

Tuesday, July 14, 1981

Plenary Lectures

13:30-15:10 h

Mechanisms for the Regulation of Coagulation

C. M. JACKSON
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St. Louis, Mo., USA

Inhibition of Coagulation and its Application in the Management of Thromboembolic Disorders

J. HIRSH
McMaster University
Hamilton, Ont., Canada

Grand Ballroom

0460

THE EFFECT OF PF-4 PEPTIDE ANALOGUES ON PLATELET ACTIVATION AND RELEASE. Daniel A. Walz, Jawed Fareed and Sandor Bajusz
Wayne State University School of Medicine, Detroit, MI, Loyola University School of Medicine, Chicago, IL USA and Institute for Drug Research, Budapest, Hungary.

The complete amino acid sequence of human PF-4 has demonstrated it to be a 70 residue protein with a lysine-rich carboxy terminal region. Chemical data, drawn from specific modification of these lysine residues, has been strongly suggestive of the dominance of these lysines in the interaction of PF-4 with heparin. We have synthesized arginyl- and lysyl- peptide analogues of the human PF-4 sequence, residues 58-68, to mimic the putative heparin binding domain. These peptides include:

1. PRO-LEU-TYR-ARG-ARG-ILE-ILE-ARG-ARG-LEU-LEU
2. LEU-TYR-ARG-ARG-ILE-ILE-ARG-ARG-LEU-LEU
3. TYR-ARG-ARG-ILE-ILE-ARG-ARG-LEU-LEU
4. ARG-ARG-ILE-ILE-ARG-ARG-LEU-LEU
5. PRO-LEU-TYR-LYS-LYS-ILE-ILE-LYS-LYS-LEU-LEU

Peptides 1 and 5 are the most effective samples in plasma for heparin neutralization. When each peptide was incubated with platelet rich plasma (PRP) in submicromolar concentrations, and followed by the addition of ADP, peptides 1-4 but not peptide 5 quantitatively blocked the release of PF-4 as judged by radioimmunoassays of the PRP supernatant. Peptide 1 also suppressed the second aggregation wave following ADP stimulation. Upon completion of aggregation of PRP, exogenously added PF-4 or these peptide analogues appeared to promote clot retraction. In other experiments, PF-4 and its peptide analogues induced a strong contraction in isolated uterus and ileum preparations. We are presently evaluating the ability of these analogues to block the interaction of PF-4 with its platelet proteoglycan carrier. This may mean that PF-4 or its analogues interact directly with cellular contractile elements and thereby modulate their contractile response.

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CHEMILUMINESCENCE IN HUMAN PLATELETS.

P. Wörner, Abtlg. f. Immunologie u. Serologie am Institut f. Hygiene u. Med. Mikrobiologie, Mannheim.
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Chemiluminescence (CL), the formation of malondialdehyde (MDA) and serotonin release were studied in suspensions of washed platelets in the presence of luminol at 37°C. CL was induced by concanavalin A, A23187, arachidonic acid, thrombin, N-ethylmaleimide (NEM) and thimerosal.

Concanavalin A- and the major part of A23187-induced CL was due to leukocytes contaminating the platelet samples. Leukocytes had no influence on CL by the other stimuli.

Arachidonic acid evoked two separate signals: the first was peaking at 7 s and decayed within 20 s, while the maximum of the second signal occurred 50 s and returned to the background 70-110 s after its addition. Thrombin induced also two signals, however, with low intensity and with a lag phase of 6 s prior to the onset of the first signal. Exposure of platelets to NEM (≥ 0.5 mM) lead to a single signal after a lag phase, depending on its concentration, between 20 and 50 s. Thimerosal-induced CL consisted also of a single signal peaking after 35-120 s.

ASA and indomethacin prevented CL, formation of MDA and release promoted by NEM or thimerosal. They interfered with MDA-generation and the first signal of CL induced by arachidonic acid or thrombin. ASA and indomethacin enhanced and NEM (50 μ M) suppressed the second signal of CL induced by arachidonic acid or thrombin.

It is concluded that CL promoted by NEM and thimerosal as well as the first signal induced by arachidonic acid or thrombin depends on prostaglandin synthesis. The second signals indicate arachidonic acid turnover by lipooxygenase. Sulphydryl reagents such as NEM interfere with platelet lipooxygenase resulting in only one signal of CL. Measurement of CL offers a useful tool with the advantage to allow monitoring of the time course of prostaglandin synthesis and lipooxygenase pathway in intact platelets.