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**2<sup>nd</sup>**

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SPECIFIC BINDING OF SALMONID GROWTH HORMONE IN RAINBOW TROUT  
(*Onchorhynchus mykiss*) OVARY.

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Scarce data have shown that growth hormone (GH) may act on gonad function, especially steroidogenesis (Singh et al, 1988). More recently, GH specific binding sites have been detected in various rainbow trout tissues (Yao et al., 1991), and receptors have been characterized in trout testis in relationship with GH steroidogenic effects (Le Gac et al., 1991). The present study was undertaken in order to investigate the GH role in the ovarian function regulation.

Ovarian membranes were partially purified by the following procedure: complete ovaries were homogenized in a Tris buffer pH 7.2 (5 mM Tris, 100 mM NaCl, 100 mM sucrose, 0.5 mM CaCl<sub>2</sub>). The homogenate was centrifuged at low speed (600 g x 20 min) and the supernatant was centrifuged again at higher speed (30 000 g x 45 min). The pellet was resuspended in the assay buffer for binding studies (20 mM Tris, 5 mM MgCl<sub>2</sub>, 0.5 mM ascorbic acid, pH 7.5). It has been checked that such procedure retains 5'-nucleotidase activity. Specific binding has been measured using rt-GH (trout recombinant GH) labelled with I<sup>125</sup> (30 pM). Non specific binding was estimated by displacement with a large excess of rt-GH (450nM). Maximal binding (% of total I<sup>125</sup>GH) was determined by incubating increasing amounts of membranes. Affinity constants and binding capacities were calculated from a Scatchard transformation of saturation curves for specific binding of I<sup>125</sup>GH.

Ovaries have been sampled at different stages. Maximal binding was 2.2% at the beginning of vitellogenesis, and 6.25 % before spawning. Maximal bindings in immature fish ovaries were respectively 27.8 % for 1 year old females and 2.1% for 2 years old females. The affinity constant was the same for both ages ( $K_a = 2.3 \pm 0.37 \cdot 10^9 \text{ M}^{-1}$ ) and comparable to the one found in liver ( $2.4 \cdot 10^9 \text{ M}^{-1}$ ). However, capacities were respectively 29.4 fmole/mg protein (1 year) and 3.21 fmole/mg (2 years). Salmon gonadotropin, tilapia prolactin and tilapia GH did not compete significantly on rt-GH binding.

In conclusion, specific saturable binding with high affinity for GH, similar to receptor-hormone binding, have been found in rainbow trout ovary, showing that growth hormone could have a role in the regulation of trout ovarian function.

SINGH H. et al. Gen. Comp. Endocrinol. 72, 144-153 (1988)

YAO K. et al. Gen. Comp. Endocrinol. 81, 72-82 (1991)

LE GAC F. et al. 4<sup>th</sup> International Symposium on Reproductive Physiology of Fish, July 7-12 1991, Norwich (U.K.) 83 abst.