid depletion (as much as 50%) of adrenal catecholamines in normal animals but have found no change in mutants from their high levels. A possible explanation may be that mutant animals are already stressed to the point of maximal catecholamine secretion and synthesis and that additional stresses cannot evoke further enhancement. This possibility will be explored further.

This investigation was supported by a research grant, GM 05921, from the National Institutes of Health, Public Health Service.

References

Bertler, A., A. Carlsson and E. Rosengren, 1958. Acta Physiol. Scand. 44: 273-292.

Boylen, J. B. and J. H. Quastel, 1961. Biochem. J. 80:644-648.

Crout, J. R., C. R. Creveling and S. Udenfriend, 1961. J. Pharm. Exper. Therap. 132:269-277.

Danowski, T. S., Clinical Endocrinology, Williams and Wilkins Co., Baltimore, Vol. IV p. 64, 1962.

Davison, A. N. and M. Sandler, 1958. Nature. 181:186-187.

Fellman, J. H., 1956. Proc. Soc. Exper. Biol. Med. 93:413-414.

Jacobs, S. L., C. Sobel and R. J. Henry, 1961. J. Clin. Endocrin. Metab. 21:305-314.

Jervis, G. A., 1953. Proc. Soc. Exper. Biol. Med. 82:514-515.

Kaufman, S., 1958. Science. 128:1506-1508.

Kelton, D. E. and H. Rauch, 1962. Exptl. Neurol. 6:252-262.

Meister, A., S. Udenfriend and S. P. Bessman, 1956. J. Clin. Invest. 35:619-626.

Moldave, K. and A. Meister, 1957. J. Biol. Chem. 229:463-476.

Nadler, H. L. and D. Y. Hsia, 1961. Proc. Soc. Exper. Biol. Med. 107: 721-723.

Rauch, H. and M. T. Yost, 1963. Genetics. 48:1487-1495.

Searle, A. G., 1952. Heredity. 6:395-401.

Von Euler, U. S. and I. Floding, 1955. Acta Physiol. Scand. 33, suppl. 118:45-56.

Von Euler, U. S. and F. Lishajko, 1959. Acta Physiol. Scand. 45:122-132.

Weil-Malherbe, H., Blood Adrenaline and Intelligence, in Biochemistry of the Developing Nervous System, ed. H. Waelsch, New York Academic Press, pp. 458-466, 1955.

Manual of Fluorometric Clinical Procedures. G. K. Turner Associates, Palo Alto, California, pp. 11-12.