Crustacean Primary Cell Cultures and the Effects of Ecdysteroids and Gonadotropic Hormones

By

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Abstract

Modifications of crustacean organ culture systems have allowed development of crustacean long term primary cell cultures. These cell cultures have been used to resolve the influence of 20-hydroxyecdysone (20-HE), the arthropod molting hormone, on hematopoietic and testicular events that occur *in vivo* during premolt. In addition, effects of putative gonadotropic hormones were screened with testicular cell cultures for alteration of protein synthetic patterns, and the uptake of [^{3}H]-leucine.

Primary cell cultures of crayfish and lobster testicular or hematopoietic tissue remained viable for up to 15 months. The culture conditions were characterized by low Ca^{2+} (3.5 mM), low temperature (15 to 20°C), and high osmolarity. Medium 199 osmolarity was adjusted with NaCl to 1000 milliosmoles for lobster culture medium and to 400 milliosmoles for crayfish culture medium. Testes were dissociated with 200 U/ml type II collagenase.

Lobster hemocytes reacted to physiological concentrations (10^{-7} M) of 20-HE by reducing contact inhibition and increasing invasive behavior. Physiological concentrations of 20-HE caused

spermatogonial proliferation and testicular mesodermal cell death in lobster and mesodermal vacuolization in crayfish.

It is unclear which of the putative hormones from crustacean androgenic gland, thoracic ganglion, brain, eyestalk, mandibular organ or Y-organ directly affect testes. Cell cultures derived from crayfish and lobster testes increased uptake of [^{3}H]-leucine when exposed to homogenates (0.1 gland equivalent/ml) of brain, thoracic ganglion or androgenic gland. Autoradiographs of protein polyacrylamide gels following electrophoresis demonstrated a dose-response induction of specific protein synthesis and suppression of uptake of [^{3}H]-leucine by testicular cells exposed to physiological concentrations of 20-HE and its analog, RH 5849. Addition of the thymidine analog, 5-bromo-2'-deoxyuridine, allowed partial rescue of the suppression of uptake of [^{3}H]-leucine by 20-HE.