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EXCHANGE OF PARTNERS IN GLUCAGON RECEPTOR-ADENYLATE CYCLASE COMPLEXES

PHYSICAL EVIDENCE FOR THE INDEPENDENT, MOBILE RECEPTOR MODEL

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Summary

The apparent target sizes of the glucagon receptor and the catalytic unit of adenylate cyclase in rat liver plasma membranes have been measured by the technique of radiation inactivation in an electron beam. When irradiated in the uncoupled state, the apparent target size for the catalytic unit assayed by fluoride-stimulated activity was 160 000, and for the receptor assayed by specific ^{125}I-labelled glucagon binding was 217 000. The corresponding target size estimated from glucagon-stimulated activity after irradiation in the uncoupled state was 389 000. When the complexes were irradiated in the coupled state in the presence of glucagon, the apparent target sizes from ^{125}I-labelled glucagon binding, and fluoride- or glucagon-stimulated activities had similar values of 310 000, 380 000 and 421 000, respectively. However, if the complexes were allowed to uncouple by removing glucagon after irradiation and activity was then assayed after readdition of glucagon, the apparent target size from the glucagon-stimulated activity increases from 421 000 to 811 000.

The pattern of apparent target sizes obtained under these different conditions has been tested against the pattern predicted for simple models of the coupling mechanism. The only simple model that is consistent with the pattern of target sizes requires the receptors and catalytic units to be present in approximately equal numbers. On binding glucagon, the receptor forms a locking interaction with the catalytic units, so that the complex and its components are inactivated as a single target with an apparent size of about 380 000 (± 15%). After the removal and readdition of glucagon to complexes that were irradiated

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in the coupled state, the new population of complexes must contain hybrids of active and inactive partners obtained by exchange between active and inactivated complexes, to account for the doubling in apparent target size to 811 000 for glucagon-stimulated activity. This hybridization of catalytic units and receptors is the essential feature of the model that distinguishes it from others in which permanently associated complexes of the two components are activated by lateral dimersation on binding glucagon. Simple models of this type are shown to be physically improbable. It is emphasised that the models described are based only on the relationships between the apparent target sizes of components that are defined by their functions, and the apparent target sizes do not necessarily relate solely to the components that can be defined structurally as the receptor or catalytic unit.

Introduction

There is some agreement that adenylate cyclase can be stimulated either through coupling to a hormone receptor (e.g. for glucagon), or by independent action on the catalytic unit (e.g. by fluoride), and it is also generally assumed that the catalytic unit and the receptor are distinct entities (see refs. 1 and 2 for discussion). However, although many models for the coupling mechanism have been suggested, it is clear that they cannot be distinguished with any confidence from the available biochemical evidence. The original model proposed by Sutherland and co-workers [3], postulated a permanent association between the two components in which the catalytic unit is activated by conformational changes originating in the receptor when the hormone is bound. More recently, the realisation that membrane proteins may be able to undergo fast lateral diffusion, has prompted a second type of model in which the uncoupled receptor and the adenylate cyclase moieties can migrate independently in the plane of the membrane. In this model, binding of the hormone to the receptor on the outside of the plasma membrane causes a locking interaction with the catalytic unit facing the cytoplasm which is then activated [2,4,5]. The converse model, in which the receptor and catalytic unit become dissociated on binding hormone has also been suggested [6]. However, the reversible association model of independent components is particularly attractive in membranes where several different types of receptor can activate adenylate cyclase, and their coupling is apparently competitive rather than additive, for example in fat cells [2,7]. This excludes the possibility of a permanent association between each type of receptor molecule and its own catalytic unit, which would presumably be activated independently of other receptors.

We have used the technique of radiation inactivation to determine the apparent target size of the glucagon receptor and adenylate cyclase in rat liver plasma membranes, in the coupled and uncoupled states. This technique suffers from disadvantages rather similar to freeze-fracture electron microscopy in that the samples are frozen and dehydrated by lyophilising before irradiation, and it is assumed that the normal interaction between receptor and the catalytic unit is preserved. However, it provides the only available method for assessing the apparent size of identified proteins in situ, since the functions of the proteins

in the rehydrated membranes are assayed after irradiation at increasing doses.

The method has been used to provide apparent molecular weights for several membrane-bound proteins which are not incompatible with the available biochemical evidence (e.g. $(Na^+ + K^+)$-ATPase [8], mitochondrial Mg^{2+}-ATPase [8], succinate dehydrogenase [9] and acetylcholinesterase [10]). We show that the pattern of apparent target sizes that are obtained under different conditions impose severe restriction on the types of model for the coupling mechanism that are compatible with the data.

Materials and Methods

Membrane preparation and assays. Rat liver plasma membranes were prepared by the method of Pilkis et al. [11] from male Sprague-Dawley rats weighing about 200 g and stored as described previously [12]. Adenylate cyclase was assayed as described previously [12] in the presence of either fluoride ($1.5 \cdot 10^{-2}$ M), or 5'-guanylyl imidodiphosphate (GMP-P(NH)P) at 10^{-4} M, or glucagon (10^{-6} M). Assays were linear for at least 10 min at 30°C.

^{125}I-labelled glucagon was prepared by the method of Giorgio et al. [13]. Specific binding of ^{125}I-labelled glucagon to the receptor was carried out essentially as described by Giorgio et al. [13] and Rodbell et al. [14], in an assay buffer containing 1 mM theophylline, 5 mM $MgSO_4$, 1 mM EDTA, 25 mM triethanolamine · HCl, and 2.5% bovine serum albumin at pH 7.2. Membranes were incubated with ^{125}I-labelled glucagon (10^{-9} M) for 10 min at 36°C before loading 100-μl aliquots onto 250 μl of a washing buffer containing 10% sucrose, 40 mM triethanolamine · HCl, 2.5% bovine serum albumin at pH 7.2. The samples were centrifuged for 5 min in a Beckman microfuge, and the samples were then frozen in solid CO_2/acetone freezing mixture in the centrifuge tubes. The tips of the tubes were cut off and the radioactivity counted. The specific component of the binding was assessed in all experiments by adding 10^{-6} M unlabelled glucagon [14], after checking that this was sufficient to displace the saturable component of ^{125}I-labeled glucagon binding under the conditions used.

GMP-P(NH)P, ATP, triethanolamine · HCl, creatine phosphate, and creatine kinase were obtained from Boehringer Corp.; glucagon, theophylline and neutral alumina type WN3 were obtained from Sigma. Des-his glucagon was a generous gift from Eli Lilly and Co.

Irradiation procedures. The membranes were lyophilised in 1 mM $KHCO_3$, pH 7.4, as aliquots of 250 μl containing 2.5 mg of protein in thin wall glass tubes as described by Levinson and Ellory [10,15]. For irradiation in the coupled state, either glucagon or des-his glucagon were added at 10^{-6} or 10^{-7} M final concentrations and the membranes incubated for 10 min at 4°C before lyophilising.

The samples were irradiated with electrons using the 15 MeV linear accelerator (Addenbrooke's Hospital, Cambridge) under conditions designed to eliminate inactivation due to second-order effects such as free radical formation [10,15]. After irradiation the samples were normally re-hydrated with 1 mM $KHCO_3$, pH 7.2, and assayed at 30°C after 30 min at 4°C. Storage overnight (16 h) at 4°C, or freeze-thawing, had no effect on the activities obtained. Thiol

reagents and sucrose were rigorously eliminated from the membranes throughout these procedures, as they can cause anomalous inactivation effects [8,9]. To remove glucagon or des-his glucagon from pretreated samples, the membranes were washed four times with a buffer containing 2 mM EDTA, 0.05 mM GTP, 1 mM $KHCO_3$, 10 mM triethanolamine · HCl at pH 7.2. Each suspension of the membranes in washing buffer was incubated for 15 h at 4°C before centrifugation for 6 min at 14 000 × g_{av}, and the pellet was finally resuspended in 1 mM $KHCO_3$, pH 7.4.

Estimation of apparent target sizes. An estimate of the apparent molecular target size has been obtained for a number of biological molecules by the application of classical target theory to radiation inactivation data [9,16]. This analysis gives a linear relationship between log(remaining activity) and irradiation dose, the slope of which is proportional to the size of the target molecule. Kepner and Macey [8] simplified this analysis by combining empirically determined values for the energy dispersion of sparsely ionising radiations together with assumed values for the average density of protein. They obtained a relationship between molecular weight and D_{37} (that dose at which 37% of the original activity remains) given by mol.wt. = $6.5 \cdot 10^5/D_{37}$ where D_{37} is in Mrad. More detailed explanations of the theory and empirical justifications of this method are given in Okada [16] and Pollard [17].

Results

(i) Effect of lyophilisation on membrane functions

Lyophilisation had little effect on the specific activity of adenylate cyclase, with more than 90% of the activity recovered after rehydration, irrespective of the stimulating ligand used. The activities obtained after rehydration were also unaffected by heating the lyophilised membranes to 35°C for 5 min. The amount of ^{125}I-labelled glucagon bound specifically to the membranes before and after lyophilisation was 2.0 pmol/mg protein, similar to the value obtained by Rodbell et al. [14].

(ii) Removal of glucagon from pretreated membranes

The washing procedure to remove glucagon or des-his glucagon from pretreated membranes was shown to be effective in that specific ^{125}I-labelled glucagon binding to the washed membranes was very similar to the specific binding to untreated membranes (> 95%). The apparent basal activity of the washed membranes was 1.8 μunits/mg at 30°C, compared with 1.5 μunits/mg for the membranes which had not been pretreated with glucagon, and the pretreated and washed membranes were stimulated 5.5-fold by fluoride, compared with 6.0-fold for the untreated membranes.

(iii) Radiation inactivation

In all of the radiation inactivation experiments, linear, first-order inactivation plots were obtained (see Fig. 1), consistent with inactivation of a target of uniform size by a single hit process [9,19], and we have used the expression of Kepner and Macey [8] given previously to estimate the apparent target sizes.

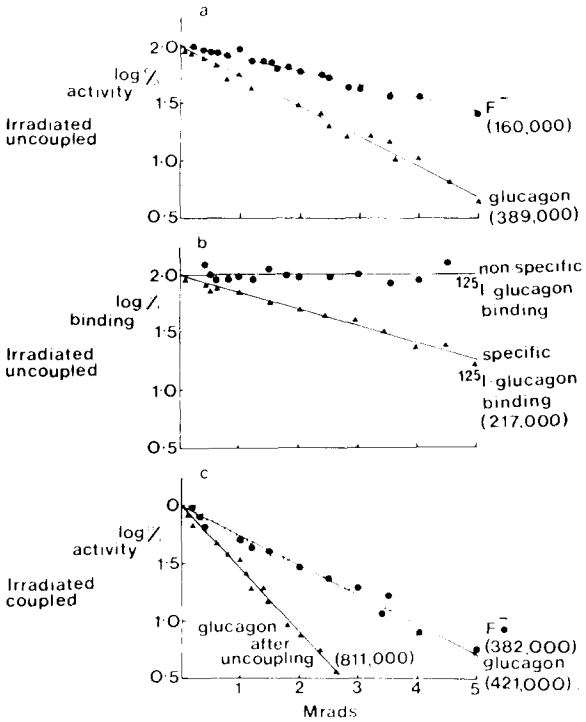

Fig. 1. Inactivation plots of adenylate cyclase activities stimulated by fluoride or glucagon, and of specific ^{125}I-labelled glucagon binding, under different conditions. The apparent target sizes given are the average values obtained from all experiments (see Table I). (a) Adenylate cyclase activities stimulated by fluoride or glucagon after irradiation in the uncoupled state. (b) Specific ^{125}I-labelled glucagon binding to membranes after irradiation in the uncoupled state. The non-specific binding is unaffected by irradiation. (c) Adenylate cyclase activities stimulated by glucagon before and after washing away the glucagon with which the membranes were pretreated for irradiation in the coupled state. The decay in fluoride-stimulated activity after irradiation in the coupled state is also shown (this assay can only be made after washing away the glucagon).

The results obtained were not affected by increasing the irradiation rate from 1 to 2 Mrad/min.

After irradiation in the uncoupled state, both fluoride and GMP-P(NH)P, which activate the enzyme independently of the glucagon receptor, gave an apparent target size of about 160 000, whereas the glucagon-stimulated activity decayed with an apparent target size of 389 000 (Fig. 1a, Table I). The apparent target size for specific ^{125}I-labelled glucagon binding to the uncoupled receptor was 217 000 (Fig. 1b, Table I). After pretreatment with either glucagon or des-his glucagon, the fluoride-stimulated activity decayed with an apparent target size of about 380 000 (Fig. 1c), and the specific ^{125}I binding to the receptor decayed at about 320 000. The glucagon-stimulated activity decayed with an apparent target size of 421 000, provided that the complex remained coupled through the presence of glucagon throughout the assay procedure after irradiation. However, if the glucagon was removed by washing and the adenylate cyclase activity was assayed after readdition of glucagon, the apparent target size increased to 811 000, or 866 000 if des-his glucagon was used in the pretreatment before irradiation (Fig. 1c). It should be emphasised that des-his

TABLE I

APPARENT TARGET SIZE FOR FLUORIDE- AND GLUCAGON-STIMULATED ACTIVITIES OF ADENYLATE CYCLASE AND SPECIFIC ^{125}I-LABELLED GLUCAGON BINDING DETERMINED BY RADIATION INACTIVATION UNDER DIFFERENT CONDITIONS

Values of the target sizes are given with standard deviations and the number of separate radiation inactivation experiments in parentheses. Each value was obtained from a semi-log plot as shown in Fig. 1, in which a minimum of twelve radiation doses were applied in each experiment. Assays in the presence of each ligand or ^{125}I-labelled glucagon were made in triplicate. Target sizes were estimated as described in Materials and Methods.

Assay	State of adenylate cyclase during irradiation			
	Uncoupled	Coupled		
		Pretreated with glucagon, not removed before assay	Pretreated with glucagon, removed before assay	Pretreated with des-his glucagon, removed before assay
Fluoride-stimulated activity	160 000 ± 20 000 (5)	—	382 000 ± 25 000 (6)	381 000 ± 36 000 (4)
Glucagon-stimulated activity	389 000 ± 51 000 (5)	421 000 ± 59 000 (4)	811 000 ± 26 000 (6)	866 000 ± 33 000 (4)
Specific ^{125}I-labelled glucagon binding	217 000 ± 6 000 (2)	—	310 000 ± 26 000 (3)	328 000 ± 40 000 (3)

glucagon caused similar effects to glucagon in all of these experiments when it was used to pretreat the membranes although it is a competitive antagonist of glucagon (see Table I).

As controls for these pretreatment experiments, membranes were irradiated in the uncoupled state without pretreatment with glucagon, and were subsequently treated with 10^{-6} M glucagon after rehydration. The glucagon was washed away by the standard procedure, and the adenylate cyclase activities in the presence of fluoride or glucagon. Linear inactivation plots were obtained giving apparent target sizes of 164 000 for fluoride-stimulated activity and 400 000 for glucagon-stimulated activity. These results do not differ significantly from those obtained for membranes irradiated in the uncoupled state (Table I), demonstrating that the increases in apparent target sizes after irradiation in the coupled state are due to the presence of glucagon or des-his glucagon before lyophylisation and irradiation. In further experiments, membranes were pretreated with 15 mM fluoride before lyophilisation and irradiation. After rehydration, the fluoride was washed away to give a basal activity of 1.6 μunits/mg. The apparent target sizes for fluoride- or glucagon-stimulated activities were unaltered by pretreatment of the membranes with fluoride. It was also found that 5′-nucleotidase activity was unaffected by pretreatment with glucagon. Finally it should be noted that while the apparent target size for the receptor assayed by specific ^{125}I-labelled glucagon binding was increased by pretreatment with glucagon (Table I), the amount of non-specific binding was unaffected by irradiation in the coupled or uncoupled states (Fig. 1b). We con-

clude that the increases in apparent target sizes obtained by pretreatment with glucagon or des-his glucagon can be attributed to the specific action of the ligands acting on the receptors.

Discussion

The pattern of apparent target sizes obtained from the adenylate cyclase activities and glucagon binding under different conditions show some obvious internal consistencies which can be tested against the pattern of target sizes expected for simple models of the coupling mechanism. For the models we have considered, (summarised diagrammatically in Table II). the assumptions required to achieve a consistent fit with the apparent target sizes differ widely in the extent to which they are physically reasonable and compatible with other biochemical evidence. We find that the simplest consistent model requires mobile receptors and catalytic units that are independent in the uncoupled state, and respond to glucagon with a locking interaction that activates the enzyme. More importantly, the results suggest a number of new physical and biochemical experiments which should provide further critical tests of the validity of the model. An extensive rationale in support of the model has been presented recently by De Haen [20].

The key observation on which our model building is based is the 2-fold increase in apparent target size obtained for glucagon-stimulated activity when complexes irradiated in the coupled state have previously been washed free of glucagon. We take this to imply that receptors and catalytic units are able to exchange partners in forming new complexes after the removal and readdition of glucagon. The new population of complexes must contain hybrids of active and inactive partners which lack glucagon-stimulated activity to account for the doubling in apparent target size. This hybridisation is the essential characteristic of an independent receptor in the uncoupled state that distinguishes the model from one in which each receptor directly activates a catalytic unit with which it remains permanently associated. The latter model cannot be accomodated simply within the observed pattern of target sizes. However, a variant in

TABLE II

MODELS CONSIDERED FOR COMPATIBILITY WITH THE PATTERN OF APPARENT TARGET SIZES ($\times 10^{-3}$)

	Model	Uncoupled	Coupled
Independent mobile receptor	(i)	(R) (217) (C) (160)	R C (~380)
Activation of permanently associated complexes by dimersiation	(ii)	(R) (C) (~200)	R R C C (~400)
	(iii)	(R) (217) (C) (160)	R R C C (~760) *

* The apparent target size after irradiation in the coupled state is about 380 000 for glucagon-stimulated activity if the complexes are not allowed to uncouple before assay: the apparent target size increases to 811 000 if the glucagon-stimulated activity is measured after removal and readdition of glucagon (see Table I and text).

which the permanently associated complexes are activated through lateral dimersation after glucagon binding will fit the experimental data. We discriminate against models of this type on the assumption, based on biochemical evidence, that a dimer will not be formed unless both complexes in the dimer have an intact glucagon receptor.

To illustrate the two main classes of simple models more clearly, we set out in detail the assumptions we have made and the requirements they impose. However, it should be emphasised first that the assignments of apparent target sizes to receptor and catalytic units are defined by functional assays of undefined structural entities that are hit as a single target. These structures may correspond to single receptor and catalytic units or to complexes of these two components, but they may also correspond to multi-subunit structures consisting, for example, of a catalytic unit associated with regulatory components binding GTP etc, and we note that the apparent target size obtained from GMP-P-(NH)P-stimulated activity of 160 000, coincides with the apparent size obtained from fluoride-stimulated activity of the uncoupled catalytic unit. The data presented do not allow the composition of the structural entities to be defined, and the models described are based only on the relationships between the apparent target sizes of the functionally defined components. We note that solubilised preparations of adenylate cyclase insensitive to glucagon have a reported molecular weight of 150 000—160 000 by gel filtration or ultracentrifugation [21], and a solubilised protein which binds glucagon specifically has an estimated molecular weight of 190 000—210 000 [13], but these estimates are derived from unpurified proteins that may not relate to the isolated receptor and catalytic units.

(i) The mobile independent receptor model

We assume that when irradiated in the coupled state, the receptor and the catalytic components decay as a single target with an apparent size of about 380 000. If glucagon is not removed before the assay of glucagon-stimulated activity, this is the apparent size of the coupled complex. However, on removal of glucagon, the components can separate and on readdition of glucagon, form complexes with new partners that may be active or inactive. The minimum assumption required is that either inactive receptors can couple to active catalytic units or vice versa. Since a receptor that cannot bind glucagon will presumably be unable to couple to an active catalytic unit, we suppose that to observe the formation of inactive hybrids, an inactive catalytic unit can couple structurally (but not functionally) to an intact receptor in the presence of glucagon. If we assume that out of 100 active receptor-catalytic unit complexes (RC) before irradiation in the presence of glucagon, n complexes are inactivated (rc) as a single target, then the number of active hybrids that will be reformed after removal and readdition of glucagon will be $(100-n)^2/100$ assuming that active receptors (R) can combine with active or inactive catalytic units (C, c) with equal probability, and that all R form new complexes RC or Rc. This gives an increase in apparent target size of 2-fold, or 760 000 compared with an observed value of 811 000.

For this model we therefore assign apparent target sizes to the receptor and catalytic unit of 217 000 and 160 000, respectively, forming a coupled com-

plex of about 380 000. The apparent size of 389 000 obtained from glucagon-stimulated activity after irradiation in the uncoupled state can be accounted for consistently by again assuming that the inactive catalytic unit can couple to an active receptor. Previous irradiation studies provide some support for the possibility that "binding interfaces" may survive when catalytic activity has been lost [9]. For example the apparent target size for invertase coincided with independent estimates of the molecular weight. However, a much smaller target (~ 14%) was obtained using the binding of a purified antibody to the enzyme to assay the integrity of the protein, and other enzymes show similar behaviour when antibody binding is compared with catalytic activity; amino acid analysis; or solubility. This may provide an analogy for the interaction of inactive cyclase units with active receptors, and the requirement is susceptible to independent biochemical analysis. We also note that the very similar pattern of target sizes obtained after irradiation in the presence of des-his glucagon imply that a structural interaction of the catalytic unit with the receptor does not require the catalytic unit to be in the activated conformation, and at least allows the possibility that catalytically inactive units may couple structurally to the receptor.

The increase in apparent target size from 160 000 to 381 000 for fluoride-stimulated activity after irradiation in the coupled state coincides approximately with the target size for the corresponding glucagon-stimulated activity of 421 000, and suggests that the receptor and catalytic unit are inactivated as a single entity in the coupled state, so that a hit in either component leads to inactivation of both. The only result that does not fit closely with this model is the apparent target size for the receptor of 310 000 after irradiation in the coupled state, which is low compared with 382 000 or 421 000 obtained from fluoride- or glucagon-stimulated activities in the same samples. This could be accounted for by a small proportion of about 30% of excess receptors over catalytic units, which would lead to a decrease in the apparent target size of the receptor in the coupled state. The inactivation plot for specific ^{125}I-labelled glucagon binding for a mixture of 70% of coupled receptors with an apparent target size of 421 000 and 30% of uncoupled receptors of 217 000 would not deviate significantly from linearity and would fit the observed target size.

However, for this model, a significant excess of independent receptors would not be consistent with the evidence that in the absence of GTP, the glucagon-stimulated activity is directly proportional to the receptor occupancy (to be published). We defer further analysis of this problem until the assay of apparent target size of the glucagon receptors has been repeated using tritiated glucagon which retains full biological activity, in the presence and absence of GTP. It is an unequivocal requirement of the present model that the apparent target sizes obtained from glucagon- and fluoride-stimulated activities and specific glucagon binding to the receptor should not differ significantly in the coupled state, under conditions where adenylate cyclase activity is directly proportional to receptor occupancy.

In summary, the independent mobile receptor model requires that the receptors and catalytic units are present in approximately stoicheiometric proportions and that inactive catalytic units can couple with functional receptors in the presence of glucagon. We now consider several alternative models based on

lateral dimerisation of permanently associated receptor-catalytic unit complexes.

(ii) The receptor-catalytic unit complex has a total target size of about 200 000 in the uncoupled state, and is activated by dimerisation

If we assume that the apparent size of the uncoupled receptor (217 000) and the uncoupled catalytic unit estimated from fluoride-stimulated activity (160 000) represent the inactivation of a single target with an apparent size of about 200 000, then in order to fit the overall pattern of apparent target sizes we have to suppose that glucagon causes the monomeric complexes to dimerise. The complex formed by dimerisation is also inactivated as a single target in the coupled state with an apparent size of about 400 000, which increases to about 800 000 by hybridisation of the dimers after the removal and readdition of glucagon. However, for this model we have to assume that an inactive monomeric complex (rc), which lacks both a glucagon binding site and an active catalytic unit, can nevertheless form a dimer with an active monomer/(RC). This association, to form an inactive dimer/(RCrc) seems very unlikely since the normal association to form an active dimer would require glucagon binding to both monomers, from the biochemical evidence that saturation of the receptors is required to achieve full glucagon activation. For this reason we regard this model, and others that require the same assumption, as physically improbable.

(iii) The receptor-catalytic complex has a total target size of about 400 000 and is activated by dimerisation

In this model we assume that the receptor and catalytic units remain permanently associated, but are inactivated as independent targets in the uncoupled state and as a single target with an apparent size of about 400 000 in the coupled state. Such a model also requires dimerisation of the monomeric complexes, since hybridisation of the monomers from the coupled complexes is required to account for the 2-fold increase in apparent target size after removal and readdition of glucagon. However, if we assume that inactive hybrid dimers can be formed from an active and inactive monomer, we find that the calculated target size for glucagon-stimulated activity after irradiation in the uncoupled state is significantly larger than the observed target size. If we consider one-hundred monomeric complexes RC that can form fifty active dimers RCRC when saturated with glucagon, then since the apparent target sizes of the uncoupled receptor and catalytic units are similar ($\sim$ 200 000), after irradiation in the uncoupled state will be nr and nc inactive components radomly distributed in Rc, rC and rc monomers. For reasons already discussed we assume that the only inactive monomers that could form inactive hybrid dimers are the Rc species which have an intact receptor, of which there are $(100-n)n/100$. If we allow Rc to form dimers with the same probability as RC, then the number of active RCRC dimers that would be formed is given by

$$0.5\left\{\frac{(100-n)^2}{100}\cdot\frac{(100-n)^2}{100}\right\}\Big/\left\{\frac{(100-n)^2}{100}+\frac{(100-n)n}{100}\right\}=0.5(100-n)^3/10^4$$

This correponds to a 3-fold increase in apparent target size to about 600 000 for glucagon-stimulated activity in the uncoupled state, compared with an

observed target size of 380 000. We can only restrict the calculated target size to the observed value if we assume that no inactive hybrid dimers containing Rc are formed, but this is incompatible with the requirement to form inactive hybrid dimers after irradiation in the coupled state. We have also been unable to rationalise the apparent target sizes of the complexes after irradiation in the coupled state into a physically consistent pattern for this model and we conclude that the model does not fit the observed pattern of target sizes. It should be noted that we were unable to eliminate this model previously [22], before the requirement for hybridisation had been implied by the data.

Conclusions

Although it is not possible to test more complex models of the coupling mechanism against the pattern of apparent target sizes without further structural information about the components of the receptor-catalytic unit complexes, it is clear that the present results severely restrict the types of simple model that are compatible with the data. Several models that have been proposed elsewhere can readily be discarded. For example, a model in which the dissociation of receptor and catalytic unit occurs on binding glucagon [6] is incompatible with the observed changes in target size. Of the two main classes of models we have considered, the essential difference is that the mobile independent receptor model requires inactivated catalytic units to couple structurally to active receptors, whereas the lateral dimerisation model requires monomeric complexes with inactive receptors to form dimers with active monomeric partners.

The similarity of the pattern of apparent target sizes obtained with des-his glucagon provides strong independent support for our previous conclusion from biochemical experiments that des-his glucagon can couple to the glucagon receptor to the catalytic unit structurally, without activating the enzyme. We have pointed out that this at least indicates that a catalytic unit which is not activated can couple to an intact receptor.

The requirement for hybridisation of partners to account for the apparent target sizes suggests that the receptor are mobile as well as independent, although we cannot exclude the possibility of clusters of receptor-catalytic unit complexes in the membrane, in which partners can exchange with their nearest neighbours after the removal and readdition of glucagon. However, the available evidence suggests that the receptors are not clustered, but are randomly distributed on the membrane surface. One test of the mobile receptor model indicated by the present work, is that in membranes modified by fusion with dimyristoyl lecithin [23], hybridisation should be inhibited after removal and readdition of glucagon below the phase separation which occurs in the modified membranes, but normal hybridisation should occur at temperatures above the phase separation. More generally, similar irradiation experiments on fat cell plasma membranes under conditions where different types of receptor compete for the catalytic units [2,7], should enable us to determine whether the receptors are permanently associated with the same catalytic unit, or whether the receptors are mobile and independent of the catalytic units in the uncoupled state, as the present work indicates.

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We are grateful to Dr. Martin Rodbell for pointing out that authentic des-His-glucagon is a partial weak agonist which yields 70% of the maximal activity given by saturating concentrations of glucagon [24]. The material used in the present study acted as a competitive inhibitor of glucagon action, with no detectable biological activity at 10^{-6} M. The conclusion from this work and a previous paper [12], that the receptor can couple structurally to the catalytic unit without activating it, is therefore unaffected.

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