Effect of Hypophysectomy on the Oxidative and Glycolytic Metabolism of Hypothalamus[1]

In previous papers[2,3] it has been demonstrated that the oxidative metabolism of hypothalamus undergoes modifications in relation with sexual activity.

Studies performed with sexual hormones in vivo[3] and in vitro[4], and with gonadotrophins in vitro[5], showed that the metabolic changes of hypothalamic metabolism are in some way related to a direct effect of gonadotrophins on the hypothalamus, and it has been proposed that such effect is connected with the hypothalamic regulation of reproductive function of pituitary[6].

The present investigation was undertaken in order to determine whether the suppression of hypophyseal gonadotrophin secretion is accompanied by modifications in the glycolytic and oxidative metabolism of hypothalamus.

Material and methods. Albino male rats fed on the standard diet of the Instituto de Fisiología and weighing between 130 and 150 g were used. Light and temperature were controlled and kept constant (25 °C; 14 h light and 10 h darkness). Hypophysectomy were performed 15–20 days before sacrifice under ether anaesthesia by parapharingeal rout. The animals were decapitated and the hypothalamus removed. The sample was placed on its dorsal surface and cut under a dissecting microscope in 3 portions by 2 frontal sections as described previously[3]. The first section was made through the optic chiasma and the second immediately behind the infundibulum; these sections divided the hypothalamus in 3 areas: a prechiasmatic region (anterior hypothalamus), a retroinfundibular region (posterior hypothalamus) and a region between the 2 sections (middle hypothalamus).

Oxygen uptake was determined by Warburg manometry in micro-Warburg vessels of 4–5 ml capacity containing 1.5 ml of Krebs-Ringer-phosphate buffer pH 7.4 and 7.7 m*M* glucose. The vessels were gassed for 5 min with 100% O_2; 15 min were allowed for equilibration and the observation period lasted 60 min. Results expressed as µl O_2/mg wet tissue/h have been compared with Student's *t*-test accordingly with FISHER and YATES[7].

Following incubations, the vessels were removed from the manometers and placed in an ice bath. Samples for lactate determination were pipetted into trichloroacetic acid (10% final concentration). Lactic acid accumulation was determined by measuring the initial and final concentration in the incubating media by the method of BAKER and SUMMERSON[8] and expressed as µmoles produced per gram of hypothalamic tissue and hour.

Results. The oxygen uptake of anterior, middle and posterior hypothalamus from normal and hypophysectomized rats is summarized in the Table. As can be seen, the anterior and posterior hypothalamic areas of hypophysectomized rats showed a significant increase ($p < 0.01$ and 0.05 respectively) in the oxygen uptake as compared with the controls. The oxidative metabolism of the middle hypothalamus was similar in both groups of rats. There were no differences between normal and hypophysectomized rats in the lactic acid production of the 3 hypothalamic areas.

Discussion. Our results showed that hypophysectomy performed in male rats produces a significant increase in the oxygen uptake of the anterior and posterior hypothalamus without modifications in the middle hypothalamus. No differences were found in the lactic acid production of the different hpothalamic areas between hypophysectomized and control rats.

It has previously been reported[3] that castration decreases the oxidative metabolism of the anterior and posterior hypothalamic areas and that the values of oxygen uptake of cerebral cortex and hypophysis are higher in castrated than in normal rats. Since testosterone was able to correct in vivo and in vitro[4] the metabolic changes of cerebral cortex and pituitary, it has been proposed that the hormone has a direct effect upon these structures. The fact that the hypothalamic modifications of castrated rats are only corrected by testosterone in vivo may indicate that the mechanisms implicated in the metabolic modifications of hypothalamus in gonadectomized rats are different from those that take place in the cerebral cortex and pituitary.

Many endocrine modifications are present in the hypophysectomized rats that could be responsible for the oxidative changes in the anterior and posterior hypothalamus described in the present paper. Nevertheless, the fact that the increases of gonadotrophin secretion are accompanied by a decreased oxygen uptake in the hypothalamus (castrated rats) and that the suppression of gonadotrophins (hypophysectomized rats) produces an increase in the oxidative activity of hypothalamus, may support the hypothesis that the metabolic changes of

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	Hypothalamus		
	Anterior	Middle	Posterior
	QO_2 (µl O_2/mg wet tissue · h)		
Control	1.60 ± 0.03[a] (15)	1.44 ± 0.07 (13)	1.41 ± 0.07 (17)
Hypophysectomized	1.80 ± 0.05 (15)	1.31 ± 0.05 (13)	1.70 ± 0.10 (17)
p value	0.01	N.S.	0.05
	Lactate production (µmoles of lactate/g wet tissue · h)		
Control	26.7 ± 1.38 (13)	25.6 ± 1.90 (13)	28.3 ± 1.60 (10)
Hypophysectomized	27.2 ± 2.13 (13)	27.0 ± 2.25 (13)	28.4 ± 1.87 (10)
p value	N.S.	N.S.	N.S.

[a] Mean standard error. In parentheses No. of determinations.

[1] Supported by a grant of the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

[2] J. A. MOGUILEVSKY and M. R. MALINOW, Am. J. Physiol. *206*, 855 (1964).
[3] J. A. MOGUILEVSKY, O. SCHIAFFINI and V. G. FOGLIA, Life Sci. *5*, 447 (1966).
[4] J. A. MOGUILEVSKY, Acta physiol. latinoam. *16*, 353 (1966).
[5] J. A. MOGUILEVSKY, N. GHITTONI and O. SCHIAFFINI, Acta physiol. latinoam. *16*, 86 (1966).
[6] J. A. MOGUILEVSKY and L. RUBINSTEIN, Neuroendocr. *2*, 213 (1967).
[7] R. A. FISHER and F. YATES, *Statistical Tables for Biological, Agricultural and Medical Research*, 5th edn (Hafner, New York 1957).
[8] S. B. BAKER and W. H. SUMMERSON, J. biol. Chem. *138*, 535 (1941).

the hypothalamus during sexual activity are related to a direct effect of gonadotrophins on this nervous structure.

Since the oxidative metabolism, in the central nervous system, is one of the principal sources of the high energy compounds which are needed for protein synthesis, and considering the probable peptide nature of the hypothalamic releasing substances[9], it has been proposed[5] that the metabolic changes of hypothalamus are directly related to the formation of these peptides. If such an hypothesis is consistent, it is probable that the increased oxygen uptake of hypothalamus of hypophysectomized rats is related to the increase of gonadotrophin-releasing factors described in such animals[10]. Nevertheless, further evidence is needed before a conclusion can be reached on this point.

Resumen. En el presente trabajo se ha estudiado el consumo de oxígeno y la producción de ácido láctico en hipotálamo anterior, medio y posterior en animales hipofisectomizados. Los resultados obtenidos indicaron que la hipofisectomía produce un significativo incremento en la actividad oxidativa del hipotálamo anterior y posterior sin modificar el hipotálamo medio.

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[9] G. W. HARRIS, M. REED and C. P. FAWCETT, Br. med. Bull. *22*, 266 (1966).
[10] R. NALLAR and S. M. MCCANN, Endocrinology *76*, 272 (1965).
[11] Research Fellow from Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.
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Autoradiographic Studies on the Neurosecretory Hypothalamo-Hypophysial System of the Grass Frog (*Rana pipiens*) After Disconnection of the Distal Hypophysis

In 1949, with the discovery of the applicability of Gomori's chromium hematoxylin phloxin method for the study of neurosecretory phenomena[1], research in neurosecretion entered a new phase. Since then 2 concepts have prevailed as to the possible origin of the neurosecretory substance: the classical concept considers the neurosecretory material to be produced in the perikaryon of the neurons of the supraoptic and paraventricular nuclei and conveyed by a proximo-distal axoplasmic flow in the neurohypophysis; on the other hand the concept of a synthesis of neurosecretory granules within the axon was put forward[2–4]. Recently, however, in the light of new electron microscopic findings, the latter hypothesis was modified to conceive a packaging and pulling together of amino acids and/or polypeptides and proteins within the neurotubules, considered to be continuous with the Golgi apparatus, to take place in the distal parts of the neurons[5–7]. SACHS and collaborators[8,9] suggest that a precursor molecule which contains vasopressin in a biologically inactive form precedes the activation of the hormone in the more distal part of the neuron. Our current experiments were designed to yield additional information on the possible existence of a distal hormone synthesis, a packaging and/or activation of a precursor molecule; they were carried out on 23 grass frogs which received 10 or 20 μc of S-35 L-cysteine hydrochloride via i.p. injection immediately after transection of the proximal hypophysis. They were sacrificed at various time intervals between 1 h and 4 days after transection; at the same time 11 control animals were sacrificed. In addition 350 experimental and 167 control animals were available for comparison studies using standard methods for the demonstration of neurosecretory material and electron microscopy.

One hour after the injection of the labeled amino acid into the control animals a slight darkening can be observed in the distal lobe of the neurohypophysis; a higher uptake is noticed in the distal and intermediate lobes of the adenohypophysis. 12 h after the injection both the distal and the proximal parts of the neurohypophysis are characterized by an increased uptake of labeled substance; as expected, due to the few neurosecretory granules which are found in neurosecretory nerve fibers of the proximal neurohypophysis and the ventral hypothalamus, there are only a few black granules observed here making it impossible to demonstrate the neurosecretory pathway by this method. At the same time a much higher uptake is observed in the distal and intermediate lobes of the adenohypophysis. A slight intensification of the phenomena observed at 12 h occurs at 24 h; conditions remain essentially the same after this period (Figure 1).

Six hours after transection of the proximal hypophysis the first appearance of radioactive material can be observed in the proximal as well as in the distal parts of the neurohypophysis, but there is a definite concentration at the distal end of the proximal stump. The distal lobe and especially the intermediate lobe of the adenohypophysis have a higher uptake than the distal neurohypophysis. 12 h after the surgical intervention a higher uptake, as compared to the control animals and

[1] W. BARGMANN, Z. Zellforsch. mikrosk. Anat. *34*, 610 (1949).
[2] J. CHRIST, F. ENGELHARDT and R. DIEPEN, in 2nd Int. Symp. on Neurosecretion (Ed. W. BARGMANN, E. HANSTRÖM and E. SCHARRER; Springer, Berlin-Göttingen-Heidelberg 1958), p. 30.
[3] J. F. CHRIST, Mem. Soc. Endocr. *12*, 125 (1962).
[4] H.-D. DELLMANN, J. Hirnforsch. *5*, 249 (1962).
[5] H. A. BERN and F. G. W. KNOWLES, in *Neuroendocrinology* (Ed. L. MARTINI and W. F. GANONG; Academic Press, New York 1966), p. 139.
[6] H.-D. DELLMANN, H. E. DALE, P. A. OWSLEY and L. F. ELDRIDGE, Experientia *24*, 383 (1968).
[7] H.-D. DELLMANN and P. A. OWSLEY, Z. Zellforsch. mikrosk. Anat. *87*, 1 (1968).
[8] H. SACHS and Y. TAKABATAKE, Endocrinology *75*, 943 (1964).
[9] H. SACHS, R. PORTANOVA, E. W. HALLER and L. SHARE, in *Neurosecretion* (Ed. F. STUTINSKY; Springer, Berlin-Heidelberg-New York 1968), p. 146.