INHIBITION OF ERYTHROCYTE HEMOLYSIS BY SOME 1,4-DIHYDROPYRIDINE DERIVATIVES

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Ability to inhibit erythrocyte hemolysis is often used as a characteristic of the membrane-stabilizing action of chemical compounds. In the investigation described below the acid erythrogram method [4] was used to determine the membrane-stabilizing activity of a number of 4-substituted 3,5-dicarbonyl derivatives of 1,4-dihydropyridine (1,4-DHP), some of which possess cardiovascular activity [3].

EXPERIMENTAL METHOD

The compounds were synthesized by known methods [1, 3]. Chromatographically pure specimens were used. A suspension of packed red cells from human blood donors was diluted 100 times with 0.9% NaCl solution. To 2.4 ml of the diluted erythrocyte suspension (about 6×10^6 cells/ml) 25 µl of a 5×10^{-3} M solution of the test substance in ethanol was added (in the control experiment 25 µl of ethanol alone was added) and the sample incubated for 20 min at 37°C. Erythrocyte hemolysis was induced by the addition of 0.1 ml of 0.05 N HCl. Changes in optical density at 576 nm were recorded on a "Spectronic-70" spectrophotometer. The inhibitory effect (IE) was estimated as the relative increase in the time taken for 50% hemolysis: $IE = \tau/\tau_0$, where τ is the 50% hemolysis time with the test substance (in min), τ_0 the same without the substance.

To determine the lipophilicity of the compounds the partition coefficients (P) were determined in an octanol–water system. Saturated solutions of the compounds were prepared in octanol (saturated with water) and in distilled water (saturated with octanol). The partition coefficient was determined by the equation:

$$P = \frac{C_O}{C_W},$$

where C_O and C_W denote the concentration of the given compound in octanol and water respectively.

EXPERIMENTAL RESULTS

The results indicate that the derivatives of 1,4-DHP tested delay erythrocyte hemolysis comparatively effectively (Table 1). The inhibitory activity when 4-furyl derivatives were used changed parallel with changes in lipophilicity of the compounds, and was a parabolic function of the partition coefficient, described by the equation:

$$\log \tau/\tau_0 = -0.13 (\log P)^2 + 1.03 \log P - 1.86;$$

$n = 6$, $r = 0.96$, $S = 0.03$. The optimum of inhibitory activity was found at the value log P = 3.9. The effect of improved lipophilicity explains why 4-substituted 1,4-DHP stabilize erythrocyte membranes, despite the fact that their antiradical and antioxidant activities are weaker than those of compounds not substituted in position 4 [2]. The change from a 4-methyl- to a 4-phenyl-substituent, accompanied by an increase in lipophilicity, also increased the inhibitory activity of the 1,4-DHP. The effectiveness of the most active compounds (Table 1, compounds 3, 5-7) was comparable with that for the widely used antioxidant

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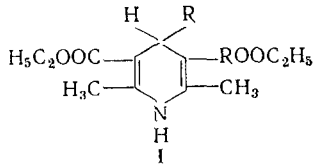
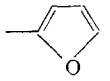
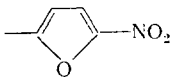
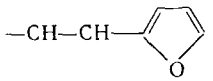
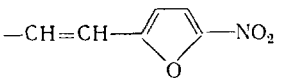
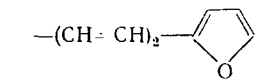
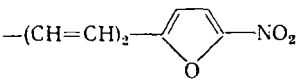
TABLE 1. Logarithms of Partition Coefficients of 1,4-DHP Derivatives and Inhibitory Effect on Erythrocyte Hemolysis



I

Compound	I. R =	lg P	τ/τ_0
1	H	3,6	1,2
2	CH_3	3,7	1,3
3	C_6H_5	4,0	1,6
4		3,7	1,5
5		3,9	1,7
6		4,7	1,6
7		3,5	1,6
8		5,4	1,0
9		3,2	1,4

ional (τ/τ_0 = 1.8). Substitution of the ethyl radical in the ester group in positions 3 and 5 of the dihydropyridine ring by the less lipophilic methyl radical reduced the inhibitory activity of the 1,4-DHP. For example, the corresponding compound 4 (the 3,5-dimethoxycarbonyl derivative) has log P = 2.6 and τ/τ_0 = 1.1.

The investigation described above showed that 4-substituted derivatives of 1,4-DHP effectively delay erythrocyte hemolysis and that this inhibition is a parabolic function of the lipophilicity of the compounds.

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