Influence of the Mouse Major Histocompatibility Complex, *H-2*, on Liver Adenylate Cyclase Activity and on Glucagon Binding to Liver Cell Membranes†

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ABSTRACT: The major histocompatibility complex of mice, the *H-2* complex, regulates the steady-state level of adenosine cyclic 3′,5′-monophosphate (cAMP) in liver. This effect of *H-2* may be due to an effect on hormone binding to receptors. Here we show that liver membranes from animals of different *H-2* types differ in their sensitivity to glucagon stimulation of adenylate cyclase and in the affinity of their receptors for glucagon. No *H-2*-associated differences are seen in basal, NaF-stimulated, or GMP-PNP-stimulated adenylate cyclase.

A single complex genetic locus, the major histocompatibility complex (MHC[1]), regulates many cellular immune responses in vertebrate [review in Götze (1977)]. It is thought that the MHC serves as a master controlling region for the immune system, regulating the association of antigens, soluble or cellular, with cell surfaces [see a recent brief review by Klein (1979)].

In mice, the MHC, *H-2*, may be readily transferred from one genetic background to another by breeding and selection (Snell, 1948; Klein, 1975). This results in a series of inbred strains, congenic strains, differing from one another only in the portion of chromosome 17 which includes *H-2*. These strains allow examination of possible *H-2* regulation of many physiological and biochemical functions. Comparison of functions in congenic strains of different *H-2* types can, under uniform environmental conditions, give a strong indication of linkage of particular traits with *H-2*. These observations may be followed with breeding experiments and the use of antibodies to products of the MHC to alter or modify the function under study.

In humans a statistical approach is used to detect association of the MHC, *HLA*, and particular physiologic functions or diseases. The *HLA* types of patients with particular diseases are determined, and their incidence is compared to that of a control population. An excess of particular *HLA* types suggests a functional association between *HLA* and disease [review by Bias & Chase (1977)].

These two approaches have shown associations between the MHC and a number of physiological and biochemical functions which do not appear to be part of the immune system. The entire range of traits affected by *H-2* is summarized by McKenzie (1977).

All MHC regulation, including that of immune responses, may be via effects of MHC products on recognition at the cell surface, whether recognition of ligands (including antigens and hormones) or recognition of other cells. General models of MHC-linked regulation have been discussed by McKenzie (1977), Snell (1979), and Meruelo & Edidin (1979). McKenzie and Snell suggest that MHC products are themselves receptors or recognition units for a wide range of functions, while Meruelo & Edidin consider MHC products as modifiers of specific cell surface receptors. Another, more limited model suggests that *HLA* antigens function as peptide hormone receptors (Svejgard & Ryder, 1976).

Study of the regulation by MHC of recognition at the cell surface is best done at the cellular or biochemical level with experimental systems that can be readily quantitated. Some years ago our laboratory found an association between the level of liver cAMP and *H-2* type. The differences observed were no more than twofold, but they were consistent and segregated as a unit factor with *H-2* in crosses (Meruelo & Edidin, 1975). We felt that cAMP levels should reflect the average of the effects of many hormones binding to liver parenchymal cells. Though the association of cAMP levels with *H-2* could also be due to *H-2* effects on othe steps in cAMP metabolism, for example, on nucleotide cyclic phosphodiesterase activity, it appeared that kinetic studies could define the point in cAMP metabolism regulated by *H-2*. Preliminary results indicated that basal and fluoride-stimulated adenylate cyclase activities were similar in mice of all *H-2* types tested, while glucagon-stimulated adenylate cyclase was highest in animals with high steady-state levels of cAMP and lowest in animals with low levels of cAMP. There were no significant differences in nucleotide cyclic phosphodiesterase activities when *H-2* congenic strains were compared (Meruelo, 1974; Lafuse & Edidin, 1978).

In this paper we confirm the initial observations on association of *H-2* haplotype with glucagon-stimulated adenylate cyclase activity. We further show that the differences in glucagon-stimulated adenylate cyclase correlate with differences in the affinity of receptors for glucagon and to differences in the manner in which these receptors interact in the plasma membrane.

Materials and Methods

Breeding stock of *H-2* recombinant congenic strains B10.A (2R), B10.A (4R), B10.A (5R), and C3H.OH was generously supplied by Dr. Donald Shreffler and bred in our animal house. The remaining strains used in this study (obtained from the

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[1] Abbreviations used: MHC, major histocompatibility complex; cAMP, adenosine cyclic 3′,5′-monophosphate; GMP-PNP, 5′-guanylyl imidophosphate; ATP, adenosine triphosphate; Ia antigens, immune response associated antigens.

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Jackson Laboratory, Bar Harbor, ME) represent two groups of congenic strains, one with different *H-2* types inserted in the C57BL/10 genetic background and the other with different *H-2* types inserted in the C3H genetic background.

ATP, cAMP, 2-phosphoenolpyruvate (PEP), nucleotide cyclic 3′,5′-phosphodiesterase, theophylline, and glucagon were obtained from Sigma. Pyruvate kinase was obtained from Sigma as a crystalline suspension in 2.2 M $(NH_4)_2SO_4$. The suspension was centrifuged at 10000*g* for 10 min, and the pellet was diluted in 0.01 M Tris-HCl, pH 7.5, with 10 mM KCl to a specific activity of 400 units/mL. 5′-Guanylyl imidophosphate (GMP-PNP) was purchased from International Chemical and Nuclear Corp. [^{3}H]cAMP (24–30 Ci/mmol) and monoiodinated [^{125}I]glucagon (100–200 μCi/μg) were obtained from New England Nuclear Corp.

Preparation of Crude Liver Membranes. Livers from 11–15-week-old mice were homogenized in 1 mM $NaHCO_3$ buffer, pH 7.5, with a Dounce homogenizer (loose pestle). The homogenate was filtered through two layers and then through four layers of cheesecloth and centrifuged at 1200*g* for 15 min. The pellet was resuspended in $NaHCO_3$ buffer, homogenized a second time with the Dounce homogenizer (loose pestle), and recentrifuged. The pellet was resuspended with $NaHCO_3$ buffer, divided into aliquots, and stored at −70 °C. Membrane protein was estimated by the method of Lowry et al. (1951).

Adenylate Cyclase Assay. The adenylate cyclase assay medium was 25 mM Tris-HCl, pH 7.5, 2 mM ATP, 5 mM $MgCl_2$, 1 mM theopylline, 5 mM PEP, and 4 units of pyruvate kinase. The reaction was initiated by the addition of 20 μL of a membrane suspension, containing 25–100 μg of membrane protein, to 80 μL of assay medium. After incubation at 30 °C for 10 min, the reaction was stopped by the addition of 0.40 mL of 62.5 mM sodium acetate buffer, pH 4.0. Aliquots of 20, 40, 60, 80, and 100 μL of the mixture were assayed for cAMP content by the Gilman binding protein assay (Gilman, 1970). In our assay, ATP and $MgCl_2$ were at saturating concentrations and adenylate cyclase activity was linear throughout the 10-min incubation. Treatment of samples with nucleotide cyclic 3′,5′-phosphodiesterase for 30 min before assaying removed more than 99% of the cAMP detected in the binding assay.

Glucagon Binding Assay. The assay mixture was 25 mM Tris-HCl, pH 7.5, 1% BSA, 1.25 nM [^{125}I]glucagon, and 85–90 μg of membrane protein in a total volume of 100 μL. In competition experiments, varying amounts (0–60 pm) of unlabeled glucagon from freshly prepared stock solutions were included in the assay. After 20 min of incubation at 30 °C, the reaction was stopped by the addition of 1 mL of 25 mM Tris-HCl, pH 7.5, followed by immediate filtration through Millipore EGWP filters previously soaked at least 1 h in the same buffer containing 0.1% BSA and then washed with 25 mM Tris-HCl buffer, pH 7.5. After filtration the filters were washed again with the same buffer and counted in a Beckman γ counter. Nonspecific binding in the presence of excess unlabled glucagon (5×10^{-6} M) was 5–10%; it was subtracted from total glucagon binding to give specific glucagon binding.

Scatchard plots (Scatchard, 1949) were obtained by competitive displacement of [^{125}I]glucagon with 0.50–500 nM unlabeled hormone. Affinity constants were estimated from curvilinear Scatchard plots by the curve-fitting method described by Kahn et al. (1974). The glucagon low-affinity constant was obtained from the slope of a linear least-squares fit of data from glucagon concentrations of 100–400 nM. The intercept of this line on the *x* axis is the total number of glucagon binding sites. The contribution of the low-affinity sites was then subtracted from points obtained with glucagon concentrations less than 100 nM. The resulting points were fitted by the method of least squares. The high-affinity constant was obtained from the slope of this fitted line. The intercept on the bound axis is the concentration of high-affinity sites. The concentration of low-affinity sites was obtained by subtracting the concentration of high-affinity sites from the total concentration of glucagon binding sites.

Table I: cAMP Levels and Basal Adenylate Cyclase Activity in Liver of Various Congenic Mouse Strains[a]

strain	*H-2*	pmol of cAMP per mg of liver (wet weight ± SD)[b]	basal adenylate cyclase act. (pmol of cAMP per min per mg of membrane protein)[c]
B10.A	*a*	1.49 ± 0.31	9.67 ± 4.38
B10	*b*	1.29 ± 0.18	9.77 ± 1.60
C3H.SW	*b*	1.25 ± 0.37	11.00 ± 0.87
B10.D2	*d*	1.27 ± 0.25	10.2 ± 2.12
B10.Br	*k*	0.95 ± 0.20	11.2 ± 1.94
C3H/HeSn	*k*	0.99 ± 0.28	11.2 ± 2.57

[a] Statistical analysis: an analysis of variance at the 5% confidence level indicated no significant differences in basal adenylate cyclase activity with a computed *F* value of 0.378. [b] Lafuse et al. (1979). [c] Mean plus or minus standard deviation.

Dissociation of [^{125}I]Glucagon. Negative cooperative site–site interactions among glucagon receptors were assessed by a kinetic method (Demeyts et al., 1973, 1976). Low concentrations of [^{125}I]glucagon were bound to liver membranes such that only a small percentage of the receptor sites were occupied. Dissociation of the [^{125}I]glucagon was effected by diluting 100-fold with buffer or buffer containing excess hormone.

In these experiments, 0.950 nM [^{125}I]glucagon was incubated with membranes at 30 °C for 20 min. Duplicate 50-μL aliquots containing 88 μg of membrane protein were diluted in 5 mL of 25 mM Tris-HCl, pH 7.5, or 25 mM Tris-HCl, pH 7.5, with 500 mM unlabeled glucagon and incubated at 30 °C. At intervals samples were filtered through EGWP filters. The filters were then washed with 5 mL of 25 mM Tris-HCl, pH 7.5, and counted in a Beckman γ counter.

Degradation of Glucagon. Degradation of glucagon by liver plasma membranes was measured in terms of the ability of the glucagon remaining after incubation to bind to fresh membranes. Eighty-eight micrograms of liver plasma membranes was incubated at 30 °C with 1.25 mM [^{125}I]glucagon for 0–60 min. Membranes were sedimented by centrifugation at 1200*g* for 10 min. Intact glucagon in the supernatant was then determined by incubation of the supernatant with 176 μg of fresh liver plasma membranes for 20 min at 30 °C. After incubation the samples were filtered through EGWP filters. The filters were washed with 5 mL of 25 mM Tris-HCl, pH 7.5, and counted.

Results

Adenylate Cyclase Activity. Liver plasma membranes prepared from mouse strains differing at the *H-2* region and in liver cAMP level do not differ significantly in basal adenylate cyclase activity (Table I).

NaF in concentrations ranging from 2 to 15 mM stimulated adenylate cyclase. Concentrations greater than 15 mM inhibited adenylate cyclase. Double-reciprocal plots of NaF stimulation of adenylate cyclase of liver plasma membranes from *H-2* congenic strains showed no differences in the NaF concentrations needed for stimulation nor any differences in the adenylate cyclase activity produced by NaF stimulation. V_{max} ranged from 452 to 500 pmol of cAMP per min per mg

Table II: Kinetic Constants for GMP-PNP or Glucagon Stimulation of Adenylate Cyclase[a]

		GMP-PNP		glucagon	
strain	*H-2*	K_m[b] (nM)	V_{max} [pmol/ (min mg)]	K_m[b] (nm ± SD)	V_{max} [pmol/ (min mg)]
B10.A	*a*	266	109	23.3 ± 2.9	110 ± 20
B10	*b*	252	83	35.7 ± 2.7	164 ± 49
B10.D2	*d*	244	93	35.7 ± 2.7	178 ± 79
B10.Br	*k*	231	97	50.1 ± 7.3	138 ± 40
C3H.SW	*b*	-[c]	-	38.9 ± 0.5	129 ± 1.8
C3H/HeSn	*k*	-	-	50.7 ± 0.9	126 ± 3.5

[a] No difference could be seen between K_m for GMP-PNP stimulation. An analysis of variance at the 1% level of confidence indicated significant differences in K_m for glucagon in the strains shown with a computed F value of 37.52. A Duncan's multiple range test divided the strains into three different subsets: one formed by B10.A and one formed by B10.Br and C3H/HeSn, with a third subset of C3H.SW, B10, and B10.D2 intermediate between these subsets. An analysis of variance at the 5% confidence level comparing the V_{max} of these strains showed no significant differences in V_{max} with an F value of 0.583. [b] Concentration required for half-maximal stimulation. [c] (-) Not done.

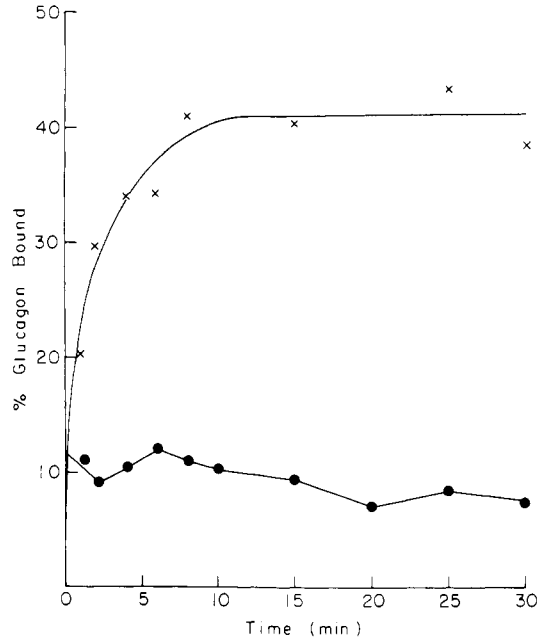

FIGURE 1: Time course of glucagon binding to liver plasma membranes. Total binding (×) and nonspecific binding (●) were plotted as a function of time of incubation. 73.2 μg of B10.A membranes in a volume of 100 μL was incubated with 1.2 nM [^{125}I]glucagon at 30 °C for varying times. Nonspecific binding was determined by including 5 × 10^{-6} M unlabeled glucagon.

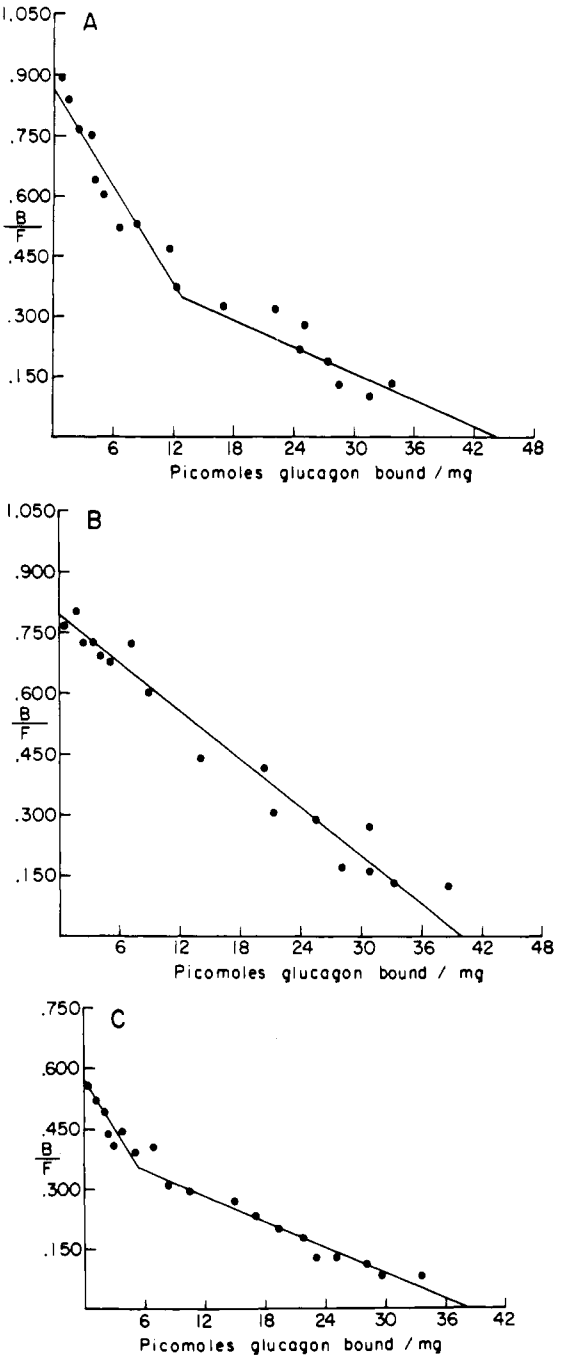

FIGURE 2: Scatchard plots of glucagon binding to liver plasma membranes from *H-2* congenic resistant strains. (A) B10.A; (B) B10.Br; (C) (B10 × B10.Br)F1.

of protein for the *H-2* types listed in Tables I and II.

Recent reports (Londos et al., 1974; Lefkowitz, 1974) have demonstrated that GMP-PNP, a synthetic analogue of GTP which is not hydrolyzed by nucleotide phosphohydrolases, stimulates adenylate cyclase in the absence of hormone. GMP-PNP activation of adenylate cyclase of liver plasma membranes of different *H-2* types gave no significant differences in K_m or V_{max} (Table II).

Significant differences were observed in the concentration of glucagon required for half-maximal stimulation of adenylate cyclase, though not in the V_{max} (Table II). The differences were observed both in Lineweaver–Burk plots and in Eadie–Hofstee plots. Strains with lower K_m were those with higher steady-state cAMP levels (Table I). These results suggest that *H-2* control of liver cAMP content is influenced by the level of hormonal stimulation of adenylate cyclase.

Glucagon Binding to Liver Plasma Membranes. A time course of glucagon binding is shown in Figure 1. Equilibrium is reached within 10 min, and binding is stable for at least 30 min. In all subsequent experiments, incubation was for at least 20 min. Nonspecific binding measured in the presence of excess unlabeled glucagon (5 × 10^{-6} M) was 7–12%. There was no effect of 5 × 10^{-6} M unlabeled insulin on glucagon binding.

Scatchard plots for glucagon binding to membranes of various *H-2* congenic strains were produced by competitive displacement of [^{125}I]glucagon with increasing concentrations of unlabeled glucagon. Scatchard plots for membranes of strains B10.A (*H-2*^{a}), B10.D2 (*H-2*^{d}), and B10 (*H-2*^{b}) cannot be fit by a single straight line; they are curvilinear. The

Table III: Equilibrium Constants for Glucagon Binding to Liver Plasma Membrane[a]

strain	*H-2*	high affinity		low affinity	
		$K \times 10^{-7}$ (M^{-1})	capacity[b]	$K \times 10^{-6}$ (M^{-1})	capacity[b]
B10.Br	*k*	–[c]	–	27.7 ± 0.58	34.3 ± 3.9
C3H/HeSn	*k*	–	–	20.8 ± 1.4	31.0 ± 7.4
B10.A	*a*[d]	4.50 ± 0.30	13.7 ± 2.5	12.5 ± 0.11	32.6 ± 1.1
B10	*b*	4.67 ± 0.19	14.5 ± 5.5	8.49 ± 0.40	32.5 ± 15.4
C3H.SW	*b*	4.14 ± 0.02	12.7 ± 2.6	8.45 ± 0.46	41.2 ± 9.1
C3H.NB	*p*	4.33 ± 0.30	13.7 ± 0.99	8.41 ± 0.69	33.9 ± 4.5
B10.D2	*d*	4.54 ± 0.34	15.1 ± 1.7	5.21 ± 0.89	47.5 ± 6.3
B10.M	*f*	4.67 ± 0.42	12.8 ± 0.35	5.40 ± 0.15	56.6 ± 4.7
(B10.D2 × B10.Br)F1	*d/k*	4.47 ± 0.39	6.32 ± 2.3	8.69 ± 1.5	23.5 ± 7.3
(B10 × B10.Br)F1	*b/k*	4.59 ± 0.33	5.76 ± 3.1	11.5 ± 1.3	25.5 ± 6.7
C3H.OH	*02*[d]	–	–	23.5 ± 7.9	32.0 ± 8.0

[a] Values = mean plus or minus standard deviation of two to four measurements. Statistical analysis: analysis of variance at the 1% confidence level indicated no significant differences in the high-affinity constants ($F = 0.474$) and the capacity of low-affinity binding ($F = 3.45$). Analysis of variance of high-affinity binding capacity showed significant differences ($F = 12.0$). A Duncan's multiple range test revealed that the high-affinity binding capacity is significantly lower in (B10.D2 × B10.Br)F1 and (B10 × B10.Br)F1 mice. Differences were also observed in low-affinity constants ($F = 42.8$). A Duncan's multiple range test divided the strains into four groups: (1) B10.Br and C3H/HeSn; (2) B10.A and (B10 × B10.Br)F1; (3) B10, C3H.SW, C3H.NB, and (B10.D2 × B10.Br)F1; (4) B10.M and B10.D2. [b] Capacity = picomoles of glucagon bound per milligram of membrane protein. [c] (–) No high-affinity site. [d] Recombinant *H-2* type.

Scatchard plot for strain B10.Br (*H-2*^{k}) membranes is linear (parts A and B of Figure 2). Affinity constants and the concentration of binding sites computed from the plot are listed in Table III. C3H/HeSn and B10.Br, *H-2*^{k}, appear to lack a high-affinity glucagon-binding component; their single affinity constants are lower than the high-affinity constants of the other strains, and their total number of binding sites is about equal to the number of low-affinity sites of the other strains (Figure 2, Table III). Membranes from (B10 × B10.Br)F1 and (B10.D2 × B10.Br)F1 mice have approximately half the number of high-affinity binding sites of B10 or B10.D2 (Figure 2C).

The low-affinity constants of the strains also differ significantly from one another. B10.Br (*H-2*^{k}), C3H/HeSn (*H-2*^{k}), and B10.A (*H-2*^{a}) have higher constants than B10.D2 (*H-2*^{d}) and B10.M (*H-2*^{f}). Low-affinity constants of B10 (*H-2*^{b}), C3H.SW (*H-2*^{b}), and C3H.NB (*H-2*^{p}) are intermediate between extreme values. The low-affinity constant of (B10 × B10.Br)F1 mice is intermediate between the low-affinity constants of parental strains B10 and B10.Br. Likewise, the low-affinity constant of (B10.D2 × B10.Br)F1 mice is intermediate between the low-affinity constants of parental strains B10.D2 and B10.Br (Table III).

The differences observed in glucagon binding could be due to *H-2*-dependent differences in the degradation of hormone by these membranes. The degradation of hormone by the membranes was examined by measuring the ability of glucagon remaining in the supernatant after incubation with membranes to bind to fresh membranes. Only 10–15% of the added glucagon was degraded by the liver plasma membranes during the 10 min required for binding to reach equilibrium. There were no strain differences in the degradation of glucagon.

Congenic strains bearing recombinant *H-2* complexes, that is, with different portions of the MHC derived from different parental MHC's, were examined for glucagon binding. At least two genes associated within *H-2* affect liver cAMP levels (Lafuse et al., 1979), and, similarly, two or more genes of the *H-2* complex influence glucagon binding. One gene, influencing high-affinity binding, maps to the *H-2D* region or to the immediate right of this region since C3H.OH, which has the *k* haplotype only in the *H-2D* region, has a linear Scatchard plot with an affinity constant equal to the affinity constant for binding of glucagon to *H-2*^{k} liver plasma membranes (Table III). A second gene located to the left end of the complex affects low-affinity binding since B10.A, B10.A (2R), B10.A (4R), and B10.A (5R) have *k* or *b* haplotype in this region and have higher low-affinity constants than B10.D2, which is *d* in this region (data not shown).

Dissociation of Glucagon from Liver Plasma Membranes. Curvilinear Scatchard plots may be explained by the existence of several classes of binding sites or by negatively cooperative interactions between sites. Demeyts et al. (1973, 1976) proposed that if such site–site interactions occur then, when only a small number of sites are occupied, dissociation by dilution in the presence of excess hormone will be greater than by dilution alone. The dissociation of tracer amounts of bound glucagon from mouse liver plasma membranes is very slow but is almost twice as fast when membrane–hormone complexes are diluted with buffer containing excess glucagon as when they are diluted with buffer only (data not shown). If site–site interactions occur, then the dissociation of bound hormone by dilution should depend upon binding site occupancy, with increased occupancy resulting in increased dissociation. Binding site occupancy was varied by adding unlabeled hormone to tracer amounts of [^{125}I]glucagon and incubating with membranes. Dissociation of these mixtures was compared for dilution with buffer alone vs. dilution with buffer and excess glucagon. Dissociation of bound glucagon by dilution is dependent on binding site occupancy, increasing with increased occupancy (Figure 3).

The dependence of the dissociation of tracer amounts of [^{125}I]glucagon on hormone concentration was examined in several *H-2* congenic strains. Data were plotted as double-reciprocal plots of percent dissociation vs. the concentration of hormone (Figure 4). Native glucagon did not enhance the dissociation of [^{125}I]glucagon from B10.Br membranes; the dissociation by dilution with excess hormone from B10.Br membranes was identical with dissociation by buffer only. This is consistent with the linear Scatchard plot of glucagon binding to B10.Br membranes. Dissociation of labeled glucagon was enhanced by dilution of membranes of B10, B10.A, and B10.D2 strains in buffer. The concentrations of glucagon which result in half-maximal enhancement of dissociation were significantly different in B10, B10.A, and B10.D2 membranes.

Discussion

We have measured the adenylate cyclase activity of and the glucagon binding to liver plasma membranes in an attempt to determine the biochemical basis for differences observed in liver cAMP levels of mice differing at the major histocom-

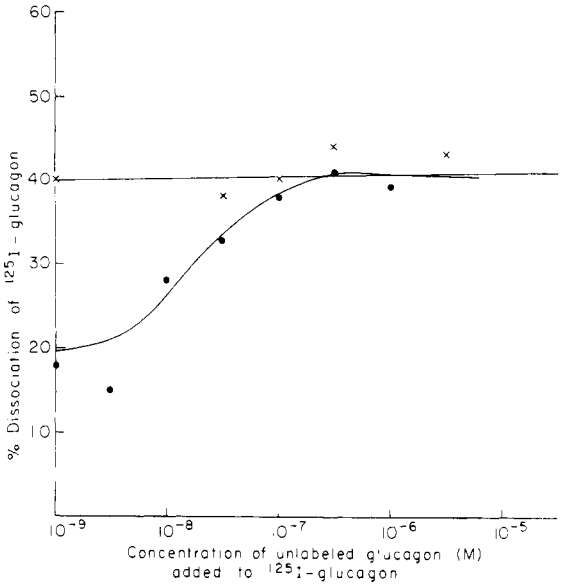

FIGURE 3: Effect of changing binding site occupancy on the dissociation of [^{125}I]glucagon from liver plasma membranes. B10 membranes were incubated with 0.950 nM [^{125}I]glucagon and varying concentrations of unlabeled glucagon for 20 min at 30 °C. Duplicate aliquots containing 86 μg of membrane protein were diluted 100-fold with 25 mM Tris-HCl buffer, pH 7.5 (●), or 25 mM Tris-HCl, pH 7.5, with 5×10^{-6} M glucagon (×) and incubated at 30 °C. After 4 h of incubation, samples were filtered through EGWP filters and counted.

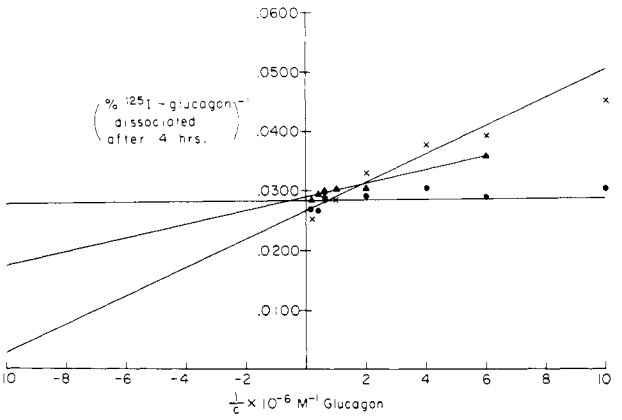

FIGURE 4: Effect of glucagon concentration on dissociation of [^{125}I]glucagon from liver plasma membranes from various *H-2* congenic resistant strains. Data are plotted as double-reciprocal plots of percent dissociation vs. concentration of hormone. B10.A, B10.Br, and B10.D2 membranes were incubated at 30 °C with 0.950 nM [^{125}I]glucagon for 20 min. Aliquots containing 88 μg of membrane protein were diluted 100-fold with varying concentrations of unlabeled glucagon in 25 mM Tris-HCl buffer, pH 7.5, and incubated at 30 °C. After 4 h of incubation, samples were filtered through EGWP filters and counted. B10.A (×); B10.D2 (▲); B10.Br (●).

patibility complex, *H-2*. Adenylate cyclase, basal and GMP-PNP or NaF stimulated, did not vary with *H-2* haplotype. Although the mechanism for NaF and GMP-PNP stimulation is poorly understood, recent studies (Ross & Gilman, 1977; Ross et al., 1978) have shown that several regulatory proteins besides the catalytic component of adenylate cyclase are involved. The failure to find any differences associated with *H-2* haplotype in basal and NaF- or GMP-PNP-stimulated adenylate cyclase suggests that the *H-2* complex does not exert its influence on either the catalytic component of adenylate cyclase or the regulatory proteins involved in NaF and GMP-PNP stimulation.

H-2-associated differences were observed in the ability of glucagon to stimulate adenylate cyclase. These differences correlated well with the level of cAMP in the liver. B10.A ($H\text{-}2^a$), the strain with the highest cAMP levels, reached half-maximal stimulation of adenylate cyclase activity at a significantly lower concentration of glucagon than did B10.Br ($H\text{-}2^K$), the strain with the lowest liver cAMP levels. Strains with haplotypes *b*, *d*, and *f*, which are intermediate in liver cAMP levels, were also intermediate in the K_m for glucagon stimulation.

The differences in glucagon stimulation of adenylate cyclase were paralleled by *H-2*-dependent differences in the binding of glucagon liver plasma membranes of various strains. Differences between strains were found in both the high- and low-affinity components of glucagon binding.

At least two loci within the *H-2* complex affect the binding of glucagon. One locus mapping to the *H-2D* region or the immediate right of this region is defined by the linear Scatchard plot of $H\text{-}2^k$ mice. A second locus is defined by differences in glucagon low-affinity constants; B10.Br ($H\text{-}2^k$), C3H/HeSn ($H\text{-}2^k$), and B10.A ($H\text{-}2^a$) have significantly higher constants than B10.D2 ($H\text{-}2^d$) and B10.M ($H\text{-}2^f$). Affinity constants of B10 ($H\text{-}2^b$), C3H.SW ($H\text{-}2^b$), and C3H.NB ($H\text{-}2^p$) are intermediate between those of these strains. Scatchard plots of liver plasma membranes from *H-2* recombinant mice position this locus to the *H-2K* end (*H-2K* or *I-A* regions) of the *H-2* complex.

The affinity constants for glucagon binding correlate with the *H-2*-associated differences in glucagon stimulation of adenylate cyclase. The affinity of B10.A, B10.D2, and B10 membranes for glucagon is higher than the affinity of B10.Br membranes. The same strains have lower K_m values for glucagon stimulation of adenylate cyclase than B10.Br. Also, the affinity of B10.A membranes is higher than that of B10 and B10.D2. Again, B10.A has a lower K_m for glucagon stimulation of adenylate cyclase than do B10 and B10.D2. In addition, the same *H-2* regions which are reflected in differences in glucagon binding also are reflected in differences in liver cAMP levels.

Recently, Lin et al. (1976) have shown that iodination of the tyrosyl residues of glucagon may alter the binding of glucagon to liver plasma membranes. They found that at pH 7.0 and below iodinated glucagon bound to the receptor with an affinity about threefold higher than that with native glucagon. At pH 8.5, the affinity of the receptor for native glucagon was the same as at pH 7.0. However, the affinity of the iodinated glucagon decreased with increasing pH. They suggest that this difference between native and iodinated glucagon results from increased ionization of the hydroxyl group of tyrosine following iodination ($pK_a = 8.2$). At pH 7.5, at which this study was done, 15% of the iodinated tyrosines are ionized. This and denaturation of carrier glucagon in the labeled preparation may account for the 10–100-fold discrepancy between the K_m for glucagon stimulation of adenylate cyclase and the affinity constants for glucagon binding. It has also been suggested by Taylor (1975) that curvilinear Scatchard plots arise from differences in receptor affinity between native and iodinated hormone. This does not seem to be the case in our experiments. Both curvilinear and linear plots were obtained with the same preparation of [^{125}I]glucagon. The only difference resulted from the *H-2* haplotype of the animal from which the membranes were obtained.

The physical meaning of Scatchard plots for membrane receptor–ligand interactions is ambiguous. Curvilinear plots may reflect (1) two or more classes of receptors with different affinities or (2) negatively cooperative site–site interactions.

These two models cannot be distinguished by steady-state binding experiments, and we have used the formalism of (1) in discussing most of our data.

Demeyts et al. (1973, 1976) used dissociation kinetics to show that insulin binding to receptors in lymphocytes and liver plasma membranes induces site–site interactions consistent with negative cooperativity. Similar dissociation experiments with nerve growth factor (Frazier et al., 1974a,b), thyroid stimulating hormone (Kohn & Winand, 1975), and a β-andrenergic antagonist, [^{3}H]dihydroalprenolol (Limbird et al., 1975; Limbird & Lefkowitz, 1976), suggest that site–site interactions are also involved in the binding of these ligands to their target tissues. Some of our experiments can be interpreted in terms of a site–site interaction model for glucagon receptors.

It is evident from data presented in this paper that genes in the *H-2* complex affect the binding of glucagon to the liver plasma membrane and that differences in the binding of glucagon result in *H-2*-associated differences in the glucagon stimulation of adenylate cyclase. We have also found *H-2*-associated differences in the binding of insulin to liver plasma membranes (Lafuse, 1978). This suggests that *H-2* influence on hormone binding is a general phenomenon and that *H-2*-associated differences may exist in the binding of other hormones. At his stage we can only speculate on how genes mapping in the *H-2* complex influence hormone binding. It is possible that genes coding for hormone receptors are located in the *H-2* gene complex. It is also possible that *H-2* genes act by modifying the interaction of hormone with specific receptors.

While *H-2* could affect steady-state cAMP levels in liver at many points, for example, in determining cell levels of GTP (Rodbell et al., 1974) or calcium-dependent regulatory proteins (Wang et al., 1975), the correlation between in vitro binding of glucagon and stimulation of adenylate cyclase by glucagon and steady-state levels of cAMP leads us to suggest that the *H-2* effect on liver cAMP is primarily due to its modifying effects on peptide hormone binding at the cell surface.

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