Characterization and GtH regulation of microsomal ovarian aromatase activity in rainbow trout

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CHARACTERIZATION AND GtH REGULATION OF MICROSOMAL OVARIAN AROMATASE ACTIVITY IN RAINBOW TROUT

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Introduction

The maturational gonadotropin (GtH) has been claimed to depress aromatase activity in fish ovary, especially at the end of the sexual cycle. These conclusions were based on in vivo analysis of plasma oestradiol (E2) levels , in vitro measurements of E2 output (Fostier & Jalabert, 1986) and in vitro studies of androstenedione metabolism by ovarian tissues (Sire & Depeche, 1981).

To confirm GtH action, we partially isolated aromatase activity by subcellular fractionation for further characterization and kinetic studies.

Experimental

Microsomes were obtained by differential centrifugation of homogenates from preovulatory or vitellogenic (oocyte mean diameter : 2.5 mm) ovaries. They were incubated 10 min. with NADPH and 3H androstenedione ($\Delta 4$) or testosterone (T). Oestradiol and oestrone production was measured after extraction and chromatography. Oestrogens identity was checked in two cases by crystal lization to constant isotopic ratio.

To study aromatase activity regulation, ovaries were preincubated with or without GtH (60 ng/ml) for 24 h before their processing for subcellular fractionation. Each set of experiment was performed with the pooled ovaries of one (preovulatory) or two (vitellogenic) animals. Kinetic constants were estimated from experimental data by a non-parametric fit to the Michaelis-Menten equation (Eisenthal & Cornish-Bowden, 1974) and compared using a Mann-Whitney test.

Results

The aromatase specific activity was compared in the various fractions collected during subcellular fractionation. It was mainly located in the microsomal pellet (195000g pellet : 92 fmoles oestrogens/min. mg ; total homogenate : 0.2 fmoles/min.mg). Aromatization was therefore studied in the microsomes.

The activity was linear with time during at least 10 minutes. Specific activity was not affected by microsomal protein concentration between 1 and 5 mg. Androstatrienedione, known as an aromatase inhibitor in mammals, was also a powerful inhibitor of oestrogens synthesis in our preparations (I 50 : 90 nM, for a 200 nM $\Delta 4$ concentration).

Kinetics of aromatization was investigated in microsomes issued from GtH treated or control ovaries. In most cases, the Michaelis constant (Km) was not affected while Vmax was lowered (table) by GtH treatment.

Table : Effect of GtH pretreatment (60ng/ml) of trout ovaries on their microsomal aromatase activity.
* : significantly different from the control

Sexual cycle period	substrate	Vmax (pmoles/min-animal) control	+GtH
preovulation	$\Delta 4$	8	8
		20	7 *
		11	7 *
		4	3 *
vitellogenesis	$\Delta 4$	46	47
		39	25 *
	T	46	31 *

Conclusion

As in mammals, trout ovarian aromatase appears to be mostly located in microsomes. Its partial isolation provides a new tool to study the regulation of its activity.

We confirm GtH inhibitory effect on ovarian aromatase activity during the second half of the sexual cycle.

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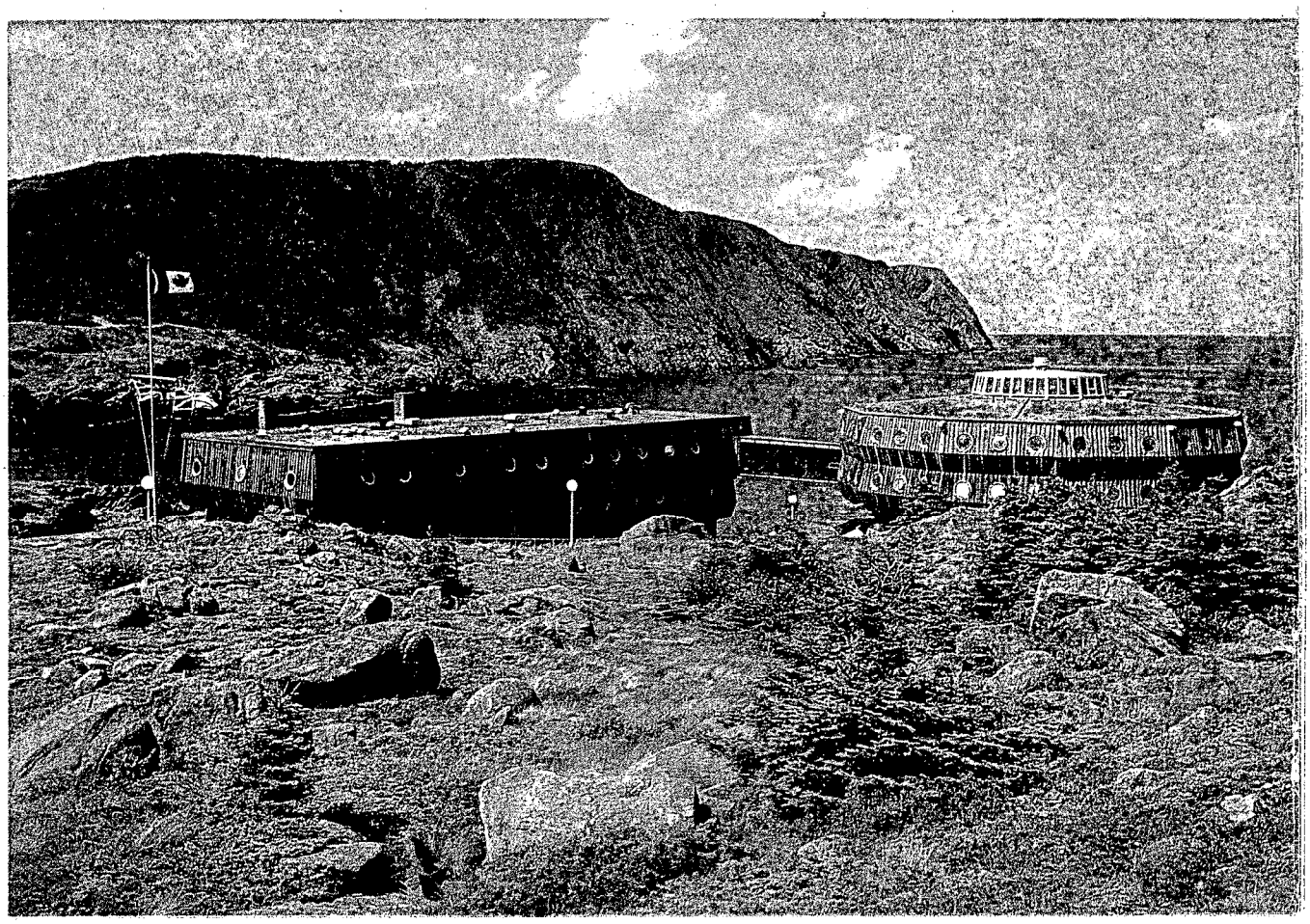

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