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CHOLINESTERASE AND LACTIC DEHYDROGENASE ACTIVITY IN THE RAT BRAIN[1]

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Previous studies have demonstrated the existence of reliably measurable individual, strain, and age differences in cholinesterase (ChE) activity of the rat brain (BENNETT, et al., 1958). These individual differences in ChE activity have also been shown to be related to the adaptive behaviour of the rat (ROSENZWEIG, KRECH, and BENNETT, 1958). This latter relationship has been assumed to reflect the fact that the acetylcholine-ChE system is of peculiar significance in the metabolism of the brain because of its contribution to synaptic transmission. However, since the acetylcholine-ChE system is only one of the numerous biochemical systems which are important in the metabolism of the brain, the correlations which have been found between ChE activity of the cortex and the behaviour of the rat may be non-specific; that is, ChE activity may simply reflect the general enzyme and metabolic levels in the rat brain. It is therefore of interest to determine what relationships exist between ChE and other enzyme systems. In this paper ChE activity is compared with lactic dehydrogenase (LDH) activity in the cerebral cortex and subcortical brain of the strains of rats we have used in many of our previous studies.

Lactic dehydrogenase carries out the interconversion

pyruvic acid ⟷ lactic acid

which is the last step in the glycolytic formation of lactic acid from glucose. Normally, the brain oxidises about 85 per cent of the glucose it utilises, while it metabolises only about 15 per cent of the glucose by the glycolytic route to lactate (McILWAIN, 1955). However, during periods of maximal nerve activity, the glycolytic pathway may assume increased importance. Therefore, if it is assumed that high levels of ChE are associated with a potentially high general activity of the brain, a positive correlation might be expected between ChE and LDH activity. On the other hand, the same assumption that high levels of ChE are associated with a potentially high general activity of the brain might lead one to expect an inverse relationship between ChE and LDH. This would follow from the argument that the formation of lactic acid from glucose represents less efficient utilisation of glucose than does the complete oxidation via the tricarboxylic acid cycle. Thus the less adaptive rats with low ChE activity in the cortex would be expected to have high LDH activity representing the less efficient utilisation of energy sources. Finally, it is possible that the two systems are quite unrelated. If no correlation were found to exist between LDH and ChE this would support the hypothesis that the observed correlations between cortical ChE activity and adaptive behaviour did not reflect a correlation between general metabolic rate in the brain and adaptive behaviour, but, rather, was specific to the acetylcholine-ChE cycle.

METHODS

The LDH and ChE activities were determined for the visual (V) and somesthetic (S) areas of the cerebral cortex and for the subcortical brain (brain with dorsal cortex removed). To give a single value for cortical

enzyme activity, the determinations for the V and S areas were averaged [(V + S)/2]. Fifty-nine male rats of the S_1 strain and 47 male rats of the S_3 strain were tested. These rats were descendants of the two strains selectively bred by TRYON (1929) for high and low maze-learning ability. They range in age from 30 to 148 days. These animals were included among 458 rats whose ChE values were reported in a previous paper (BENNETT, et al, 1958).

Lactic dehydrogenase activity was estimated by the spectrophotometric measurement at 340 mμ of the rate of formation of DPNH (NEILANDS, 1955). The assay mixture consisted of 2 ml of 0.1 M glycine buffer, pH 9.2; 0.5 ml of 0.5 M sodium D,L-lactate, pH 9.2; and an aliquot portion of the same tissue sample homogenate used for the ChE determination. (Fifty μl of the homogenates containing 100 μg wet weight of the cortex, or 100 μl or a 1/10 dilution of the subcortical brain homogenates containing 150 μg of tissue was used.) The mixture was pre-incubated in 1 cm Beckman cells at 37° in a thermostated cell compartment of a Beckman DU spectrophotometer. After 10 minutes, 0.4 ml of DPN (10 mg/ml, Sigma 95-100% pure) was added to each sample, and the solutions were mixed and replaced in the cell compartment of the spectrophotometer. After 5 minutes when temperature equilibrium was re-obtained, the optical density was determined at 340 mμ for 20 minutes, at 5 minute intervals. The change of optical density/minute was calculated by the formula:

$$\Delta \text{ OD/minute} = \frac{N\Sigma XY - (\Sigma X)(\Sigma Y)}{N\Sigma X^2 - (\Sigma X)^2}$$

where X is time, and Y is the density reading. The values for LDH activity have been expressed in terms of moles of DPN reduced x 10^9/minute/mg wet tissue, using 6220 as the molecular extinction coefficient for

DPNH (HORECKER AND KORNBERG, 1948).[4] Duplicate analyses were made of each sample and the results averaged.

Cholinesterase activity was determined by the rate of hydrolysis of acetylcholine in an automatic titrater. The methods have been reported in detail (ROSENZWEIG, KRECH, and BENNETT, 1958).

RESULTS

The results of the analyses of the two enzymes are summarized in Tables I and II and Fig. 1. They show several significant differences between the ChE and LDH activities in the rat brain:

1). The distribution of LDH in the cortex and subcortex is quite unlike that of ChE. We have consistently found with both strains that the ChE activity of the V section of the cortex is 15 to 20 per cent less than that of the S section (see Fig. 1), and the average ChE activity of the V and S sections is only 35 to 40 per cent of the activity in the subcortical brain. These findings are repeated with the present sample. On the other hand, the present data show that the LDH activity of the V section is the same or slightly higher than that of the S section, and the averaged LDH activity of the V and S sections is 110 to 120 per cent of the activity in the subcortical brain (Tables I and II).

2). The effect of age differs upon ChE and LDH activity. ChE activity per unit of weight of cortex has been shown to increase with the age of the rat to a maximum at about 100 days; thereafter the activity declines slowly (BENNETT, et al., 1958). The early increase is reflected in Tables I and II and in Fig. 1; the tables do not include animals old enough to show the decline in ChE at later ages. On the contrary, the LDH activity of the cortex is essentially the same at all the ages tested,

as can be seen from Tables I and II. (For subcortical brain, not enough values are available for both ChE and LDH to permit determinations of comparative age functions.)

5). ChE and LDH differ in relations between strains. We have previously reported (BENNETT, et al., 1958) that the variability of ChE activity is approximately 5 per cent when strain, brain area, and age are held constant. A similar result for the variability of LDH activity is indicated by the small values of the standard deviation in relation to the means and by the ranges (Tables I and II). This is further evidence for the low biochemical variability of the brain as compared with other tissues. Despite this low variability, we have consistently found a statistically significant difference between the ChE levels of the S_1 and S_3 strains such that the S_1 strain is found at every age and for every brain area measured (see Fig. 1). However, no such strain differences are found for LDH activity. As can also be seen from Fig. 1, the LDH activity per unit weight is very much the same for both the S_1 and the S_3 strains. This is true for every age and for every brain area tested.

When, however, we consider total enzyme activity instead of activity per unit weight, a different picture emerges. The brain weight of the S_3 strain is significantly greater than that of the S_1 strain (BENNETT, et al., 1958), and brain weight of the rat increases steadily with age. When total activity is calculated, taking into account the brain weight, the S_1 strain shows a higher total ChE activity than the S_3 strain in the cortex but a lower total in the subcortex. For LDH, on the other hand, the S_1 strain shows a lower level total activity than the S_3 strain in both the cortex and the subcortex.

4). The generality within the brain of ChE and LDH activity differs

significantly. As shown in Table III, fairly high, statistically significant, and positive correlations exist between ChE activity in the V sections of the cortex and in the S sections. This holds for either strain. The size of the over-all correlation, 0.67, indicates that this relationship accounts for 45 per cent of the variance; that is, knowing an animal's ChE value for one cortical region, the uncertainty in the value for the other region is reduced by 45 per cent. In previous work (ROSENZWEIG, KRECH, and BENNETT, 1958), it has been shown that the ChE activity in the V and in the S sections is also highly correlated with ChE activity in the motor section of the rat's cortex. Thus it appears that the level of the ChE activity in the rat cortex is a general characteristic of the cortex. Table II also shows that there is a positive, but considerably lower, correlation between the ChE activity in the cortex and in the subcortical brain. Here, however, none of the correlations for either strain is significantly greater than zero. However, the correlation for both strains combined (as indicated in the table) is significant at the 5 per cent level of confidence. The corresponding correlational picture for LDH is radically different. The LDH activity in the V section does not correlate significantly with that in the S section, nor do the cortical values correlate significantly with the subcortical ones. The correlations which do appear are not consistent in sign as we go from one age group to another, or from one strain to another. In other words, the level of LDH activity at one locus of the brain seems unrelated to the activity at other loci.

5). There appears to be a moderate positive relationship between the ChE and LDH activity levels of the cerebral cortex (see lower right-hand column in Table III). Although only one of the three subgroups shows a significant relationship, the correlations for all groups combined are

significant at the 1 per cent level of confidence. The size of the correlation, 0.87, indicates that this relationship accounts for 14 per cent of the variance. In the subcortical brain, however, there is no consistent relationship between ChE and LDH levels. It appears, therefore, that whatever metabolic process is reflected by the level of ChE activity, it is somewhat related in the cortex to the process governed by LDH, but the two processes are unrelated in the rest of the brain.

DISCUSSION

The results of the present study clearly indicate that the distribution of LDH and ChE activities in the cortex and subcortical brain of the rat differ in many significant respects. Of particular interest, in the light of our major program of investigating the enzymatic control of behaviour, is the low correlation between the ChE and LDH levels within the individual rat, and the lack of any difference in LDH levels between the S_1 and S_3 strains. The low correlation suggests that while the ChE level of the rat brain is somewhat related to metabolic systems quite removed from the acetylcholine-ChE system, most of the variance (about 85 per cent) is independent of LDH. To this extent, then, the observed correlations between ChE and adaptive behaviour cannot be ascribed to some general metabolic level. The fact that there is no difference between the LDH levels of the S_1 and S_3 strains, while there is a difference between the ChE levels of the two strains, further supports this conclusion. In almost every behavioural test we have used thus far, the S_1 strain has been consistently superior to the S_3 strain. Thus, as far as strain comparisons are concerned, we find a relationship between ChE and performance on our behavioural tests, but no relationship between LDH and performance.

Quite aside from this consideration, however, analysis of the cyto-architectural distribution of brain enzymes supports the general conclusion of a large degree of independence between ChE and LDH. The rat cortex is a complex of cell types and layers, the proportions of which change with the age of the animal and the areas of the cortex. POPE (1952) has shown that the ChE activity of the rat somesthetic cortex varies from layer to layer, generally decreasing by a factor of 2 from layer I to layer VI and the underlying white matter. This suggests that ChE is localized at the surfaces of dendrites and axones including synaptic terminals.

KUHLMAN and LOWRY (1956) have studied the changes in concentration of four dehydrogenases in the cortex of the newborn, the 10-day old, and the adult rat. Lactic dehydrogenase concentration, unlike that found by POPE for ChE, increases from layer I to layer II, and then remains fairly constant through layer VII.

These observations also suggest that some of the differences we have observed between the activity levels of the two enzymes, ChE and LDH, as well as the differences in ChE between the two strains, may be related to variations in architectonic structure of the cortex. Further investigations on this problem are now in process.

SUMMARY

Cholinesterase and lactic dehydrogenase activities were determined for 106 male rats of two strains and several ages. Both enzymes show low inter-individual variability. The values for the two enzymes differ in the following respects:

1. ChE activity shows a more sharply differentiated pattern of regional distribution within cortex and between cortex and subcortex than

does LDH activity.

2. ChE shows a clear rise from 30 to 100 days while LDH does not.

3. ChE shows significant differences between our strains while LDH does not.

4. ChE values are significantly correlated from locus to locus within the brain, while LDH values are independent from locus to locus.

5. ChE and LDH activity show some correlation with each other in the cortex but not in the subcortex.

These results are interpreted to mean that in the previously observed correlations between ChE activity and behavior, ChE activity is not simply an index to general metabolic level of the brain.

FOOTNOTES

1. This research was made possible by grants from the National Science Foundation and the U.S. Public Health Service, and was also supported in part by the U.S. Atomic Energy Commission. Mr. Alvin Halevy did developmental work on the LDH procedure. We also want to acknowledge our debt to Professor Melvin Calvin, who has maintained a continued interest in the problem and has made a number of helpful suggestions throughout the program of work.

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4. Preliminary experiments indicated that the change in specific activity of LDH was less than 3 per cent with a 25 per cent change in the weight of samples used in the range of 50 to 150 μg of cortex. Less than 10 per cent change in specific activity was observed when the amount of DPN added was varied from 2 to 8 mg or the volume of 0.5 M lactate added was varied from 200 μl to 600 μl.

Table I

Comparisons between Cholinesterase and Lactic Dehydrogenase Activities in Brain of S_1 Rats at Six Different Ages

Age in Days	N		Cholinesterase Activity[1] V	S	$\frac{V+S}{2}$	Sub-cortex	Lactic Dehydrogenase Activity[2] V	S	$\frac{V+S}{2}$	Sub-cortex
30	6	$\bar{X}$	48.1	59.5	53.8	153.3	77.4	73.8	75.5	63.9
		SD	2.89	1.54	2.07	5.09	2.23	1.09	1.62	1.19
		Range	43.9-51.9	57.4-61.6	50.6-56.7	144-159	75.6-81.7	72.6-76.1	74.2-78.9	65.1-66.4
44	6	$\bar{X}$	55.9	66.4	61.1	165.8	75.0	74.5	74.8	65.1
		SD	1.73	4.40	2.80	5.89	2.86	3.25	1.01	0.90
		Range	53.0-58.1	57.5-71.6	55.8-64.8	161-173	72.3-80.8	69.1-78.0	73.5-76.6	64.0-66.9
92	6	$\bar{X}$	57.6	65.6	61.6	*	69.8	68.6	69.7	*
		SD	1.75	1.97	1.74		2.55	1.63	1.95	
		Range	54.0-59.3	62.1-68.1	58.0-63.3		65.8-71.9	67.2-72.3	66.7-71.9	
110	20	$\bar{X}$	59.2	69.2	64.2	163.3	72.2	73.6	72.9	64.5
		SD	3.21	3.39	5.12	11.28	3.64	2.98	2.33	3.08
		Range	55.1-67.2	65.2-76.3	59.8-71.3	175-192	67.6-81.3	67.2-78.0	68.1-78.4	57.8-69.7
129	15	$\bar{X}$	58.3	65.4	61.9	171.1	77.7	72.5	75.2	65.7
		SD	2.48	3.22	2.47	6.40	1.66	4.36	2.40	1.98
		Range	54.0-63.7	58.2-70.3	56.8-65.5	158-182	75.6-80.8	66.7-80.8	70.9-79.4	62.1-69.7
147	6	$\bar{X}$	57.5	64.6	61.1	*	69.3	70.0	69.7	*
		SD	1.64	2.67	1.61		2.57	1.49	1.89	
		Range	54.6-59.6	60.5-69.1	59.0-64.2		65.8-72.8	68.6-71.9	67.2-72.3	

1 Expressed in terms of moles x 10^{10} acetylcholine hydrolyzed/min/mg tissue.

2 Expressed in terms of moles x 10^{9} DPNH formed/min/mg tissue.

* Subcortical values are not available for these groups.

Table II

Comparisons between Cholinesterase and Lactic Dehydrogenase Activities in Brain of S_3 Rats at Five Different Ages

Age in Days	N		Cholinesterase Activity[1] V	S	$\frac{V+S}{2}$	Subcortex	Lactic Dehydrogenase Activity[2] V	S	$\frac{V+S}{2}$	Subcortex
51	6	$\bar{X}$	45.5	56.8	50.0	149.5	75.8	75.1	75.5	65.2
		SD	1.68	3.09	1.71	5.25	2.99	3.09	0.89	1.67
		Range	41.4-46.5	51.4-60.7	46.6-51.6	142-155	71.4-78.9	69.5-78.0	74.2-77.0	61.1-65.4
94	6	$\bar{X}$	51.0	63.1	57.0	*	71.4	69.9	70.6	*
		SD	2.63	2.45	2.55		0.54	2.46	1.04	
		Range	47.1-54.8	60.1-66.0	53.6-60.0		70.5-71.9	67.2-74.2	69.1-72.5	
108	3	$\bar{X}$	52.2	61.3	56.7	166.3	70.8	69.5	70.1	64.3
		SD	0.31	5.50	1.94	8.34	2.93	1.67	1.45	0.22
		Range	51.9-52.6	57.8-66.1	54.8-59.4	159-178	67.6-74.7	68.1-71.9	68.1-71.4	64.0-64.5
128	26	$\bar{X}$	51.0	61.8	56.3	150.7	68.9	75.5	71.2	61.7
		SD	2.62	3.70	2.87	6.57	3.55	3.58	2.51	3.02
		Range	45.8-55.1	55.4-72.4	51.2-62.7	141-161	62.0-76.1	67.6-80.8	65.8-75.6	54.5-66.4
148	6	$\bar{X}$	51.4	61.7	56.5	*	69.7	69.4	68.5	*
		SD	1.75	2.89	2.14		2.44	1.47	1.55	
		Range	48.7-54.2	56.1-65.1	52.4-58.4		66.2-74.2	66.7-70.9	66.7-70.8	

1 Expressed in terms of moles x 10^{10} acetylcholine hydrolyzed/min/mg tissue.

2 Expressed in terms of moles x 10^{9} DPNH formed/min/mg tissue.

* Subcortical values are not available for these groups.

Table III

Correlations between and among Cholinesterase and Lactic Dehydrogenase Activity

Correlations among Cholinesterase Values

Age in Days	Strain	N	V vs S	V vs Subcortex	S vs subcortex	$\frac{V+S}{2}$ vs subcortex
110	S_1	20	0.75***	0.42	0.15	0.30
129	S_1	15	0.54**	0.03	0.37	0.26
128	S_3	26	0.67***	0.37	0.17	0.28
Both str. combined [1]		61	0.67***	0.31*	0.21	0.28*

Correlations among Lactic Dehydrogenase Values

Age in Days	Strain	N	V vs S	V vs Subcortex	S vs Subcortex	$\frac{V+S}{2}$ vs Subcortex
110	S_1	20	0.04	0.17	-0.20	0.04
129	S_1	15	0.03	-0.17	-0.46	-0.50
128	S_3	26	-0.05	-0.04	0.26	0.17
Both str. combined [1]		61	0.00	0.00	-0.07	-0.04

Correlations between Cholinesterase and Lactic Dehydrogenase Values

Age in Days	Strain	N	V vs V	S vs S	Subcortex vs subcortex	$\frac{V+S}{2}$ vs $\frac{V+S}{2}$
110	S_1	20	0.56**	-0.05	-0.04	0.37
129	S_1	15	-0.04	0.07	0.35	0.07
128	S_3	26	0.15	0.35	-0.21	0.51**
Both str. combined [1]		61	0.26	0.16	-0.22	0.37**

* Significant at 0.05 level

** Significant at 0.01 level

*** Significant at 0.001 level

[1] Correlations for the combined strains are weighted averages using Fisher's r to z transformation

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Fig. 1. Boxes A and B give cortical lactic dehydrogenase activity for the S_1 and S_3 strains, respectively. The LDH is expressed in terms of moles x 10^9 DPNH formed/min/mg tissue. The mean LDH activity of the visual area (V) of the cortex is shown by a solid dot, the upper and lower ends of the solid bar indicating $\pm$ one standard deviation from the mean. The LDH activity of the somesthetic area (S) is similarly shown by an open dot and a dotted bar. For each age, the number of animals is indicated. Since the LDH values for visual and somesthetic areas overlap, the visual values have been displaced slightly to the left and the somesthetic values slightly to the right on the graph.

Boxes C and D give corresponding values for cortical cholinesterase activity expressed in terms of moles x 10^{10} ACh hydrolyzed/min/mg tissue.

Cortical ChE

Cortical LDH

Age in Days

Age in Days

Strain

Strain

A

B

C

D