CHEMICAL INACTIVATION OF THROMBOPLASTIN ACCORDING TO 23Na, 31P-NMR

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Thromboplastin (tissue factor, TF) is the membrane fragments of damaged cells, which in the presence of calcium ions are able to activate Factor YII of blood clotting system. From all amount of membrane proteins the only integral protein - apoproteid III plays the determinant role in membrane thromboplastic activity (1). Excess of TF in blood vessels causes one of the most dangerous disturbances in the the blood coagulation - so called disseminated intravascular coagulation. Still now there are no effective chemical agents for inactivation of TF in vivo. At the present study we have studied inactivation of TF from the human brain in vitro and vivo. The chemical inhibitors of TF glutaric aldehyde, glycerol and dimexidum were used (2,3).31P and 23Na-NMR spectra were analysed on spectrometers CXP-100 and WM-250 (Bruker, FRG).

Thromboplastin from the human brain is presented by a lipoproteid complex, phospholipid component of which is organized into the hexagonal (HII) lyotropic mesophase (4). Analysis of 31P-NMR spectra showed the following. Phospholipids in the structure of TF at 274- 370 K in the presence of glutaric aldehyde, glycerol and dimexidum retain the hexagonal (HII) form of packing. Heating up to 370 K and the following cooling up to 310 K don't significantly influence onto the lipids phase state in the structure of TF. Removing a protein component essentially changes lipids state, since in the case of total lipids fraction at 274-350 K some part of lipids is reorganised into the lamellar mesophase. In a water solution of NaCL NMR signal of 23Na has the Lorentz form with a halfwidth nearly 7 Hz. In the water suspension of TF 23Na-NMR signal can be presented as a superposition of two Lorentz lines with a different halfwidth, but with the same resonance frequency. This form of spectra indicates that binding of natrium is described by the model of rapid two-positional exchange between free (natrium ions are in a water solution) and bound conditions (natrium ions are bound with the surface of TF).In the presence of dimexidum the line of bounded natrium ions becomes wider. This change we explain as the result of protein plunging (with bound natrium ions) into the lipid matrix of thromboplastin.

Intravascular injection of dimexidum with the following intravenous infusion of letal dose of thromboplastin prevents the animals death.

Analysis of proteolytic (5), thermal (6) and chemical inactivation of TF permitted us to work out requirements to drug properties wich may be used for inactivation of thromboplastin in vivo.I.Common properties: a) have no toxic, allergenic and enzymatic activity b) should not disturb the biosynthesis of procoagulants and reduce the activity of plasma factors of blood clotting system. II. Membranetropic properties: a) the drug should not change the phase state of lipids and destroy the physical integrality of lipid and protein components of membranes b) the drug has to increase reversibly the hydrophobic properties of apoproteid III.

The following scheme of thromboplastin inactivation is suggested. Factor YII-binding center consists of the apopropteid III and 2-3 surrounding bilamellar layers of phospholipids. Apoproteid III specifically orients lipids around itself in such a way that this part of membrane in the presence of calcium ions acquires affinity to the Factor YII. The increase of hydrophobic properties of the apoproteid III (plunging into the lipid matrix) reduces its influence on the surrounding lipids. Induced lipids orientation is disturbed and the affinity of membrane to Factor YII decreases, resulting in thromboplastic reaction inhibition.

References

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