HORMONE ACTION AND MEMBRANE FLUIDITY: EFFECT OF INSULIN AND CORTISOL ON THE HILL COEFFICIENTS OF RAT ERYTHROCYTE MEMBRANE-BOUND ACETYL-CHOLINESTERASE AND $(Na^+ + K^+)$-ATPase.

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SUMMARY: The influence of insulin and cortisol on the Hill coefficients for the inhibition by F^- of rat erythrocyte membrane-bound acetylcholinesterase and $(Na^+ + K^+)$-ATPase was studied "in vitro". The study was carried out with erythrocyte membranes exhibiting a high or low fatty acid fluidity, which were obtained from rats fed a corn oil or lard supplemented diet, respectively. The hormone-induced changes in the Hill coefficients of both enzymes are interpreted to mean that insulin decreases and cortisol enhances membrane fluidity.

As has been discussed by Coleman (1), the properties of many membrane-bound enzymes may be very sensitive indicators of several aspects of membrane ultrastructure. The role of membrane fluidity in the regulation of cooperative enzymes from mammalian and bacterial membranes has been previously reported (2-4). The evaluation of changes in the Hill coefficients of membrane-bound enzymes has been suggested as a useful tool for recording changes in membrane conformation (2,3,5). The Hill coefficients for the inhibition by F^- of the erythrocyte membrane-bound acetylcholinesterase (EC 3.1.1.7) and $(Na^+ + K^+)$-ATPase (EC 3.6.1.3) from rats fed diets differing in their fat supplement, have been shown to correlate with the membrane fluidity expressed as the ratio double bond index/saturated fatty acids. An increase in the fatty acid fluidity of the membrane was accompanied by a parallel increase in the cooperativity of acetylcholinesterase and a decrease in the cooperativity of $(Na^+ + K^+)$-ATPase (2). These correlations raised the possibility to evaluate changes

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in membrane fluidity through changes in the cooperativity of these membrane-bound enzymes. In the present report, this experimental approach has been used to investigate whether membrane-hormone interactions could result in modification of membrane fluidity. Membrane-hormone interaction is considered to be the initial step in the series of reactions leading to regulation of target cell metabolism by a variety of hormones (6-11). Modifications in physicochemical properties of erythrocyte and liver membranes upon interaction with growth hormone have been demonstrated (12,13).

In this communication, the effect of insulin and cortisol on the Hill coefficients for the inhibition by F^- of rat erythrocyte acetylcholinesterase and $(Na^+ + K^+)$-ATPase are shown. As will be seen, the results suggest that these hormones induce opposite changes in membrane fluidity.

Materials and Methods: Male Sprague-Dawley rats (220-320 g) grown after weaning on basic diet supplemented with 5 % lard or corn oil were used in order to obtain erythrocyte membranes exhibiting low or high fatty acid fluidity, respectively (2). Details concerning erythrocyte ghost preparation, assays of the enzymatic activities and calculation of the kinetic parameters were given in preceding papers (2,14,15). Enzymatic activities were measured (under "initial velocity" conditions) when varying the concentration of F^- in the presence of insulin or cortisol at the concentrations indicated in Figues and Tables. The values of n (Hill coefficient) for acetylcholinesterase and $(Na^+ + K^+)$-ATPase were determined from the slopes in the Hill plots as previously described (2).

Porcine insulin (single compound, sodium form) from Eli-Lilly and Co., Indianapolis (24 units per mg, Lot. IDG 04-94204), was dissolved in N/300 HCl and stored frozen until used. For the enzymatic assays, appropiate dilutions from the stock solution were made in sodium phosphate buffer for acetylhholinesterase and in Tris-HCl buffer for $(Na^+ + K^+)$-ATPase. Cortisol (Sigma Chemical Co., St. Louis) was dissolved in 30 % ethanol and stored at 4°. When diluted in buffer to the final concentrations indicated for the enzymatic assays, ethanol was present at 0.1 % or lower which had no effect on the kinetic parameters of acetylcholinesterase or $(Na^+ + K^+)$-ATPase.

Results and Discussion: As can be seen in Fig. 1A, when the specific activity of acetylcholinesterase was plotted against the concentration of F^-, the shape of the curves was different between both groups of rats. The values of n were 1.5 for corn oil-fed rats and 1.0 for lard-fed rats, in agreement with previously reported data (2). In the presence of

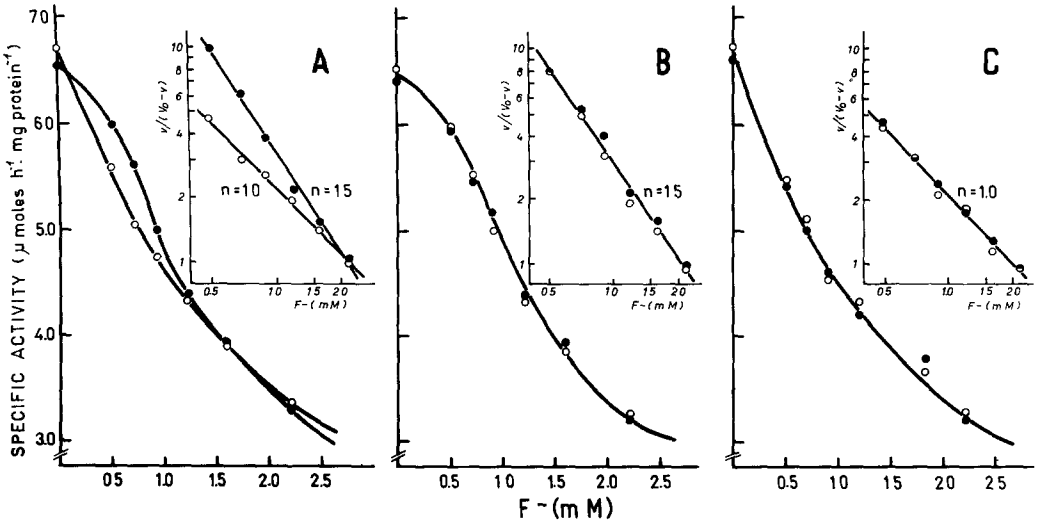

Fig.1. Specific activity of erythrocyte membrane-bound acetylcholinesterase as a function of F^- concentration. Inset: Hill plots. Experimental conditions were as described in the text. 0-0, enzyme from lard-fed rats; ●-●, enzyme from corn oil-fed rats. A: curves in the absence of hormone (controls); B: curves in the presence of 10^{-6} M cortisol; C: curves in the presence of 10^{-9} M insulin. The same membrane preparation was used for the control and hormone tests in each diet group. When two different curves are made with the same enzymatic preparation under the same experimental conditions, the individual points and the slopes (n values) obtained show a maximum variability of 5 %.

cortisol (Fig. 1B), the shape of the curve and the value of n of lard-fed rats became equal to that of corn oil-fed rats. The situation was the opposite in the presence of insulin (Fig. 1C), since the shape of the curve and the value of n of corn oil-fed rats became equal to that of lard-fed rats.

The Hill coefficients for the inhibition by F^- of acetylcholinesterase were studied as a function of insulin (Fig.2A) and cortisol (Fig.2B) concentration. Insulin at 8.5×10^{-10} M or higher gave maximal effect on the values of n for rats fed a corn oil-supplemented diet, while cortisol at 10^{-6} M or higher gave maximal effect on those for lard-fed rats. Half-maximal effects were obtained at 4.5×10^{-10} M (63 μunits/ml) insulin and 4×10^{-7} M cortisol, which are within the range of concentrations that occur in plasma (16-18). Neither the values of n for lard-fed rats were affected by the presence of insulin nor also were those

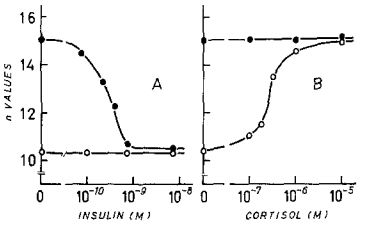

Fig.2. Hill coefficient (n) for the inhibition by F^- of erythrocyte membrane-bound acetylcholinesterase from rats fed a lard (O-O) or corn oil (●-●) supplemented diet as a function of insulin (A) and cortisol (B) concentrations. Conditions were indicated in the text. The same membrane preparation was used for each dosis-response curve.

for corn oil-fed rats in the presence of cortisol within the whole range of concentrations studied.

Rat erythrocyte membrane-bound acetylcholinesterase can be solubilized by the non-ionic detergent Triton X-100. Hill coefficients of 1.6 have been reported for the soluble enzyme (15). It was important to determine whether insulin and cortisol were able to modify the values of n when the enzyme was released from the membrane. As can be observed in Table I, the enzyme from solubilized membranes presented the same value of n in both groups of animals, and this value was not modified by either of the two hormones. In membrane-like reconstituted material, control values of n were equal to those from native membranes, and the effects of insulin on the enzyme from corn oil-fed rats and of cortisol on that from lard-fed ones were recovered. The effects were reproduced in three separate experiments. These results are a strong argument against a direct action of insulin or cortisol on the cooperative behaviour of acetylcholinesterase, and they are an evidence supporting the concept of an action mediated by the membrane.

If the effect were mediated by a change in membrane fluidity, one could expect an inverse change in the values of n for $(Na^+ + K^+)$-ATPase (2). Table II summarizes the statiscal data for the effects of insulin and cortisol on n values of acetylcholinesterase and $(Na^+ + K^+)$-ATPase from rats fed a lard or corn oil supplemented diet. Control values were in accordance with those previously reported (2). As can be observed, insulin provoked significant inverse changes in the n values of acetylcholinesterase and $(Na^+ + K^+)$-ATPase from corn oil-fed rats, without affecting the correspondent values from lard-fed rats. On the other hand

TABLE I

EFFECT OF INSULIN AND CORTISOL ON THE VALUES OF n FROM ACETYLCHOLINESTERASE THAT IS EITHER SOLUBLE OR BOUND TO MEMBRANE FROM CORN OIL AND LARD-FED RATS.

Enzyme preparation	Corn oil-fed rats		Lard-fed rats	
	Control	Insulin 8.5×10^{-10} M	Control	Cortisol 10^{-6} M
Native membrane	1.52 ± 0.05	1.05 ± 0.04	1.07 ± 0.03	1.50 ± 0.04
Solubilized membrane	1.58 ± 0.04	1.55 ± 0.07	1.50 ± 0.06	1.52 ± 0.05
Membrane-like reconstituted material	1.54 ± 0.06	1.07 ± 0.05	1.13 ± 0.03	1.48 ± 0.06

Solubilization of membranes was carried out by mixing 2 ml of erythrocyte ghost (8.5 mg protein) with 1 ml of 10 mM sodium phosphate buffer (pH 8.0) containing 1% Triton X-100 and 0.1 mM $MgCl_2$. After 15 min at room temperature, the supernatant was obtained by centrifuging at 100,000 x g for 1h at 4°C. The Hill coefficients for the soluble enzyme were determined in the presence of 0.002% Triton X-100. This concentration did not affect the response of the membrane-bound enzyme to the hormones. Reconstitution of membrane-like structures was carried out by dialysis (for 20h at 4°C) of solubilized membranes against one renewal of 1 l of 5 mM sodium phosphate buffer (pH 6.5) containing 10 mM $MgCl_2$. The dialyzed material was then centrifuged at 30,000 x g for 20 min at 4°C and the supernatant discarded. 50-60% of the enzymatic activity present in the erythrocyte ghost was recovered in the reconstituted membranes. Other experimental conditions were described in the text. Each value represents the mean $\pm$ S.E. of three separate experiments.

cortisol action on the n values for both enzymes was totally opposite from that of insulin. Neither the specific activity (in the absence of F^-) nor the Ki of either enzyme was modified by the presence of insulin or cortisol nor also was the % of $(Na^+ + K^+)$-activation in the ATPase system (not shown).

All the above findings are consistent with an hormone action mediated by a change in membrane fluidity, and could be interpreted assuming that insulin decreases and cortisol enhances membrane fluidity. In later preliminary studies we have found that another steroid hormone (10^{-7} M progesterone) decreases the value of n for acetylcholinesterase

TABLE II

EFFECT OF INSULIN AND CORTISOL ON THE HILL COEFFICIENTS OF MEMBRANE-BOUND ACETYLCHOLINESTERASE AND $(Na^+ + K^+)$-ATPase FROM RATS FED A LARD OR CORN OIL-SUPPLEMENTED DIET[a].

Hormone	Corn oil-fed rats		Lard fed-rats	
	Acetyl-cholinesterase	$(Na^+ + K^+)$-ATPase	Acetyl-cholinesterase	$(Na^+ + K^+)$-ATPase
None	1.51 ± 0.03^d	2.39 ± 0.04^d	1.04 ± 0.02^d	3.14 ± 0.04^d
Insulin[b]	1.03 ± 0.02^e	2.95 ± 0.07^e	1.07 ± 0.09^d	2.98 ± 0.12^d
Cortisol[c]	1.50 ± 0.05^d	2.22 ± 0.10^d	1.46 ± 0.03^e	2.05 ± 0.09^e

a. Each value represents the mean $\pm$ S.E. of five animals. The same membrane preparation was used for the control and hormone tests in each diet group. Conditions were given in the text.

b. Insulin concentration was 8.5×10^{-10} M in the case of corn oil-fed rats and 8.5×10^{-9} M in the case of lard fed-rats.

c. Cortisol concentration was 10^{-5} M in the case of corn oil-fed rats and 10^{-6} M in the case of lard-fed rats.

d,e. Values in the same column followed by different letters were significantly different ($p < 0.001$) when compared by the Student's t test for paired samples.

from corn oil-fed rats (from 1.5 to 1.1). This effect is the opposite of that of cortisol and similar to that of insulin. Therefore it is not possible thus far to relate the effect of insulin or cortisol with its peptide or steroid nature, respectively. To find out a possible general relationship between hormone structures and changes in membrane fluidity further and more exhaustive studies should be undertaken.

The effect of the hormones on membrane fluidity may involve changes in the ordering or the state of compression of the lipids. This phenomenon should be related to membrane lipids in general since it is reflected in the behaviour of acetylcholinesterase and $(Na^+ + K^+)$-ATPase which have different lipid dependency and spatial distribution (19). A local change in the state of compression of the lipids in a bilayer might be transmitted appreciable distances away in a mosaic membrane, and such change might produce small but significant structural changes in the proteins embedded in the mosaic at distances well removed from the point of iniciation of the effect. The effect

may be iniciated either at the lipid portions or the protein portions of the mosaic (20).

Specific insulin receptors have been demonstrated in plasma membrane of target cells (8). Insulin-receptor interaction could expand the receptor protein causing a compression in the lipid molecules of the belayer; this would be only a special case in the general mechanism proposed by Weiss (21). Major changes in membrane conformation, which could then alter various metabolic processes in the cell, have been proposed as a hypothesis to explain all the biological effects of insulin by a single common mechanism (6,8). As far as we know, the findings that we reported here are the first evidence for an action of insulin on membrane fluidity. The physiological significance of our findings is at present a matter of speculation. However, there is an appealing agreement between the range of hormone concentrations reported in plasma (16,17) and that in which the effect on the Hill coefficient is observed (Fig.2).

Specific intracellular binding of glucocorticoids has been demonstrated (22). So far, cortisol receptors in cellular membranes have not been described. However, this steroid influences the permeability of model lipid structures an natural membranes; in general, it appears to stabilize membranous structures (23).

Our findings indicate that acetylcholinesterase and $(Na^+ + K^+)$-ATPase respond to insulin or cortisol in a fashion dependent on the membrane fatty acid composition, which in turn depends on the lipidic nature of the diet. A similar observation has been reported by Counis who stated that the activation of membrane-bound adenylyl cyclase by noradrenaline is related to the linoleic acid content of the diet on which the rats are fed (24). Since the n values for acetylcholinesterase and $(Na^+ + K^+)$-ATPase denpend on the fluidity of the membrane (2), the effect of insulin and cortisol on the Hill coefficients of these enzymes can only be observed in membranes exhibiting an appropiate fatty acid fluidity. No effects were found (Table II) for low fluidity membrane-insulin interaction (lard) or high fluidity membrane-cortisol interaction (corn oil). It is interesting to note that this does not necessarily imply that insulin or cortisol is unable to modify the fluidity of "lard-membranes" or "corn oil-membranes", respectively, since in these membranes the cooperative transitions of the enzymes are at their minimal or maximal expression and by this probe no additional changes in membrane fluidity can be evaluated. This theoretical point has been discussed in detail elsewhere (25,26).

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