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EFFECT OF THIOL-REACTIVE REAGENTS ON THE PERMEABILITY OF FISH ERYTHROCYTE MEMBRANE FOR SPIN-LABELLED NON-ELECTROLYTES

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Carp erythrocytes were treated with *p*-chloromercuribenzoate or *N*-ethylmaleimide. It was observed that these thiol-group inhibitors decreased the transport of spin-labelled hydrophilic compound, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl, and increased the transport rate of more hydrophobic 2,2,6,6-tetramethylpiperidine-1-oxyl.

Transport of small hydrophilic molecules across biological membranes is inhibited by certain thiol-reactive reagents, such as NEM[1], DTNB, PCMB and other organomercuric compounds (Macey & Farmer, 1970; Sha'afi *et al.*, 1971; Naccache & Sha'afi, 1974; Sha'afi, 1977). Application of small nitroxide molecules is a very useful method for studying the transport across the erythrocyte membrane. Nitroxide compounds incubated with red blood cells penetrate the cell membrane and are reduced to non-paramagnetic hydroxylamines (Ross & McConnell, 1975). The reduction capacity of erythrocytes is usually in excess compared to the amount of the spin label used.

We have observed that thiol-reactive reagents produce a decrease of the permeability of membranes for a spin-labelled non-electrolyte in bovine erythrocytes (Gwoździński *et al.*, 1983). In the present work the effect of thiol-reactive reagents on the permeability of fish erythrocyte membrane to 2,2,6,6-tetramethylpiperidine-1-oxyl and 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl was examined.

[1] Abbreviations used: DTNB, 5,5'-dithio-bis-2-nitrobenzoate; NEM, *N*-ethylmaleimide; PCMB, *p*-chloromercuribenzoate; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl; TEMPOL, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl; EPR, electron paramagnetic resonance.

MATERIALS AND METHODS

Adult carps (*Cyprinus carpio*) were kept in an aquarium for two weeks before the experiment. Fish blood was drawn by tail vein puncture with a heparinized syringe containing 0.6% NaCl and heparine as anticoagulant. Erythrocytes were separated according to the method previously reported (Gwoździński, 1983). Erythrocytes were incubated with thiol inhibitors for 30 min at room temperature. TEMPOL and TEMPO were synthetized according to Rozantsev (1970). The transport of spin-labelled compounds into erythrocytes was studied by determining their concentration after appropriate time intervals by ESR spectroscopy. ESR measurements were performed with an SE/X-28 ESR spectrometer (Wrocław Technical University).

RESULTS AND DISCUSSION

The rate of disappearance of the ESR signal from the erythrocyte suspension incubated with TEMPO or TEMPOL was a measure of the rate of transport of the spin label across the membranes. The decay of signal intensity was monophasic (Fig. 1) and could be described for both spin labels by the equation:

$$c = C + c_o \exp(-kt)$$

where c is the extracellular concentration of the label, c_o is the fraction of the label which decays exponentially with a permeation constant of k, t is the time and C is the fraction of the label subject to a rapid reduction (presumably by cellular constituents released into the extracellular medium), (Gwoździński *et al.*, 1981). In order to compare the effect of thiol inhibitors on the transport rate of TEMPOL and TEMPO, the reduction half-times of the labels were computed. The half-time parameter was calculated on the basis of the ESR signal height. On comparing the respective half-times of the reduction rate of untreated and inhibitor-treated erythrocytes it was found that 0.2 mM-PCMB caused a decrease of the TEMPOL penetration rate by approximately 25% and an increase of the more hydrophobic label TEMPO by approximately 20%. The effect of NEM was less pronounced than that of PCMB but had the same character: the rate of TEMPOL transport was decreased by approximately 16% and that of TEMPO increased by about 16% (Table 1). Similar results were obtained on studying the transport of some spin labels across carp erythrocyte membrane after exposure *in vitro* to gamma radiation (Gwoździński, 1983). This similarity is most likely due to the operation in both cases of the same mechanism, since the ionizing radiation destroys thiol groups by oxidizing them.

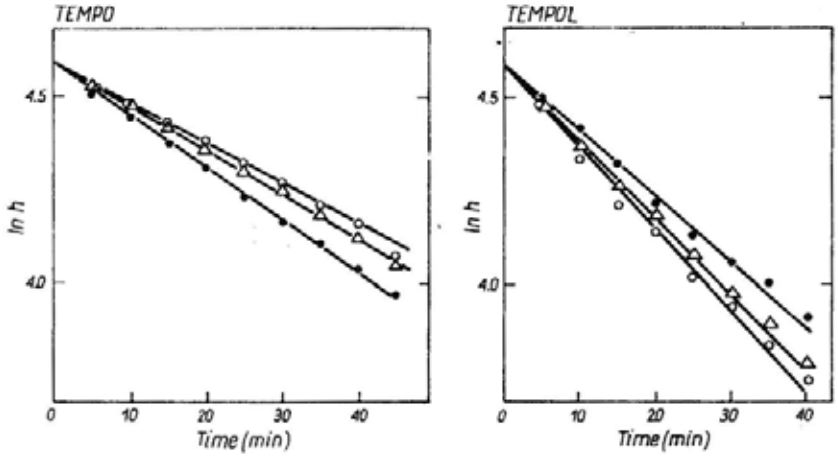

Fig. 1. Kinetics of permeation of TEMPOL and TEMPO into carp erythrocytes. ●, Control; ○, 0.2 mM-PCMB; Δ, 0.2 mM-NEM.

Table 1

The effects of PCMB and NEM on kinetic parameters of the transport of TEMPO and TEMPOL into carp erythrocytes

Concentration of the thiol-group inhibitor was 0.2 mM, temperature 21°C ± 1°C; c_0 is expressed as percentage of the initial inhibitor concentration; $T_{1/2}$ is the resultant half-time of decay of the spin label; k is the permeation constant. The results are mean values ± S.D.; p was estimated by paired Student's t test, n = 6.

Inhibitor	k (min^{-1})	c_0 (%)	$T_{1/2}$ (min)
	A. TEMPO transport		
None	0.0042 ± 0.0004	100.6 ± 1.7	39.2 ± 4.4
PCMB	0.0049 ± 0.0004[c]	98.9 ± 4.7	31.2 ± 3.5[a]
NEM	0.0048 ± 0.0005[d]	100.0 ± 2.2	33.0 ± 2.9[a]
	B. TEMPOL transport		
None	0.0034 ± 0.0002	100.6 ± 2.1	48.3 ± 4.1
PCMB	0.0028 ± 0.0002[c]	101.2 ± 1.4	60.2 ± 5.2[a]
NEM	0.0030 ± 0.0002[b]	100.6 ± 2.6	55.3 ± 5.1[c]

[a] $p < 0.001$; [b] $p < 0.002$; [c] $p < 0.01$; [d] $p < 0.02$.

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