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Research Note

MONOCLONAL ANTIBODIES AGAINST BRAIN NEUROSECRETORY A CELLS OF *PANSTRONGYLUS MEGISTUS* INHIBIT MOULTING AND EGG PRODUCTION IN TRIATOMINES

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The processes culminating in ecdysis and egg deposition in insects are complex. In the moulting process the first endocrine event is the release of a neurohormone, prothoracicotropic hormone (PTTH), by neurosecretory cells from the *pars intercerebralis*. PTTH stimulates prothoracic glands to secrete the moulting hormone (ecdysteroids) during the head critical period (L. I. Gilbert et al., 1980, *Recent Prog. Horm. Res., 36:* 401-449). Oogenesis is controlled by juvenile hormone which is produced by the corpus allatum that in turn is influenced by the neurosecretory cells from the brain (K. G. Davey, 1980, The physiology of reproduction in *Rhodnius* and other insects: some questions, p. 325-344. In M. Locke & D. S. Smith (eds), *Insect Biology in the Future*. Academic Press, New York).

A. F. Furtado (1979, *J. Insect Physiol., 9:* 561-572) by ablation of the prothoracic glands and selective ablation of neurosecretory A cells of the brain, showed that in *Panstrongylus megistus* there were two separate factors from the brain required for moulting: one secreted by the A cells related to the process of mitosis preceding apolysis, and the other, probably PTTH secreted by A' cells, that activates the prothoracic glands. In 5th-instar female larvae of *P. megistus* the neurosecretory A cells are related to the mitosis of the ovaries and the A' cells are responsible for the initiation of gonial meiosis during oogenesis (A. F. Furtado, 1979, *Experientia, 35:* 1123-1126).

We describe here for the first time that a monoclonal antibody with striking specificity against neurosecretory A cells of the brain of *P. megistus* and *R. prolixus* causes delay of moulting, inhibits oviposition of triatomines and disturbs ecdysteroid secretion in these insects. In addition, we show that the monoclonal antibody against the brain neurosecretory A cells affects the moulting process only if applied before the head critical period.

We used in our experiments 4th- and 5th-instar larvae of *R. prolixus* and *P. megistus* as well as adult females of these species. Monoclonal antibodies which specifically react with neurosecretory A cells of the brain of *Rhodnius* and *Panstrongylus* were produced using only *pars intercerebralis* of *P. megistus* as described elsewhere (Furtado et al., in preparation). Culture medium supernatant from each monoclonal antibody was added to the bloodmeal or injected into the hemocele of larvae and adult insects. Ecdysteroids were measured by radioimmunoassay (RIA) as previously described by E. S. Garcia et al. (1987, *J. Insect Physiol., 10:* 729-732).

Table I shows that insects which fed on blood containing the monoclonal antibody experienced, (5% of hybridoma culture medium, approx. 5 μl of monoclonal antibody culture supernatant per insect), drastically inhibited moulting of 5th-instar larvae, delayed and inhibited moulting of the 4th-instar larvae of both insect species. However, if the inoculation of monoclonal antibody was carried out after

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TABLE I

Effects of monoclonal antibody against neurosecretory A cells at the time from feeding to ecdysis in 4th-instar and 5th-instar larvae of *Rhodnius prolixus* and *Panstrongylus megistrus*. For administration by ingestion, the supernatant of monoclonal antibody culture was diluted 5% in the bloodmeal. For inoculation it was used 1-2 μl of the supernatant culture

Monoclonal antibody	Treatment by	Instar	Number of insects	Days from feeding to moulting (range)	Percentage of moulting First 4 days	Total
			Rhodnius prolixus			
None	–	4th-	40	14 – 17	80	100
None	–	5th-	45	22 – 26	75	96
B5c	Ingestion	4th-	40	16 – 24	20	70
B5c	Injection	5th-	30	24 – 32	25	56
			Panstrongylus megistus			
None	–	5th-	40	15 – 20	80	100
B5c	Injection	5th-	36	21 – 33	20	46

the head critical period of 5th-instar larvae of *R. prolixus*, (i. e., 7 days after feeding), the insects underwent normally moulting (data not shown).

To determine whether monoclonal antibodies against neurosecretory A cells from the *pars intercerebralis* of *P. megistus* disturb the egg deposition of *R. prolixus* and *P. megistus*, we injected 1-4 μl of the hybridoma culture medium supernatant and compared the rate of oviposition of these insects with that of control groups receiving only injection of culture medium (Table II). It is clear from Table II that the oviposition was drastically affected by the monoclonal antibody used. Furthermore, the inhibition of egg deposition was proportional to the concentration of monoclonal antibody inoculated in the mature females. Preliminary experiments have shown that the monoclonal antibody induces a shift of the ecdysteroid peak in 4th-instar larvae of *R. prolixus* and that in adult females the ecdysteroid levels were significantly diminished (data not shown).

As far as we know this is the first report showing that monoclonal antibody which reacted specifically with brain neurosecretory A cells of *P. megistus* was able to arrest the moulting and to inhibit the egg production in *P. megistus* and *R. prolixus*. The interpretation of the results is complicated by the fact that it is not yet clear exactly how neurosecretion influences the prothoracic glands and ovaries,

TABLE II

Number of eggs deposited by adult females of *Rhodnius prolixus* and *Panstrongylus megistus* treated with monoclonal antibody against brain neurosecretory A cells of *P. megistus*. The insects were inoculated with 1 – 4 μl of the supernatant of monoclonal antibody culture 1 day before the bloodmeal. The oviposition was computed for 30 days after feeding

Species	Monoclonal antibody	Number of females	Cumulative number of eggs per female
R. prolixus	–	40	26.5 ± 5.0
	B5c	40	8.5 ± 1.5
P. megistus	–	15	75.0 ± 5.0
	B5c	15	6.5 ± 1.5

the organs which secrete ecdysteroids in insects. However, our data have shown that these effects were related to the release of neurosecretion from the brain since inoculation of monoclonal antibody after the head critical period did not cause any effects on the moulting cycle. Taken together, these data show that it may be possible to use monoclonal antibodies against neurosecretory cells of the brain as powerful tools for investigating the neuroendocrine regulation of insect growth and reproduction.

A detailed description of these data will be published elsewhere.