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THE EFFECT OF TEMPERATURE ON THE ACTIVITY OF THE ADENYLATE CYCLASE SYSTEM OF LIVER PLASMA MEMBRANES

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SUMMARY

The rat liver adenylate cyclase system shows a discontinuity in the Arrhenius plots at 20°C in the nonstimulated activity (basal) with activation energies of 16 and 28 Kcal/mole. The discontinuity disappears when the enzyme is stimulated either by glucagon, sodium fluoride, 5'guanylyl-imidodiphosphate or glucagon plus 5'guanylyl-imidodiphosphate and the energy of activation was the same with all the compounds tested. If the activator was initially in contact with the membranes at 0°C the energy of activation was similar to that observed below the break (26 Kcal/mole) but it changed to that above the break if the compound contacted the membranes at temperatures above the break (22-24°C). We discuss the possibility of two different conformations of the enzyme; both conformations can be "frozen" by any of the compounds tested, "isolating" the enzyme from any subsequent physical change of the membrane due to temperature.

A model has been proposed for the structure of the plasma membrane (1). In such a model, the phospholipids are arranged in a bilayer and the globular proteins are immersed in the lipid phase. It is not a rigid structure but a liquid phase in which the proteins operate independently although influenced by the physical properties of the lipids.

The adenylate cyclase system is an intrinsic component of membranes and therefore we would expect it to be influenced by the lipids just like any other protein of the membrane. There are several cations that influence the effect of hormones on the activity of the adenylate cyclase system (2,3), and it is thought that their effect is due to actions on the structure of the membrane rather than on the enzyme itself. It has been shown that detergent dispersed membranes lose the hormonal response but retain the ability to re-

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act to the fluoride ion (4-8). Previous studies suggest the participation of specific lipids on the hormonal action (8-11). We have presented some evidence that acidic phospholipids are required for glucagon and nucleotides to affect the rat liver adenylate cyclase (8); also we showed a clear relationship between the presence of these lipids and the binding either of the glucagon histidine residue or of nucleotides to a site that controls the activity of this regulatory enzyme system.

In several studies the discontinuities in the Arrhenius plots of membrane bound enzyme activities have been correlated with lipid phase changes (transitions and separations) and have intended to establish a physiological relationship with changes in the Arrhenius activation energy (12-15).

The present study describes the effect of temperature on the rat liver membrane adenylate cyclase activity with and without the stimulation of various compounds.

MATERIALS AND METHODS

Glucagon was supplied as a gift by Lilly. [α-^{32}P]ATP, Gpp(NH)p[1] and cyclic AMP were supplied by the International Chemical and Nuclear Corporation and used without any further purification. Creatine phosphate and creatine phosphokinase were purchased from Sigma Chemical Co. All other reagents were of analytical grade.

Partially purified plasma membranes from rat liver (male Wistar rats) were prepared according to Neville's procedure (16) with a modification described by Pohl et al. (17), and frozen and stored in liquid nitrogen. For further use the membranes were suspended in 20 mM Tris-HCl, pH 7.5. Adenylate cyclase activity was measured by the method described by Salomon, Londos and Rodbell (18). Unless specified otherwise, the assay medium contained 3.2 mM [α-^{32}P]ATP (20-40 cpm/pmole), 5 mM $MgCl_2$, 25 mM Tris-HCl pH 7.5,1 mM cyclic AMP and an ATP regenerating system consisting of 20 mM creatine phosphate and 100 U/ml creatine phosphokinase. Either glucagon, sodium fluoride, Gpp(NH)p or Glucagon plus Gpp(NH)p was added to stimulate the enzymatic activity. Incubations were carried out for ten minutes at the temperature indicated. Unless stated otherwise, the reaction was initiated by the addition of the membranes to give 30-40 micrograms of proteins, which were measured by the procedure of Lowry et al. (19) using bovine serum albumin as standard. The slopes of the Arrhenius plots and the existence and locations of break points were determined by linear regression analysis, and the activation energies of the basal and stimulated activities were compared to each other by the Student test.

RESULTS AND DISCUSSION

Under the conditions of the assay for basal and stimulated activities

[1]The abbreviations used are: Gpp(NH)p, 5'guanylyl-imidodiphosphate; Cyclic AMP, adenosine 3':5'monophosphate.

with 10^{-6}M glucagon and 10 mM sodium fluoride, we found that the adenylate cyclase activity is linear in time during the ten minutes of incubation and the specific activity was of the same order of the values previously reported (5,12). We were able to measure the basal activity at temperatures as low as 5°C.

The temperature affected the basal and stimulated activities differently, as shown in Fig. 1, where it is seen that the Arrhenius plot of the basal activity presents a discontinuity at approximately 20°C, whereas the plot of the activities stimulated either by glucagon, sodium fluoride or glucagon plus Gpp(NH)p do not present such a break. The activation energy is essentially the same for each compound and very similar to that of the basal activity below the break point (Table I, condition I).

The results described above were obtained through initiating the reaction by adding the membranes to the incubation medium, that in the case of stimulated activities already contained the activators, and this addition was performed at 0°C.

In the case of the basal activity the break of the Arrhenius plot seems to be the result of a conformational change of the enzyme due to a change in the lipids and in the case of the stimulated activities the activator added seems to "freeze" the conformation below the break point.

To test this hypothesis, we repeated the above experiments, but keeping the membranes at temperatures between 22 and 24°C for 15 minutes and then added to the activator solution. The activation energy of the reaction under these conditions was very similar to that found for the basal activity above the break point (Fig 2) (Table I, Condition II).

To assure that this reaction did not have a different activation energy due only to the temperature but also to the stabilizing action of the activators on an enzyme with two different active conformations, we performed the following experiments: first, we kept the membranes above 20°C (22-24°C) for 15 minutes, then we added the activator and returned the mixture to an

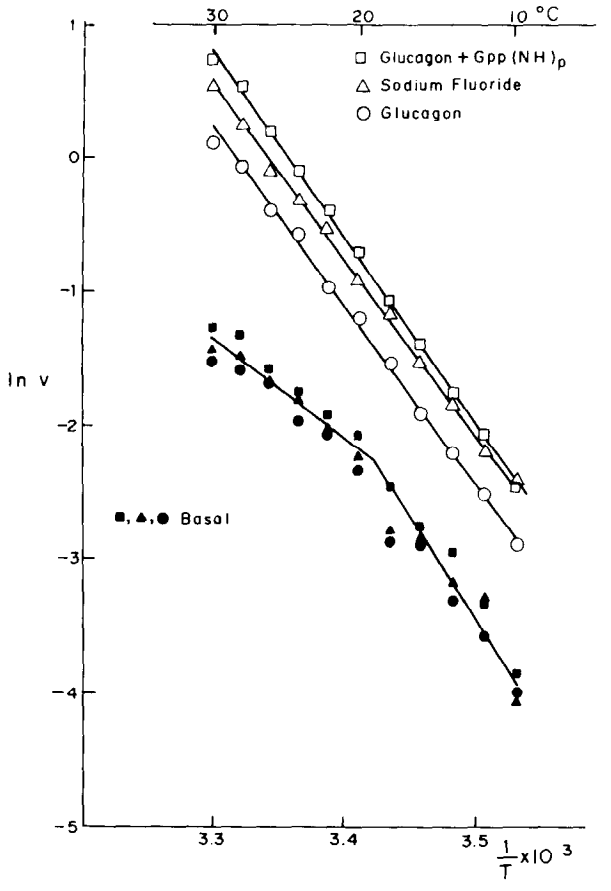

Fig. 1 Arrhenius plot of the adenylate cyclase activity (nmoles cyclic AMP/mg protein in ten minutes) Effect of 10 mM Sodium fluoride, 10^{-6}M glucagon and 10^{-6}M glucagon combined with 10^{-5}M Gpp(NH)p. The membranes at 0°C were added to the incubation medium (see methods) containing the activators (Condition I). The different symbols for the basal activity indicate 3 different experiments with membranes frozen in liquid nitrogen one year (■) 6 months (▲) and one week (●) before the experiment.

ice bath and at this point the substrate was added to start the reaction. The activation energy was, as we expected, the same as in the case of the basal activity above the break point (Table I, Condition III-B). The condition III-A (Table I) is a control in which the reaction was started by adding the substrate to the mixture membrane-activator which had been kept in an ice-bath; again, as we expected, the activation energy was similar to that observed under the basal conditions below the break point.

Finally, an experiment was performed that probed that the change in

TABLE I

THE EFFECT OF VARIOUS COMPOUNDS ON THE ENERGY OF ACTIVATION (Kcal/mol) OF ADENYLATE CYCLASE

COMPOUND ADDED	CONDITION I*	CONDITION II*	CONDITION III*	
			A	B
NONE (BASAL)	27.9 ± 2.5(12)	-	-	-
	15.6 ± 0.98(11)**	-	-	-
GLUCAGON	26.0 ± 2.8(11)	17.0 ± 0.73(10)	-	-
FLUORIDE	25.5 ± 1.2(15)	17.1 ± 1.6(6)	25.5 ± 0.50(2)	17.7 ± 0.60(3)
Gpp(NH)p	24.9 ± 2.0(4)	18.5 ± 0.12(3)	-	-
GLUCAGON+ Gpp(NH)p	26.4 ± 1.6(7)	18.5 ± 0.91(5)	24.7 (1)	17.5 (1)

The results are expressed as mean ± S. D. The figures in parentheses are the number of experiments performed. The results that gave a determination coefficient < 0.9 were discarded.
The range of temperature studied was 10° - 30°C.
* For definition of conditions I, II and III see the text.
** The basal activity showed a change in the slope at 19.7° ± 0.99°.

the enzyme due to temperature is reversible: the membranes without the activator standed at 23°C for 15 minutes, and after that time were slowly returned to the ice-bath temperature. Once there, the activator was added and the experiments were performed as described in condition I. The results (not shown) were those expected for such a condition: the activation energy was similar (27 Kcal/mole) to that observed for the basal below the break point.

The Student test with all the data of the activation energies was indicative that the results of the experiments done under conditions I and III-A and the basal activation energy below 20°C had a high probability of being identical. The probability was also high to be the same for the activation energies of the experiments done under conditions II and III-B and for activity above 20°C.

This study shows that the adenylate cyclase enzyme from a homeothermic animal presents a biphasic behavior in the Arrhenius plots. This is not

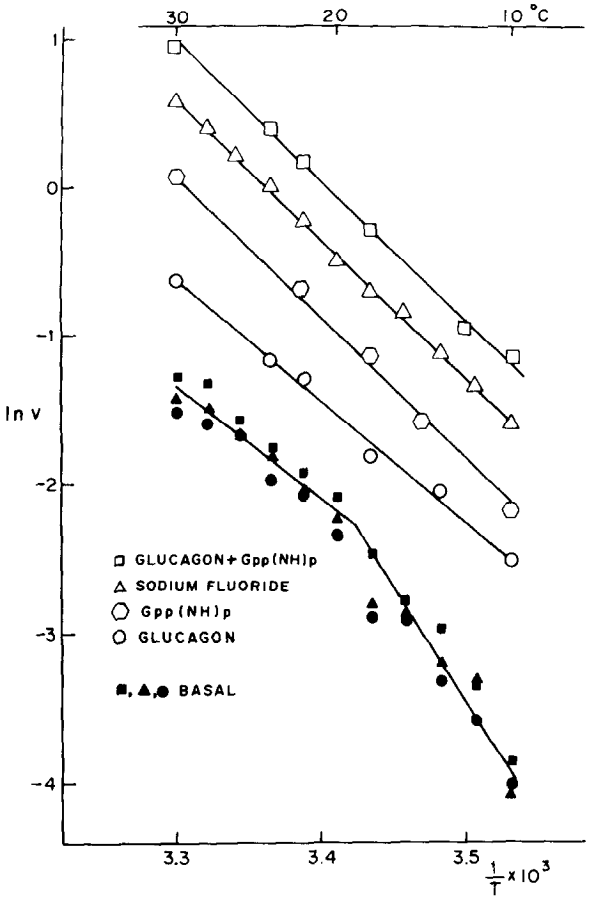

Fig. 2 Arrhenius plot of the adenylate cyclase activity (nmoles cyclic AMP/mg protein in ten minutes). Effect of 10 mM sodium fluoride, 10^{-6}M glucagon, 10^{-5}M Gpp(NH)p and 10^{-6}M glucagon combined with 10^{-5}M Gpp(NH)p. The plasma membranes were incubated at 23°C for 15 minutes and then were added to the medium (see methods) containing the activators (Condition II).

surprising since various membrane enzymes in studies already published have shown the same behavior (20). This behavior has been usually interpreted as a result of a change in the membrane lipid phase (12-15, 20). The use of viscosity probes of the membrane lipid core (21) and some kind of photoactive covalent labeling of the membrane components (22) could help to elucidate the features of that change in the membrane and its possible relationship with the changes observed on the enzyme. Although all the activators used here act

at different sites of the complex (5, 23) they do not modify the activation energy, but instead all of them "stabilize" the system with the very same activation energy; this suggests the existence of a high cooperativity among the components of the system, and this "stabilization" isolates it from subsequent physical changes of the lipids.

In conclusion, the adenylate cyclase catalizes the conversion of ATP to cyclic AMP with two activation energies. It is proposed that this is due to a conformational change of the enzyme possibly as a result of a modification of the physical state of the membrane. Also, it is proposed that the activators tested are able to stabilize the enzyme conformation they find when added to the membranes.

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