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STUDY OF THE SECRETORY ACTIVITY OF SINGLE SHORT MALPIGHIAN TUBULES (FORMICA CUNICULARIA) : PRELIMINARY RESULTS (Hymenoptera, Formicidae)

by
R. WELTENS and E. VAN KERKHOVE

L.U.C., Dept. WNF,
Universitaire Campus,
3610 Diepenbeek (Belgium)

Summary : The secretory activity of isolated Malpighian tubules of worker ants was studied. These tubules are rather short and thus have a low secretory rate. Nevertheless the tubules could be studied by adapting the methods. It was shown that the secretion rate depended on the K concentration and that the tubules concentrated chloride.

Key-Words : Formicidae, *Formica cunicularia*, hemolymph, Malpighian tubule, secretion rate, Cl transport.

Activité sécrétoire des tubules courts de Malpighi isolés de fourmis (*Formica cunicularia*) : résultats préliminaires

Résumé : L'activité sécrétoire de tubules de Malpighi isolés d'ouvrières de fourmi a été étudiée. Ces tubules sont assez courtes et ont dès lors une vitesse de sécrétion assez basse. En adaptant les méthodes nous avons pu nèanmoins les étudier. Il a été montré que la vitesse de sécrétion dépend de la concentration de K dans le bain et que les tubules arrivent à concentrer le Cl.

Mots-clés : Formicidae, *Formica cunicularia*, hémolymphe, tubule de Malpighi, vitesse de sécrétion, transport de Cl.

INTRODUCTION

Formica cunicularia has about 15 Malpighian tubules (MT) which are 1.8 to 2.5 mm long (unpublished results). Until now isolated, single tubules shorter than 3 mm with secretion rates smaller than 0.5 nl/min have only rarely been studied (Phillips and Maddrell, 1974, for ex. used several short tubules of *Aedes Campestris* to collect enough secretory fluid). By miniaturising collecting and analysing techniques one such tubule of a worker ant could be isolated and studied.

MATERIALS AND METHODS

Na concentration in the hemolymph was determined with an Eppendorf emission flame photometer. Cl concentration was calculated from the Cl content measured in 0.5 nl droplets with a WPI microtitrator (cfr. infra).

The dissecting solution (normal Ringer) contained 22 mM NaCl, 5 mM KCl, 10 mM Hepes, 2 mM $CaCl_2$, 13 mM $MgCl_2$, 17,8 mM Na_2-fumarate, 9 mM Na_3-citrate, 15.4 mM Na_2-succinate, 11.2 mM alanine, 10.6 mM Trehalose, 11.7 mM maltose and 137 mM glucose, pH = 7.20. In Na Ringer KCl was replaced by NaCl. In K-Ringer NaCl and Na_3-citrate were replaced by KCl and K_3-citrate respectively.

In high KCl-Na-free (or high NaCl-K-free) Ringer all K and Na salts were replaced by KCl (or NaCl respectively).

The M.T. were cut as close to the gut as possible and transferred to a plastic petri-dish in a bathing droplet, under paraffin oil. This petri-dish was placed on a Zeiss invertoscope (ICM 405) (fig. 1).

By applying negative pressure the cut end was sucked into a micropipet filled with paraffin oil. About 0.2 mm is lost in this holding pipet. The tubule is then pulled out of the droplet for a short distance (0.3 to 0.4 mm). A constriction in the holding pipet prevents the secreted fluid to leave the tubule and so the lumen widens. With a broken glass tip a hole is made in the short

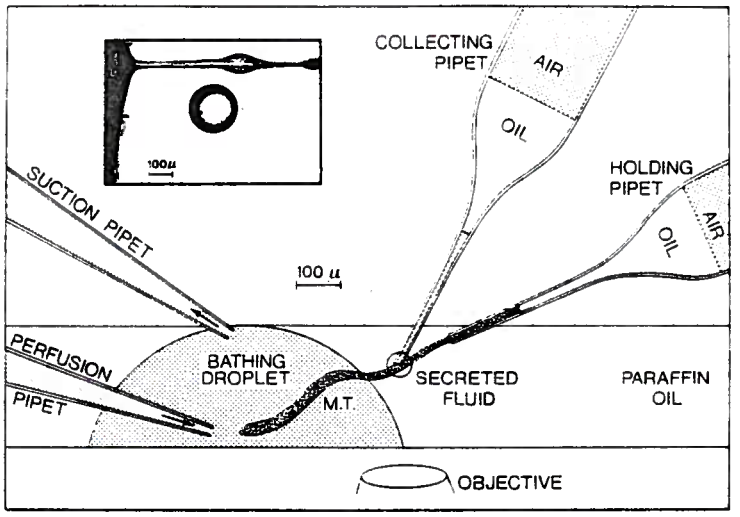

Fig. 1.

length exposed to the oil and a droplet appears. Another well siliconized micropipet is used to collect the secreted fluid at predetermined time intervals (10 to 20 min). At the end of each collection period the collected fluid is blown out of the pipet under oil so as to form a sferical droplet (see inset fig. 1). Photographs are made of the droplet and a calibrated meter at two different magnifications. So the radius can be measured to calculate volume and secretion rate.

Changing bath solutions and/or continuously perfusing the bath droplet was realised by placing a perfusion and suction pipet in the bathing droplet.

Cl-content of the collected fluid was measured on 0.5 nl samples by microtitration (W.P.I. F.T. 2230) 2 to 5 measurements were made for each collected droplet and compared to a calibration curve.

Results are given as mean value $\pm$ S.E. (n = number of droplets t = number of tubules).

RESULTS

[Na] in the hemolymph was 113 mM ± 4.6 (16 ants), [Cl] was 37 mM ± 1.9 (16 ants). [Na] and [Cl] in the dissecting Ringer were chosen accordingly.

Secretion rate and secreted [Cl] were first measured in K-Ringer (51 mM K). The rate was 60 pl/min ± 7.5 S.E. (n = 30, t = 12). [Cl] was almost tripled : from 57 mM in the bathing solution to 139 mM ± 5.4 S.E. (n = 10, t = 2) in the secreted fluid. The secretion virtually stopped when K-Ringer was replaced by Na-Ringer (t = 6) ; the effect was reversible.

In high KCl-Na-free Ringer ([K] = 103 mM, [Cl] = 133 mM) secretion rate rose to 183 pl/min ± 14 S.E. (n = 40, t = 7) and virtually stopped in high NaCl-K free Ringer (t = 2) ; the effect was reversible. [Cl] was almost doubled : from 133 mM to 230 mM ± 3.2 S.E. (n = 56, t = 20).

In two experiments (t = 2) the bathing [Cl] was kept constant (133 mM) but [K] was halved from 103 mM to 51 mM by mixing the high NaCl and high KCl Ringer. This did not significantly influence the [Cl] in the secreted fluid : 236 mM/l ± 5.0 S.E. (n = 12, t = 2) in 103 mM K; 228 mM/l ± 5.4 S.E. (n = 8, t = 2) in 51 mM K ($p > 0.10$). The secretion rate however fell to 79 %. The effect was reversible.

DISCUSSION

As in many other insect species K may be the prime mover in the secretory activity of Malpighian tubules of the worker ant. During the secretion process the chloride ion is concentrated. Measurement of [K] in secreted fluid and of transepithelial electrical potential difference is necessary to determine the electrochemical gradient for K and Cl and to decide which ion is actively transported.

REFERENCES

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