Parallel Modulation of Catecholamine Activation of Adenylate Cyclase and Formation of the High-Affinity Agonist·Receptor Complex in Turkey Erythrocyte Membranes by Temperature and *cis*-Vaccenic Acid†

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ABSTRACT: Catecholamine stimulation of adenylate cyclase in turkey erythrocyte membranes is significantly reduced when assayed below physiological temperatures. This inhibition appears to involve the coupling of β-adrenergic receptor occupancy to adenylate cyclase activation rather than the catalytic activity of the enzyme, because the sodium fluoride stimulated activity of adenylate cyclase was not reduced to the same extent. Receptor binding characteristics at 20 and 37 °C were compared by using radioligand binding studies in order to more precisely determine the effects of low assay temperatures on the coupling process. Although binding of the antagonist (−)-[^{3}H]dihydroalprenolol (DHA) to the β-adrenergic receptor was not altered significantly, a dramatic change in the properties of agonist binding was observed in (−)-isoproterenol competition for [^{3}H]DHA binding at 20 °C. At this temperature the receptor did not form the high-affinity nucleotide-sensitive complex with agonist which has been observed at 37 °C and shown to be a necessary intermediate in the activation of adenylate cyclase by agonist. Agonist activation of adenylate cyclase and the ability of the β-adrenergic receptor to form the high-affinity nucleotide-sensitive complex with agonist at 20 °C were both restored when the membranes were treated with *cis*-vaccenic acid, which may act by increasing the fluidity of the membrane. These observations demonstrate that an appropriate membrane environment is required for the formation of the high-affinity nucleotide-sensitive agonist·receptor complex and that the inability of the receptor to form this complex is a major factor in the low-temperature inhibition of catecholamine stimulation of adenylate cyclase in turkey erythrocyte membranes.

The membrane-bound enzyme adenylate cyclase can be stimulated by many drugs and hormones, including β-adrenergic catecholamines. This transmembrane regulatory system is composed of at least three distinct molecular components, the hormone receptor, the catalytic unit of the enzyme, and a guanine nucleotide regulatory component (Limbird & Lefkowitz, 1977; Haga et al., 1977; Pfeuffer, 1977; Ross et al., 1978). The latter component appears to mediate the effects of guanine nucleotides on the system by functioning as a coupling unit between the receptor and the catalytic moiety (Pfeuffer, 1979; Limbird et al., 1980; Cassel & Selinger, 1978).

Hormone stimulation of adenylate cyclase is strongly influenced by the phospholipid membrane environment. Receptor–enzyme coupling can be significantly altered or abolished by manipulations which change the fluidity or lipid composition of the membrane (Sinha et al., 1977; Klein et al., 1978; Puchwein et al., 1974; Rubalcava & Rodbell, 1973; Bakardjieva et al., 1979). Several investigators have reported that catecholamine stimulation of adenylate cyclase of turkey erythrocyte membranes was remarkably decreased when assayed below physiological temperatures, while NaF stimulation was much less affected (Orly & Schramm, 1975; Rimon et al., 1978). Treatment of the membranes with unsaturated fatty acids such as *cis*-vaccenic acid led to a return of the ability of the agonist to stimulate the enzyme. Orly & Schramm (1975) suggested that the temperature-sensitive event involved guanine nucleotide regulation of enzyme activity. Rimon et al. (1978) reported that *cis*-vaccenic acid increased the apparent fluidity of the membrane and concluded that a fluid membrane environment was a prerequisite for lateral diffusion in the membrane and successful coupling of the β-adrenergic receptor and adenylate cyclase.

It has been demonstrated in frog erythrocyte membranes that agonists form a high-affinity slowly dissociable complex with the β-adrenergic receptor which appears to facilitate guanine nucleotide activation of the cyclase (Williams & Lefkowitz, 1977; Lefkowitz & Williams, 1978). This complex probably contains the agonist, the receptor, and the guanine nucleotide regulatory protein (Limbird et al., 1980). Interaction of guanine nucleotides with this complex destabilizes it, releasing free agonist and receptor and activating adenylate cyclase. This model is supported by observations of nucleotide regulation of receptor affinity for the agonist in a variety of different experimental systems, including the β-adrenergic receptor in S49 cells (Ross et al., 1977), the α-adrenergic receptor in platelets (Tsai & Lefkowitz, 1979), and the glucagon receptor in the liver (Rodbell et al., 1971). Although not observed in previous investigations (Brown et al., 1976; Tolkovsky & Levitzki, 1978), such a high-affinity nucleotide regulated agonist·receptor complex has recently been described in the turkey erythrocyte membrane and shown to be a necessary intermediate step in the activation of adenylate cyclase (Stadel et al., 1980).

In the present studies we have utilized the turkey erythrocyte membrane, perturbed by low temperature and *cis*-vaccenic acid treatment, as a model for investigating the importance of membrane fluidity in modulating the formation of the high-affinity nucleotide-regulated complex of agonist and receptor. The observations presented here indicate that an appropriate membrane environment is crucial to the formation of this complex in the turkey erythrocyte membrane and that this high-affinity agonist·receptor complex is required for catecholamine stimulation of adenylate cyclase.

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Experimental Procedures

Materials. The [α-^{32}P]ATP,[1] [^{3}H]cAMP, and the radioligand [^{3}H]DHA (41 or 45 Ci/mmol) were obtained from New England Nuclear. *cis*-Vaccenic acid was obtained from Sigma Chemical Co. The sources, purity, and biological activity of the radioactive substances, as well as the other materials used, have been previously reported (Mukherjee et al., 1975).

Turkey Erythrocyte Membranes. Turkey erythrocyte membranes were prepared as described previously (Stadel et al., 1980). In brief, the cells were lysed in hypotonic buffer and centrifuged. The membrane pellets were homogenized with a Brinkman polytron and Dounce homogenizer, layered over a 35% (w/w) sucrose cushion, and centrifuged at 1200*g* for 20 min. The supernatant was centrifuged at 30000*g* and resuspended in 75 mM Tris-HCl, 12.5 mM $MgCl_2$, and 1.5 mM EDTA, pH 7.5 at 25 °C. Adjusting the pH of the buffer to 7.5 at the 20 or 37 °C assay temperatures did not significantly alter the results. *cis*-Vaccenic acid was dissolved at 100 mM concentration in absolute ethanol. Turkey erythrocyte membranes were incubated with 1 mM *cis*-vaccenic acid or an equal volume of ethanol for 30 min on ice. There was no significant difference in the results obtained with membranes treated with ethanol and untreated membranes. Protein concentration was determined by the method of Lowry et al. (1951).

Adenylate Cyclase Assay. Assays were performed in a 50-μL volume containing 30 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 0.6 mM EDTA, 0.1 mM cAMP, 0.1 mM ATP, 2.6 mM phosphoenolpyruvate, 13 μg/mL pyruvate kinase, and 10 μg/mL myokinase. The assays were incubated for 10 min at 37 °C or for 60 min at 20 °C and were terminated with the addition of 1 mL of a stop solution containing [^{3}H]cAMP (15 000 cpm/mL), 100 μg of ATP, and 50 μg of cAMP. The [^{32}P]cAMP generated was determined as described by Salomon et al. (1974). Activators of adenylate cyclase were 10 mM NaF or 0.1 mM (−)-isoproterenol and 0.1 mM Gpp(NH)p.

[^{3}H]DHA Binding Assays. Turkey erythrocyte membranes (0.3–0.5 mg of protein/mL) were incubated in a final volume of 0.5 mL with [^{3}H]DHA (3–6 nM) for either 10 min at 37 °C or 60 min at 20 °C. Binding equilibrium was attained during these intervals (data not shown). Competition for [^{3}H]DHA binding by (−)-isoproterenol was measured by adding increasing concentrations of (−)-isoproterenol to the assay mixtures in the absence or presence of 0.1 mM Gpp(NH)p. The incubation was terminated by adding 5 mL of cold buffer, pouring the samples over Whatman GF/C glass fiber filters, and washing the filters under vacuum with two additional 5-mL aliquots of cold buffer. Nonspecific binding, defined as the amount of [^{3}H]DHA binding in the presence of 10 μM (±)-propranolol, was 5–10% of the total binding.

Data Analysis. The competitive binding assay data for (−)-isoproterenol in the presence or absence of Gpp(NH)p[1] were analyzed simultaneously by a weighted nonlinear least-squares curve-fitting procedure using a generalized model for complex ligand-receptor systems (Feldman, 1972; Hancock et al., 1979; Kent et al., 1980; Munson & Rodbard, 1979). For each competition binding curve fitted parameter estimates were derived on the basis of models in which the receptor could exist in either one or two afffinity states. The term "state" or "binding state" rather than "site" or "binding site" is used to signify that the β-adrenergic receptor is capable of binding β-adrenergic agonists with several different apparent affinities for the ligands. Statistical analysis comparing "goodness of fit" between one and two affinity state models was also provided and was used to determine the most appropriate model for the ligand being examined. A two-state model was accepted as appropriate to explain the binding data only after it was demonstrated to significantly improve the computer fit of the data when compared to a one-state model.

We have previously found in frog and turkey erythrocytes that antagonist binding to the β-adrenergic receptor exhibited a single binding affinity (K_D). In contrast, interaction of agonists such as (−)-isoproterenol with the receptor in the absence of Gpp(NH)p was best described by two states of binding. R_H was defined as the population of receptors which bound the ligand with high affinity (high affinity state receptors) when compared with the remaining lower affinity receptors R_L (Kent et al., 1980). The dissociation constants for these two affinity states were defined as K_H and K_L, respectively. Receptor binding of (−)-isoproterenol in the presence of Gpp(NH)p exhibited a single binding affinity (K_D), which could in all cases be set equal to the K_L derived from the (−)-isoproterenol curve in the absence of Gpp(NH)p without significant worsening of the computer "fit".

Slope factors ("pseudo" Hill coefficients) were determined by fitting the data to a four-parameter logistic equation as previously described (De Lean et al., 1978). Because indirect rather than direct binding was evaluated in this study, these slope factors are not true Hill coefficients. A slope factor close to 1.0 suggests that a single affinity state exists, whereas a slope factor lower than 1.0 indicates that more than one state may exist. Fitted estimates of the slope factors and statistical analysis were provided. All computations were performed by using iterative programs in PL/1 for a PDP 11/45. Results were reported as the mean ± SEM for each parameter estimate, determined from several experiments analyzed individually.

Results

Adenylate cyclase in the turkey erythrocyte membrane can be stimulated at 37 °C by sodium fluoride or by (−)-isoproterenol plus the guanine nucleotide analogue Gpp(NH)p, as shown in Figure 1A. When the enzyme was assayed at 20 °C, however, stimulation by (−)-isoproterenol plus Gpp(NH)p was reduced to an extremely low level (Figure 1B). The 40-fold stimulation of adenylate cyclase over basal activity induced by (−)-isoproterenol and Gpp(NH)p at 37 °C was reduced to a one- to sixfold stimulation at 20 °C. A much smaller decrease in NaF activation was observed at 20 °C. Addition of the fatty acid *cis*-vaccenic acid to turkey erythrocyte membranes had two effects on adenylate cyclase activity. One was a one- to twofold temperature-independent increase in the catalytic activity of the enzyme, demonstrated by the NaF activity at 37 or 20 °C and the (−)-isoproterenol stimulation at 37 °C. A far more dramatic increase, however, was observed in the (−)-isoproterenol-stimulated adenylate cyclase activity of treated membranes assayed at 20 °C, which was 15–25-fold higher than that in untreated membranes. These observations basically confirm the previous findings of Orly & Schramm (1975) concerning the effects of temperature and *cis*-vaccenic acid on adenylate cyclase activity.

Radioligand binding and agonist competition studies were conducted at 37 and 20 °C in order to delineate the effects of low temperature and *cis*-vaccenic acid treatment on the

[1] Abbreviations used: [^{3}H]DHA, (−)-[^{3}H]dihydroalprenolol; Gpp(NH)p, guanyl-5′-yl imidodiphosphate; ATP, adenosine 5′-triphosphate; cAMP, adenosine cyclic 3′,5′-phosphate; [^{125}I]HYP, [^{125}I]hydroxybenzylpindolol.

Table I: Computer-Modeled Parameters of Agonist Interaction with β-Adrenergic Receptors in Turkey Erythrocyte Membranes[a]

temp (°C)	*cis*-vaccenic acid treatment	K_H (M)	K_L (M)	R_H (%)	R_L (%)	K_L/K_H	slope factors −Gpp(NH)p	slope factors +Gpp(NH)p	N
37	−	$4.7 \pm 1.6 \times 10^{-8}$	$4.0 \pm 0.3 \times 10^{-7}$	55 ± 7	45 ± 7	9	0.83 ± 0.06[d]	1.06 ± 0.07[e]	6
37	+	$2.4 \pm 1.1 \times 10^{-8}$ [b]	$4.8 \pm 1.6 \times 10^{-7}$ [b]	58 ± 11[b]	42 ± 11[b]	20	0.71 ± 0.09[d]	1.06 ± 0.14[e]	3
20	−	[c]	$3.2 \pm 0.8 \times 10^{-8}$	0[c]	100		0.94 ± 0.09[e]	0.98 ± 0.07[e]	7
20	+	$4.0 \pm 1.0 \times 10^{-9}$	$4.4 \pm 1.1 \times 10^{-8}$	48 ± 9[b]	52 ± 9[b]	11	0.73 ± 0.10[d]	1.11 ± 0.14[e]	7

[a] The parameters were determined by computer analysis of individual experiments as described under Experimental Procedures. The values reported are the mean ± SEM of the results from a number of experiments. N = number of experiments. [b] Not significantly different from the parameters at 37 °C in the absence of *cis*-vaccenic acid ($p > 0.05$). [c] A single affinity state of receptors was observed and designated K_L (see text). [d] Significantly different from 1 ($p < 0.05$). [e] Not significantly different from 1 ($p > 0.05$).

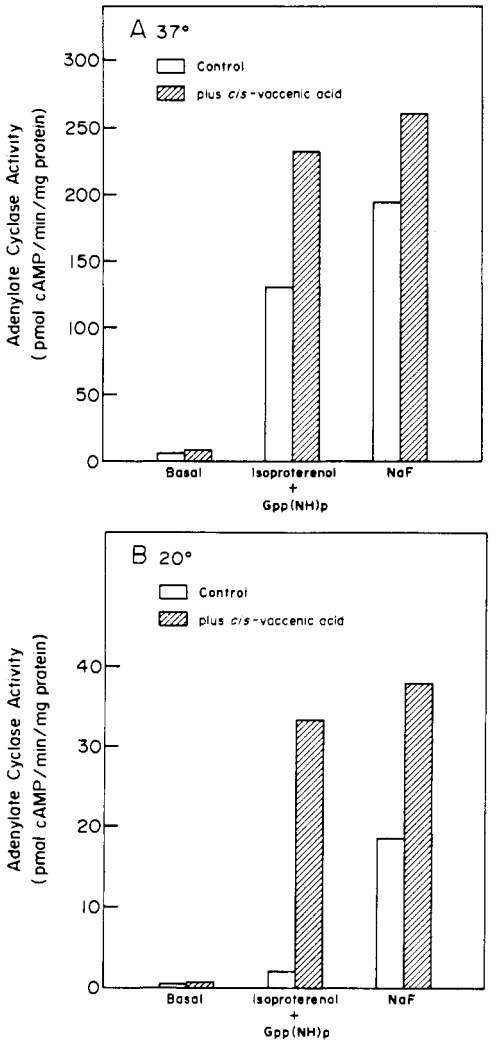

FIGURE 1: Adenylate cyclase activity in control and *cis*-vaccenic acid treated membranes at (A) 37 °C and (B) 20 °C. The *cis*-vaccenic acid treatment and the assays were performed as described under Experimental Procedures, with 0.1 mM (−)-isoproterenol plus 0.1 mM Gpp(NH)p or 10 mM NaF as activators. Note that different scales were used for panels A and B. The values are the means of duplicate determinations, which agreed within 5%, in a representative experiment. The experiment was repeated six times with comparable results.

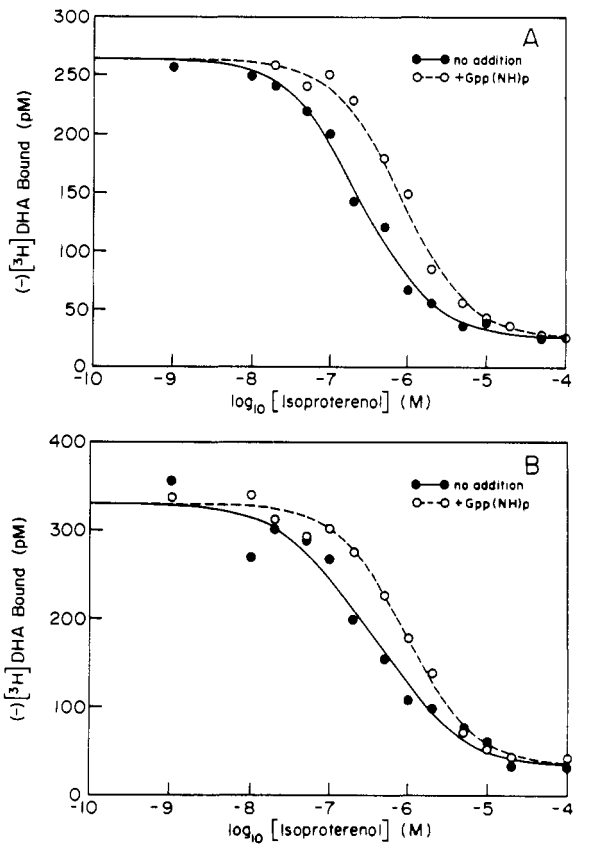

FIGURE 2: [^{3}H]DHA competition curves for (−)-isoproterenol and (−)-isoproterenol plus 0.1 mM Gpp(NH)p at 37 °C in (A) control and (B) *cis*-vaccenic acid treated membranes. The data points are the means of triplicate determinations in a representative experiment. The experiments were repeated six (A) or three (B) times with comparable results. The K_D for DHA (5 nM) was identical in control and treated membranes. The lines through the data points are the "best fits" obtained by the computer modeling procedures. The parameter estimates are summarized in Table I.

interactions among the β-adrenergic receptor, the nucleotide regulatory protein, and the adenylate cyclase catalytic unit. Binding of the antagonist (−)-[^{3}H]DHA to turkey erythrocyte β-adrenergic receptors and (−)-isoproterenol competition curves have been described previously. Typical (−)-isoproterenol competition curves at 37 °C in the absence and presence of 0.1 mM Gpp(NH)p are shown in Figure 2A. These experiments were analyzed with a nonlinear least-squares curve-fitting procedure (as described under Experimental Procedures) and the parameters summarized in Table I. The competition curves for (−)-isoproterenol were shallow (slope factor < 1) and best described by a model with two distinct states of receptor affinity for the agonist. The dissociation constants were $K_H = 4.7 \times 10^{-8}$ M and $K_L = 4.0 \times 10^{-7}$ M, with ~55% of the receptors in the higher affinity state. In contrast, the agonist competition curves in the presence of Gpp(NH)p were steeper (slope factor ≅ 1), shifted to the right, and best explained by a single affinity state with a dissociation constant of 4.0×10^{-7} M. The affinity of the receptor for (−)-isoproterenol in the presence of Gpp(NH)p was virtually identical with the lower affinity (K_L) obtained for (−)-isoproterenol in the absence of Gpp(NH)p. Essentially the same curves were observed when *cis*-vaccenic acid treated membranes were used (Figure 2B). These results are very similar

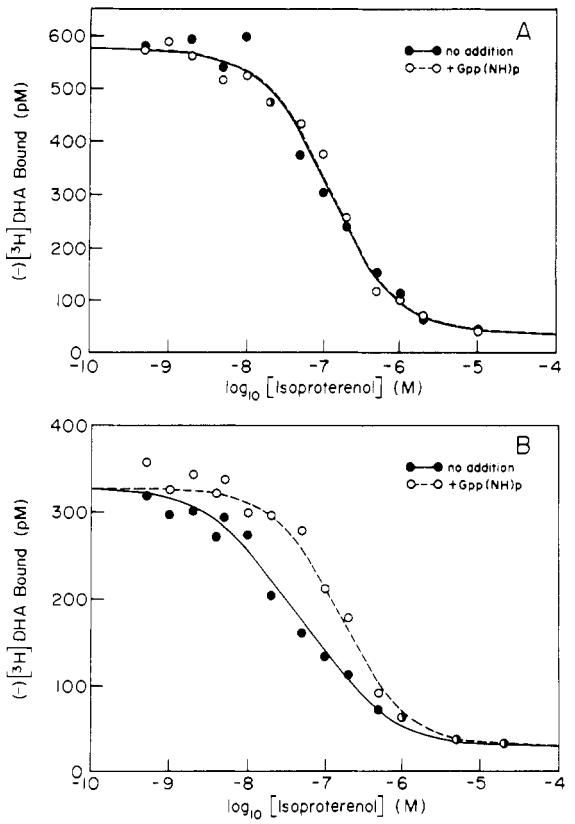

FIGURE 3: [^{3}H]DHA competition curves for (−)-isoproterenol and (−)-isoproterenol plus 0.1 mM Gpp(NH)p at 20 °C in (A) control and (B) *cis*-vaccenic acid treated membranes. The data points are the means of triplicate determinations in a representative experiment. The experiments were repeated seven times with comparable results. The K_D for DHA at 20 °C (5 nM) was identical in control and treated membranes. The lines through the data points are the "best fits" obtained by computer modeling procedures. The parameter estimates are summarized in Table I.

to those reported by Stadel et al. (1980).

Agonist binding to the receptor was significantly altered at 20 °C, as shown in Figure 3. The competition curves obtained with untreated membranes (Figure 3A) in the presence and absence of Gpp(NH)p were virtually superimposable. These steep curves modeled to one affinity state with a K_D for (−)-isoproterenol in the presence or absence of Gpp(NH)p of 3.2×10^{-8} M. Thus the receptor exhibits no nucleotide-induced shift in affinity at 20 °C as it does at 37 °C (cf. Figure 2A). The receptor concentration and the affinity of the β-adrenergic receptor for antagonists such as [^{125}I]HYP (Hanski et al., 1979; Weiland et al., 1979) or [^{3}H]DHA were not significantly different at 37 and 20 °C (Pike & Lefkowitz, 1978; Insel & Sanda, 1978; M. M. Briggs and R. J. Lefkowitz, unpublished experiments). However, receptor affinity for (−)-isoproterenol plus Gpp(NH)p was found to be ~10-fold higher at 20 °C than at 37 °C. This observation is consistent with previous reports (Pike & Lefkowitz, 1978; Insel & Sanda, 1979; Weiland et al., 1979) that β-adrenergic receptor affinity for agonists increased as the assay temperature was lowered (see Discussion).

Figure 3B shows that *cis*-vaccenic acid treatment of turkey erythrocyte membranes caused an apparent reversion of agonist binding characteristics to those observed at 37 °C. The competition curves in Figure 3B closely resemble those obtained at 37 °C, in that the (−)-isoproterenol curve was again shallow (slope factor < 1) and was shifted to the left compared to the curves obtained with (−)-isoproterenol plus Gpp(NH)p. It was best fit by two receptor states with $K_H = 4.0 \times 10^{-9}$ M and $K_L = 4.4 \times 10^{-8}$ M, with ~48% of the receptors in the higher affinity state. The competition curve in the presence of Gpp(NH)p was steep (slope factor ≅ 1) with a K_D corresponding to that of the lower affinity state (K_L) in the curves with (−)-isoproterenol alone (Figure 3B) and to the K_D observed in both competition curves obtained with untreated membranes at 20 °C (Figure 3A). The virtual identity of the K_L observed in the *cis*-vaccenic acid membranes with the single K_D observed in the absence of *cis*-vaccenic acid at 20 °C suggests that the receptor can form the nucleotide-sensitive high-affinity state of binding at 20 °C only in the presence of *cis*-vaccenic acid.

Discussion

Stimulation of the turkey erythrocyte adenylate cyclase by β-adrenergic agonists such as (−)-isoproterenol is severely impaired at low temperatures (Orly & Schramm, 1975). This effect cannot be attributed solely to inhibition of the adenylate cyclase catalytic unit itself because the decrease in basal and NaF activities is much less profound than the decrease in agonist stimulation. Treatment of turkey erythrocyte membranes with *cis*-vaccenic acid reverses the low-temperature inhibition of agonist stimulation of adenylate cyclase activity, whereas basal and NaF activities are increased only slightly. Because no change in receptor number or affinity for antagonists is observed at the lower temperature, the cold-induced inhibition appears to involve one or more of the steps in the coupling of agonist occupancy of the receptor to the activation of adenylate cyclase.

The decrease in adenylate cyclase activation by the agonist at 20 °C can be correlated with a significant temperature-dependent alteration in agonist binding properties. (−)-Isoproterenol competition for [^{3}H]DHA binding to the β-adrenergic receptor at 37 °C was analyzed with a nonlinear least-squares curve-fitting procedure (Kent et al., 1980; Munson & Rodbard, 1979). The data are best explained by a model in which the receptor can have a high-affinity state of agonist binding which is converted to lower affinity by guanine nucleotides. This high-affinity nucleotide-regulated agonist binding state has been reported previously (Kent et al., 1980; Williams & Lefkowitz, 1977) and shown to be a requisite step in the coupling of the β-adrenergic receptor to the activation of adenylate cyclase (Stadel et al., 1980). When the binding studies were conducted at 20 °C, however, only one affinity state for (−)-isoproterenol was observed and there was no regulation of agonist affinity by Gpp(NH)p (Figure 3A), indicating that no detectable amount of the high-affinity nucleotide-sensitive receptor state is formed at 20 °C. Presumably a very small amount of the high-affinity state must be formed at 20 °C, because very low (−)-isoproterenol-stimulated adenylate cyclase activity is observed at that temperature. However, the amount of high-affinity agonist·receptor complex is probably so small that it is not detectable with our binding and analytic techniques. It is interesting to note that the receptor affinity for (−)-isoproterenol in the presence of Gpp(NH)p was ~10-fold higher at 20 °C than at 37 °C (Table I). This observation is in accord with several previous reports of temperature-dependent changes in β-adrenergic receptor affinity for agonists (Pike & Lefkowitz, 1978; Insel & Sanda, 1978; M. M. Briggs and R. J. Lefkowitz, unpublished experiments), but the explanation for this phenomenon on a molecular level remains unclear. It is unlikely that this effect is related to our observation that the formation of the nucleotide-sensitive agonist·receptor complex is inhibited at 20 °C. The finding that the competition curves at 20 °C in the presence of *cis*-vaccenic acid resemble those at 37 °C, but

are shifted farther to the left, indicates that two independent effects are being observed.

Further evidence that the formation of the high-affinity agonist state of the receptor is inhibited at 20 °C is provided by the competition curves obtained with *cis*-vaccenic acid treated membranes at 20 °C. Under these conditions, the (−)-isoproterenol plus Gpp(NH)p competition curve (Figure 3A) was virtually identical with the curves in the absence of *cis*-vaccenic acid (Figure 3B), but the (−)-isoproterenol curve in the absence of Gpp(NH)p was shifted to the left of the other curves and exhibited two states of receptor affinity. Gpp(NH)p shifted the receptor population to a single low-affinity state with a K_D virtually identical with both the K_L observed in the (−)-isoproterenol curve in the absence of Gpp(NH)p and the K_L observed at 20 °C in the absence of *cis*-vaccenic acid (Table I). Thus at 20 °C *cis*-vaccenic acid appears to facilitate the formation of the high-affinity state of the receptor previously shown to be required as an intermediate for stimulation of adenylate cyclase (Stadel et al., 1980). These observations further suggest that the inability of the β-adrenergic receptor to form this high-affinity guanine nucleotide regulated complex at 20 °C is a major factor in the low-temperature inhibition of catecholamine stimulation of adenylate cyclase in turkey erythrocyte membranes.

Nucleotide regulation of receptor affinity for agonists appears to be a general property shared by a number of hormone receptors coupled to adenylate cyclase, including the β-adrenergic receptors from a variety of sources (Williams & Lefkowitz, 1977; Stadel et al., 1980; Ross et al., 1977; Hegstrand et al., 1979, Lucas & Bockaert, 1977). There are several indications that an interaction between the nucleotide regulatory component and the β-adrenergic receptor may be involved in the regulation. These include the following: (1) An agonist-specific increase in the apparent Stokes radius of the β-adrenergic receptor was observed in solubilized preparations derived from frog erythrocytes and rat reticulocytes (Limbird & Lefkowitz, 1978; Limbird et al., 1980). The increase was prevented when the receptor was incubated with the agonist in the presence of guanine nucleotides. (2) The larger molecular weight form of the receptor coelutes on gel filtration columns with a 42 000-M_r protein which is ADP-ribosylated by cholera toxin and thus appears to be the guanine nucleotide regulatory protein of the adenylate cyclase system (Limbird et al., 1980). (3) As determined by computer modeling of agonist affinity data, a ternary complex model involving the interaction of agonist, receptor, and a third molecular component is the simplest model which can accurately explain competition curves in frog and turkey erythrocyte membranes (Kent et al., 1980; De Lean et al., 1980).

In this context the present data suggest that in turkey erythrocyte membranes lowered temperatures may impair the actual physical interaction between the β-adrenergic receptor and the nucleotide regulatory protein. This is consistent with the suggestion by Orly & Schramm (1975) that the temperature-sensitive event involves guanine nucleotide regulation of enzyme activity and provides a more precise localization of a major limiting step in the activation of adenylate cyclase. There are several possible mechanisms which could account for limited interaction at low temperatures. It has been well documented that lowering the temperature of membrane and model phospholipid systems decreases the fluidity of the membrane phospholipids and apparently slows the lateral diffusion of integral membrane proteins (Cherry, 1976; Edidin, 1974; Poo & Cone, 1974). Lateral diffusion within the plane of the membrane may be necessary for the interaction between the receptor and the nucleotide regulatory component to occur and is probably extremely slow at 20 °C. This explanation would be consistent with the observations that *cis*-vaccenic acid increases the fluidity of the membrane (Rimon et al., 1978), allows agonist stimulation of adenylate cyclase to occur, and leads to a reappearance of the characteristic high-affinity nucleotide-sensitive state of the receptor. It is conceivable that these effects do not extend over the entire membrane but rather may be limited to distinct membrane domains in which coupling may be regulated by more localized changes in membrane environment. Another possibility is that the formation of high-affinity agonist·receptor binding requires conformational changes in either the receptor or the nucleotide regulatory protein, and such changes in structure may be restricted at lower temperatures (Kimelberg, 1977).

The decrease in adenylate cyclase stimulation by the agonist at 20 °C has been correlated with the inability of the β-adrenergic receptor to form the high-affinity nucleotide-sensitive intermediate with the agonist. The addition of *cis*-vaccenic acid to the membrane preparation reversed the cold-induced inhibition, allowing the high-affinity complex to be formed and adenylate cyclase to be stimulated by the agonist. These observations indicate that an appropriate membrane environment is required for the formation of this high-affinity agonist·receptor complex and that the inability of the β-adrenergic receptor to form this complex at 20 °C is a major factor in the low-temperature inhibition of catecholamine stimulation of adenylate cyclase in turkey erythrocyte membranes.

Acknowledgments

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High Mobility Group Chromosomal Proteins Isolated from Nuclei and Cytosol of Cultured Hepatoma Cells Are Similar†

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ABSTRACT: Using sequential chromatography on columns containing immobilized double-stranded DNA and single-stranded DNA, we have purified a protein from the cytosol of an established line of cultured rat hepatoma cells that, by several criteria, is a high mobility group (HMG) protein. Analyses of DNA binding properties, electrophoretic mobilities, amino acid compositions, and immunochemical reactivities reveal that the cytosolic protein is the same protein as HMG-1 isolated from the purified chromatin of the same cell line. Thus, authentic HMG-1 appears to be at least partially responsible for the cytoplasmic fluorescence observed when mammalian cells are stained with fluorescent-labeled, affinity-purified antibodies against HMG-1 [Bustin, M., & Neihart, N. K. (1979) *Cell 16*, 181-189]. We suggest that HMG-1 can shuttle between nucleus and cytoplasm, perhaps in response to the nucleus' need for helix destabilizing proteins.

The high mobility group (HMG)[1] proteins are a class of nonhistone chromatin proteins that can be released from chromatin with 0.35 M NaCl and that are soluble in 2% trichloroacetic acid (Goodwin et al., 1973). The term HMG, which was first applied to proteins from calf thymus, refers to the high mobility that the proteins exhibit in an acid-urea gel electrophoresis system (Goodwin et al., 1973). The calf thymus HMG proteins have very distinctive amino acid compositions with high contents of both acidic and basic amino acid residues (Johns et al., 1975). Proteins that are similar in physical, chemical, or immunochemical properties to the calf thymus HMG proteins have been found in phylogenetically diverse organisms (Watson et al., 1977; Sterner et al., 1978; Spiker et al., 1978; Romani et al., 1979).

The HMG proteins were first isolated from chromatin, and they have therefore been thought of as nuclear components. Recently, however, Bustin & Neihart (1979) presented evidence that HMG-1 or proteins immunologically cross-reactive with HMG-1 occur in the cytoplasm of several types of cultured mammalian cells as well as in the nuclei of the same cells. That evidence was obtained by microscopic observations of

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[1] Abbreviations used: HMG, high-mobility group; HTC cells, hepatoma tissue culture cells.

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