Types of response of insects on treatment with juvenile hormone active insect growth regulators

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Summary. The reactions of immature insects of more than 40 species placed in continuous contact with high doses of juvenile hormone active insect growth regulators were analyzed. 4 different types were recognized: Inhibition of both ecdysis and metamorphosis, defective metamorphosis, defective adult emergence and defective embryogenesis. The reactions are understood as a consequence of JH/ecdysone antagonism. The limits for the practical application of these substances are discussed.

Juvenile hormone (JH) active insect growth regulators (IGRs) are highly specific and the type of reaction upon their application varies considerably in different insects. The comprehension of this reaction is important for the evaluation of chances that this type of compound has for the control of the given species or group of insects. In this report the development responses of more than 40 species belonging to 8 orders of insects are examined. To recognize the specific types of reaction, the insects were exposed during the whole development to high doses of different JH-active IGRs. An attempt is made to sort the examined insects according to their reaction to the treatment, to explain the physiological mechanism of the observed effects and to draw conclusions for the practical application of JH-active IGRs. The contact with reduced dosages or single topical application causes very complicated reactions, the analysis of which is beyond the scope of this study.

Material and methods. The insects were reared in continuous contact with various JH-active IGRs that were coated or sprayed on the diet or substrate upon which they lived in the highest nontoxic dosages, these being not higher than 100 µg/cm^2 or 100 ppm. When untreated control insects had matured and deposited eggs, any treated experimental insects which were still alive were transferred to untreated conditions and allowed to continue development in order to observe whether they could recover. We report the experimental results of 10 JH-active IGRs (table 1) used by us or other cited authors.

Results. 4 different reactions of immature forms of the examined insects after treatment with JH-active IGRs are distinguished. The results of our observations are described below and summarized in 3 tables along with references to similar results existing in the literature.

A. Inhibitions of both ecdysis and metamorphosis. Young larval stages are less sensitive to the treatment with JH-active IGRs than the last instars or pupae, but in some insects such as the German cockroach and scale insects, treated larvae died regularly during the first larval ecdysis (table 2, A-1). In many holometabolous insects, only the last larval stage is sensitive to JH-active IGRs. 2 responses were noted. In insects belonging to 1 group (A-2), represented mainly by stored product pests, exposure to JH-active IGRs completely blocked the development and produced true permanent larvae of the last instar. When transferred to untreated medium, the permanent larvae resumed their development and formed fertile adults. In the last group of insects (A-3), represented by crop pests, exposure to JH-active IGRs results in a prolonged last larval stage, but metamorphosis is not completely stopped and proceeds at least in the most sensitive tissues. The result is a delayed and usually abortive ecdysis into a larval-pupal intermediate.

B. Defective metamorphosis. The ecdysis of many insects cannot be stopped or delayed even by high titres of JH-

Table 1. JH-active IGRs employed

Number	Chemical name or symbol
1	Methyl 10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate, cis/trans mixture of JH I.
2	6,7-Epoxy-3-ethyl-1-(p-ethylphenoxy)-7-methylnonane (cis/trans mixture). Ro 10-3108/000 (a constituent of epofenonane).
3	6,7-Epoxy-1-(p-ethylphenoxy)-3,7-dimethyl-2-octene (cis/trans mixture). R-20458.
4	6,7-Epoxy-1-[3,4-(methylenedioxy)-phenoxy]-3,7-dimethyl-2-nonene (mixture of isomers). Ro 20-3600.
5	Isopropyl 11-methoxy-3,7,11-trimethyl-2-cis/trans, 4-trans-dodecadienoate. ZR 515 = methoprene.
6	Ethyl 3,7,11-trimethyl-2,4-dodecadienoate (cis/trans mixture). ZR 512 = hydroprene.
7	Ethyl trans-3-methyl-5-(p-phenoxyphenyl)-2-pentenoate. CGA 34301.
8	Farnesylmethylether.
9	Ethyl 10,11-epoxy-3,7,11-trimethyl-2,6-tridecadienoate. Ro 7-6049.
10	6,7-Epoxy-1-[3,4-(methylenedioxy)-phenoxy]-3,7-dimethyl-2-octene (mixture of isomers).

Tables 2–4. Effects of high doses of JH-active IGRs (substance numbers as in table 1) on postembryonic development and capacity of treated insects to recover when transferred to uncontaminated environment. References to similar results from the literature are included, where available.

Table 2

Inhibition of ecdysis and metamorphosis	Substance No.	Reference
A-1. Death in the first larval ecdysis		
Blattella germanica	1,3	1
Aonidiella aurantii	1,3–6	2–4
Quadraspidiotus perniciosus	2	4
Planococcus citri	2	5
Parthenolecanium corni	7	6
Psylla piri	2	42
A-2. Permanent last larval stage, recovery to fertile adults in uncontaminated environment		
Blattella germanica	1,3	7
Chilo suppressalis	1	8,10–12
Ephestia kuehniella	4,8	9
Plodia interpunctella	1	13
Caryedon gonagra	1,10	14
Dermestes vulpinus	1	15
A-3. Prolonged last larval stage, death as larva-pupa intermediates in uncontaminated environment		
Adoxophyes reticulana	2	4
Bombyx mori	1	16
Clysia ambiguella	2	4
Laspeyresia pomonella	2	4
Lobesia botrana	2	4
Pieris brassicae	1,4	17
Spodoptera littoralis	2	4
Ostrinia nubilalis	1	18
Sphinx ligustri	1	19
Cerura vinula	8	20
Leptinotarsa decemlineata	2	4
Epilachna chrysomelina	2	4

active IGRs. Metamorphosis, however, is more or less inhibited, and this may cause a secondary disturbance in ecdysis. The insects showing this type of response to JH-active IGRs may be divided into 3 groups depending upon their developmental response. Insects in the 1st group (table 3, B-1), represented mostly by stored product pests, showed the largest response, namely a complete inhibition of metamorphosis. When such insects are treated with JH-active IGRs, last stage larvae moult into perfect supernumerary larvae, i.e. they bear no pupal characteristics and resume development into fertile adults when transferred into the untreated medium.

The 2nd group (B-2) consists of hemimetabolous insects, the metamorphosis of which can be only partially stopped. The treated last instar larvae moult into 1 or 2 supernumerary instars which are characterized by considerable growth and partial metamorphosis. The insects die as larval-adult intermediates.

The females of some insects do not undergo any conspicuous metamorphosis (table 3, B-3). The treatment with JH-active IGRs does not stop the formation of fully grown adults, but the females are permanently sterile. The differentiation of the follicular epithelium is impeded and the oogenesis stops at the end of previtellogenesis.

C. Defective adult emergence. The larvae of Diptera exposed to JH-active IGRs usually pupate normally (table 4). An exception are mosquitoes, the pupae of which may retain some larval characters when reared in the treated water. However, the adult emergence is disturbed. The adults fail to emerge and die inside the pupal cuticle or puparium.

D. Inhibition of embryogenesis. Both embryogenesis and embryonic ecdysis are inhibited in eggs of many insect species exposed to JH-active IGRs in the female's body or after the egg deposition[35].

Discussion. The JH treated larvae of the German cockroach[36] and last instar larvae of *Pieris*[37] show a pronounced deficiency of ecdysone. The inhibited development can be induced again through the injection of ecdysone. These results indicate an active role of JH in the regulation of ecdysone titre in the insect body. Williams[38] suggested a very plausible explanation for this control mechanism in which chronic exposure to JH during the final instar prevents the brain prothoracotropic hormone from turning on the prothoracic glands.

The effects of JH-active IGRs described in this paper can be understood as a consequence of the effect of JH on the ecdysone titre. Some insects, notably pests of stored products, seem to possess a life-saving mechanism which prevents abnormal development under unfavorable conditions of a high JH titre. According to this concept, the ecdysone titre of treated last stage larvae is suppressed below the level necessary for metamorphosis when the perfect supernumerary larvae are produced (e.g. *Tineola*) or even below the lower level required for ecdysis in an insect reacting by the formation of permanent larvae (e.g. *Blattella*). The larvae resume their development only when the JH titre has decreased to a level which no longer causes harmful effects on differentiation, and fertile adults are then formed. As far as stored product pest control is concerned, the employed JH-active IGRs may be useful in preventing an increase in infestation but not in the reducing the number of an existing population.

The long-lasting suppression of adult development through JH can hardly be an efficient way to save life for crop pests, where the life cycle is limited by the vegetation season. Ecdysis of last stage larvae exposed to a JH-active IGR is considerably postponed but most of them eventually try to pupate. According to our concept, a slowly augmenting ecdysone titre causes a partial metamorphosis in the most sensitive tissues and finally ecdysis into non-viable larva-pupa intermediary forms. Thus, the JH-active IGRs may control an existing population but, unfortunately, only after the major damage caused by the feeding larvae has been done.

The hemimetabolous insects and flies lack the described life-saving mechanism. The last stage larvae of bugs exposed to a JH-active IGR moult at the same time or even earlier than the control insects. However, the amount of ecdysone inducing the ecdysis is too low for metamorphosis, which therefore remains incomplete. The insects moult into intermediary forms between the larvae and adults, which often resemble supernumerary larvae but they always bear some adult characteristics. In Diptera mainly the emergence of adults out of pupae is affected. In any case, no viable permanent or supernumerary larvae were observed in these insects, a fact which makes them good targets for JH-active IGRs.

The insect eggs contain rather large quantities of ecdysone[39,40,41] which may play an important role in regulation of embryonic differentiation and ecdysis. Their derangement, as observed in JH-treated eggs of many insects, may be the consequence of a modified ecdysone titre.

Table 3

Inhibition of metamorphosis	Substance No.	Reference
B-1. Supernumerary larval moults, recovery to fertile adults on untreated medium		
Diatraea grandiosella	5	21
Galleria mellonella	2	4
Tineola bisselliella	2	4
Trogoderma granarium	1,10	14
Tenebrio molitor	8	4,23
Tribolium castaneum	2	24
B-2. Defective metamorphosis in the last larval stage, death as larva-adult intermediates		
Locusta migratoria	1	25
Dysdercus cingulatus	2	4
Aphis fabae	9	26
Planococcus citri	2	5
Quadraspidiotus perniciosus	2	4
Psylla piri	2,7	27,42
Nephotettix virescens	2	4
B-3. Defective differentiation of ovaries, permanently sterile females		
Thermobia domestica	1,10	22
Quadraspidiotus perniciosus	2	4
Planococcus citri	2	5

Table 4

Inhibition of adult emergence	Substance No.	Reference
C-1. Defective adult emergence, death as pharate adults		
Aedes aegypti	3,5	4,28
Anopheles albimanus	3-5	29
Culex pipiens	3,5	4,28
Drosophila melanogaster	1	30
Stomoxys calcitrans	1	31
Sarcophaga bullata	1	32,33
Rhagoletis pomonella	4,5	34
Musca domestica	5	4,44
Ceratitis capitata	5	44
Calliphora erythrocephala	5	44
Dacus oleae	5	43
D. Inhibition of embryogenesis		
Many insect species	Many substances	44

The comparative study of the reaction of many insect species on the JH-active IGRs makes the limits for their practical application apparent. However, selectivity, low activity against parasitoids in eggs and larvae[6,45], low toxicity and general environmental safety[46] increase their real pesticide value.

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Circadian variation of the streptozotocin-diabetogenic effect in mice[1]

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Summary. Streptozotocin was injected in normal mice every 4 h, during the day. Greatest number of diabetic animals were obtained at 16.00 h (95%) and lowest at 08.00 h (50%). Magnitude of hyperglycemia also showed similar distribution. This effect might be considered when planning its use for both experimental and clinical purposes.

The induction of experimental diabetes by the use of β-cytotoxic agents[3] is an useful tool for people working in the field of diabetes, both for research and clinical purposes in the treatment of severe cases of hyperinsulinism[4,5]. Although alloxan was previously extensively employed in animals, its use is no longer encouraged, mainly on account of its high toxicity[6]. For this reason, in the last few years streptozotocin (SZ)[7], a less toxic compound, has been almost the only drug used to induce chemical pancreatectomy.

Recently, it has been demonstrated that normal rats modify during the day their susceptibility to alloxan diabetogenic effect, despite the prior fasting period[8]. On the other hand, the doses of SZ employed to induce diabetes in mice seems to be considerably higher than the one used in other species[9]. This fact might represent a larger risk of secondary lesions due to the toxic effects of the drug. Since the final purpose of using β-cytotoxic drugs is to have the largest number of diabetic animals with the smallest doses and consequently the lowest incidence of extrapancreatic alterations, we assume that it would be of interest to search for a circadian rhythm of susceptibility of SZ. With this idea in mind, normal mice were injected at different times of the day with different doses of SZ.

Materials and methods. Normal female mice from the C3Hs strain, caged in groups of 8, were used. They were maintained in a room at a constant temperature of 23 °C and with free access to food and water. Lights were automatically switched on and off at 06.00 and 18.00 h, respectively. Under this light regime, animals engage in exercise and foold intake only during the darkness period[10].

Streptozotocin (Upjohn U-9889, lot No.60140) was diluted in cold citrate buffer pH 4.5 and immediately injected, solutions being discarded 5 min after its preparation. Several doses (0, 50, 100, 175, 200 and 250 mg/kg b.wt) were injected i.v. using one of the tail veins, at 04.00, 08.00, 12.00, 16.00, 20.00 and 24.00 h on different days. After the injection, urine glucose was controlled daily (Gluco-Cinta, Lilly) and b.wt once a week. 21 days after the injection, all the animals were sacrificed at 12.00 h and blood samples were obtained from each animal for serum glucose[11] determination. Statistical analysis of the data was done accord-