Relationship of ornithine decarboxylase activity to HCG induced androgen production by rabbit testis[1]

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Summary. In the rabbit testicular ornithine decarboxylase activity and androgen content as well as serum androgens were increased 4 h after the injection of HCG. D,L-α-difluoromethylornithine (α-DFMO) alone or together with HCG inhibited enzyme activity although testicular and serum androgens were significantly increased after α-DFMO and HCG. These data suggest that ODC activity is not required for testicular androgen production.

Since the induction of ornithine decarboxylase (ODC, E.C. 4.1.1.17) occurs after trophic hormone stimulation it has been suggested that this enzyme may be a useful marker for hormone response[2]. Ornithine decarboxylase activity is the rate limiting enzyme in polyamine synthesis[2]. In ovarian tissue gonadotropins can increase ODC activity[3-7] as well as steroid synthesis[8-11]. The enzyme can also be stimulated in the testes of neonatal mice[12] and immature rat[13] but no correlation was made with testosterone synthesis. It was therefore of interest to determine whether such a correlation occurred in the male rabbit.

Materials and methods. 29 male New Zealand White rabbits weighing 3.33 ± 0.09 kg were studied at least 2 weeks after acclimatization to laboratory conditions. Human chorionic gonadotropin (HCG) was obtained from Sigma. DL-1-^{14}C-ornithine monochloride (51.3 mCi/mmole) was purchased from New England Nuclear. D,L-α-difluoromethylornithine (α-DFMO) was generously donated by Centre de Recherche Merrell International, Strasbourg, France.

Blood was withdrawn from the ear vein and the animal was anesthetized. The left testis was removed, weighed, a piece fixed for histological examination and the rest homogenized in 2-3 vols of ODC buffer A[14]. The left testis served as control for each animal studied.

The right testis was injected with either 100 µl of normal saline, HCG (2 mg/ml saline), α-DFMO (100 mM) or HCG plus α-DFMO. Whenever α-DFMO was used it was injected at 2 points in the testis. 2 h later the testis was again injected with α-DFMO or saline and the animal sacrificed 2 h after the 2nd injection. Blood was taken at 2 and 4 h after the left testis was removed. At the time of sacrifice the right testis was removed and homogenized in enzyme buffer A (1 mM $MgCl_2$, 1 mM dithioerythritol, 0.01% NaN_3, 250 mM sucrose and 20 mM Tris-HCl, pH 7.8). The homogenate was centrifuged at 100,000 × g for 30 min. The supernatant was used for ODC activity[14], protein determination by the method of Lowry et al.[15] and androgen content.

For ODC activity, there was a linear relationship with temperature (maximum 37 °C), protein concentration (0.2-2.0 mg protein) and time (15-90 min). The K_m for the enzyme activity was calculated to be 0.4 mM. Buffer B used in the assay contained 9 mM dithioerythritol, 0.4 mM pyridoxal phosphate, 2 mM L-ornithine, 80 mM Tris HCl pH 7.0, 0.3 µCi DL-1-^{14}C-ornithine. Enzyme activity was done the same day or after less than 1 week storage at −70 °C.

Serum androgens were determined by the method of Moor and YoungLai[16]. The 100,000 × g supernatant of testicular tissue was diluted 1:25, 1:50 and 1:100 and assayed directly for androgens against a testosterone standard curve. Parallel curves were obtained with the testicular homogenate and the standard curve.

Results and discussion. Histologically, all testes had well developed spermatogenic elements with sperms present within the tubules. Testicular weights were 2.36 ± 0.1 g (n = 58).

In a series of experiments up to 2 h, ODC activity was not significantly altered after intratesticular injection of HCG (1280 ± 57 pmoles $^{14}CO_2$/h/mg protein at 0 h and 928 ± 160 pmoles $^{14}CO_2$/h/mg protein at 1 h, n = 3). For the 2-h study the ODC activity of control testes was 1740 ± 733 pmoles $^{14}CO_2$/h/mg protein and HCG injected 2327 ± 1054 pmoles $^{14}CO_2$/h/mg protein (n = 3). At 4 h ODC activity had increased by 99.7% (table). This table also shows that ODC activity was significantly depressed by α-DFMO even in HCG injected testes.

HCG also caused a significant increase in testicular androgen concentration and serum androgen concentrations at 4 h. α-DFMO had no effect on the HCG induced increase in testicular androgen content or serum androgens. α-DFMO seemed to depress serum androgens.

These results demonstrate that intratesticular injections of HCG can stimulate ODC activity after 4 h. This interval is similar to that observed in male rats[13,17] after trophic hormone administration and in neonatal mice testes after the injection of epidermal growth factor[12].

α-DFMO, is an enzyme-activated irreversible inhibitor of ODC[18]. At the concentrations used in the present study enzyme activity was not completely inhibited. The percent inhibition was similar in testes treated with α-DFMO alone and in testes treated with HCG and α-DFMO. It was assumed that the α-DFMO injected at 2 points in the testis would have diffused rapidly enough to influence activity in the whole testis. In preliminary experiments a single injection of 100 mM α-DFMO was not sufficient to inhibit ODC activity for the 4-h interval studied. From the table it is clear that α-DFMO significantly inhibited ODC activity

Effects of HCG and α-DFMO on ornithine decarboxylase activity and androgen content of testicular tissue and serum androgens

	Testicular ornithine decarboxylase activity (pmoles $^{14}CO_2$/h/mg protein)		Testicular androgen content (ng androgen/mg protein)		Serum androgens (ng/ml)	
	0 h	4 h	0 h	4 h	0 h	4 h
Saline (n = 5)	840 ± 158	581 ± 107	4.37 ± 1.79	1.17 ± 0.33	1.90 ± 0.55	0.67 ± 0.21
α-DFMO (n = 4)	1064 ± 46	484 ± 76*	9.99 ± 3.97	3.02 ± 1.33	2.46 ± 0.41	1.79 ± 0.31***
HCG (n = 8)	826 ± 184	1560 ± 395*	3.75 ± 1.40	7.84 ± 0.87*	1.94 ± 0.31	4.08 ± 0.59**
α-DFMO + HCG (n = 6)	1185 ± 96	539 ± 72**	1.38 ± 0.25	6.51 ± 0.65**	1.37 ± 0.27	2.37 ± 0.15****

Each animal served as its own control (0 h). Results were expressed as mean ± SEM. Numbers in parenthesis refer to number of animals sacrificed. Statistical analysis was done using Student's paired t-test and compares results at 0 h with those at 4 h. * $p < 0.025$; ** $p < 0.001$; *** $p < 0.05$; **** $p < 0.005$.

compared to saline injected controls and also completely suppressed the HCG stimulated increase in activity. However, steroidogenesis as measured by testicular and serum androgen concentrations was not suppressed in the presence of α-DFMO. The observed decrease in serum androgen after α-DFMO is not necessarily due to the inhibitor.

In view of the long half-life[19] of polyamines it is not possible to conclude that the depletion of polyamines can allow the expression of more differentiated cell function. Although we did not demonstrate an actual reduction in testicular polyamine concentrations following α-DFMO treatment the data suggest that the stimulation of ODC activity by HCG is not a requirement for androgen production.

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Somatostatin inhibits in vitro release of luteinizing hormone releasing hormone from rat mediobasal hypothalamic slices

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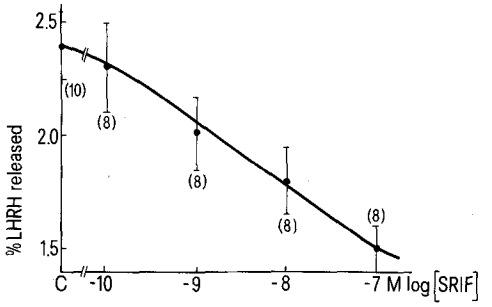
Summary. Somatostatin, in concentrations ranging from 10^{-10} M to 10^{-7} M, induces a dose-dependent inhibition of LHRH release from mediobasal hypothalamic slices incubated in vitro. In contrast, VIP, secretin, glucagon, substance P, neurotensin and arginine-vasotocin do not affect spontaneous release of LHRH.

It has been shown recently that a great number of peptides can be involved in the regulation of pituitary hormone release[2]. It is the case for instance for the known hypothalamic hypophysiotropic hormones such as thyrotropin-releasing hormone (TRH), luteinizing hormone releasing hormone (LHRH) and somatostatin (SRIF) as well as for a variety of peptides isolated both from the brain and the periphery, among them, neurotensin, substance P, secretin and the vasoactive intestinal peptide (VIP)[3]. The aim of the present study was to test whether various neuropeptides can affect luteinizing hormone (LH) secretion by modulating the release of LHRH from mediobasal hypothalamus (MBH).

Materials and methods. Adult male Wistar rats (280–300 g b.wt) were killed by decapitation and the MBH was rapidly dissected on ice. 250 µm thick slices of MBH were cross-cut with a Mc Ilwain tissue chopper and incubated under constant gassing with 95% O_2–5% CO_2 in a modified Locke medium (NaCl 154 (mM); KCl 5.6; $CaCl_2$ 2.2; $MgCl_2$ 1; $NaHCO_3$ 6; glucose 10) buffered to pH 7.2 with 2 mM Hepes (Calbiochem). Bacitracin (2.10^{-5} M, Sigma) was added to prevent degradation of neuropeptides. The experimental procedure included a washing of the slices in cold medium until a clear supernatant was obtained, and a 40-min preincubation step at 37 °C. After the incubation for 10 min, slices were separated from the medium by 2-min centrifugation in an Eppendorf microcentrifuge. The slices and the medium were immediately extracted with 0.1 N HCl; the slices were sonicated for 15 sec and samples were frozen until LHRH was determined. LHRH was measured by radioimmunoassay as previously described[4]. LHRH release was expressed as the percentage of LHRH secreted in the medium over LHRH content in the tissue. Statistical significance was assessed by analysis of variance.

Results and discussion. As shown on the figure, addition of SRIF to the incubation medium induced a dose-dependent inhibition of LHRH release from MBH slices. A significant decrease of LHRH secretion was observed with a concentration of SRIF as low as 10^{-9} M, and a significant inhibition, which represents 60% of the control value, was obtained with 10^{-7} M; half-maximum inhibition was around 4×10^{-9} M. In contrast, under similar experimental conditions, VIP, secretin, glucagon, substance P, neurotensin and arginine-vasotocine (AVT) did not affect spontaneous release of LHRH (table).



Effect of increasing concentrations of SRIF on spontaneous LHRH release from MBH slices. Number of experimental points in each group is given in parentheses. Values are mean ± SEM. LHRH release expressed as percentage of LHRH tissue content against the logarithm of the concentration of SRIF.