INFLUENCE OF PHOSPHOLIPASE A AND THE "DIRECT" HEMOLYSIN FROM COBRA VENOM ON ARTIFICIAL BIMOLECULAR MEMBRANES

L. Ya. Yukel'son and O. V. Krasil'nikov

UDC 591.105:577.15.598.126

It has been shown previously [1] that the components of cobra venom that cause the hemolysis of erythrocytes are the "direct" hemolytic factor (DHF) and phospholipase A. The synergistic nature of the action of these components on biological membranes has been established. In the present work we discuss how the DHF and phospholipase A of cobra venom affect artificial bimolecular membranes (ABMs), which represent a simplified model of biological membranes.

The direct hemolytic factor and phospholipase A were obtained from the venom of the Central Asian cobra in the pure state [3, 4]. The artificial bimolecular membranes were made from the phospholipids of human erythrocytes by a published method [5]. In the experiments we determined the mean lifetime (MLT), i.e., the stability, of the ABM and its permeability in the presence of the DHF and phospholipase A (Table 1).

Phospholipase A in concentrations of $7.7 \cdot 10^{-7}$ and $3.85 \cdot 10^{-6}$ M decreases the stability of the ABM but scarcely affects its permeability. The addition of DHF to the phospholipase A enhances its action on the MLT of the membrane and the permeability of the ABM increases by an order of magnitude. DHF itself does not affect the stability but increases the permeability of the ABM, which correlates with its action on biological membranes [6, 7]. The summation of the two processes is probably the basis of the synergistic hemolytic effect of DHF and phospholipase A taken together.

The ions Ca^{2+}, which are well-known activators of phospholipase A [1], inhibit its effect on an ABM, which is apparently due to their capacity for stabilizing the membrane. On the simultaneous addition to an ABM of the three ingredients (phospholipase A, DHF, and Ca^{2+}) the MLT of the membrane remains the same as in the presence of phospholipase A alone in a concentration of $3.85 \cdot 10^{-6}$ M, but the permeability rises. Ca^{2+} somewhat reduces the increase in the permeability of the ABM caused by DHF. An analogous effect of the inhibition by calcium of the action of DHF has been demonstrated on biological membranes [2].

TABLE 1

Parameters of the membrane	Phospholipase A		Phospholipase A ($3.85 \cdot 10^{-6}$ M) and 10^{-7} M DHF	Phospholipase A ($3.85 \cdot 10^{-6}$ M) and 4mM Ca^{2+}	Phospholipase A ($3.85 \cdot 10^{-6}$ M), 10^{-7} DHF, and 4 mM Ca^{2+}
	$7.7 \cdot 10^{-7}$ M	3.85×10^{-6} M			
MLT, min	102	27±2,4	11±2,8	50±6,3	21±1,5
Conductivity, mho/cm^2	$1,75 \times 10^{-9}$	$4,55 \times 10^{-9}$	$3,0 \times 10^{-8}$	$8,3 \times 10^{-9}$	$1,8 \times 10^{-8}$

Note. In all cases the medium contained 5mM tris and 150 mM NaCl, pH 7.6.

LITERATURE CITED

1. D. N. Sakhibov, V. M. Sorokin, and L. Ya. Yukel'son, The Chemistry and Biochemistry of Snake Venoms [in Russian], Tashkent (1972).
2. P. G. Lankisch, B. Lege, H. D. Oldigs, and W. Vogt, Biochem. Biophys. Acta, 239, 267 (1971).

Institute of Biochemistry, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 5, p. 687, September-October, 1974. Original article submitted February 25, 1974.

3. L. Ya. Yukel'son, É. S. Sadykov, and V. M. Sorokin, Uzb. Biol. Zh., No. 4, 5 (1973).
4. D. N. Sakhibov, V. M. Sorokin, and L. Ya. Yukel'son, Biokhimiya, 35, 13 (1970).
5. P. Mueller, D. O. Rudin, H. T. Tuen, and W. C. Wescott, J. Phys. Chem., 67, 534 (1963).
6. C. C. Chang and C. Y. Lee, Br. J. Pharmac. Chemother., 28, 172 (1966).
7. B. S. Meldrum, Br. J. Pharmac. Chemother., 25, 197 (1965).