Biochimica et Biophysica Acta, 596 (1980) 180—185

BBA 78639

ANTAGONISTIC EFFECT OF INSULIN ON GLUCAGON-EVOKED HYPERPOLARIZATION

A CORRELATION BETWEEN CHANGES IN MEMBRANE POTENTIAL AND GLUCONEOGENESIS

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(Received May 8th, 1979)

Key words: Insulin; Glucagon; Hyperpolarization; Gluconeogenesis; Valinomycin; (Membrane)

Summary

In the perfused rat liver, administration of glucagon causes a hyperpolarization of the liver cell membrane and increases gluconeogenesis. Insulin, a hormone which is known to antagonize the effect of glucagon on gluconeogenesis also blocks the hyperpolarizing effect of glucagon. Because of this inhibitory effect of insulin of the glucagon-evoked hyperpolarization, a systematic study of possible correlation between changes in membrane potential and gluconeogenesis was undertaken. The membrane potential was changed by valinomycin, tetracaine, or by varying the ionic composition of the perfusate. A highly significant correlation between changes in membrane potential and the rate of gluconeogenesis was noticed. The possibility was raised that changes in membrane potential might exert an influence on metabolic process by a yet unknown mechanism.

Introduction

The importance of the ionic environment in metabolic processes was first emphasized by Claude Bernard [1]. The fact that the maintenance of blood glucose level by insulin and glucagon also might be associated with changes in electrolyte concentrations became apparent shortly after the discovery of insulin. It was noticed then that administration of insulin results not only in the lowering of the blood glucose level but also in the lowering of the blood K^+ [2]. As the studies on the mechanism of action of insulin progressed, it became

apparent that insulin not only changes the permeability of target cells to glucose [3,4], but it also affects K^+ and Na^+ fluxes and the cell membrane potential [5]. Other hormones which are hyperglycemic, such as glucagon and epinephrine, increased blood K^+ level [6]; thus, their actions are opposite to those of insulin in both respects. When studied in the perfused liver rather than in the whole organism, it has been demonstrated that one of the earliest effects of glucagon is a redistribution of ions associated with hyperpolarization of the cell membrane [7—9]. Interference, by various means, with the ion fluxes resulted in an inhibition of the gluconeogenesis and lipolytic effects of the hormone [10].

Because insulin antagonizes the effect of glucagon on glucose production and K^+ efflux [11—13], the question arose whether or not insulin would also block the hyperpolarization evoked by glucagon. If so, a systematic study of the possible connection between changes in membrane potential and rate of gluconeogenesis was indicated. This paper presents the results obtained so far which demonstrate a correlation between changes in membrane potential and glucose and glucose production.

Materials and Methods

Liver perfusion. Livers from fasted, 100—150-g Sprague-Dawley rats were perfused in situ with Krebs-Ringer bicarbonate buffer containing 4% bovine albumin (Cohn fraction V) in a recirculating system as described in detail [14]. Hormones or drugs where indicated were added directly to the perfusate.

Composition of the various perfusates employed. Krebs-Ringer buffer contained 143 mM Na^+, 5.9 mM K^+, 2.5 mM Ca^{2+}, 1.2 mM Mg^{2+}, 128 mM Cl^-, 24.9 mM HCO_3^-, 1.2 mM $H_2PO_4^-$, 1.2 mM SO_4^{2-}. High K^+ Krebs-Ringer buffer contained: 43.4 mM Na^+, 106 mM K^+, the rest unchanged. Na^+-free choline buffer contained: 142 mM choline instead of Na^+, the rest unchanged. Na^+-free Li^+/Tris buffer contained instead of Na^+ 188 mM Li^+ and 24.9 mM Tris, no bicarbonate, 40 mM Na^+. Choline buffer contained 43.4 mM Na^+, 100 mM choline, the rest unchanged. Cl^--free isothionate buffer contained: 9.8 mM Cl^-, 188 mM isothionate, the rest unchanged.

Membrane potential determination. Liver cell membrane potential was measured in the perfused liver as described previously [15]. In short, the measurements were done with drawn pyrex glass capillary microelectrodes filled with 3 M KCl, and connected to an electrometer (WP Inst. Model M701) via an Ag|AgCl half-cell. Potentials were monitored on an oscilloscope and also recorded on paper. Individual liver cell membrane potentials were sampled at a rate of 5—20/min with each cell penetration sustained only long enough to ascertain a stable potential level.

Measurement of glucose production. The technique of liver perfusion and the method used for the determination of gluconeogenesis has been described in detail [14].

Results and Discussion

In Fig. 1, the effects of glucagon (Fig. 1A), and glucagon and insulin (Fig. 1B) on liver membrane potential are illustrated. Glucagon administration

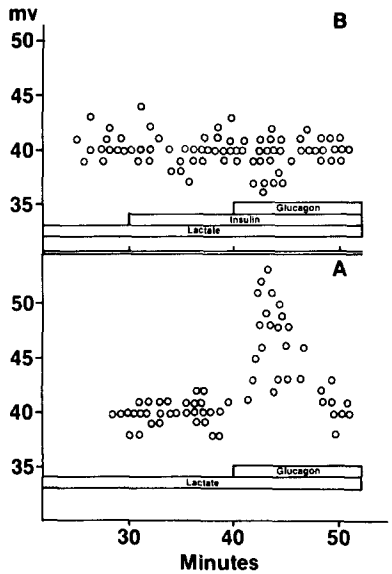

Fig. 1. The effect of 10^{-7} M glucagon and 0.05 unit/ml insulin on liver membrane potentials. Livers were perfused with 10 mM Na^+/lactate and where indicated the hormones were added to the perfusate in the reservoir. (A) Without, and (B) with insulin.

was followed by a fast and transient hyperpolarization as reported previously [8,15]. The hyperpolarization did not depend on the presence of lactate. Perfusion of the liver with insulin itself did not affect the membrane potential. However, prior insulin administration completely blocked the hyperpolarization effect of glucagon. This lack of effect of insulin on the liver membrane potential is in contrast with the hyperpolarization observed in muscle cells. The inhibitory effect is not likely to be due to an inhibitory effect on the adenylate cyclase reaction because insulin also blocked the hyperpolarization effect of 10^{-5} M cyclic AMP completely in perfused livers from fed donors (control = 40 ± 1 mV; cyclic AMP = 48 ± 1 mV, $P < 0.01$, insulin + cyclic AMP = 42 ± 2 mV). In livers from fasted donors the inhibition was less clearcut (control = 42 ± 2 mV, cyclic AMP + insulin 45 ± 3 mV).

Because insulin blocked the glucagon-induced hyperpolarization, an effort was made to determine whether a relationship can be demonstrated between changes in membrane potential and the rate of gluconeogenesis. The potential across the liver cell membrane was therefore altered in four different ways: (1) by hormones; (2) by changing the ionic composition of the perfusate, namely varying the concentration of Na^+, K^+ and Cl^-; (3) by using the local anesthetic, tetracaine, and (4) the K^+ ionophore, valinomycin. The results are given in Table I and Fig. 2. A correlation between the level of the membrane potential and the rate of glucose production was found under all conditions. The significance of this correlation is illustrated in Fig. 2.

The possibility that the observed correlation is due to nonspecific toxic effects is imposed upon the cell by the various experimental conditions was examined and appears unlikely. For example, as demonstrated in Fig. 3, the depolarizing effect of Li^+ is immediately reversible. The inhibition of glucose

TABLE I

RATES OF GLUCONEOGENESIS AND MEMBRANE POTENTIAL VALUES IN VARIOUS EXPERIMENTAL CONDITIONS

Each number represents the mean ± S.E. of at least four experiments. The membrane potential in column A was recorded between 15 and 30 min after the addition of 10 mM lactate to the perfusate, and in column B, 3—7 min after the addition of 10^{-7} M glucagon. Gluconeogenesis from 10 mM lactate was measured in the absence (column C) and in the presence (column D) of 10^{-7} M glucagon. Calculation of the correlation coefficient (r) of A versus C gave $r = 0.91$. The correlation coefficient for A and B versus C and D was also calculated and $r = 0.97$. The P values in all cases were < 0.001.

	Membrane potential (mV)		Gluconeogenesis (μmol glucose/g per h)	
	A	B	A	B
Krebs-Ringer buffer	39.0 ± 1	47.2 ± 1	35.3 ± 2	53.8 ± 3
Krebs-Ringer buffer + insulin (0.05 unit/ml)	39.7 ± 0	40.4 ± 1	30.5 ± 2	34.2 ± 3
High K^+ Krebs-Ringer buffer (106 nM)	25.3 ± 1	30.1 ± 2	15.5 ± 2	24.3 ± 2
Na^+-free choline buffer	33.0 ± 3	32.3 ± 3	26.1 ± 2	21.3 ± 1
Na^+-free Li^+/Tris buffer	22.5 ± 1	21.3 ± 3	3.4 ± 0	0.8 ± 0
40 mM Na^+/choline buffer	42.2 ± 1	53.5 ± 3	31.9 ± 1	50.6 ± 3
40 mM Na^+/Li^+ buffer	33.4 ± 3	40.1 ± 2	29.9 ± 1	43.8 ± 4
Cl^--free isothionate buffer	42.6 ± 2	58.7 ± 4	36.0 ± 2	56.3 ± 3
Krebs-Ringer buffer				
+ Tetracaine (10^{-4} M)	23.1 ± 2	24.0 ± 1	22.1 ± 1	24.8 ± 2
+ Valinomycin (10^{-6} M)	20.0 ± 2	21.3 ± 2	5.5 ± 1	7.1 ± 1
+ Valinomycin (10^{-10} M)	48.5 ± 2	59.1 ± 3	45.1 ± 2	55.6 ± 3

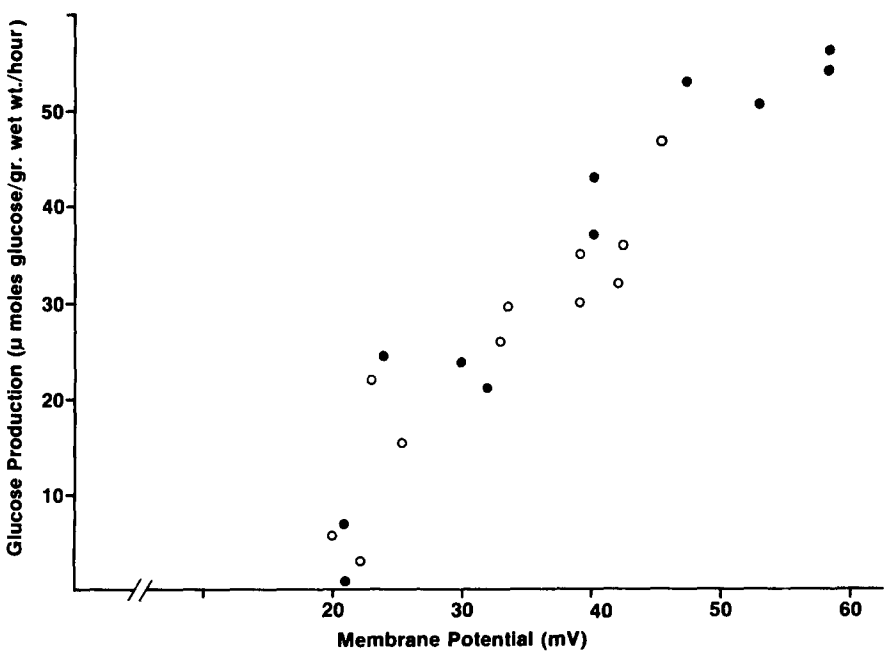

Fig. 2. Correlation between glucose production and membrane potential. Data are taken from Table I. ●, with and ○, without glucagon.

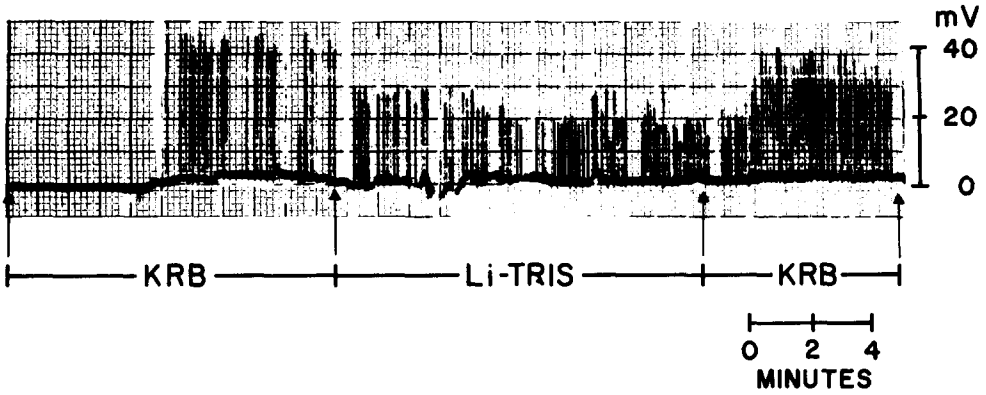

Fig. 3. The reversibility of the depolarizing effect of sodium-free Li^+/Tris buffer. The liver was perfused as usual. At the lines indicates by the arrows, the reservoir was switched to one containing the indicated perfusate. Each deflection represents one measurement.

production (not shown) is also reversible. Valinomycin, an ionophorous antibiotic with increased K^+ permeability of the cell, in high concentration inhibited and in low concentration stimulated glucose production (Table I) as previously noticed by others [16,17]. We also found that at high concentration the ionophore depolarized the liver while at low concentrations it evoked a significant hyperpolarization (Table I). The correlation between the increase in glucose production and the increase in membrane potential is therefore inconsistent with a nonspecific toxic effect.

In separate experiments not reported here, we did not find a correlation between changes in membrane potential and the rate of glycogen breakdown, measured as glucose release. Glucose by itself had no effect on the liver cell membrane potential. These results indicate a specificity of the observed effects.

The high r values reported in Fig. 2 indicate that it is unlikely that the observed correlation is coincidental. In addition to glucagon and cyclic AMP, other hyperglycemic agents, (e.g. cyclic GMP, isoproterenol, epinephrine) also hyperpolarize the liver [8]. Because of the results presented in Table I and Fig. 2, the possibility that a casual relationship might exist between the gluconeogenic rates and the changes in membrane potential has to be considered. The question then arises; by what possible mechanism(s) can a change in membrane potential influence the rate of glucogenesis? Several possibilities exist: a substance might be released from the membrane in a voltage-dependent fashion which then interacts with some other component. In many cells internal connections were shown to exist between the plasma membrane and intracellular structures extending into the cells. Such connections might exist in the liver, thus propagating the stimulus into the intracellular environment. Voltage-dependent permeability change of the cell membrane might occur, thus leading to a secondary ion redistribution. The change in the ionic environment might then influence metabolic parameters by changing enzyme activities [18], transmembrane electrical and chemical gradients [19], transport processes [20], protein conformation [21] of membrane permeability [22].

The reported data indicates a need to explore the role of membrane poten-

tial change in regulating metabolic processes in general, and raises the possibility that the inhibitory effect of insulin on the glucagon-stimulated gluconeogenesis is due to its inhibitory effect on the glucagon-evoked ion fluxes and hyperpolarization.

Acknowledgement

This work was supported by U.S.P.H. grant AM-18083.

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