Oxidative Metabolism of the Hypothalamus in Hypophysectomized-Castrated Rats[1]

In previous papers[2,3] it has been demonstrated that the oxidative activity of hypothalamus is modified in situations associated with changes in the secretion of gonadotrophins (e.g. castration, hypophysectomy), and it was postulated that these metabolic modifications are related to changes in the synthesis of the gonadotrophin releasing factors.

FSH and LH exert inhibitory effects on the synthesis and/or release of the FSH and LH releasing substances[4,5] as well as on the oxidative metabolism of the anterior and posterior hypothalamic areas[6]. In absence of these gonadotrophins (hypophysectomized rats), the FSH and LH substances are hypersecreted and become detectable in plasma[7,8]. Furthermore, there is a marked increase in the oxygen uptake of the anterior and posterior hypothalamus in hypophysectomized rats[3].

It has been demonstrated that the reduction of circulating androgens may enhance FSH releasing factor synthesis[9], and that androgens in vivo are able to modify the metabolic activity of hypothalamus[2,6]. Thus the increase in the oxygen uptake of hypothalamus observed in hypophysectomized rats[3] could reflect changes induced by the reduction in androgen levels as well as changes induced strictly by the absence of gonadotrophins. It therefore seemed to be of interest to compare the oxidative activity of different hypothalamic areas in hypophysectomized rats, in which some androgen secretion remains, with hypophysectomized-castrated rats in which the testicular androgen secretion has been completely eliminated.

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, 10 h darkness). Hypophysectomy and castration were performed simultaneously 15–20 days before sacrifice under ether anesthesia. The animals were decapitated and the hypothalamus removed. The sample was placed on its dorsal surface and cut under dissecting microscope in 3 portions by 2 frontal sections, as described previously[2]. 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 were expressed as µl of O_2/mg wet tissue/h and analyzed for variance following SNEDECOR[10]. The statistical significance of the data was determined according to TUKEY's method[11]. The minimal significant difference of the means was 0.17 in the anterior hypothalamus and 0.25 in the posterior hypothalamic areas.

Results. As can be seen in the Table, hypophysectomy increases the oxygen uptake of the anterior and posterior hypothalamus. Hypophysectomized-castrated rats showed similar values as control rats, being significantly lower than those observed in the anterior and posterior hypothalamus of hypophysectomized rats. No differences in the oxygen uptake of the middle hypothalamus were found between the groups.

Discussion. Our results demonstrated that the increase in the oxidative activity of the anterior and posterior hypothalamic areas of hypophysectomized rats described in a previous paper[3] and confirmed in this paper is abolished if the animals are castrated at the time of hypophysectomy.

In a previous paper[2] it has been demonstrated that castration depresses the oxidative metabolism of the anterior and posterior hypothalamic areas. Since testosterone corrected these changes in vivo but not in vitro[12], and since LH and FSH have a depressor effect on the respiration of the anterior and posterior hypothalamus[6], it was postulated that the hypothalamic metabolic changes observed in gonadectomized animals were connected with the increase in the gonadotrophin secretion. The elevated hypothalamic oxygen uptake observed in hypophysectomized rats further support this hypothesis.

Assuming that the lack of gonadotrophin secretion is directly connected with the increase of the oxidative metabolism in the anterior and posterior hypothalamic areas in hypophysectomized rats, it is difficult to explain how gonadectomy in these rats restored to normal the

Oxygen uptake of different hypothalamic areas of male rats

	QO_2 (µl O_2/mg wet tissue/h) Hypothalamus		
	Anterior	Middle	Posterior
A. Control	1.56 ± 0.05 (22)	1.45 ± 0.07 (12)	1.42 ± 0.05 (25)
B. Hypophysectomized	1.87 ± 0.10 (11)	1.31 ± 0.04 (11)	1.87 ± 0.11 (11)
C. Hypophysectomized-castrated	1.45 ± 0.05 (24)	1.46 ± 0.02 (17)	1.50 ± 0.09 (22)
Analysis of variance			
F ratio	8.44	0.90	6.41
P value	<0.01	NS	<0.01
Multiple comparisons test			
$P < 0.05$ between	A vs B B vs C		A vs B B vs C

Mean ± standard error. In parentheses, number of determinations.

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

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high metabolic levels of hypothalamus. Nevertheless these observations are in complete agreement with those performed by SAITO et al.[8], in which it was demonstrated that FSH releasing factor appears in the peripheral blood of hypophysectomized rats, probably as a consequence of an increased synthesis of this hypothalamic factor, and that this peripheral FSH releasing factor activity disappears after castration of hypophysectomized animals. If changes in the oxidative metabolism of hypothalamus are considered as representative of modifications in the synthesis of the releasing factors, these experiments could help explain the decrease in the oxygen uptake of the anterior and posterior hypothalamus which was observed in hypophysectomized rats after gonadectomy.

Since the only difference between hypophysectomized-castrated and hypophysectomized rats is that in the latter some androgen secretion remains, it can be postulated that the absence of the depressor effect of gonadotrophins is not solely responsible for the increase in the metabolic activity of hypothalamus; the presence of some androgenic activity is also necessary for this increase. Whether or not this finding is concerned with the fact that the presence of testes is necessary for the increase of FSH releasing factor in hypophysectomized rats remains to be studied.

Resumen. Los resultados obtenidos han indicado que el incremento del consumo de oxígeno en el hipotálamo anterior y posterior de los animales hipofisectomizados desaparece si estos animales son castrados simultaneamente con la hipofisectomía. Se discute la importancia de la presencia del testículo en la elevación de la actividad metabólica del hipotálamo luego de la hipofisectomía.

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The Karyotype of the Black Rat (*Rattus rattus* L.). Another Population with a 38-Chromosomes Complement

Following the classical observations of numerous authors[1–4], the diploid number of the black rat (*Rattus rattus* L.) is now fixed at $2n = 42$. This diploid number has also been confirmed recently by YOSHIDA et al.[5], for black rats of 2 Japanese populations, and by YONG[6] in the Malayan *Rattus rattus diardii* (Jentink). Both these recent observations were carried out by employing the bone marrow technique, which allows an excellent characterization of the morphology of the karyotype. The results of all these observations indicate, for *Rattus rattus*, a diploid number that is unmistakably fixed at $2n = 42$. In fact, this is the diploid number given for this species in the latest critical list of the diploid numbers of the eutherian mammals[7].

However, in a number of this periodical there is a report by BIANCHI et al.[8] on 2 South American populations of *Rattus rattus* showing a chromosome-complement of $2n = 38$. This fact has prompted us to publish our past observations, which were merely a preliminary report by two of us[9], on the discovery of a similar chromosome complement in an Italian population of *Rattus rattus*.

In the course of an investigation involving studies on the indigenous populations of the small Italian islands[10], we had the opportunity of studying, from the cytotaxonomic point of view, the populations of *Rattus rattus alexandrinus* Geoffroy of the islands of Giglio and Giannutri (southern Tuscan archipelago). The karyotype of the black rats captured here is constantly characterized by a diploid number of $2n = 38$.

A study was made of the chromosomes of 13 specimens from Giglio and 7 from Giannutri, using both the method of primary cultures of splenic fibroblasts and that of bone marrow. In order to check whether the karyotype anomaly was limited to the island area, we studied the populations of *Rattus rattus* of the Argentario promontory, which is connected to the mainland by 2 natural dunes and an artificial dam (see map shown in Figure 3), and of the inland area of the Tuscan Maremma. Also in these cases (14 specimens studied) the karyotype was constantly found to be characterized by $2n = 38$.

Also in our case, as in that reported by BIANCHI et al.[8], the decrease in the diploid number may be imputed to 2 Robertsonian[11] translocations, in a homozygous condition, which have produced 2 pairs of large metacentric autosomes from 4 pairs of acrocentric chromosomes. The pairs of acrocentric autosomes involved in this Robertsonian process would appear to be the same in the 2 populations (Italian and South American), at least as far as may be seen from a comparison between our figures and those published by BIANCHI et al.[8]. A precise karyometric assessment will provide more reliable information in this respect, and we are now in process of working on our karyometric data.

The interest of this report goes well beyond a mere confirmation of a strange diploid number. In fact, we find ourselves in the position of having to justify data in

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