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An insect acetylcholinesterase inhibitor from compound eyes of *Triatoma infestans* (Hemiptera)[1]

E. Wood, E. Zerba, M. de Villar, S. de Licastro and F. Melgar

Laboratorio de Entomotoxicología, CITEFA, Zufriategui y Varela, V. Martelli, Provincia de Buenos Aires 1603 (Argentina), 20 March 1979

Summary. The presence of an acetylcholinesterase inhibitor in the compound eyes of adult *Triatoma infestans* was demonstrated. The inhibitory activity was localized in the ocular pigments separated by disc gel electrophoresis. The inhibitor was selective against insect acetylcholinesterase, reversible, noncompetitive and heat stable.

The presence of naturally occurring inhibitors of enzymes in insects has been demonstrated in various species. Chymotrypsin[2], mixed function oxidase[3], glutathion S-transferase[4] and cholinesterase[5] are enzymes for which inhibition caused by endogenous insect materials has been reported. In the particular case of acetylcholinesterase (AChE) inhibitors, there are few data available about their characteristics and localization. This fact makes it difficult to study the possible physiological role of these compounds and their eventual use in the development of new structures useful as insecticides.

In previous studies on the AChE of *T. infestans* head we failed to determine any acetylcholine(ACh)-hydrolysing activity by the titrimetric[6] or the Hestrin method[7]. Later experience with the more sensitive Ellman's procedure[8] allowed us to measure the cholinesterase activity and purify the AChE present in the head of *T. infestans*[9].

During the development of the purification scheme we observed anomalies in the behaviour of the total activity, whose value increased in each step. This fact could be interpreted as being due to the presence of an endogenous inhibitor of AChE in the *T. infestans* head. The localization and characteristics of that inhibitor are the subject of this report.

Materials and methods. Adults and eggs of *T. infestans* were obtained from the colony reared in our Institute for 5 years. Specimens of other insect species were obtained from the Malbran Institute and Insher (Argentina). Commercial enzymes (erythrocytes bovine AChE and horse plasma ChE), 5,5′-dithiobis-(2-nitrobenzoate) (DTNB) and acetylthiocholine iodide (ATC) were obtained from Sigma (USA).

The enzymatic activity was determined by Ellman's colorimetric method[8], measuring changes in absorbance at 412 nm with a Varian model 634 spectrophotometer.

Polyacrylamide disc electrophoresis in tris glycine buffer (pH 8.3) was performed using a Bio Rad apparatus according to reported procedures[9]. Gel concentration was 7% and the current applied was 5 mA per tube for 1 h.

Results and discussion. The 1st step in the purification procedure of *T. infestans* head AChE developed in our laboratory[10] was to centrifuge the crude homogenate at 18,800 × g in 0.5 M NaCl-0.02 M phosphate buffer pH 7.2. After centrifugation almost all the ChE activity was recovered in the pellet. When we rehomogenized it in 15 mM sodium deoxycholate (DOC) in 0.02 M buffer phosphate pH 7.2 and centrifuged at 10,000 × g, the enzymatic activity remained in the supernatant, and a 50% increase with respect to the total activity before centrifugation was observed. This fact suggests to us the presence of a reversible ChE inhibitor present in the colored 10,000 × g pellet.

The inhibition of the AChE present in the *T. infestans* head appeared to be related to the color of the homogenate.

In fact, the rehomogenized and boiled 10,000 pellet were shown to be inhibitor of the AChE present in the supernatant and was active against the AChE of housefly head too.

As most of the color in the *T. infestans* homogenates was due to the ocular pigment, the compound eyes of the insect were excised and the AChE activity in homogenates of heads without eyes was compared with the activity in homogenate of whole heads. The hydrolysis rate of ATC determined in extract of heads with eyes in 0.5 M NaCl-0.02 M phosphate buffer pH 7.2 was 20% less. In both cases

Inhibitory action of *Triatoma infestans* eyes homogenate against cholinesterases from different sources

Source of cholinesterase	Specific activity (units*/mg of protein)	ATC hydrolysis rate (Δ abs.$_{412}$/min)	Inhibition final concentration (%) 3 eyes/ml	6 eyes/ml
T. infestans head (eyes free)	23.4	0.038	25	40
R. prolixus head	9.1	0.028	36	50
M. domestica head	71.5	0.054	17	26
P. americana head	21.5	0.051	17	31
Bovine erythrocyte	711.8	0.044	0	0
Horse serum	1352.0	0.056	0	0

* A unit of acetylcholinesterase is the amount catalyzing the hydrolysis of 1 nmole of ATC per min at 22 °C and pH 7.2.

we used the same concentration expressed in anatomical units: 18 heads per ml, and the activity was 23.4 and 18.7 units per mg of protein respectively. When the whole homogenate was reconstitued by mixing the homogenized eyes in water with the homogenate of the rest of the heads, the activity became almost the same as that measured in the extract of whole heads. The eye extract in 15 mM DOC was shown to be a good inhibitor of insect AChEs (table). Enzymes from the heads of housefly *(Musca domestica), Rhodnius prolixus,* cockroach *(Periplaneta americana)* and *T. infestans,* free of eyes, were inhibited by the colored homogenate of *T. infestans* eyes. In contrast no inhibitory activity was observed against mammalian cholinesterase as shown in the table.

We obtained an indirect proof of the localization of the inhibitor during the *T. infestans* embryogenesis. In previous work in our laboratory an embryonic development of 15 days until hatching was found[11]. The appearance of the compound eyes in the embryo was observed on the 7th day of development.

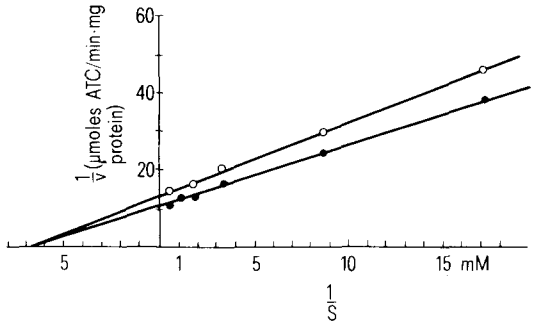
Taking into account the presence of the eyes, a boiled homogenate of 7-day-old eggs of *T. infestans* was assayed as an inhibitor. AChE from *T. infestans* heads free of eyes was inhibited 30% by a final concentration of 2.5 eggs per ml. In contrast, similar homogenates of 4–6-day-old eggs, in which the embryos lack visible eyes, did not show any inhibitory activity against insect AChE.

The eye extract in 15 mM DOC was subjected to electrophoresis in order to separate the ocular pigments present in the *T. infestans* homogenates. These pigments appear to be related to the AChE inhibition. We observed 2 partially overlapping pigments, violet and yellow respectively, with high electrophoretic mobility in our conditions.

The gel column was transversely sectioned into 10 pieces and each one was homogenized with a homogenate of housefly head (AChE activity: 71.5 units/mg protein) under similar conditions and incubated for 10 min. All the homogenates showed the same activity except the one containing both ocular pigments. In the latter case the hydrolysis rate of ATC was 70% inhibited.

With regard to the properties of the inhibitor, the K_m of housefly head AChE was determined with and without extract of *T. infestans* eyes. The results shown in the figure point out the noncompetitive characteristic of the inhibitor because of the similar K_m-value obtained in both cases (1.5×10^{-4} M).

Taking into account the above results, the inhibitor associated with the pigment from the *T. infestans* eyes could be defined as reversible, noncompetitive and selective against insect AChE.



Noncompetitive inhibition of housefly head AChE by homogenate from *Triatoma infestans* eyes. Enzyme activity was 71.5 units/mg protein and the incubation time 5 min. Results are expressed according to the method of Lineweaver and Burck (J. Am. chem. Soc. *56,* 658 (1934)). ● control, ○ inhibition (final concentration 3.5 eyes per ml).

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Time courses and refractoriness of enhanced vascular permeability induced by histamine, serotonin and bradykinin in synovialis of the rat

L.P. Bignold[1,2] and A.W.J. Lykke

School of Pathology, University of New South Wales, P.O. Box I, Kensington, (N.S.W. 2033, Australia), 15 February 1979

Summary. Enhanced vascular permeability induced in synovialis of the rat by histamine and serotonin lasts 5–15 min and that induced by bradykinin less than 5 min. Synovialis of the rat becomes refractory to the permeability effects of repeated doses of each of these substances in the hour following initial application.

Histamine, serotonin and bradykinin are known to enhance the permeability of the vasculature of synovialis in several mammalian species including the rabbit[3], monkey[4], rat[5] and dog[6]. In synovialis of the rat, serotonin is more potent than bradykinin, which is in turn more potent than histamine[5].

This paper reports an investigation of the duration of the permeability effects of histamine, serotonin and bradykinin on synovialis of the rat and of refractoriness of synovial vessels to repeated applications of these substances.

Materials and Methods. Albino rats of both sexes (b. wt 250–350 g) were used throughout and were lightly anaesthetized with ether for all injections.

Increased vascular permeability of synovialis of the stifle joints of the rats was detected by the use of i.v. injections of colloidal carbon as previously described[5]. After i.v. injection, carbon is removed from the circulation by the reticuloendothelial system within 1 h, but also collects in the walls of abnormally permeable blood vessels[7]. Each animal received colloidal carbon (Gunther Wagner, CII/1431a,