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RESEARCH BRIEF

Trypanosoma brucei: Stimulation of Adenylate Cyclase by Proventriculus and Esophagus Tissue of the Tsetse Fly, *Glossina morsitans morsitans*

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INDEX DESCRIPTORS AND ABBREVIATIONS: *Trypanosoma brucei*; tsetse fly; *Glossina morsitans morsitans*; Adenylate cyclase; GRESAGs 4.

African trypanosomes of the *Trypanosoma brucei* group have a complex developmental cycle in the bloodsucking insect, the tsetse fly (*Glossina* spp.), ending with the infective metacyclic stage in the salivary glands. This development depends on several intermediate stages at different locations in the digestive tract of the fly involving drastic morphological and biochemical changes and cell cycle switches between multiplicative and nonproliferative stages (Vickerman *et al.* 1988; Steiger 1973). It is speculated that at least some developmental changes are triggered by molecular biocommunication between competent trypanosome cells and tsetse tissues. These interactions are assumed to occur at the surface of the trypanosome to be transferred to its intracellular environment by one of the signal transduction mechanisms. Adenylate cyclases are already evidenced in African trypanosomes (Martin *et al.* 1978; Walter and Opperdoes 1982). *T. brucei* contains several isoforms of adenylate cyclase. One of them is encoded by a gene (ESAG 4) linked to that of the VSG and is only expressed in bloodstream forms while the others, encoded by GRESAGs 4, are expressed in both bloodstream and procyclic forms (Alexandre *et al.* 1990). In bloodstream forms the adenylate cyclase is down-regulated since it can be strongly stimulated upon rupturing of the cells (Voorheis and Martin 1980). The adenylate cyclase activity of bloodstream forms differs from that of procyclic forms with respect to its response to both Ca^{2+} (Rolin *et al.* 1990) and membrane perturbating agents (Rolin *et al.*, submitted for publication). Moreover, in bloodstream forms, changes in cAMP levels seem associated with events triggering differentiation from slender to stumpy forms (Mancini and Patton 1981; Reed *et al.* 1985) and from bloodstream to procyclic forms (Rolin *et al.* 1993).

In the present study we aimed at determining whether tsetse tissue compounds might be capable of activating adenylate cyclase of *T. brucei* parasites.

Bloodstream forms of *T. brucei* (stock EATRO 1125, clone AnTat 1.1) were isolated from mouse blood (Lanham and Godfrey 1970). Procyclic forms derived from the bloodstream ones were maintained routinely at 28°C by passage every 3–4 days in GLSH medium (Le Ray 1975) supplemented with 10% FCS. Tsetse fly tissues originated from male flies from our *Glossina morsitans morsitans* ITMA colony which have a high intrinsic vectorial capacity of >0.5, i.e., more than 50% metacyclogenesis of *T. brucei* AnTat 1.1. Tissues were homogenized and fractionated by centrifugation into a PBS-soluble and a crude membrane fraction. Bioassays for adenylate cyclase activity were performed on cells permeabilized by swell dialysis as previously described (Voorheis and Martin 1980; Rolin *et al.* 1993). Trypanosomes were incubated during 10 min at 28°C (and also at 37°C for bloodstream forms) with a specific tissue fraction suspended in a medium of low osmotic strength, prior to a 3-min incubation with the assay cocktail. The viability of the trypanosomes was not affected.

In a first experiment, 72-hr culture procyclics were incubated with different tsetse tissues: midgut, proventriculus/esophagus, proboscis, and salivary glands. Tissues of freshly emerged flies (8–32 hr after emergence, teneral) and 15-day-old flies were compared for stimulating activity. The PBS-soluble fraction and the crude membrane fraction (CMF) of each tissue were incubated separately with the trypanosomes. Table I shows that incubation with the crude membrane fraction of the proventriculus/esophagus tissue selectively stimulated adenylate cyclase of procyclic cells. Tissue from teneral flies (A) proved to be more active than tissue from 15-day-old flies (B). We next compared the effect of the proventriculus/esophagus CMF on adenylate cyclase activity of procyclic cells at different stages in their growth curve. The results (Fig. 1) indicated a higher adenylate cyclase stimulation in multiplicative trypanosomes (exponential growth phase) than in the nonproliferative cells (stationary and poststationary phases). Finally, we found that proventriculus/esophagus CMF increased adenylate cyclase activity not only in procyclic cells but also in blood-

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TABLE I
Adenylate Cyclase Activity (Expressed as pmole cAMP Formed/min by 10^7 Organisms) of 72-hr Culture Procyclics of *Trypanosoma brucei* (Derived from Stock EATRO 1125, Clone AnTat 1.1) Incubated with PBS-Soluble and Corresponding Crude Membrane Fractions of Tissues from Freshly Emerged (A) and 15-day-old (B) *Glossina morsitans morsitans* Males

		PBS-soluble fraction	Crude membrane fraction
Midgut	A	4.5 ± 0.5	5.9 ± 0.5
	B	4.1 ± 0.5	5.8 ± 0.7
Proventriculus/ esophagus	A	5.0 ± 0.1	13.4 ± 0.1
	B	4.9 ± 0.5	9.2 ± 1.4
Proboscis	A	4.5 ± 0.3	6.2 ± 0.1
	B	4.4 ± 0.3	7.4 ± 0.5
Salivary gland	A	4.1 ± 0.1	4.9 ± 0.3
	B	2.8 ± 0.1	4.8 ± 0.4

Note. Each value represents the mean ± SD of one representative experiment performed in triplicate. Procyclic trypanosomes expressed a basic adenylate cyclase activity of 4.2 ± 0.1 pmole cAMP/min 10^7 tryp.

stream forms (Table II). Incubation of the bloodstream forms at 37°C yielded a remarkable stimulation of more than six times the basic level.

While different treatments have been reported to induce adenylate cyclase activation specific to the bloodstream forms, these data represent the first evidence for a stimulation of trypanosomal adenylate cyclase by tsetse fly components. We acknowledge that in bloodstream forms we should be cautious about interpretation of the stimulation since the activity can be strongly stimulated under stress. However, this is definitely not the case in procyclic forms whose cyclase activity resists all experimental treatments assayed to date. The level of activity between bloodstream forms and procyclic forms is similar at 28°C, suggesting that the activation by the ligands from the fly is not stronger in bloodstream forms. The higher activation at 37°C may simply reflect increased catalytic activity at high temperature. Although our results did not allow us to discriminate which adenylate cyclase isoform is actually stimulated by the tsetse fraction, the comparable stimulation in both bloodstream and procyclic forms is in complete agreement with our evidence that some of the cyclase isoforms (GRESAGS 4) are expressed in both forms (Alexandre *et al.* 1990).

A similar vector–parasite communication mechanism has been recently observed between *T. cruzi* and its *Triatoma* hematophagous insect vector (Fraidenraich *et al.* 1993): a

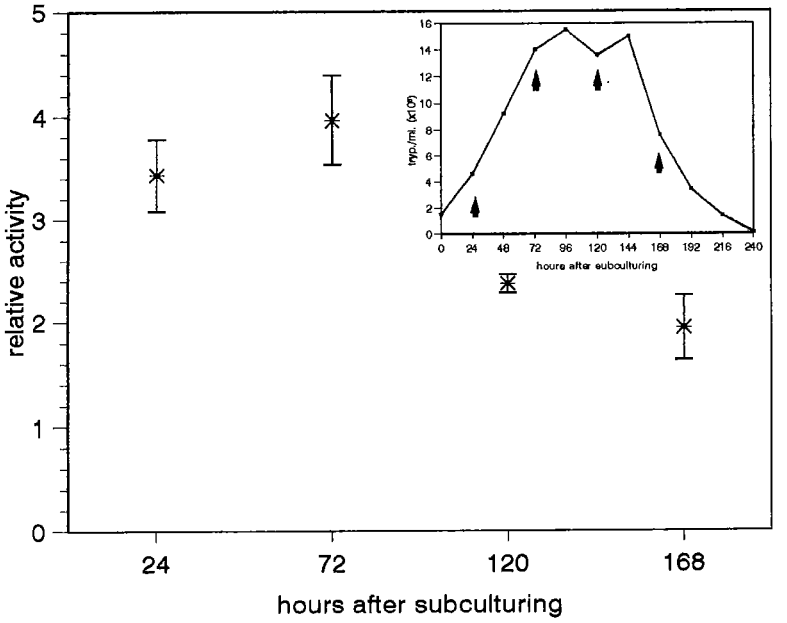

FIG. 1. Stimulation of adenylate cyclase of procyclic *Trypanosoma brucei* (derived from stock EATRO 1125, clone AnTat 1.1) sampled at four different points of the growth curve (↑). Trypanosomes were incubated with crude membrane fraction of proventriculus/esophagus from *Glossina morsitans morsitans* male flies. Measurements were performed in triplicate. Adenylate cyclase activity (mean ± SD) is expressed relative to the basic activity in corresponding control trypanosomes. The value obtained is a measure of stimulation.

TABLE II
Stimulation of Adenylate Cyclase Activity of 72-hr Procyclic (PROC-) and Bloodstream Forms (BSF-) of *Trypanosoma brucei* (Stock EATRO 1125, Clone AnTat 1.1) Incubated with Crude Membrane Fraction of Proventriculus/Esophagus of *Glossina morsitans morsitans* Male Flies

	Relative activity
PROC-28	3.8 ± 0.1
BSF-28	4.2 ± 0.3
BSF-37	6.7 ± 0.7

Note. Procyclic trypanosomes were incubated at 28°C only; bloodstream forms, at 28°C and at 37°C. Measurements were performed in triplicate. Adenylate cyclase activity (mean ± SD) is expressed relative to the basic activity in corresponding control trypanosomes. The obtained value is a measure of stimulation.

hemoglobin-derived peptide present in the hindgut of the bug activates adenylate cyclase in *T. cruzi* epimastigote membranes and it stimulates the *in vitro* differentiation of epimastigotes to metacyclic trypomastigotes. Based on the amino sequence of this peptide these authors constructed a synthetic peptide which activated adenylate cyclase in *T. cruzi* epimastigote membranes. This peptide, however, was not capable to activate adenylate cyclase in procyclic *T. brucei* trypanosomes (S. Rolin and E. Pays, unpublished results).

In our experiments stimulation of adenylate cyclase of *T. brucei* is highly tissue-specific, i.e., only crude membrane fraction of the tsetse proventriculus/esophagus was effective. This stimulation was particularly directed to proliferating procyclic trypanosomes. Microscope observations of trypanosome populations associated with these tissues in the infected tsetse fly showed radical morphological changes of the trypanosomes and a switch in their cell cycle to a nonmultiplicative stage (Van Den Abbeele, unpublished results; Steiger 1973; Vickerman *et al.* 1988). We hypothesize that at least some of these changes are triggered by an interaction of the parasite with a specific membrane-bound compound present in these tissues. However, until now, none of these changes could be mimicked *in vitro* by incubating culture procyclic forms with proventriculus/esophagus tissue (Van Den Abbeele, unpublished results). This suggests that, besides a primary trigger, specific biochemical conditions and/or cell competence of the trypanosome determine whether a molecular communication between vector and parasite will induce appropriate parasite changes.

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