PHYSIOLOGY

Inverter Mechanism of the Influence Exerted by Insulin on the Plasma Membranes of Cardiac Myocytes in Rats of Different Age

V. V. Frol'kis, O. K. Kul'chitskii, and E. N. Gorban'

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 8, pp. 122-125, August, 1995
Original article submitted September 7, 1994

In the plasma membranes of cardiac myocytes from old rats, Na,K-ATPase activity and phosphatidylinositol levels were lower and cardiolipin levels higher than in those from younger (adult) animals. Insulin injected into adult rats elevated Na,K-ATPase activity and phosphatidylethanolamine levels and caused a sharp fall in phosphatidylinositol levels. In old rats, insulin had no effect on Na,K-ATPase activity, but lowered phosphatidylethanolamine levels. In experiments with cellular hybrids (cytosol+plasma membranes), cytosol from adult rats activated Na,K-ATPase in both adult and old rats, whereas cytosol from old rats failed to activate the enzyme both in old and in adult rats. Actinomycin D prevented the stimulatory effect of insulin on Na,K-ATPase activity.

Key Words: *insulin; myocardium; membranes; aging*

During aging, less and less insulin is produced in the body, alterations occur in its secretion by B cells and its concentration in the blood, the number of insulin receptors diminishes, the tolerance to carbohydrates decreases, and a number of other associated events take place [2]. The mechanism of action of insulin is associated, in particular, with its effects on the plasma membrane (PM) and with changes in the transmembrane transport of glucose and ions and in cell excitability [7]. Effects of insulin on myocardial metabolism and vessel walls in health and in diabetes have been described [6].

The objectives of the present study were to find out how insulin might affect the phospholipid composition and Na,K-ATPase activity of cardiac myocyte PM in animals of different age and to elucidate the possible role played in the mediation of insulin effects by inverters - intracellular PM regulators synthesized under genomic control [4,10].

Laboratory of Physiology and Laboratory of Metabolic Regulation, Institute of Gerontology, Ukrainian Academy of Medical Sciences, Kiev

MATERIALS AND METHODS

Adult (aged 9 months) and old (aged 28 months) male Wistar rats were used. Test rats were injected with insulin intraperitoneally (1.6 U/kg body weight) 40 min before sacrifice by decapitation. Control rats were given physiological saline by the same route. Some of the test rats also received actinomycin D in a dose of 50 μg/kg body weight 30 min prior to insulin, while others were given only actinomycin D in the indicated dose.

PM were isolated as described by Sazontova [3] and assayed for protein [9] and phospholipids (PL). These were extracted according to Bligh and

0007-4888/95/0008-0763$12.50

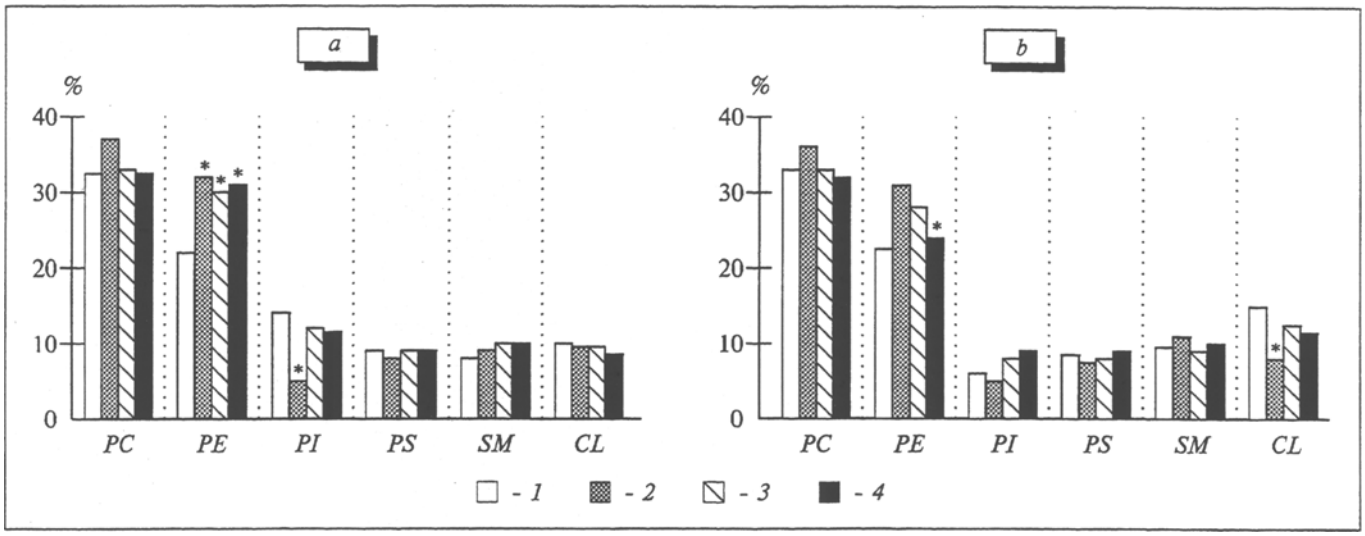

Fig. 1. Effects of insulin and actinomycin D on the phospholipid composition of cardiac myocyte plasma membranes in adult and old rats. *Ordinate*: levels of individual phospholipids. *a*) adult rats; *b*) old rats. *PC* = phosphatidylcholine; *PE* = phosphatidylethanolamine; *PI* = phosphatidylinositol; *PS* = phosphatidylserine; *SM* = sphingomyelin; *CL* = cardiolipin. *1*) controls; *2*) insulin–injected rats; *3*) actinomycin D–injected rats; *4*) rats injected with both insulin and actinomycin D. *$p<0.05$.

Dyer [8] and separated by two-dimensional microthin-layer chromatography [11] in the solvent system chloroform:methanol:28% ammonia (65:35:5) in the first direction and chloroform:acetone:methanol:glacial acetic acid:water (30:40:10:10:5) in the second. PL were identified on the chromatograms using appropriate markers. Individual PL fractions were identified by the method of Vaskovsky *et al.* [12]. Na,K-ATPase activity was measured as described by Potapenko [1]. Isolated PM of cardiac myocytes were incubated with the cytosol of these cells or with blood serum in a medium containing 150 ml of cytosol or serum and 100 μg of protein; after incubation, the test tubes were kept in an ice bath for 40 min with continuous stirring.

RESULTS

Cardiac myocyte PM of old rats differed from those of adult animals in several respects, the greatest difference being recorded for phosphatidylinositol, whose content in the old rats amounted to only 6.3±0.8% of total phospholipids *vs.* 14.5±0.8% in the adult rats. Na,K-ATPase activity in the cardiac myocyte PM of old rats was also markedly decreased (by 49%).

Significant age-related changes were detected in the reaction of cardiac myocyte PM to insulin. In adult rats, the injected insulin caused a significant lowering of the phosphatidylinositol level and a 48% elevation of the phosphatidylethanolamine level; in old rats, it raised phosphatidylethanolamine by 21% and lowered cardiolipin by 41% (Fig. 1). Na,K-ATPase activity after insulin injection rose by 73% in adult rats and remained almost unchanged in old animals (Fig. 2).

Inspection of Figs. 1 and 2 shows a lack of correspondence between the insulin-induced changes in PL and Na,K-ATPase activity. Thus, the activity of this enzyme in adult rats rose despite a fall in phosphatidylinositol, although the latter is known to be a modulator of Na,K-ATPase activation [13]. Presumably, the mechanisms of the changes we detected operate not only at the membrane level but at the general cellular level as well. The considerable reduction of phosphatidylinositol in adult animals suggests activation of its metabolism, which should lead to an intensification of intracellular metabolism, including biosynthetic processes, via second messengers (polyphosphoinositides/diacylglycerol). On the other hand, there is evidence that changes in membrane functioning and in Na,K-ATPase activity in particular under the action of insulin may be determined by cytosolic factors of protein nature, known as inverters [4]. If so, then the considerable activation of phosphatidylinositol metabolism by insulin in adult animals may stimulate enhanced synthesis of cytosolic factors that activate Na,K-ATPase. This would explain, in part at least, the age-related differences in responses to insulin.

Insulin exerts a pronounced regulatory influence on the rates of biosynthetic processes in cells. Evidence has been gathered for the existence of a link between the activity of protein biosynthesis and the state of PM, in particular the level of their polarization [5]. To gain better knowledge of this possible link, we used actinomycin D - a

blocker of transcription in the protein biosynthesis system. Preinjecting rats with actinomycin D was found to prevent the stimulatory effect of insulin both on PL (Fig. 1) and on Na,K-ATPase activity (Fig 2). Injection of actinomycin D alone elevated only the phosphatidylethanolamine level (by 24%) and had little or no effect on Na,K-ATPase activity. This suggests a connection between protein biosynthetic activity and shifts in the state of PM, a connection which presumably exists because intracellular regulators of cardiac myocyte PM are synthesized under the influence of insulin.

To check this possibility, several *in vitro* experiments were undertaken to create cellular hybrids by adding PM of cardiac myocytes from intact rats to cytosol of cardiac myocytes from insulin-treated rats. Moreover, in a separate experiment, heterochromic hybrids were produced by adding cardiac myocyte cytosol from adult rats to PM of old ones, and vice versa. *In vitro* incubation of insulin with isolated cardiac myocyte PM failed to activate Na,K-ATPase (Fig. 2). Nor did the cardiac myocyte cytosol from adult or old intact rats alter the activity of this enzyme in isolated cardiac myocyte PM. In contrast, cardiac myocyte cytosol from insulin-injected adult rats increased by 111% Na,K-ATPase activity in isolated cardiac myocyte PM from adult rats upon *in vitro* incubation with these membranes. Similar cytosols from insulin-injected old rats, however, failed to activate the Na,K-ATPase of cardiac myocyte PM from old rats, whereas such cytosol from insulin-injected adult rats raised Na,K-ATPase activity by 83% upon incubation with cardiac myocyte PM from old rats. No increase in enzyme activity was recorded when cardiac myocyte cytosol from insulin-injected old animals was incubated with cardiac myocyte PM from adult animals. Finally, cardiac myocyte cytosol from adult animals injected with actinomycin D 30 min before insulin also failed to activate Na,K-ATPase. These results indicate that the production of regulatory factors is linked with protein biosynthesis.

It may therefore be hypothesized that the insulin-induced stimulation of protein biosynthesis in adult rats results in the accumulation of a factor, inverter [4], that activates the Na,K-ATPase of cardiac myocyte PM. The failure of insulin to influence enzyme activity in old animals is probably attributable to altered biosynthesis of this factor. However, the enzyme still retains its potential for adequately responding to the factor, as is attested by the finding that cytosol from insulin-treated adult rats activates the Na,K-ATPase of cardiac myocyte PM from old animals.

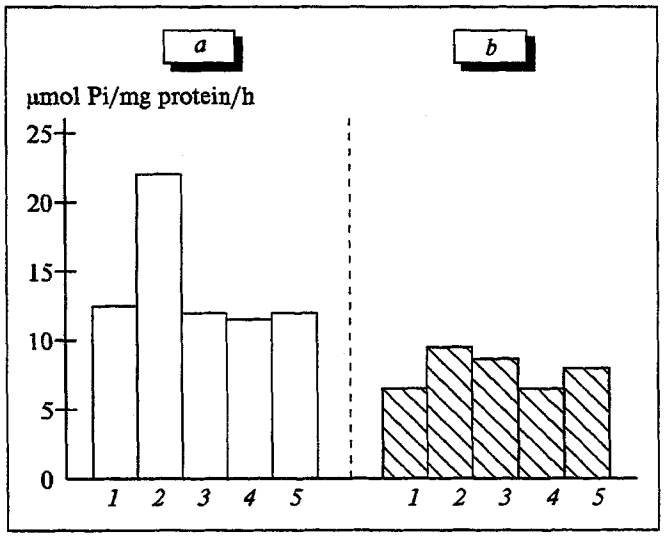

Fig. 2. Effects of insulin and actinomycin D on Na,K−ATPase activity of cardiac myocyte plasma membranes (PM) in adult and old rats. *a*) adult rats; *b*) old rats. *1*) controls; *2*) insulin−injected rats; *3*) actinomycin D−injected rats; *4*) rats injected with insulin and actinomycin D; *5*) cardiac myocyte PM incubated with insulin *in vitro*. P_i = inorganic phosphorus. *$p<0.05$.

Presumably, the inverters synthesized inside cells under the action of insulin enter the circulation. Indeed, *in vitro* incubation of isolated cardiac myocyte PM from adult rats with serum of insulin-treated adult rats resulted in a 79% rise in Na,K-ATPase activity. However, as shown in Fig. 3, serum of adult rats administered actinomycin D before insulin injection failed to activate the Na,K-

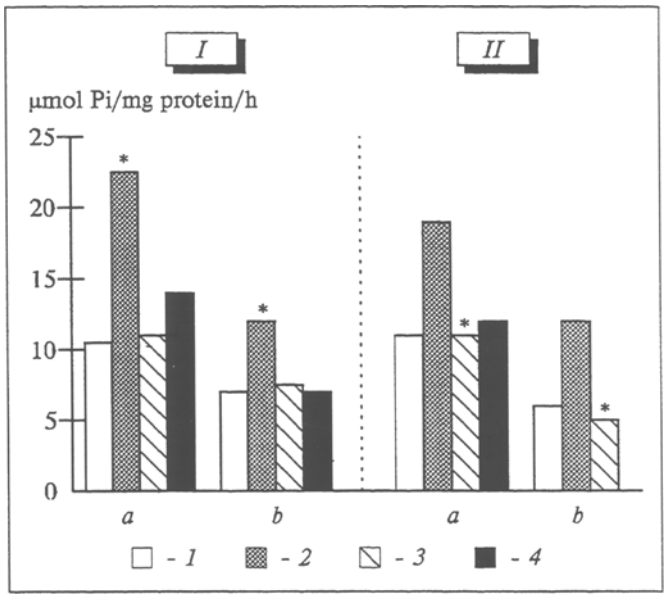

Fig. 3. Effects of cardiac myocyte cytosol (*I*) and blood serum (*II*) from rats injected with insulin or with both insulin and actinomycin D on the Na,K−ATPase activity of cardiac myocyte plasma membranes from adult (*a*) and old (*b*) rats. *1*) control; *2*) cytosol (*I*) or serum (*II*) from insulin−injected adult rats; *3*) cytosol (*I*) or serum (*II*) from adults rats injected with both insulin and actinomycin D; *4*) cytosol (*I*) or serum (*II*) from insulin−injected old rats.

ATPase of isolated cardiac myocyte PM from adult animals. Nor was the enzyme activated by serum of old animals upon *in vitro* incubation with isolated cardiac myocyte PM from either adult or old rats, whereas serum of insulin-treated adult rats caused a 112% rise in the activity of cardiac myocyte PM Na,K-ATPase from old animals. Lastly, serum of adult or old control rats did not alter Na,K-ATPase activity in cardiac myocyte membranes.

These results suggest that, as in the case of cardiac myocyte cytosol, a factor associated with insulin-induced activation of protein biosynthesis accumulates under the action of this hormone in the serum of adult rats, and that this factor is responsible for the observed activation of cardiac myocyte Na,K-ATPase.

To conclude, the action of insulin on the state of the cardiac myocyte plasma membrane and, in particular, on its phospholipids and Na,K-ATPase activity is mediated by the cell cytoplasm. A factor of protein nature, inverter [4], that activates Na,K-ATPase accumulates in the cardiac myocyte cytosol and blood serum under the influence of insulin. The failure of insulin to stimulate Na,K-ATPase activity in old animals is presumably associated with altered biosynthesis of this insulin-induced inverter.

REFERENCES

1. R. I. Potapenko, *Neirokhimiya*, 5, No. 1, 57-59 (1986).
2. J. Roth, *Fiziol. Zh.*, **36**, No. 5, 82-90 (1990).
3. T. G. Sazontova, *Byull. Eksp. Biol. Med.*, **102**, No. 12, 685-687 (1986).
4. V. V. Frol'kis, *Dokl. Ross. Akad. Nauk*, **324**, No. 1, 229-232 (1992).
5. V. V. Frol'kis, *Fiziol. Zh.*, No. 4, 102-104 (1981).
6. V. V. Frol'kis, V. V. Bezrukov, and V. G. Shevchuk, *Circulation and Aging* [in Russian], Leningrad (1984).
7. D. L. Baly, J. S. Scheneiderman, and A. L. Garsia-Welsh, *J. Nutr.*, **120**, No. 1, 1075-1079 (1990).
8. E. G. Bligh and W. I. Dyer, *Canad. J. Biochem. Physiol.*, **37**, 911-917 (1959).
9. M. M. Bradford, *Anal. Biochem.*, **72**, 248-254 (1976).
10. V. V. Frolkis and A. L. Shkapenko, *Mech. Aging Dev.*, **60**, No. 1, 55-60 (1991).
11. V. I. Svetashev and V. E. Vaskovsky, *J. Chromatogr.*, No. 2, 376-378 (1972).
12. V. E. Vaskovsky, E. Y. Kostesky, and J. M. Vasendin, *J. Chromatogr.*, No. 1, 376-378 (1975).
13. I. Yguerable and E. E. Yguerable, *Enzymes Biol. Membr.*, New York - London, Vol. 1 (1985), pp. 393-420.