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An insulin-like system involved in the control of Pacific oyster *Crassostrea gigas* reproduction: hrIGF-1 effect on germinal cell proliferation and maturation associated with expression of an homologous insulin receptor-related receptor

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Abstract

The putative involvement of insulin-like peptides in the control of the reproduction of the Pacific oyster *Crassostrea gigas* was investigated using different approaches. In conjunction with a monthly histological analysis of the oyster reproductive cycle, in vitro biological effects of the human recombinant IGF-1 (hrIGF-1) on dissociated germinal cells were mesured over 1 year using [^{3}H]-thymidine and [^{14}C]-amino acid mixture as tracers for DNA and protein synthesis. DNA synthesis was stimulated by hrIGF-1 in November ($114 \pm 11\%$ for 10^{-7}M), December ($46 \pm 6\%$ for 10^{-7} M) and January, which was identified as the highest gonial mitosis period. A clear dose–effect was observed in January with a maximum activation of $68 \pm 7\%$ for 10^{-12} M. Germinal cell protein synthesis was also stimulated in March ($20 \pm 1\%$ for 10^{-10} M), April ($41 \pm 5\%$ for 10^{-13} M), May ($25 \pm 4\%$ for 10^{-13} M), and by almost all of hrIGF-1 doses in June ($21.5 \pm 2\%$ for 10^{-13} M) and July ($34 \pm 1\%$ for 10^{-13} M). This suggests the involvement of insulin-like substances in gonadal tubule rebuilding (December), as well as in the development of germinal cells (March, April), and in the summer maturation of gametes (May, June, July). These insulin-like effects conform with the expression pattern of the recently identified *C. gigas* insulin receptor-related receptor (CIR): It appeared highly expressed in the gonadal area during gonial mitosis phase, but also in maturating oocytes, suggesting the involvement of an insulin-like system in gonial proliferation and maturation. Moreover, CIR showed differential expression during embryogenesis and larval developmental stages. The expression of maternal CIR during the embryonic and early larval development, followed by the increasing zygotic CIR expression from D larvae to 11-day-old veliger larvae, then a decrease until metamorphosis, also suggest that insulin-like peptide is involved in organogenesis.

Keywords: IGF-1; Insulin-like receptor; Regulation; Reproduction; Gametogenesis; Development; *Crassostrea gigas*; Pacific oyster

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1. Introduction

Even if *Crassostrea gigas* oysters are reared in natural open environment, a part of their developmental cycle may be realised in controlled conditions in hatcheries. At the present time in France, the main part of the spat is issued from natural collecting; however, the hatchery production increases year by year in order to improve growing efficiencies. Thus, to ensure quality and quantity of larvae production, it appears essential to increase our knowledge about optimal rearing conditions, as well as mechanisms of growth, reproduction and development, and their regulatory processes, in controlled conditions. In bivalve species, these mechanisms remain poorly understood; however, previous works have shown that environmental factors such as temperature or food supply, as well as endocrine and neuroendocrine factors, may be involved in control of the different metabolic pathways. Concerning reproduction, the occurrence of an internal control has been clearly demonstrated in various mollusc species, using organ culture experiments (Lubet and Mathieu, 1982), in vitro effects of heterologous molecules (Monnier and Bride, 1995; Pazos and Mathieu, 1999), or by identifying endogenous active molecules (Mathieu et al., 1988). Among the putative active molecules, the insulin-like family, one of the most widely distributed peptide families even among invertebrate species (Table 1), acting by binding to tyrosine kinase receptors, may have a key role in the regulation of reproduction.

Several studies have indicated the role of the insulin system in invertebrate reproduction. The relationship between the insulin signalling pathway and reproductive physiology was particularly well studied in numerous insect species. In the silkmoth *Bombyx mori*, high levels of insulin-like peptides, bombyxins, were found in the female hemolymph to stimulate prothoracic glands in secreting ecdysteroids, inducing meiosis in the ovary, and regulating ovarian and embryonic development (Nagasawa et al., 1984; Saegusa et al., 1992; Orikasa et al., 1993; Iwami et al., 1996b). In *Locusta migratoria*, the *locusta* insulin-related peptide (LIRP) and ecdysteroids concentrations in the hemolymph were shown to be closely correlated with an increase during oogenesis then a decrease at ovulation (Lagueux et al., 1977; Sevala and Loughton, 1992). In ovaries isolated from unfed mosquitoes *Aedes aegypti*, Graf et al. (1997) showed that ecdysteroidogenesis and protein synthesis were stimulated by porcine insulin and insulin-like molecules released from the mosquito brain. However, even if insulin-like peptides were detected in the hemolymph of various species, the paracrine/autocrine involvement of these molecules could not be ruled out (Kappler et al., 1986; Orikasa et al., 1993). Indeed, in most species as in *B. mori* (Fugo et al., 1987; Tanaka et al., 1995; Iwami et al., 1996b; Fullbright et al., 1997), *Drosophila melanogaster* (Garofalo and Rosen, 1988) or *A. aegypti* (Graf et al., 1997; Helbling and Graf, 1998), insulin-related peptide and specific receptor expression were detected in reproductive organs.

In molluscs, the involvement of insulin-like substances in reproduction is poorly documented: in *Planorbarius corneus* (Sonetti et al., 1992) and *Lymnaea stagnalis* (van Minnen and Schallig, 1990), substances immunologically related to insulin-like peptides were detected in neuroendocrine cells of the central nervous system, possibly acting in the hormonal control of reproduction (Geraerts, 1976; Wijdenes et al., 1987). Gomot (1993) hypothesised that the central nervous system of the male snail, *Helix aspersa*, could both stimulate and inhibit the germinal cell proliferation. Monnier and Bride (1995) showed that vertebrate insulin stimulates [^{3}H]-thymidine incorporation by 69% and protein synthesis by 57% in isolated gonadal cells of *H. aspersa*, suggesting that insulin-like peptides may be involved in the regulation of germinal cell proliferation and maturation. Molluscan insulin-related peptide sequences were also determined in the freshwater pond snail *L. stagnalis* (Smit et al., 1988, 1998 for review; Li and Geraerts, 1992; Li et al., 1992a,b) and in the marine mollusc *Aplysia californica* (Floyd et al., 1999). In molluscs, as in other invertebrates, these ligands were usually encoded by a large gene family whereas an unique insulin receptor-related receptor was present (Roovers et al., 1995; Lardans et al., 2001).

In *C. gigas*, no ligand has been identified, but the existence of an insulin-like system was attested by the identification and characterisation of *C. gigas* insulin receptor-related receptor (CIR) (GenBank accession no. AJ535669, Gricourt et al., 2003). Here, we report the potential in vitro effect of the human recombinant insulin-like growth factor of type 1