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**In vitro effects of hormones upon the lysosomal
enzymes in the hypothalamo-hypophyseal system of
the male frog**

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Biologia. — *In vitro effects of hormones upon the lysosomal enzymes in the hypothalamo-hypophyseal system of the male frog* (*).
Nota di MARIO MILONE, MARIA F. CALIENDO, MARINA GRECO e RAKESH K. RASTOGI, presentata (**) dal Socio G. MONTALENTI.

RIASSUNTO. — L'attività *in vitro* della β -glucuronidasi e della fosfatasi acida è variamente influenzata nel sistema ipotalamo-ipofisario di maschi adulti di *Rana esculenta* dagli ormoni endogeni (prodotti dalle ghiandole periferiche). Gli Autori discutono in particolare l'influenza del testosterone sull'attività di questi due sistemi enzimatici.

INTRODUCTION

The importance of the hypothalamo-hypophyseal system in neuroendocrine activities has been widely recognized in vertebrates.

The presence of acid phosphatase and β -glucuronidase has been demonstrated in the hypothalamo-hypophyseal system of frog, both histochemically and biochemically (Rastogi and Milone, 1974; Milone and Rastogi, 1976). The activity of these two hydrolases has been shown to manifest an annual pattern and surgical procedures like castration and androgen replacement therapy have profound effects in the *in vivo* enzyme activity (Milone *et al.*, 1975; Iela *et al.*, 1974). It has also been considered that lysosomal hydrolases play a role in the transformation of hormone molecules from the precursor to the functional stage thus regulating the mechanisms of action of hormones (see also Pitot and Yatvin, 1973). Thus this work was undertaken to elucidate the *in vitro* effects of some hormones (produced in the peripheral target endocrine glands) on acid phosphatase and β -glucuronidase activity in the pituitary gland and hypothalamus of the green frog, *Rana esculenta*.

MATERIALS AND METHODS

Adult males of *Rana esculenta* were used in this study. After decapitation, the pituitary gland and hypothalamus (from median eminence to preoptic area) were taken, weighed and homogenized in deionized water at 0°-4 °C. Organs from three animals were pooled for each determination.

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(**) Nella seduta dell'11 marzo 1978.

TABLE I

Effects of hormones on the β -GLR and ACP activities in the pituitary gland and hypothalamus of Rana esculenta.

ACP			β -GLR		
Hormone concentration	Pituitary Gland	Hypothalamus	Hormone concentration	Pituitary Gland	Hypothalamus
T (*)			T		
1,5 pM	469 \pm 35	329 \pm 27	0,1 pM	3,84 \pm 0,90	4,39 \pm 0,80
7,5 pM	467 \pm 40	361 \pm 73	1,0 pM	3,88 \pm 0,68	5,10 \pm 0,87
15 pM	477 \pm 47	386 \pm 21	10 pM	4,08 \pm 0,75	5,78 \pm 1,21
75 pM	500 \pm 43	403 \pm 37	100 pM	5,05 \pm 0,93	6,00 \pm 1,68
150 pM	540 \pm 46	406 \pm 41	1,0 pM	5,77 \pm 0,92	5,87 \pm 1,24
750 pM	469 \pm 33	380 \pm 24	10 nM	5,87 \pm 1,06	5,76 \pm 1,33
Ref.	33 \pm 28	296 \pm 24	Ref.	3,52 \pm 0,42	3,96 \pm 0,56
17 β -E ₂			17 β -E ₂		
2 pM	307 \pm 25	315 \pm 31	0,1 pM	2,87 \pm 0,36	2,44 \pm 0,28
10 pM	274 \pm 28	259 \pm 27	1,0 pM	2,84 \pm 0,45	2,33 \pm 0,39
20 pM	175 \pm 17	214 \pm 26	10 pM	3,19 \pm 0,37	2,16 \pm 0,24
100 pM	155 \pm 19	161 \pm 19	100 pM	2,49 \pm 0,45	2,00 \pm 0,33
200 pM	115 \pm 12	124 \pm 19	1,0 nM	3,15 \pm 0,30	1,77 \pm 0,26
1000 pM	72 \pm 8	102 \pm 12	10 nM	3,08 \pm 0,28	1,55 \pm 0,19
Ref.	336 \pm 28	296 \pm 24	Ref.	3,52 \pm 0,42	3,96 \pm 0,56
CST			CST		
10 pM	356 \pm 45	231 \pm 18	10 pM	3,29 \pm 0,34	1,92 \pm 0,34
40 pM	227 \pm 20	200 \pm 6	40 pM	3,40 \pm 0,37	1,88 \pm 0,37
80 pM	206 \pm 17	184 \pm 6	80 pM	3,35 \pm 0,35	1,94 \pm 0,34
160 pM	196 \pm 16	174 \pm 6	160 pM	3,42 \pm 0,30	2,24 \pm 0,31
240 pM	193 \pm 16	153 \pm 5	240 pM	3,48 \pm 0,52	2,52 \pm 0,47
320 pM	179 \pm 14	151 \pm 4			
Ref.	336 \pm 28	296 \pm 24	Ref.	3,52 \pm 0,42	3,92 \pm 0,56
T ₄			T ₄		
1 pg	355 \pm 23	296 \pm 32	1 pg	3,50 \pm 0,36	3,79 \pm 0,92
10 pg	313 \pm 39	341 \pm 36	10 pg	3,49 \pm 0,41	4,21 \pm 0,81
50 pg	361 \pm 15	363 \pm 39	50 pg	3,54 \pm 0,28	4,41 \pm 0,46
100 pg	279 \pm 8	387 \pm 20	100 pg	3,22 \pm 0,30	4,56 \pm 1,02
500 pg	269 \pm 22	474 \pm 52	500 pg	3,46 \pm 0,35	4,38 \pm 0,76
1,0 μ g	237 \pm 19	400 \pm 32			
Ref.	336 \pm 28	296 \pm 24	Ref.	3,52 \pm 0,42	3,96 \pm 0,56

(*) T = testosterone; 17 β -E₂ = 17 β -estradiol; CST = corticosterone; T₄ = thyroxine.

Acid phosphatase (ACP) activity was determined by the method taken from Chieffi *et al.* (1966) as modified by Milone *et al.* (1975). The enzyme activity was expressed as pmol paranitrophenol liberated/ μ g protein/min. For β -glucuronidase (β -GLR) the Lin and Fishman (1972) method was employed and the enzyme activity expressed as nmol phenolphthalein liberated/ μ g protein/min. Proteins were determined according to the method of Lowry *et al.* (1951).

RESULTS (Table I)

Effects of testosterone. Administration of increasing concentrations of testosterone to the incubation medium containing tissue homogenate tended to stimulate ACP and β -GLR activities in both the pituitary gland and the hypothalamus.

Effects of estradiol-17 β . Contrary to the effect of testosterone, estradiol-17 β inhibited the activity of both hydrolases in the pituitary gland and hypothalamus. A dose-response relationship was observed as far as the estrogen-dependent inhibition of the enzyme activity was concerned.

Effects of corticosterone. As far as the acid phosphatase activity is concerned, corticosterone had effects quite similar to those obtained with estradiol-17 β in both the pituitary and the hypothalamus homogenates. This hormone did inhibit β -GLR activity too, but to a lesser extent in the hypothalamus. Interestingly enough, no dose-response relationship was observed in the pituitary homogenate but in the hypothalamus lower concentrations of corticosterone showed a greater inhibitory potentiality.

Effects of thyroxine. This hormone showed variable effects. In fact it tended to inhibit acid phosphatase activity in the pituitary gland and stimulated the enzyme activity in the hypothalamus. A regular dose-response relationship in both directions was observed. The β -GLR activity remained unchanged to a large extent in the pituitary gland, but in the hypothalamus a slight stimulation (dose-dependent) was found.

DISCUSSION

The main conclusion that emerges from this study concerns the effects of testosterone upon the acid phosphatase and β -GLR activity of the hypothalamo-hypophyseal system. The present findings further strengthen the evidence that hydrolytic enzymes in the male frog (at least in the target organs) are under androgen control. In addition it should be emphasized that the *in vivo* and *in vitro* effects of testosterone are similar i.e. this androgen stimulates the acid hydrolases of the pituitary gland and hypothalamus, thus intervening in the hormonal regulatory mechanism (see Milone and Rastogi, 1976 and Iela *et al.*, 1974). Estradiol-17 β , present in trace amounts in the plasma of male *Rana esculenta* only in the spring months, behaves in our experiments

as an "antiandrogen". This implicates that the homogenates of pituitary gland and hypothalamus contain a certain amount of endogenous active androgen molecule. Otherwise it could simply be stated that estrogens inhibit the hydrolytic enzymes of the hypothalamo-hypophyseal system of frog. A similar *in vitro* study in the female frogs showed that here too estradiol-17 β inhibits the acid hydrolases (unpublished results).

Corticosterone has been found to be the most potent corticosteroid in amphibians (Mehdi, 1971), and thus its effects upon the enzymatic activity of the tissues in question can be considered in relationship to the feed-back machinery. Similarly, thyroxine also acts upon the pituitary gland and hypothalamus.

These results thus indicate that the circulating hormones, originated in the peripheral endocrine glands, do act upon the enzymatic activity of the hypothalamo-hypophyseal system in correlation with the feed-back system that should be operating in a way quite similar to that of higher vertebrates.

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