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Probable endocrine role of midgut tissue in stimulation of digestive enzyme secretion in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae)

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Abstract. The effect of midgut epithelial extract on digestive enzyme secretion in the third instar larva of *Oryctes rhinoceros* was studied. Midguts dissected out from third instar larvae were cut at both ends, emptied of their contents, washed in insect saline, ligated at the open ends and incubated in a medium containing extract of 2 midgut epithelia/10 ml incubation solution. In control experiments midgut preparations were incubated in insect saline without midgut epithelial extract. After 30 min of incubation, the contents of the midgut preparations incubated with midgut epithelial extract showed increased secretion of protease and amylase when compared with controls. Digestive enzyme release into gut lumen appears to be due to the action of a hormone present in the midgut epithelium.

Keywords. Coconut rhinoceros beetle; *Oryctes rhinoceros*; digestive enzymes; gut hormone.

1. Introduction

Secretagogue, neural and endocrine mechanisms have been variously postulated in different insects in the regulation of digestive enzyme secretion (House 1974). Reports on endocrine mechanism mostly deal with the role of the brain median neurosecretory cells in the secretion of digestive enzymes (Briegel and Lea 1979; Muraleedharan and Prabhu 1979). By injecting midgut extracts into haemocoel of the cockroach *Periplaneta americana*, Rounds (1968) found that a substance in the midgut epithelium which stimulated digestive enzyme secretion could act as a possible intermediate link in the regulatory mechanism between the central nervous system and the midgut tissue. Recent ultrastructural studies have shown the presence of endocrine cells in the midgut epithelium of insects (Endo and Nishiitsutsuji-Uwo 1981; Nishiitsutsuji-Uwo and Endo 1981). Immunological techniques have, meanwhile, provided additional evidence for the occurrence of several vertebrate-like gut peptides in the insect gut epithelium (Raabe 1983). These findings indicate that in insects the midgut itself can be a source of hormonal principles regulating digestive enzyme secretion. However, this possibility is yet to be conclusively demonstrated. The *in vivo* method of Rounds (1968) referred to above has the drawback that the results obtained could as well be secondary, the immediate action of the midgut epithelial extract being on other target organs. Hence, an *in vitro* method was devised to find out the effectiveness of extracts of midgut epithelia on digestive enzyme secretion in *Oryctes rhinoceros* which is a highly suitable animal for the study (Sreekumar and Prabhu 1988). The findings have been briefly reported elsewhere (Sreekumar and Prabhu 1987) and the present paper is a detailed report on the findings.

2. Material and methods

Third (final) instar larvae of *O. rhinoceros* reared in the stock colony as described by Mini and Prabhu (1986) but on sterilised cowdung, were used for the study.

2.1 *Preparation of midgut epithelial extract*

Midguts from third instar larvae of *O. rhinoceros* were dissected out in insect saline (Starratt and Steele 1980). The gut contents were removed by opening the midgut and washing the tissues in several changes of insect saline. The tissues were homogenised in ice-cold insect saline. To remove protease and amylase from the homogenate, 50 mg casein and 50 mg starch were added to the solution. The homogenate was first filtered through glass wool and then centrifuged at 10,000 *g* for 10 min. The concentration of the homogenate was adjusted to 2 midgut epithelia/10 ml insect saline.

2.2 *Preparation of midgut for incubation*

Midguts dissected out from third instar larvae were cut at both ends and gut contents were removed. The midguts were then washed thoroughly in 5–6 changes of insect saline. Next, the two ends of the midgut were ligated using hair. The entire procedure took less than 10 min.

2.3 *Bioassay*

A glass tube of 6 × 2·4 cm internal diameter, open at both ends, one end of which was fitted with a rubber stopper through which a hypodermic needle was inserted served as the incubation chamber. Oxygen was delivered through the hypodermic needle. The bioassay apparatus was kept in a water bath at 37°C. The incubation chamber of the bioassay apparatus contained 10 ml midgut epithelial extract. The midgut preparation was suspended in it and incubated for 30 min under a small stream of oxygen bubble. After incubation, the midgut preparation was taken out, washed in 3–4 changes of insect saline and opened and the contents were collected in 1 ml ice-cold distilled water for quantitative estimation of protease and amylase.

2.4 *Quantitative determination of digestive enzymes*

The procedure of Birk *et al* (1962) with some modifications was followed for the determination of protease activity. The reaction mixture consisted of 0·2 ml enzyme extract, 0·2 ml glycine-NaOH buffer (pH 9) and 0·4 ml 1% casein solution. Enzyme activity was terminated after 30 min incubation at 37°C by adding 1·2 ml of 5% trichloroacetic acid. It was centrifuged at 13,000 *g* for 15 min. The supernatant was made up to 4 ml and read at 280 μm with a spectrophotometer. A 0·005% solution of tyrosine was used as standard for calculating μg of tyrosine liberated.

For determining amylase activity, the method of Noelting and Bernfeld (1948) was modified. A reaction mixture containing 0·2 ml enzyme extract, 0·2 ml Tris-HCl buffer (pH 8·2) and 0·4 ml 1% starch solution was incubated at 37°C for 20 min. The reaction was stopped by adding 1·2 ml 3,5-dinitrosalicyclic acid reagent and heating at 100°C for 5 min. The absorbancy of the solution after diluting it to 4 ml was read at 550 μm and calculated μg of maltose equivalents liberated using 0·01% maltose solution as standard.

2.5 *Time-course and dose-response studies*

The time-course of digestive enzyme secretion by midgut epithelial extract was studied by incubating the midgut preparations in a medium containing extract of 2 midgut epithelia/10 ml solution for 10, 30 and 60 min. Digestive enzyme secretion with respect to homogenate concentration was studied by incubating the midgut preparations in incubation solutions of varying concentrations of midgut epithelia (1, 2, 4 and 6) for 30 min. In both cases, the contents of the midgut preparations were collected for quantitative estimation of protease and amylase.

2.6 *Control experiments*

Casein (50 mg) and starch (50 mg) were added to 10 ml insect saline, mixed well and filtered through glass wool. The supernatant after centrifugation at 10,000 *g* for 10 min was used to incubate midgut preparations in control experiments.

3. Results

The contents of midgut preparations incubated in a medium containing extract of 2 midgut epithelia/10 ml incubation solution for 30 min showed significant increase in protease (3·61 ± 0·81) and amylase (11·03 ± 1·44) levels when compared with controls (protease: 1·86 ± 0·85; amylase: 7·95 ± 1·74). When incubation period was increased from 10–60 min, increased secretion of protease and amylase was noticed (table 1). Secretion of protease and amylase also increased with increasing concentration of the midgut epithelial extract i.e, from 1–6 midgut epithelia/10 ml solution (table 2).

4. Discussion

In vertebrates, hormones secreted by the gastro-entero-pancreatic system are involved in several digestive processes. The existence of a comparable system in insects is supported by ultrastructural and immunological studies. Basal granulated cells with ultrastructural characteristics of endocrine cells of the vertebrate gastro-

Table 1. Time-course of digestive enzyme secretion by midgut epithelial extract of *O. rhinoceros*.

	Incubation time (min)					
	10		30		60	
	Control	Test	Control	Test	Control	Test
Protease activity[a]	1·33 ± 0·60 (8)	2·55 ± 1·50 (10)	1·86 ± 0·85 (10)	3·61 ± 0·81[c] (10)	2·43 ± 0·81 (8)	5·77 ± 1·67[c] (8)
Amylase activity[b]	7·16 ± 1·20 (8)	8·67 ± 2·23 (8)	7·95 ± 1·74 (10)	11·03 ± 1·44[c] (10)	8·67 ± 1·54 (8)	15·48 ± 3·76[c] (8)

[a] μg of tyrosine liberated.
[b] μg of maltose equivalents liberated.
[c] Significance at 0·001 level.
No. of determinations given in parentheses.

Table 2. Effect of midgut homogenate concentration on digestive enzyme secretion.

	Homogenate concentration (No. of midgut epithelia per 10 ml incubation solution)				
	Control	1	2	4	6
Protease activity[a]	1·86 ± 0·85 (10)	2·39 ± 0·63 (8)	3·61 ± 0·81[c] (10)	3·49 ± 0·79[c] (10)	3·71 ± 0·56[c] (6)
Amylase activity[b]	7·95 ± 1·74 (10)	9·86 ± 1·61 (8)	11·03 ± 1·44[c] (8)	12·12 ± 1·97[c] (8)	13·51 ± 2·25[c] (6)

Same notations as in table 1.

intestinal tract were identified in 7 species of Lepidoptera (Endo and Nishiitsutsuji-Uwo 1981). *P. americana* (Nishiitsutsuji-Uwo and Endo 1981), *Oryctes nasicornis* (Bayon 1981) and *Aedes aegypti* (Brown *et al* 1985). Similarly, the midgut epithelium of insects also revealed the presence of peptides immunologically related to those of the vertebrate gastrointestinal tissue such as insulin-like peptide in Hymenopteran insects (Ishay *et al* 1976), glucagon-like peptide in *Manduca sexta* (Tager and Kramer 1980) and pancreatic polypeptide-, somatostatin- and enteroglucagon-like immunoreactive materials in *P. americana* (Iwanaga *et al* 1981). In fact with an estimated 500 endocrine cells, the midgut of *A. aegypti* constituted the largest endocrine organ in an adult mosquito (Brown *et al* 1985). However, only very few reports are available on the probable functional role of the insect gut endocrine system.

In vitro studies revealed that in *Calliphora* 5-hydroxytryptamine stimulated fluid secretion by isolated salivary glands. The brain of this insect contained 5-hydroxytryptamine (Berridge and Patel 1968). Removal of salivary glands of 10 day old adult *P. americana* resulted in a gradual decline in midgut invertase secretion. No such effect occurred when salivary ducts were severed. Injection of salivary gland extract restored enzyme secretion to a large extent in salivarectomised insects, indicating the presence of a hormone like inducing factor (Agrawal and Bahadur 1981). In the adult *P. americana* the midgut extracts also contained a substance which increased protease in the midgut lumen 25–30 min after injection of the extract into the haemocoel, although significant values were obtained only around sun set (Rounds 1968). The results presented here revealed that in the third instar larvae of *O. rhinoceros* extracts of midgut epithelium were effective in increasing digestive enzymes in gut contents. The effect of the midgut epithelial extract on digestive enzyme secretion was both time and dose dependent. This observation suggests that in *O. rhinoceros* the midgut itself is functioning as an endocrine organ, regulating the secretion of digestive enzymes without the intervention of other endocrines or nervous components. The secretory process of a midgut endocrine cell may take place in response to chemical stimulation in the gut lumen or mechanical stimulation in the epithelium (Iwanaga *et al* 1981). Endocrine cells of the 'open type' were described in most of the insects studied (Nishiitsutsuji-Uwo and Endo 1981; Brown *et al* 1985). Such cells may be specialised for the reception of molecular signals from the food ingested by the animal (Brown *et al* 1985). In the endocrine cells of the midgut of *A. aegypti* phenylalanine-methionine-arginine-phenylalanine-amide (FMRFamide) and pancreatic polypeptide (PP) like immunoreactivity decreased within 6 h after ingestion of blood suggesting release of hormones (Brown *et al* 1986). The hormones (peptides or

monoamines) released into the haemolymph by gut endocrine cells are believed to be performing a regulatory role in many digestive and metabolic processes. While some of these hormones may have paracrine effects on regenerative cell differentiation, enzyme production by the digestive cell and working of the inner circular muscle of the midgut, others may have endocrine effects on the activity of more distant target tissues such as the central nervous system and fat body (Iwanaga *et al* 1981; Brown *et al* 1985). The enhanced activity of protease and amylase in the contents of midgut preparations incubated with midgut epithelial extract, observed in the present study, thus appears to be due to the action of a midgut hormone stimulating digestive enzyme release into the lumen of the gut. Both the source and the target organ of this hormone were found to be the midgut epithelium and for this reason, it may come under the group of paracrine hormones.

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