

Monday, July 13, 1981

Poster Presentations

Coagulation – IV

Thrombin, Thrombin-like Enzymes

11:00–12:30 h

Simcoe Room Boards 149–160

0126

THE REACTION MECHANISM OF "LIMULUS TEST". T. Morita, S. Tanaka, T. Nakamura, M. Ohki and S. Iwanaga. Dept. of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan

The principle of the so-called "Limulus test" for detection of bacterial endotoxins (LPS) is based on the LPS-induced coagulation reaction, using horseshoe crab amebocyte lysate. To establish the overall molecular events in such a reaction system, we have previously studied on the complete amino acid sequence of a clottable protein, coagulogen, and the enzymatic properties of active clotting enzyme. We also developed a new chromogenic substrate method for assay of LPS, using Limulus amebocyte lysate. During these investigations, we found that proclotting enzyme, which has been characterized as a LPS-sensitive protein, is not sensitive to LPS but that another unknown factor sensitive to LPS is involved in the reaction sequence which mediates the activation of the known proclotting enzyme. The evidences were as follows.

When amebocyte lysate prepared from Tachypleus tridentatus (Japanese horseshoe crab) was applied to a column of heparin-Sepharose CL-6B, two components beside coagulogen, all of which are associated with the coagulation system, were separated into the breakthrough fraction (fraction A) and the adsorbed fraction (fraction B). Among them a component eluted in fraction A was identified as the known proclotting enzyme, but this proenzyme did not show any clotting activity even after preincubation with LPS. The other component, tentatively named factor B, eluted in fraction B showed a very weak LPS-dependent amidase activity towards Boc-Leu-Gly-Arg-pNA. However, when a catalytic amount of factor B treated with LPS was added to fraction A, a strong clotting activity appeared, indicating that both components are essential to develop the clotting activity in the presence of LPS. The same experiments as made for the lysate from T. tridentatus were performed using the lysate from Limulus polyphemus. The data was the same as those described above. These results indicate that the LPS-mediated coagulation system in the amebocyte consists of a multi-enzyme system and that this cascade system may provide an extremely high sensitivity of the lysate to endotoxins.

0127

GYKI 14,451, A SPECIFIC INHIBITOR OF THROMBIN: "IN VITRO" AND "IN VIVO" STUDIES. E. Tremoli, P. Maderna, S. Colli, G. Morazzoni and R. Paoletti. Institute of Pharmacology and Pharmacognosy, University of Milan, Milan, Italy.

The effects of a synthetic tripeptide, Gyki 14,451 (Boc-D Phe-Pro-Arg-H) have been studied in vitro on human platelet aggregation and arachidonic acid (AA) metabolism and in vivo on experimentally induced venous thrombosis in the rat.

1 μ M Gyki 14,451 concentration selectively inhibits thrombin induced platelet aggregation as well as malondialdehyde (MDA) and thromboxane B₂ (TXB₂) formation by platelet rich plasma (PRP) stimulated with thrombin. Far higher concentrations (400 μ M) of the peptide are required to exert an inhibitory effect when collagen, ADP and AA are used to stimulate platelets. No effect has been observed on the conversion of ¹⁴C AA to metabolites using unstimulated platelets. Kinetic studies of MDA production by platelets stimulated with thrombin and its inhibition by Gyki 14,451 (0.15, 0.3, 0.6 μ M) suggest that the peptide interacts with thrombin by an apparently competitive mechanism.

4 mg/Kg of Gyki 14,451, intravenously injected in the rat caudal vein, completely inhibited the occurrence of venous thrombosis, obtained by vena cava ligation.

The oral administration of the peptide (50 mg/Kg by gastric intubation) failed to reduce the percentage of incidence of venous thrombosis (88% in controls versus 90% in treated rats) resulting only in a reduction of the thrombus weight. These data suggest that the anticoagulant peptide Gyki 14,451, given intravenously, possesses a consistent activity in the prevention of experimentally induced venous thrombosis.