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INHIBITION OF NA^+- AND K^+-ACTIVATED ATPase BY THE DIRECT LYTIC FACTOR OF COBRA VENOM (*NAJA NAJA*)

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SUMMARY

The Mg^{2+}-dependent, Na^+–K^+-activated ATPase of ox brain was inhibited by the direct lytic factor of cobra venom at concentrations of 10^{-7} g/ml or higher. Only weak inhibition was seen in ghosts of human red cells. Haemolysis of guinea-pig red cells by phospholipase A was not enhanced when the erythrocyte ATPase had been blocked by ouabain. It is concluded that direct lytic factor-induced haemolysis is not dependent on an ATPase inhibiting effect.

The direct lytic factor of cobra venom (*Naja naja*) and other basic peptides with disulphide bonds strongly enhance phospholipase A haemolysis of red cells. As SH reagents like *p*-chloromercuribenzoate and *N*-ethylmaleimide similarly potentiate the lytic effect of phospholipase A it has been suggested that the disulphide peptides also react with SH groups of membrane proteins[1]. Blockade of these groups increases membrane permeability and inflow of water and ions[2]. Swollen cells are more readily attacked by phospholipase A[3]. SH reagents inhibit Mg^{2+}-dependent, Na^+–K^+-activated ATPase [4,5] an effect which also leads to accumulation of Na^+ in the cells and might cause swelling. This effect could possibly contribute to the lytic action. We have therefore studied whether direct lytic factor inhibits the transport ATPase and whether this effect is essential for the synergistic action with phospholipase A.

Na^+–K^+-activated ATPase was purified from ox brain[6]. Its activity and the interaction of direct lytic factor[7] was tested by spectrophotometric assay[6]. As shown in Fig. 1, direct lytic factor inhibits the ox brain ATPase. The threshold concentration is about 10^{-7} g/ml; 10^{-4} g/ml produce nearly 50 % inhibition.

This effect possibly explains the finding of Wolff *et al.*[8] that cobramine B (identical with direct lytic factor) inhibits iodide accumulation by thyroid and parotid slices or by isolated choroid plexus preparations. Other studies have shown that Na^+–K^+-activated ATPase from ox brain is inhibited by whole *Naja naja* venom and by phospholipase A[9,10]. Our results indicate that part of the inhibition by whole venom is due to direct lytic factor.

To see whether inhibition of ATPase might be involved in direct lytic factor-induced haemolysis the effect of direct lytic factor on Na^+–K^+-activated ATPase

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of red cells was studied. Two preparations of human erythrocyte ghosts [11] — much less active than the ox brain ATPase — were indeed inhibited by direct lytic factor. However, the effect was detectable only at very high concentrations of direct lytic factor (about 25 % inhibition by 10^{-3}g/ml direct lytic factor). The transport ATPase of guinea-pig red cells was even less sensitive. A weak inhibitory effect of direct lytic factor was also directed against the Mg^{2+}-dependent ATPase of red cells.

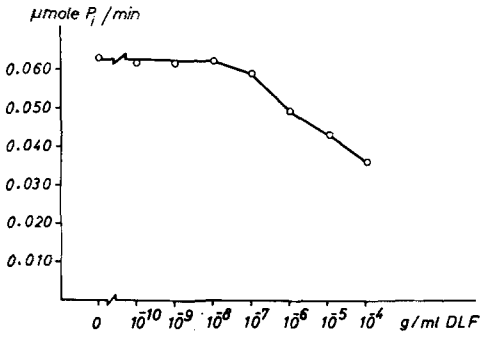

Fig. 1. Inhibition of Na^+–K^+-activated ATPase from ox brain by direct lytic factor (DLF). Abscissa: concentration of DLF. Ordinate: release of inorganic phosphate (µmole/min per mg protein).

Guinea-pig red cells were incubated with ouabain (10^{-4} M) for up to 3 h. This treatment did not enhance the lytic activity of phospholipase A of bee venom[1] checked subsequently. The blockade of the ATPase was also without effect on the lytic activity of direct lytic factor, alone or in combination with phospholipase A.

Our results demonstrate that direct lytic factor inhibits the Na^+–K^+-activated ATPase. However, this effect does not contribute to the synergistic action of direct lytic factor and phospholipase A on red cells. The direct lytic and phospholipase A potentiating effect of direct lytic factor is rather brought about by increased passive permeability. The inhibitory effect of direct lytic factor on transport ATPase may, however, be relevant for actions on other cell systems.

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REFERENCES

1 W. Vogt, P. Patzer, L. Lege, H.-D. Oldigs and G. Wille, *Naunyn-Schmiedebergs Arch. Pharmak.*, 265 (1970) 442.
2 H. S. Jacob and J. H. Jandl, *J. Clin. Invest.*, 41 (1962) 779.
3 P. G. Lankisch and W. Vogt, *Acta Pharmacol. Toxicol.*, 29, Suppl. 4 (1971) 29.
4 J. Chr. Skou, *Biochem. Biophys. Res. Commun.*, 10 (1963) 79.
5 P. Emmelot and C. J. Bos, *Biochim Biophys. Acta*, 120 (1966) 369.
6 W. Schoner, C. v. Ilberg, R. Kramer and W. Seubert, *Eur. J. Biochem.*, 1 (1967) 334.
7 P. G. Lankisch, L. Lege, H.-D. Oldigs and W. Vogt, *Biochim. Biophys. Acta*, 239 (1971) 267.
8 J. Wolff, H. Salabé, M. Ambrose and P. Reed Larsen, *J. Biol. Chem.*, 243 (1968) 1290.
9 K. Taniguchi and Y. Tonomura, *J. Biochem. Tokyo*, 69 (1971) 543.
10 K. Taniguchi and S. Iida, *Biochim. Biophys. Acta*, 233 (1971) 831.
11 B. Roelofsen, H. Baadenhuysen and L. L. M. van Deenen, *Nature*, 212 (1966) 1379.