MEMBRANE COOPERATIVE ENZYMES: INTERPLAY OF INSULIN, GLUCAGON AND EPINEPHRINE ON RAT ERYTHROCYTE ACETYLCHOLINESTERASE SYSTEM

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Received 10 May 1978

1. Introduction

Insulin, at physiological plasma levels, affects the cooperativity of membrane-bound enzymes [1,2]. This action was detected in vitro through changes in the Hill h coefficient of rat erythrocyte membrane-bound acetylcholinesterase and (Na\(^+\) + K\(^+\))-ATPase [1], and in Escherichia coli membrane-bound Ca\(^{2+}\)-ATPase [2]. Insulin decreased the values of h of erythrocyte acetylcholinesterase [1] and E. coli Ca\(^{2+}\)-ATPase [2] and enhanced it in the erythrocyte (Na\(^+\) + K\(^+\))-ATPase system [1]. The correlation between the membrane fluidity and the h values of the former enzymes were positive whereas for the latter enzyme it was negative [3,4]. Based on these findings we postulated that insulin decreases membrane fluidity [1,2].

In general insulin modifies metabolic activities in an opposite manner to that induced by epinephrine or glucagon [5], so it was of interest to study the actions of these hormones on a membrane-bound cooperative system. The present report provides the first evidence of a hormonal interplay between insulin, epinephrine, and glucagon on rat erythrocyte membrane-bound acetylcholinesterase (EC 3.1.1.7). Epinephrine and insulin showed opposite effects on the Hill coefficient. Insulin $10^{-9}$ M decreases the values of h, whereas epinephrine $10^{-5}$ M raises them. Glucagon $10^{-10}$ M has a blocking action on the insulin effect. The interplay between insulin, glucagon and epinephrine is in agreement with current ideas about the actions of these hormones on metabolic fuels.

Part of the present work was reported earlier in preliminary form [6].

2. Materials and methods

Male Sprague-Dawley rats (220–320 g) raised after weaning on a basic diet supplemented with 5% lard or corn oil were used throughout. With these diets erythrocyte membranes can be obtained with low or high fatty acid fluidity parameters and low or high values of h for the allosteric inhibition by F\(^-\) of the membrane acetylcholinesterase [3]. The correlation between the membrane fluidity and the values of h was highly positive ($r = 0.90$) [3]. Details concerning erythrocyte preparation, assay of acetylcholinesterase activity and calculation of kinetic parameters have been given in [1–3]. Inhibition of F\(^-\) of the acetylcholinesterase was obtained (under 'initial velocity' conditions) with whole red cells or isolated membrane by a method reported in [7]. Insulin, glucagon and catecholamine solutions were prepared immediately prior to use. For the enzymatic assays, appropriate dilutions were made in sodium phosphate buffer 150 mosM, pH 8.0. Preincubation of 10 min at room temperature was carried out before the determination of h (unless otherwise specified).

3. Results

Table 1 shows the interplay between insulin, glucagon, and epinephrine on the values of h for the inhibition by F\(^-\) of the membrane-bound acetylcholinesterase from rats fed corn oil or lard supplemented diets. According to a previous report [3], values of 1.6 for corn-oil-fed rats (row 1) and of 1.0 for lard-fed rats (row 8) were obtained. In the presence of
Table 1

Interplay of insulin, glucagon and epinephrine on the Hill coefficient for the inhibition by fluoride ions of membrane-bound acetylcholinesterase

<table>
<thead>
<tr>
<th>Rows</th>
<th>Diets</th>
<th>Hormone additions (M)</th>
<th>h^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Insulin</td>
<td>Glucagon</td>
</tr>
<tr>
<td>1</td>
<td>Corn oil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>10^{-9}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>10^{-7}</td>
<td>-</td>
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<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>10^{-4}</td>
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<tr>
<td>6</td>
<td>10^{-9}</td>
<td>10^{-4}</td>
<td>10^{-5}</td>
</tr>
<tr>
<td>7</td>
<td>10^{-9}</td>
<td>-</td>
<td>10^{-5}</td>
</tr>
</tbody>
</table>

|  8   | Lard   | -  | - | - | 1.00 ± 0.03 |
|  9   | -      | 10^{-9} | - | - | 0.93 ± 0.07 |
| 10   | -      | -  | 10^{-5} | - | 1.00 ± 0.07 |
| 11   | -      | -  | 10^{-5} | - | 1.55 ± 0.08 |
| 12   | -      | 10^{-9} | 10^{-5} | - | 1.53 ± 0.10 |
| 13   | -      | 10^{-9} | -  | 10^{-5} | 1.06 ± 0.10 |
| 14   | -      | 10^{-9} | 10^{-5} | 10^{-4} | 1.56 ± 0.03 |
| 15   | -      | -  | 10^{-9} | 10^{-4} | 1.53 ± 0.02 |

^a The data are the average of at least 3–5 independent experiments (Mean ± SEM)

Insulin 10^{-9} M the values of h changed from 1.6 to 1.0 in acetylcholinesterase from rats fed a corn oil supplemented diet (ref. 1 and row 2) and in the presence of glucagon 10^{-7} M (row 3) or epinephrine 10^{-4} M (row 4) the values of h remained unmodified (about 1.6). However, when glucagon (row 5) or epinephrine (row 6) were added together with insulin to the reaction mixture, the effect of insulin was inhibited. If the concentration of epinephrine was decreased from 10^{-4} M to 10^{-5} M, the insulin effect could be observed again (row 7). Epinephrine 10^{-5} M was able to increase the values of h from 1.0 to 1.5 in rats fed a lard supplemented diet (row 11), whereas insulin 10^{-9} M (row 9) or glucagon 10^{-7} M (row 10) did not affect the values of h in this membrane preparation. Depending on the epinephrine concentrations (row 12 and 13), insulin 10^{-9} M antagonized the action of this catecholamine. This insulin action was blocked by the presence of glucagon 10^{-7} M (row 14). These results suggest that, in the interplay of the effects of insulin, glucagon and epinephrine on the membrane-bound acetylcholinesterase, the effects of insulin and epinephrine were of an antagonistic nature since both hormones have an inverse action on the values of h while the effect of glucagon on the insulin action was of a blocking nature since glucagon did not affect the values of h neither in corn-oil nor in lard-fed rats.

Thriiodothyronine 10^{-9} M also decreases the value of h of acetylcholinesterase from rats fed a corn oil supplemented diet [8,9]; however, glucagon did not block the thriiodothyronine effect (not shown). In addition, glucagon did not affect the epinephrine-induced changes on the values of h of the enzyme from rats fed a lard supplemented diet (row 15). These facts suggest a specific blocking action of glucagon on the insulin effect. The concentration able to block the insulin effect decreased from 10^{-7} M (table 1) to 10^{-10} M when the membrane preparations were preincubated for 15 min at room temperature with glucagon before insulin addition. This glucagon concentration is within the order of mammalian physiological ranges [10].

The effect of epinephrine appears to be mediated by a β adrenergic receptor since L-isoproterenol 10^{-7} M (a typical β agonist) increased the values of
Fig. 1. Effect of the concentration of $F^-$ on the acetylcholinesterase from rats fed a lard supplemented diet in the absence (o-o), in the presence of isoproterenol $10^{-7}$ M (e-e) and propranolol $10^{-10}$ M (o-o). (A), direct plot of relative reaction rate as function of $F^-$ concentration. (B), plot of $v/(V_o-v)$ as function of $F^-$ concentration on logarithmic coordinates. The slope of each line is indicated in the figure. The same whole red cells preparation was used for control and hormone test.

$h$ from 1.0 to 1.6 in membranes from lard fed rats and the presence of propranolol $10^{-10}$ M (a typical $\beta$ antagonist) inhibited this action (fig.1). The effective participation of a $\beta$ receptor in this membrane-bound system is under study.

4. Discussion

Since the values of $h$ for the acetylcholinesterase depend on the fluidity of the membrane [1,2] the effect of insulin on the Hill coefficient of this enzyme can only be observed in membranes exhibiting an appropriate fatty acid fluidity. That is, the insulin action was found only in membranes with high fatty acid fluidity (from corn-oil-fed rats), but not on membranes with low fatty acid fluidity (from lard-fed rats) (row 2 and 9). This point has been discussed in detail elsewhere [1,11]. The present paper shows that when epinephrine action increases the values of $h$ of the enzyme from lard fed animals the decreasing action of insulin on the values of $h$ can also be seen in this 'lard membrane' system (rows 11 and 13). The blocking effect of glucagon (rows 5 and 14) or the antagonistic action of epinephrine (rows 6-7 and 12-13) on insulin action were found in the membrane-bound system from both groups of animals.

Insulin did not modify the cooperativity of the soluble acetylcholinesterase; $h$ values of 1.6 were obtained in the presence or in the absence of insulin [1].

The homeostatic regulation of metabolic fuels involves the general opposing actions of insulin and glucagon and catecholamines, e.g.: glucagon and epinephrine accelerate glucogenolysis, gluconeogenesis and lipolysis whereas insulin has the opposite action [5]. Another antagonistic effect is illustrated by changes in the intracellular 3',5'-cyclic-AMP concentration since insulin can suppress the increased levels of cyclic AMP in fat [12] and liver [13] cells that occur on stimulation by glucagon and catecholamines. Also insulin and glucocorticoids (cortisol) are known to have an antagonistic effect on peripheral tissues [5]. As was shown previously [1] for cortisol, and in this paper for epinephrine, these hormones show an effect on the Hill coefficient systems that is opposed to the insulin action. The mutually antagonistic behavior of insulin and glucagon is present in the system in the form of a blocking action of glucagon on the insulin effect. Glucagon reverses the inhibition by insulin of the adenylyl cyclase from Neurospora crassa [14].

Could these facts be an accident or are they of physiological relevance? The influence of other hormones on the values of $h$ is intended to be the next step in our studies to try to find a possible general biological principle in relation to the action of the hormones on the kinetic changes of membrane cooperative enzymes which are regulated by the membrane fluidity [15].

Acknowledgements

This work was supported by a grant from the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina). R.N.F. is a career investigator of the Consejo Nacional de Investigaciones Científicas y Técnicas. Special thanks are due to B. Bloj and F. Siffreriz for their assistance in the preparation of the English manuscript and the secretarial aid of J. C. Artero.

References


