Isolation from Cobra Venom of a Factor Inhibiting Glycolysis in Ehrlich Ascites Carcinoma Cells

Inhibition of anaerobic glycolysis (EMBDEN-MEYERHOF pathway) by some snake (mostly *Najas*) venoms has been known for many years. As far back as 1934, MELLANBY[1-3] pointed out that glycolysis and respiration of Jensen's sarcoma cells are completely inhibited by very small amounts of cobra venom.

CHAIN[4-6] confirmed the inhibition of glycolysis in systems containing cell-free muscle extracts and in yeast fermentation maceration juice. The author[5] tried without success to purify the inhibiting factor by fractional precipitations. He ascribed[6] the action to the destruction of NAD, an essential coenzyme of the dehydrogenases implicated in the degradation of glucose.

In 1948 GHOSH and CHATTERJEE[7], working with suspensions of pigeon brain cells, confirmed this inhibition with venom concentrations of about 30 μg/ml; on the other hand, they showed that the succinic dehydrogenase is also inhibited by venom, but to a lesser degree.

They suggested therefore that destruction of NAD was not the only mechanism of inhibition.

While investigating brain homogenates, BRAGANCA[8] noticed that cobra venom, heated at 100 °C for 10 min causes an activation of glycolysis while intact venom causes a total inhibition, which the author ascribed to the DPNase and the ATPase of the venom.

YANG[9] investigated the action of the venom on glyceraldehyde 3 phosphate dehydrogenase isolated from rabbit muscle and noticed an inhibition of this enzyme; this inhibition, however, was complete only at venom concentrations of about 700 μg/ml.

Within the frame of the investigations conducted in our laboratory on the physiological activities of cobra venom constituents[10], the purification of an inhibitor of anaerobic glycolysis was obtained in a two-step chromatographic process: a fractionation of Formosan *Naja naja atra* venom on sulphoethyl Sephadex (Figure 1) followed by a molecular gel filtration of the inhibitory fraction on Sephadex G 50 (Figure 2).

The inhibitory activities were tested directly on anaerobic glycolysis of Ehrlich ascitic cells in a WARBURG apparatus[11]. The substance obtained, far less toxic than the total venom, has a proteinic constitution and appears to be homogeneous by electrophoresis on acrylamide gel. No nucleotidasic (NADase) or lecithinasic (Lecithinase A) activity could be detected in the isolated product.

Figure 3 shows the inhibitory activity curves of the substance as a function of the concentration (0–70 μg dry weight/ml range) for various incubation times. Studies made, up to now, in the field of glycolysis inhibition show no certainty with respect to the site and mechanism of that inhibition.

This is easy to understand since the authors, working with total venom, could not discriminate between the different simultaneous inhibitory actions.

In the first analysis, the separation of the previously described glycolysis inhibitor shows that the nucleotide

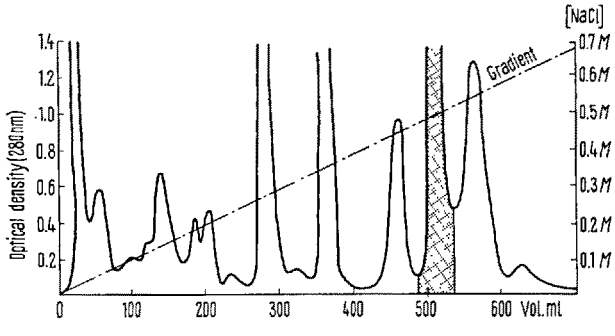

Fig. 1. Chromatography of *Naja naja atra* venom on SE Sephadex C 25. Column: height = 40 cm, ∅ = 1.5 cm. Elution linear gradient NaCl 0 → 0.7 *M*. Phosphate buffer pH 6, 0.05 *M*. Flow rate: 0.3 ml/min. Desalting: ultrafiltration on collodion membrane.

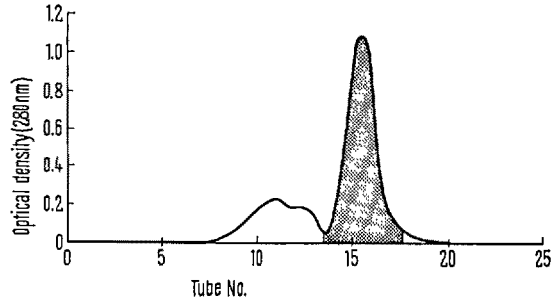

Fig. 2. Molecular gel filtration on Sephadex G 50 medium of the twelfth fraction obtained from SE Sephadex. Column: height = 200 cm, ∅ = 1 cm. Elution: ammonium acetate 0.1 *M*. Flow rate: 0.3 ml/min.

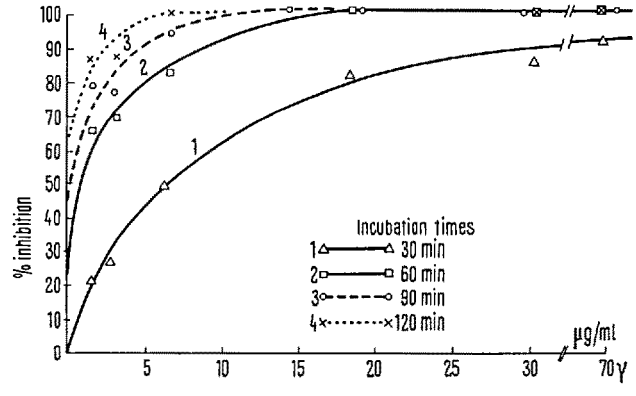

Fig. 3. Inhibition percentage of anaerobic glycolysis of ascitic Ehrlich carcinoma cells for different concentrations (μg/ml dry weight) of the isolated substance and for different incubation periods. Anaerobic glycolysis (Q lac. N_2) was measured manometrically in a WARBURG apparatus, at 39 °C in a *N* 95%, CO_2 5% atmosphere. The main flask contained 3 ml Krebs-Ringer 0.25% $NaHCO_3$, 0.1% glucose, pH 7.4, 20 × 10^6 cells. The side attachment contained 0.5 ml saline solution of venom. Saline solution was substituted for venom solution in control flasks and the final volume was always 3.5 ml.

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pyrophosphatase of the venom is not the essential factor responsible for the inhibition of that mechanism.

Investigations on the characterization of the isolated substance and on the nature and the site of its action are in progress in our laboratory[12].

Résumé. Un inhibiteur de la glycolyse anaérobie est purifié à partir du venin de *Naja naja atra* par un procédé couplant la chromatographie sur SE Sephadex et la filtration sur gel moléculaire. L'inhibition complète de la glycolyse anaérobie est obtenue sur une suspension cellulaire en 60 min pour une concentration inférieure à 20 μg/ml du produit purifié. L'inhibiteur est de nature protéinique et ne présente aucune activité nucléotidasique ni lécithinasique A.

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[12] These experiments were carried out in the laboratory of Biochemistry of the I.I.F.-I.M.C., CERIA, Brussels. We thank the Province de Brabant, the F. W. Breth Foundation, New York, for the financial and moral support given to this work. This work is a part of investigations made by one of us (L. B.) to obtain a doctorate degree.

Positive Correlation of Responsiveness to Catecholamines of the Rat Liver Glycogenolytic Receptor with Other α-Receptor Responses

Current views on the nature of the rat liver glycogenolytic adrenergic receptor are markedly divergent. Some investigators have concluded it is an α-receptor[1-4], while others[5-12] have suggested that it has not yet been adequately characterized. Accordingly, we were prompted to apply the correlation procedure utilized earlier[13,14] which had shown clearly that the adipose tissue lipolytic receptor exhibited the same profile of activity in response to catecholamines as did the cardiac receptor. This had shown lipolysis to be a β-receptor mediated response. In addition, the comparisons yielded clear-cut differentiation of the lipolytic-cardiac receptor from the vasodepressor-bronchodilator one. For simplicity of reference, it was suggested[14] that these different but closely related adrenergic receptors might be termed β-1 and β-2, respectively. More recently, LANDS et al.[15] have provided evidence for an additional β-1 adrenergic receptor, that of rabbit jejunum, while rat diaphragm and uterus were shown to manifest β-2 adrenergic receptor mediated responses.

Data on the delineation of the rat liver glycogenolytic adrenergic receptor are summarized below.

Procedure. For these studies Sprague-Dawley strain male rats, weighing 200–250 g, were maintained on feed and water ad libitum. The test compounds, in 0.01% ascorbic acid stabilized solution, were administered i.p. An hour later blood samples were taken by cardiac puncture and the glucose content, utilized as the index of liver glycogenolysis, determined by the glucose oxidase procedure[16]. In this procedure the glucose is enzymatically oxidized to yield H_2O_2, the oxygen liberated from the latter by catalase being estimated colorimetrically with *o*-dianisidine as the chromogen. The glucostat (Worthington) applied procedure[17] suggested for this series of reactions required modification in our hands to obtain a straight line reference curve. The modifications followed those of FALES et al.[18], utilizing pH 7.0 0.05 *M* phosphate buffer as the reagent diluent and final acidification with 2 drops 4 *N* HCl.

Results. The relative hyperglycemic activities of L-norepinephrine, L-nordefrin, DL-N-methyl-α-methylnorepinephrine and L-N-ethylnorepinephrine compared with a composite epinephrine response (= 100) based on multiple-level dose: response effects are shown graphically in the Figure. Norepinephrine and nordefrin were 1/3 as active as the reference, and N-methyl-α-methylnorepinephrine (as the DL form) and N-ethylnorepinephrine were about 1/4 as active as epinephrine. While epinephrine was active in the same range as that given earlier by ELLIS[19], norepinephrine was considerably more active. ELLIS reported norepinephrine to be 1/15 as hyperglycemic as epinephrine in contrast to the value of 1/3 given here. The reason for the discrepancy is not immediately evident.

The liver receptor was insensitive to L-isoproterenol, to DL-N-tert.butylnorepinephrine and to DL-indolyl-isopropyl-norepinephrine (DL-3,4-dihydroxy-α-([(2-(indol-3-yl)-1-methylethyl)amino]methyl)benzyl alcohol) at

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