INSULIN DECREASES BACTERIAL MEMBRANE FLUIDITY. IS IT A GENERAL EVENT IN ITS ACTION?

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SUMMARY: The influence of insulin on the Hill coefficient during inhibition by Na^+ of Escherichia coli membrane-bound (Ca^{2+})ATPase was studied in vitro. In the presence of insulin, the values of n change from 1.9 to 1.1. Half-maximal effect was obtained at 1×10^{-9}M (140 μunits/ml). The hormone did not affect the allosteric behavior of the soluble enzyme. The hormone-induced-changes in the Hill coefficients are interpreted as a decrease in membrane fluidity produced by insulin. The general occurrence of this event in insulin action is suggested in this paper.

Regulation of allosteric membrane-bound enzymes from mammalian and bacterial membranes through changes in their lipid composition has been reviewed (1). The thermodynamic considerations of the extreme sensitivity of the membrane-bound allosteric enzymes to detect changes in the membrane where they are imbedded was also previously reported (2). Variations in the interaction energies as low as 700 cal/mol would be enough to trigger a significant changes in the Hill coefficient (n values).

The molecular basis of insulin action is one of the most attractive problems in the field of hormonal control of metabolism. Major changes in the structural

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organization of the membrane have been proposed as one possible mechanism by which insulin exerts its effects on other metabolic events in the mammalian cell (3-5). It was pointed out by our laboratory, that insulin decreases the mammalian membrane fluidity (6). This action was evaluated through changes in the cooperativity of the erythrocyte membrane-bound acetylcholinesterase and (Na^+ + K^+)ATPase in rats fed different fat-supplemented diet (6).

To determine whether the above-mentioned events due to insulin on the membrane fluidity could be generalized to cells others than mammalian, studies on the effect of this hormone on the cooperativity behavior of the membrane-bound enzyme were performed with bacterial cell membranes. It was demonstrated that the allosteric inhibition by Na^+ of the membrane-bound (Ca^{2+})ATPase (E.C. 3.6.1.3) from Escherichia coli was dependent upon the fatty acid composition of the cell membrane (7). High correlations (r = 0.94) were obtained at 36ºC and 19ºC between the values of n and the membrane fluidity expressed as the ratio double-bound-index/saturated fatty acids (8,9). These correlations raised the possibility of evaluating changes in the membrane fluidity through changes in the cooperativity of this enzymatic system.

In this work, evidence is presented showing that the insulin effect previously found in the animal cell membrane can be extended to the membrane from procaryotic cells. The effect of insulin on the decrease of the membrane fluidity is suggested as a general mechanism in its action.

MATERIALS AND METHODS: The bacterial strains used were E.coli K-12 Hfr M_1 (pho^-, alkaline phosphatase) and E.coli K-12 K 1061, an unsaturated fatty acid auxotroph (fab B) which is also defective in fatty acid oxidation (fab E) (10). The standard Medium A (11) was used, where 1 % glicerol is the only carbon source. The Medium A contained in addition 0.05 % Triton X-100 and 0.03 % linolenic or vaccenic acid as unsaturated fatty acid supplement for growth of the E.coli K 1061. The growth conditions (unless otherwise specified), preparations of the membrane and of the soluble enzyme, the (Ca^{2+})ATPase assays, as well as the calculation of the kinetic parameters were previously described (12).

For the assay of the inhibition by Na^+ of the (Ca^{2+})ATPase, the reaction mixture consisted of 20 mM Tris-HCl buffer pH 9.0, 1 mM $CaCl_2$, 2.5 mM Tris-ATP, 1 mM cysteine and increasing amounts of NaCl as indicated in Fig. 1.

Porcine insulin (single compound sodium form) from Eli-Lilly Co Indianapolis (24 units per mg, Lot LDG 04-94204) was dissolved in N/30 HCl and stored frozen until use. For the enzymatic assays, appropriate dilutions from the stock solution were made in 20 mM Tris-HCl buffer pH 9.0

RESULTS: Fig. 1A shows the inhibition by Na^+ in the presence or in the absence of insulin for membrane-bound (Ca^{2+})ATPase of E.coli M1 performed at 36ºC. The presence of insulin changed the Na^+ inhibition curve from a sigmoidal to a hyperbolic shape. The statistical treatment of the 6 separate enzymatic preparations showed a significant difference in the values of n, both in the absence (mean $\pm$ S.E.)(1.96 $\pm$ 0.04) and in the presence (mean $\pm$ S.E.) (1.14 $\pm$ 0.06) of the hormone ($P < 0.001$). The specific activity (in the absence of Na^+) was not modified by insulin. When similar experiments were carried out with the unsaturated fatty acids auxothroph grown with linolenic or vaccenic acid as supplement, values of 2.2 and 1.6 were obtained, respectively, in agreement with previous reports (8). The presence of insulin also produced a

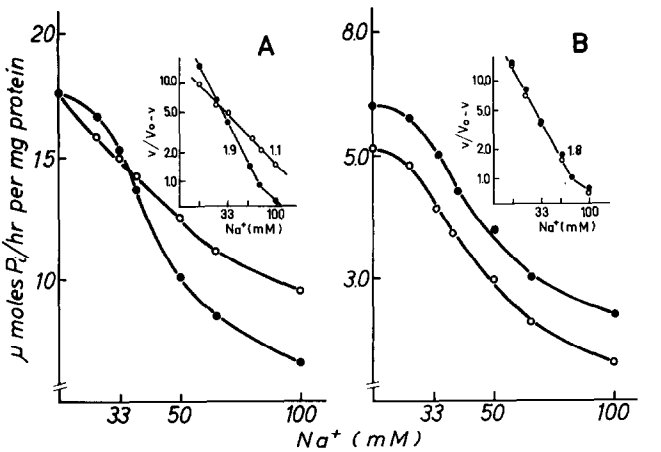

Fig. 1. Inhibition by Na^+ of the membrane-bound (A) and soluble (B) (Ca^{2+})ATPase from E.coli K-12 M1 in the absence (●-●) and in the presence (o-o) of insulin 3×10^{-9} M. Inset shows Hill plots of the same data. The inhibition were measured at 36ºC in the membrane and at 19ºC in the soluble preparation. The same enzymatic preparation was used for the control and hormone test. When two different curves are made with the same enzymatic preparation under the same enzymatic conditions, the individual points and the slopes (n values) obtained show a maximun variability of 5 %.

decrease in the values of n (from 2.2 to 1.3 and from 1.6 to 1.1, respectively).

To rule out the possibility that insulin affected the enzyme directly, an experiment was carried out with the soluble form of the enzyme. A complication of this approach is that when the assay was performed at 36ºC, there was a decrease in the values of n between the membrane-bound (1.9) and the soluble (1.1) enzyme (12). In contrast, at 19ºC, when the enzyme was released from the membrane, an increment in the values of n, from 1.1 to 1.8, was apparent (12). Thus, the decreasing action of insulin on the values of n in the soluble enzyme at 36ºC or in the membrane (Ca^{2+})ATPase at 19ºC can not take place in these case because this parameter is in its minimal expression (for theoretical discussion, see Ref.2). In fact, in the latter conditions, the values of n about 1.0 were not modified by the presence of insulin. When the Hill coefficient was determined at 19ºC in the soluble enzyme of E.coli M1, in the presence or in the absence of insulin, values of n around 1.8 were obtained in both cases (Fig. 1B).

The fact that insulin affects only the allosteric behavior of the enzyme when it is bound to the membrane, suggested that the insulin action is mediated by the membrane. However, an objetion to this conclusion, is that the action of the hormone on membrane-bound and soluble enzyme was performed at different temperatures (36ºC and 19ºC, respectively). To obviate this, the following experiment was carried out with E.coli M1 grown at 20ºC instead of a 37ºC, as in the above experiment. An increase in unsaturated fatty acid with the lowering of the temperature at which the bacteria is grown was reported (13). When the inhibition by Na^{+} was evaluated, the membrane-bound (Ca^{2+})-ATPase from the bacteria grown in this condition showed values of n of 2.7, at 36ºC, and of 1.6 at 19ºC, whereas the soluble form of the enzyme maintained a similar behavior as that presented by E.coli grown at a higher temperature. On the basis of these facts, we tested at 19ºC the action of insulin in the membrane-bound and soluble enzyme of E. coli M1 grown at low temperature. The insulin 3×10^{-9} M decreased the values of n

of the membrane-bound enzyme from 1.6 to 1.0, whereas the allosteric behavior of the soluble enzyme was no affected by the presence of the hormone. Values of n about 1.6 were found.

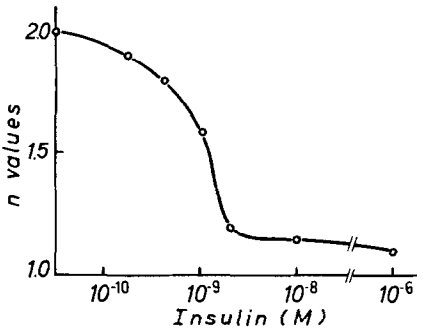

Fig. 2. Hill coefficients of the membrane-bound (Ca^{2+})ATPase as a function of insulin concentration. Experimental conditions were as described in Fig. 1.

As it can be seen in Fig. 2, the Hill coefficient was studied as a function of insulin concentration. Insulin at 3×10^{-9}M, or higher, gave the maximal effect on the values of n. Half-maximal effect was obtained at 1×10^{-9}M (140 μunits/ml). As previously found with erythrocytes enzymes (6), these insulin concentrations fall within the range of concentration that occur in normal mammalian plasma (14-15).

DISCUSSION: The positive relationship between the values of n for the enzyme and membrane fluidity (8), the finding that insulin decreased the values of n and that this action was observed only in membrane-bound enzyme constitute strong evidence for the concept that insulin effect is mediated by the membrane fluidity. In other words, insulin decreases the bacterial membrane fluidity.

It was reported that insulin modifies the activity of the membrane-bound adenylate cyclase (16-17) and ($Na^+ + K^+$)ATPase (18-19). The results reported, here showed that the specific activity of the membrane-bound (Ca^+)ATPase remained unchanged. In the presence of insulin, only the cooperative behavior was changed. This observation is

similar to that one found in the erythrocyte membrane-bound enzymes with other hormones (6). Up to now, this insulin action was investigated in membrane from rat erythrocyte (6) and in this paper, in membrane from E.coli. These membranes differ largely in properties, functions and composition, e.g., the bacterial membrane does not contain sterol, whereas the erythrocyte membrane has one of the highest cholesterol/phospholipid ratios. Similary, the enzymes used as tests in these studies differ in several aspects. The acetylcholinesterase and $(Na^+ + K^+)$ATPase from erythrocyte and (Ca^{2+})-ATPase from E.coli have different localization in the membrane, have different dependence on the lipids for their enzymatic activity and differ also in their metabolic functions (1), e.g., ATPase from erythrocyte is an integral enzyme whereas ATPase from E.coli membrane is a peripheral protein. The insulin action recorded by erythrocyte and E.coli membranes, and the consequent kinetic changes observed in the respective membrane-bound enzymes with such unrelated characteristics as described above (both membranes and enzymes), are striking,indeed. In addition, insulin decreased the values of n of the erythrocyte acetylcholinesterase (6) and E.coli (Ca^{2+})ATPase, and enhanced it in the erythrocyte $(Na^+ + K^+)$ATPase system (6). The correlations between the membrane fluidity and the n values of the former enzymes were positive (8-21) whereas for the latter enzyme, it was negative (21).

The results obtained with preocaryotic and eucaryotic cell membrane suggest that the decreasing effect of the membrane fluidity by insulin could be a general characteristic of its action. Membranes other than rat erythrocyte and E.coli, deserve further investigatior to confirm this hypothesis. This work illustates, once more, the use of this novel enzymatic sensitivity method for detecting changes in the membrane structure, that is, the use of the membrane-bound cooperative enzymes as a probe to record these events (6,22-23).

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