The influence of the steroid hormones on the physical state of human erythrocyte membranes[1]

L. Lacko and B. Wittke

Gustav-Embden-Zentrum der biologischen Chemie, Abteilung für physikalische Biochemie, Theodor-Stern-Kai 7, D–6000 Frankfurt am Main (Federal Rebublic of Germany, BRD), 9 July 1976

Summary. Steroids alter the physical state of the regions in the human erythrocyte membrane, where glucose transport is occurring.

In the course of recent years, attention has been focussed on the relationship between the biological activity and the physical state of biomembranes. It has been pointed out that, not only the transport velocities[2,3] of some substances entering the cells, but also the enzymatic activities[4] and the properties of some inhibitors of membrane-bound enzymes[5], depend on the fluidity of membranes. Since an effect of cholesterol[6] on membrane fluidity has been described, we have investigated whether steroids inhibiting the glucose transport in human erythrocytes[7] have any impact on the physical state of the membrane-region, where the glucose transport is located. As indication for its change, the following parameters were determined: the temperature of the phase transition and the apparent activation energy of the glucose transport in the presence of the steroids, and the Hill coefficients for the inhibition of the glucose transport by the steroid hormones.

Materials and methods. Erythrocytes were concentrated by centrifugation of freshly collected blood of healthy donors in an ACD solution (11 g sodium-citrate, 35 g glucose, 4 g citric acid with aqua bidest. ad 1000 ml). The erythrocytes were preloaded by 4 washings with an isotonic NaCl solution containing 200 mM glucose. Then 150 microliters of those preloaded cells were incubated in 10 ml phosphate buffer of pH 7.5 with 2% ethanol containing C^{14}-glucose concentrations from 0.75 to 3.0 mM (0.5 K_m-values for the different temperatures). The steroid concentrations were 0.3 mM cortisol and 0.1 mM 5α-androstane-3,17-dione for the Arrhenius plot. For the Hill plots, the glucose concentration was 0.033 mM;

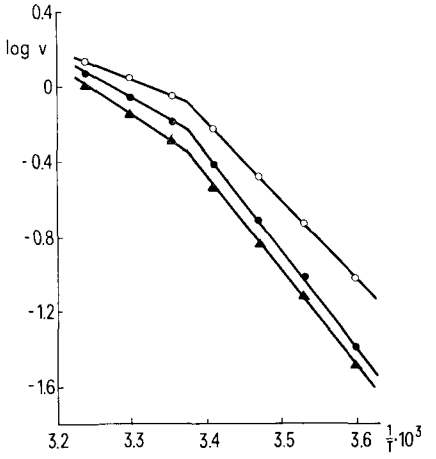

Fig. 1. Arrhenius plot of the effect of the steroids on the velocity of glucose uptake (v). Erythrocytes, preloaded with 200 mM glucose were incubated in phosphate buffer at pH 7.5 with 2% ethanol and glucose concentrations of the 0.5 K_m-values for different temperatures. The logarithm of the velocity(μmoles/sec·ml erythrocytes) was plotted against the reciprocal value of the absolute temperature. (○: control; ●: 0.3 mM cortisol; ▲: 0.1 mM 5α-androstane-3,17-dione.)

1 The work was supported by the Deutsche Forschungsgemeinschaft. We thank Dipl. Phys. P. Geck for the aid in statistical evaluation.

2 B. D. Read and R. N. McElhaney, J. Bact. *123*, 47 (1975).
3 C. D. Linden, K. L. Wright, H. M. McConnell and C. F. Fox, Proc. Nat. Acad. Sci. USA *70*, 2271 (1973).
4 H. K. Kimelberg and D. Papahadjopoulos, J. Biol. Chem. *249*, 1071 (1974).
5 B. Bloj, R. D. Morero, R. N. Farias and R. E. Trucco, Biochim. Biophys. Acta *311*, 67 (1973).
6 E. Oldfield and D. Chapman, FEBS Lett. *23*, 285 (1972).
7 L. Lacko, B. Wittke and P. Geck, J. Cell. Physiol. *86*, 673 (1975).

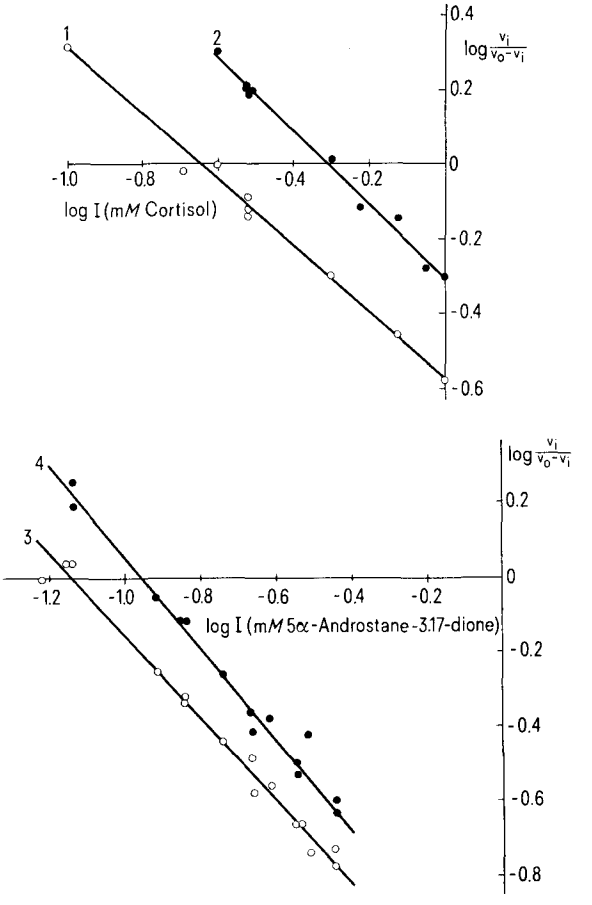

Fig. 2a, b. Hill plots for the inhibition of the glucose transport by cortisol and 5α-androstane-3,17-dione. Erythrocytes from 3 different donors (3 different experiments) were preloaded with 200 mM glucose and incubated in phosphate buffer at pH 7.5 with 2% ethanol for 10 sec at 10 °C (○) and at 25 °C (●). The concentration of glucose was 0.033 mM, that of cortisol from 0.25 to 1.0 mM and that of 5α-androstane-3,17-dione from 0.06 to 0.35 mM. The regression line for Line 1 was $y = -0.57 - 0.87x$; the correlation coefficient $r = 0.99$; for Line 2: $y = -0.30 - 0.98x$; $r = 0.99$; for Line 3: $y = -1.28 - 1.11x$; $r = 0.99$; for Line 4: $y = -1.17 - 1.22x$; $r = 0.99$; v_i = the velocity of the inhibited reaction, v_o = the velocity of the uninhibited reaction.

the cortisol concentration was varied from 0.25 mM to 1.0 mM and the 5α-androstane-3,17-dione concentration from 0.06 mM to 0.35 mM.

After 10 sec, the incubation was stopped by pouring the suspension into ice-cold stopping solution (2 mM $HgCl_2$, 310 mM NaCl, 1.25 mM KJ). The further experimental procedures have been described before[7,8].

Results and discussion. As has been described, the regions of human erythrocytes, where the glucose transport is occurring, change their physical state at a temperature of about 20 °C[8]; this leads to different apparent activation energies (μ) above and below the transition temperature. Figure 1 shows that a phase transition can be observed as well in the presence of the investigated steroid hormones cortisol and 5α-androstane-3,17-dione. The transition temperature does not change by addition of the steroids into the incubation medium. From the slopes of the Arrhenius relation it follows, however, that the hormones have an influence on the μ-values of the glucose uptake both above and below the transition temperature. The data in the table show that, below the transition temperature, the influence of the two steroids on the increase of the apparent activation energy is small and similar (about 20%). Above the transition temperature, the effect is markedly higher. The increase of the apparent activation energy by 5α-androstane-3,17-dione is about 85%, whereas that of cortisol is about 55%. An increase in μ for reactions catalyzed by membrane-bound enzymes has been attributed to a reduction of membrane fluidity[9].

Parameters for the change of the physical state of the erythrocyte membrane caused by 2 steroid hormones

Steroid in the medium	Apparent activation energy (kcal) of glucose transport	Hill coefficients for the inhibition of glucose transport with hormones
	Above the transition temperature:	
0	7.3	
Cortisol	11.1	−1.0 (at 25 °C)
5α-androstane-3, 17-dione	13.5	−1.2 (at 25 °C)
	Below the transition temperature:	
0	20.0	
Cortisol	24.3	−0.9 (at 10 °C)
5α-androstane-3, 17-dione	23.5	−1.1 (at 10 °C)

It is easy to realize that the influence of the steroids on the decrease of the membrane fluidity is higher above the transition temperature, since at this temperature-range the membrane fluidity is enhanced (in the absence of these compounds). At lower temperature-range, on the other hand, where membrane fluidity is anyway reduced, a further marked reduction cannot be achieved by the hormones. The fact that cortisol is a competitive and 5α-androstane-3,17-dione is a non-competitive inhibitor of glucose transport[7] obviously does not play any role. The Hill coefficients of the inhibition of some membrane enzymes were used as a criterion of the membrane fluidity of rat erythrocytes[10]. From our experiments (figure 2a, b and table), it can be seen that the Hill coefficients of the glucose transport inhibition by the two steroid compounds also show statistically different values (for 10 °C and 25 °C $p < 0.0005$).

Above the transition temperature, the absolute values of the Hill coefficients are higher than below (for cortisol $p < 0.0005$, for 5α-androstane-3,17-dione $p < 0.01$). This points to a higher membrane fluidity above the transition temperature. The interpretation of the relationship between the Hill coefficient and membrane fluidity is still unclear[11].

8 L. Lacko, B. Wittke and P. Geck, J. Cell. Physiol. *82*, 213 (1973).
9 H. K. Kimelberg, Biochim. Biophys. Acta *413*, 143 (1975).
10 M. G. Galo, B. Bloj and R. N. Farias, J. Biol. Chem. *250*, 6204 (1975).
11 E. M. Massa, R. D. Morero, B. Bloj and R. N. Farias, Biochem. biophys. Res. Commun. *66*, 115 (1975).

Heavy water intake in tissues. II. H_2O-D_2O exchange in the myelinated nerve of the frog[1]

V. Vasilescu, D.-G. Mărgineanu and Eva Katona

Department of Biophysics, Faculty of Medicine, 8, Dr. Petru Groza Blvd., Bucharest (Romania), 29 March 1976

Summary. The kinetic parameters of D_2O intake in the frog sciatic nerve are obtained by means of infrared spectroscopy. 3 aqueous compartments: a non-exchangeable one (≈29%) and 2 compartments of quasi-free exchangeable water: ≈50% intracellular and ≈21% extracellular, are revealed.

A great deal of experimental studies have been devoted to the effects of heavy water on various biological systems[2], mainly because the use of D_2O is the easiest way to replace a normally occurring isotope (^1H) with its heavy isotope (^2H or D), whose mass ratio is the greatest among those of all stable isotopes. At the same time, this considerable attention is motivated also by the assumption that the replacement of light water with D_2O could reveal the participation of water in various biological processes[3]. However, no molecular understanding of deuterium isotope effects is so far available; large discrepancies and debates still persist with respect to the significance of certain data[4]. In our opinion, this may be partly due to the insufficient knowledge of the time course of D_2O incorporation into each biological object. While not dealing at all with deuterium isotope effects on the nerve, the aim of the present investigation is twofold: a) to obtain the kinetical parameters of heavy water intake in the nerve, and b) to reveal in a proper way the

1 The first communication in this series is V. Vasilescu, D.-G. Mărgineanu and Eva Katona, Naturwiss. *62*, 187 (1975).
2 J. J. Katz and R. L. Crespi, in: Isotope Effects in Chemical Reactions. Ed. C. J. Collins and N. S. Bowman. Van Nostrand, New York 1971.
3 S. Lewin, Displacement of Water and its Control of Biochemical Reactions, ch. 8. Academic Press, London 1974.
4 L. Bass and W. J. Moore, Prog. Biophys. mol. Biol. *27*, 145 (1973).