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The Effect of Starvation, Restricted Feed Intake and Refeeding on Acid Phosphatase Activity of the Hypothalamus and Frontal Cerebral Cortex of the Rat

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Acid phosphatase (EC: 3.1.3.2) activity at the hypothalamus and frontal cerebral cortex has been studied in male rats maintained on different dietary regimes.

The enzyme activity in the cerebral cortex did not undergo any observable changes in any of the experimental groups. Enzyme activity in the hypothalamus, rose after food deprivation in both acutely and chronically starved rats, while the enzyme activity returned to physiological levels in refed animals. The hypothalamus neuroendocrine role and a possible interregional heterogeneity for acid phosphatase in rat brain might explain the two enzymic activity patterns observed in the present experiment.

In the lysosomes, a battery of hydrolytic enzymes which react most efficiently at an acidic pH have been monitored in different tissues. The role of these so-called «lysosomal enzymes» included the degradation of all major classes of biological macromolecules (2, 3, 5, 9, 10, 12), as well as to digest whole organelles and substructures of cells (11, 16).

Under conditions of severe food restriction, the specific activity of acid phosphatase and other lysosomal enzymes in the liver increased, whereas the organ weight and the protein content decreased (6). «Lysosomal enzymes» exhibiting latency properties have been monitored in different brain regions during development (14, 20-22), but their role under normal or pathological conditions is poorly

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understood. In the present investigation, the acid phosphatase activity was studied as an index of lysosomal activity (8) in the hypothalamus and frontal cerebral cortex of rats subjected to acute and chronic starvation, as well as after refeeding.

Materials and Methods

Adult male Sprague-Dawley rats (270 ± 35 g) from our breeding colony were housed individually in metabolic cages in a temperature controlled room (23 ± 1° C) and a 12 h light/dark schedule. The animals were allowed several days to acclimatize to these conditions with food pellets (Biona, Las Palmas, Spain) and tap water, available *ad libitum*. The daily intake of the granulated food was carefully recorded during this period. Thereafter animals were randomly divided as follows (n = 10 rats per group). Control (C) fed *ad libitum* during the 28 days, acutely starved (AS) fed *ad libitum* during 21 days, and thereafter complete food removal for 7 days; chronically starved animals (CS) were fed *ad libitum* for 7 days, starved for 7 days, followed by 1/4 normal diet for 14 days; refed group (R) were subjected to 7 days total food deprivation, followed by 1/4 normal diet for 14 days and complete return to *ad libitum* feed for 7 days (15). After the 28 day experimental period, animals were killed by decapitation and tissue samples were taken immediately from the frontal cerebral cortex and weighed. The brain was then placed on its dorsal face and the entire hypothalamus removed and weighed. On the same day, the tissues were homogenized in ice-cold saline and centrifuged at 50,000 × *g* for 30 min at 4° C.

Acid phosphatase activity [ortophosphoric-monoester phosphohydrolase (acid optimum) EC 3.1.3.2] in the supernatant fluid was assayed spectrophotometrically under strictly linear conditions. The assay mixture consisted of a medium (1 ml) containing (final concentration) 50 mM citrate buffer and 5.5 mM p-nitrophenylphosphate at pH = 4.8. After the addition of 200 μl of supernatant containing 0.5-1.0 mg protein, the reaction mixture was incubated at 37° C for 30 min. The reaction was stopped by adding 3 ml NaOH-glycine buffer pH = 10.4 (22). Proteins were determined by the method of LOWRY *et al.* (13). Results were compared by analysis of variance (ANOVA) and the least significance (LSD) method. The level of significance was chosen as $p < 0.05$.

Results and Discussion

Experimental results for enzyme activity and protein concentration in the hypothalamus and frontal cerebral cortex are summarized in table I. Acid phosphatase indicates lysosomal activity (8), and increased activity of this enzyme in the liver is a well-known event in rats subjected to food deprivation (3, 9), whereas no changes under the same conditions have been reported in the kidney and other tissues (1).

Results from the present investigation showed that in the hypothalamus acid phosphatase activity increased in the acutely and chronically starved groups and refeeding restored enzyme activity to normal levels. In the cerebral cortex, however, no differences in enzyme activity between groups could be observed, whether expressed per mg tissue or mg protein.

These different patterns observed for enzyme activity in two different brain structures could be due to the neuroendocrine role of the hypothalamus. Increased acid phosphatase activity in the pituitary of starved rats have been related to a raised intrapituitary degradation of peptide hormones (17) which resulted in the low circulating levels of anterior pituitary hormones reported for animals subjected to a food schedule similar to ours (4, 7, 18).

Table 1. *Effect of starvation, restricted food intake and refeeding on acid phosphatase activity of the hypothalamus and frontal cerebral cortex of male rats.*
Animals were subjected to the feed regimens described in the Materials and Methods section. Results are means ± S.E. n = 10 animals per group.

Experimental groups	Hypothalamus			Cerebral cortex		
	Enzyme activity		Protein content mg/g tissue	Enzyme activity		Protein content mg/g tissue
	mU/mg tissue	mU/mg protein		mU/mg tissue	mU/mg protein	
Controls (C)	1.55±0.10	196±11	4.26±0.17	0.94±0.09	113±20	5.32±0.11
Acutely Starved (AS)	1.88±0.08[1,2]	285±17[1,2,3]	3.92±0.20	0.87±0.06	105±14	5.87±0.21
Chronically Starved (CS)	1.98±0.06[1,2]	371±22[1,2]	3.85±0.07	0.85±0.09	110±13	5.52±0.13
Refed (R)	1.48±0.07	198± 6	4.14±0.13	0.86±0.07	108±19	5.51±0.21

[1] Significant difference as compared to C group, $p < 0.05$.
[2] Significant difference as compared to R group, $p < 0.05$.
[3] Significant difference as compared to CS group, $p < 0.05$.

In the same way, high lysosomal activity during starvation in the hypothalamus of the rat could be related to low levels of hypothalamic hormones in these animals (15, 18, 19). Alternatively, the different patterns of acid phosphatase activity in different brain regions may be due to the acquisition of different cell types containing the enzyme at different concentrations. Both possibilities are not incompatible, and the interregional heterogenity for this enzyme in the two structures of the brain studied may be due to the neuroendocrine role of the hypothalamus.

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Resumen

Se estudia la actividad de la fosfatasa ácida (E.C. 3.1.3.2) en el hipotálamo y corteza cerebral frontal de ratas macho sometidas a distintos regímenes alimentarios.

No se han observado cambios en la actividad de la fosfatasa ácida en la corteza cerebral frontal entre los distintos grupos experimentales. En el hipotálamo se incrementa significativamente la actividad enzimática en los grupos sometidos a ayuno agudo y crónico, resultando la realimentación en una actividad enzimática similar a la encontrada para los controles.

Las diferencias observadas en la actividad enzimática bajo las condiciones experimentales estudiadas podrían atribuirse al papel neuroendocrino del hipotálamo y sugieren una heterogeneidad para ese enzima en el cerebro de rata.

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