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Action of juvenile hormone on the follicle cells of *Rhodnius prolixus:* Evidence for a novel regulatory mechanism involving protein kinase C

V. L. Sevala* and K. G. Davey

Department of Biology, York University, North York (Ontario, Canada M3J 1P3)
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Summary. Juvenile hormone (JH) is known to act on the membranes of the follicle cells of *Rhodnius,* activating a specific Na^+, K^+-ATPase. This leads to a decrease in volume of the cells and the appearance of spaces between them (patency). The addition of an inhibitor of protein kinase C, 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7), to the medium in vitro inhibits the action of JH on the follicle cells. PDBU (phorbol-12,13-dibutyrate) mimics the action of JH in vitro and the response of the follicle cells to PDBU is blocked by ouabain. It is concluded that the activation of protein kinase C is a required step in the chain of events leading to activation of the JH-dependent ATPase and set in train by the binding of JH to the membrane.
Key words. Juvenile hormone; protein kinase C; Na^+, K^+-ATPase; follicle cells; patency; *Rhodnius.*

In many insect species, JH, a product of the corpus allatum, plays a significant role during vitellogenesis. While JH is known to initiate and sustain the synthesis and release of vitellogenin by the fat body [1], it also governs the uptake of vitellogenin by the oocyte from the hemolymph. Vitellogenin gains access to the oocyte surface via intercellular spaces of the follicular epithelium. The appearance of these spaces in *Rhodnius* is governed by JH [2]. The effect of JH on the follicle cells can be duplicated in vitro [3], and the opening of the spaces (patency) is rapid, reversible and unaffected by inhibitors of macromolecular synthesis [4]. Similarly, the effect of JH on patency, which involves a reduction in cell volume [5], is inhibited by ouabain, a specific inhibitor of the important membrane enzyme Na^+, K^+-ATPase [6]. Juvenile hormone stimulates Na^+, K^+-ATPase activity when applied directly to a microsomal preparation from follicle cells [7], and JH binding sites have been demonstrated in such membrane preparations [8]. These findings imply that JH specific receptors occur on the membrane of the follicle cells. The link between the binding of JH to the putative receptors and the activation of the JH-sensitive ATPase remains enigmatic. The present study provides an insight into the nature of that link.

Materials and methods. A modified method of Davey and Huebner [3] was used to determine the patency of the follicular epithelium. Ovaries were removed from 14-day-old mated females, fed on the 10th day of adult life. The vitellogenic oocytes were separated from the ovary and the ovarian sheath covering the follicle cells was removed. The follicles were incubated in Schneider's *Drosophila* medium (Gibco Laboratories) for 45 min at room temperature, with gentle mechanical agitation. Subsequently, JH I (Sigma Chemical Co.,) (10^{-7} M); JH I plus H-7 (10^{-4} M); PDBU (10^{-7} M); and PDBU plus ouabain (10^{-3} M) were added to the incubation medium (table). After additional 1-h incubation, the follicles were placed in a few drops of physiological saline containing 1% Evans' Blue dye on a microscope slide. The staining solution was removed and the degree of patency, as revealed by the penetration of the dye into the extracellular spaces, was estimated according to the scale devised by Davey and Huebner [3].

Results and discussion. The results, presented in the table, clearly show that exposure of vitellogenic follicles to JH in medium causes a significant increase in the patency index compared to those that were exposed to medium alone ($p < 0.001$). Addition of H-7, a potent inhibitor of protein kinase C [9, 10], to the medium prevents the JH-dependent rise in patency index ($p > 0.01$). This implies

The effect of JH, PDBU, ouabain and H-7 on the patency index of follicle cells in vitro

Treatment	Patency index ± SEM
Control (medium only)	0.50 ± 0.30 (n = 27)
JH	2.00 ± 0.38 (n = 15)
JH + H-7	0.67 ± 0.21 (n = 15)
PDBU	2.66 ± 0.37 (n = 15)
PDBU + Ouabain	0.75 ± 0.35 (n = 12)

n = total number of oocytes examined.

that JH acts on the follicle cells through the activation of protein kinase C. Incubation of follicles with PDBU, which is known to activate protein kinase C[11,12], increases the patency index ($p > 0.001$). This effect was inhibited by the inclusion of ouabain in the medium ($p > 0.001$). Thus, PDBU, like JH, appears to stimulate patency via Na^+, K^+-ATPase. These facts taken together suggest the following scheme for the action of JH on the follicle cells of *Rhodnius*. JH binds to the receptor on the membrane and initiates a series of events which stimulate protein kinase C. This leads to the phosphorylation of one or more membrane proteins, including, possibly, the JH specific Na^+, K^+-ATPase, thereby increasing the activity of that enzyme, leading to a reduction in volume of the follicle cells and the appearance of spaces between them. Experiments are currently under way to test this hypothesis. A similar membrane protein mediated effect of JH involving protein kinase C has been reported in the male accessory gland of Drosophila[13].

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* Author for correspondence.

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Relationship between the absolute configuration and the biological activity of juvenile hormone III

H. Kindle, M. Winistörfer, B. Lanzrein and K. Mori[a]

University of Berne, Zoological Institute, Division of Animal Physiology, Erlachstrasse 9a, CH-3012 Berne (Switzerland), and [a]University of Tokyo, Department of Agricultural Chemistry, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113 (Japan)
Received 10 November 1988; accepted 23 November 1988

Summary. The activity of the pure 10R (= natural) and 10S enantiomers of juvenile hormone III (JH III) was determined in 3 different bioassays, and the relative binding affinity of the 2 enantiomers to the haemolymph JH-binding protein of the cockroach *Nauphoeta cinerea* was measured. In the *Galleria* wax test, a local morphogenetic assay, the 10R enantiomer was 5240 times more active than the 10S enantiomer, 1 *Galleria* unit corresponding to 0.42 pg of 10R-JH III as compared to 2.2 ng for 10S-JH III. In a systemic morphogenetic assay with the cockroach *Nauphoeta cinerea* 380 times less 10R enantiomer was necessary in order to induce detectable juvenilisation (58 ng 10R and 22 µg 10S) and in a systemic gonadotropic assay with *Nauphoeta cinerea* 255 times less 10R was needed to induce vitellogenin synthesis in 50% of the insects (6.7 ng 10R and 1710 ng 10S). In the JH-binding protein assay 10R-JH III had an affinity for the JH-binding protein (lipophorin) which was approximately 46 times higher than that of 10S-JH III.
Keywords. Juvenile hormone; natural enantiomer; juvenilising activity; vitellogenin induction; lipophorin; cockroach; *Galleria*.

Juvenile hormone III [methyl (2*E*,6*E*)-10,11-epoxy-3,7,11-trimethyl 2,6-dodecadienoate, JH III] plays a major role in regulating development and reproduction in many insect species. The absolute configuration of the natural hormone has been established as 10R[1]. Owing to difficulties in preparing the 10R and 10S enantiomers in a state of very high purity, their biological activity has not yet been determined. Nevertheless, it has been shown that the 10R enantiomer of JH III binds to haemolymph binding proteins with higher affinity than the racemate in *Manduca sexta*[2], *Locusta migratoria*[3] and *Leptinotarsa decemlineata*[4], and to cytosolic and nuclear receptors of the fat body in *Leucophaea maderae*[5]. In addition, in the *Galleria* wax test a 12-fold higher activity was observed for 10R-JH III than for 10S-JH III (both 92% pure)[2]. A method of synthesising the pure ($\approx$ 100% enantiomeric excess) enantiomers of JH III has recently been developed[6] and we report here on their biological activity. Using the waxmoth *Galleria mellonella* we carried out a local morphogenetic assay (*Galleria* wax test), and, using the cockroach *Nauphoeta cinerea*, a systemic morphogenetic (induction of juvenile characteristics) and a gonadotropic (induction of vitellogenin synthesis) assay. Furthermore we present data on the affinity of the two