Inhibition of Nucleoside-3′:5′-Monophosphate Phosphodiesterase

A) Beef heart

Compound	I_{50} (μM)
AY-17,605	44
Theophylline	108
AY-22,252	150
AY-17,611	320
Digitoxin	350
AY-22,241	540

Final concentration of cyclic-AMP: $1.16 \times 10^{-7} M$

B) Rat brain

Compound	% Inhibition at $1 \times 10^{-4} M$
Theophylline	35
AY-17,605	24
Digitoxin	9
AY-22,252	7
AY-17,611	7
AY-22,241	3

Final concentration of cyclic-AMP: $0.58 \times 10^{-7} M$

yields a more active derivative than the natural lactone in the case examined.

When the various compounds were examined at 1×10^{-4} M for their inhibitory effect on the rat brain PDE, theophylline exhibited a moderate inhibition (35%) (Table B). None of the other compounds, i.e. AY-17,605, digitoxin, AY-22,252, AY-17,611 and AY-22,241, exhibited a greater activity.

The LINEWEAVER-BURK plot showed the K_m for the beef heart PDE was 33.3×10^{-7} M and 6.25×10^{-7} M for the rat brain PDE. With the beef heart PDE, and the cyclic-AMP at substrate concentrations of 0.875 and 1.75×10^{-7} M, AY-17,605 exhibited a K_I of 0.64×10^{-4} M as determined from the Dixon plot and its inhibition was of the non-competitive type. The K_I for theophylline was 1.3×10^{-4} M and the inhibition was also of a non-competitive nature.

The type of inhibition with theophylline apparently can vary according to the nature of the enzyme preparation utilized and the assay conditions employed as theophylline has also been reported to exhibit a non-competitive type in studies with dog heart and frog erythrocytes[8,9], competitive type with rat brain and beef heart[10,11] and mixed type with beef heart and rat erythrocytes[4,12].

In comparison with theophylline, the above factors appear to be of importance with regard to the inhibitory activity of AY-17,605 as indicated by the findings that AY-17,605 was more potent than theophylline with respect to the beef heart PDE but not to the rat brain PDE.

AY-17,605 could act to prevent the degradation of cyclic-AMP through its inhibitory action on heart PDE with higher levels of cyclic-AMP thereby resulting. This activity of AY-17,605 might be of importance with respect to its actions as a cardiotonic agent. Of interest in regard to the present findings was the demonstration that a different type of compound, i.e. 4-(3,4-dimethoxybenzyl)-2-imidazolidinone (RO 7-2956), which exhibited actions of a cardiotonic agent[13], was also an inhibitor of PDE[12], i.e. rat erythrocyte PDE.

Résumé. Des composés isomères aux glycosides cardiaques naturels ont démontré l'inhibition in vitro de l'enzyme nucleoside-3′:5′-monophosphate phosphodiesterase (PDE) d'origine cœur du bœuf exercée sur le cyclic-AMP, le composé le plus actif étant un dérivé de la lactone isomérique contenant une fraction 3-acetyl (AY-17,605); le K_I du composé AY-17,605 était de 0.64×10^{-4} M et le procédé inhibiteur était d'une nature non-compétitive. Aucun des composés n'a exhibé une activité supérieure autre qu'une inhibition modérée du PDE dans le cerveau du rat.

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Amino Acids in the Excreta of the Tsetse Fly, *Glossina palpalis*

The excretion of nitrogenous waste products has been investigated in many insects. Our knowledge on the subject has been reviewed by WIGGLESWORTH[1] and CRAIG[2]. But little information is available regarding the excretory products of hematophagous insects[3], particularly the tsetse fly[4]. The present work has been undertaken to determine the number and quantity of amino acids in the faeces of *Glossina palpalis*, and to provide a basis for further metabolic investigations.

Materials and methods. The flies used in this study were *Glossina palpalis* obtained as pupae from the Nigerian Institute for Trypanosomiasis Research, Kaduna, where enclosed colonies have been maintained for many generations. The pupae were reared at the Tsetse Research Laboratory, Langford, Bristol, and maintenance techniques of emergent flies were essentially the same as those described by NASH et al.[5]. Environmental conditions were maintained at about 25 °C and 80% r.h. Flies were kept in Geigy 10 type of cages and were fed on lop-eared rabbit blood. Immediately after feeding, the fly cages were

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placed in clean enamel trays in the fly room and collections of excrement were made at 24-h intervals from the trays as discrete pellets. These were sifted through a mesh sieve to screen out as much of the extraneous materials as possible.

Samples of faeces for analyses of free amino acids were prepared according to a slightly modified method of BURSELL[4]. A known weight of excreta (about 40 mg) was dissolved in 1 ml of 0.4% lithium carbonate. This was followed by acidification with 1 *N* HCl to precipitate almost all the uric acid and hematin in the sample. The precipitate was spun down and the supernatant evaporated to dryness at about 35 °C on a rotary evaporator. The dried residue was taken up in 1 ml of 80% methanol and undissolved residues removed by centrifugation.

The precipitate obtained by acidification of the original lithium carbonate extract of the excreta was hydrolyzed with 4 ml of 6 *N* HCl in a sealed glass tube at about 120 °C for 24–48 h. The contents of the tube were subsequently transferred to a 50-ml flask and evaporated to dryness at about 30 °C on a rotary evaporator to remove the HCl. The dried residue was treated the same way as described above for free amino acids sample, prior to analysis of the sample for protein-bound amino acids.

Preliminary analyses of unhydrolyzed and hydrolyzed excreta of the flies for amino acids were carried out using one-dimensional high voltage paper electrophoretic technique[6]. The extracts, about 10 µl each, were applied to Whatman No. 1 filter paper, 20 × 40 cm. The buffer used was 0.75 *M* formic acid, pH 1.9; voltage, 3000–3200 V; current, 30–35 mA; temperature, −1 to −2 °C; running time, 30 min. After the run, the paper was dried in a stream of air for about 2 h at 65 °C and developed in a 0.2% solution of ninhydrin in acetone to locate the spots.

The final technique employed for the separations and determinations of the amino acids in the faecal extracts of the flies was two-dimensional paper chromatography on No. 1 Whatman filter paper with 70% *n*-propanol as the first solvent in the ascending direction and water-saturated phenol as the second solvent (descending[7]). After drying, the chromatograms were developed by dipping in a 0.5% solution of ninhydrin in acetone and the ninhydrin-positive spots were identified with a prepared reference chromatogram. For quantitative estimation the spots were cut out and eluted individually in 5 ml of a copper nitrate complex reagent[8]. The optical density of each eluate was measured at a maximum absorption wavelength of 525 µm with a Unicam SP500 Series 2 Spectrophotometer, and estimates of the concentrations of amino acids detected in the excreta were determined with standard curves.

Results and discussion. The following amino acids were detected in *G. palpalis* excreta by the preliminary one-dimensional high voltage paper electrophoresis. These were: arginine, histidine, methionine, valine, α-alanine, leucine/isoleucine, lysine, cystine, glutamic acid, serine, glycine and proline. By use of two-dimensional paper chromatography, the amino acids were positively identified and their amounts determined, except serine, glycine and proline which were present in quantities too small to be estimated by the spectrophotometric method used in this study.

The results of quantitative analyses of the amino acids detected in the unhydrolyzed and hydrolyzed excreta of *Glossina palpalis* are presented in the Table. Arginine and histidine occur in largest amounts, together making up about 78% of the free amino acids detected in the excreta of the flies. The results agree with the findings of BURSELL[4] that arginine and histidine occur in unusually large amounts in the excreta of *Glossina morsitans*. In addition to arginine and histidine, valine and cystine are also present in relatively substantial amounts as free amino acids in the excreta of *G. palpalis* (Table).

Acid hydrolysis of the excreta also showed that arginine and histidine occurred in the highest concentrations of the protein-bound amino acids detected in the fly excreta. Methionine, alanine, leucine/isoleucine, lysine and glutamic acid which were detected in very small quantities in the free form occurred in increased amounts as protein-bound amino acids (Table).

The results of the present study clearly show that amino acids are present in the excreta of tsetse flies and they form a measurable part of their nitrogenous waste products. It would seem that these amino acids, particularly arginine and histidine detected in the excreta of *Glossina palpalis* are excretory products, as reported by BURSELL[4] for *G. morsitans*, rather than food ingested in excess of the insects' requirements[9].

Quantities of amino acids detected in the excreta of *Glossina palpalis* (µg/100 µl and % total)

Amino acid	Free amino acids		Protein-bound amino acids	
	µg/100 µl	% total	µg/100 µl	% total
Arginine	26.88	28.17	32.76	28.54
Histidine	47.22	49.50	53.71	46.79
Methionine	0.68	0.71	3.72	3.24
Valine	9.14	9.58	8.16	7.11
α-Alanine	1.86	1.95	5.17	4.50
Leucine/isoleucine	1.24	1.30	2.89	2.52
Lysine	1.68	1.76	3.18	2.77
Cystine	6.31	6.61	1.66	1.45
Glutamic acid	0.40	0.42	3.54	3.08
Total (µg)	95.41		114.79	

Each value represents the average of 4 determinations.

Summary. The faeces of the tsetse fly, *Glossina palpalis*, were examined for free and protein-bound amino acids. By use of chromatographic techniques 12 amino acids were detected, positively identified and their quantities estimated. Arginine and histidine occurred in largest amounts of all the amino acids detected in unhydrolyzed and hydrolyzed excreta extracts of *Glossina palpalis*.

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