Vasopressin V2-Receptor Mobile Fraction and Ligand-dependent Adenylate Cyclase Activity Are Directly Correlated in LLC-PK1 Renal Epithelial Cells

David A. Jans, Reiner Peters, Patricia Jans, and Falk Fahrenholz
Max-Planck-Institut für Biophysik, Frankfurt am Main 71, Federal Republic of Germany

Abstract. The role of hormone receptor lateral mobility in signal transduction was studied using a cellular system in which the receptor mobile fraction could be reversibly modulated to largely varying extents. The G-protein-coupled vasopressin V2-type receptor was labeled in LLC-PK1 renal epithelial cells using a fluorescent analogue of vasopressin, and receptor lateral mobility measured using fluorescence microphotolysis (fluorescence photobleaching recovery). The receptor mobile fraction (f) was ~0.9 at 37°C and <0.1 at 10°C, in accordance with previous studies. When cells were incubated for 1 h at 4°C without hormone, and then warmed up to 37°C and labeled with the vasopressin analogue, f increased from ~0.9 to 0.8 over ~1 h. The apparent lateral diffusion coefficient was not markedly affected by temperature pretreatment.

Various models have been proposed for the activation of adenylate cyclase (AC) by hormones binding to specific membrane integral receptors on the external surface of the plasma membrane lipid bilayer (Cuatrecasas, 1974; Kahn, 1976; Tolkovsky and Levitzki, 1978). All models must account for the mediating role of the GTP-binding (G-) protein complexes, which are responsible for modulating AC activity through their stimulating (Gs-protein) or inhibiting (Gi-protein) action. The fact that the Gs polypeptide component appears capable of redistribution from the membrane, where it is probably anchored by the β-γ-complex, to the cytosolic fraction upon agonist binding to receptor (Stryer and Bourne, 1986; Lynch et al., 1986; McArdle et al., 1988; Ransnäss et al., 1988; Ransnäss and Insel, 1989) implies that the rate-limiting steps in signal transduction are likely to be the protein–protein interactions occurring within the plasma membrane lipid bilayer fraction (Chabre, 1987; Peters, 1988). The collision coupling or "mobile receptor" hypothesis (Cuatrecasas, 1974; Kahn, 1976; De Meyts et al., 1976; Tolkovsky et al., 1978) and variations thereof (e.g., Swillens and Dumont, 1980; Swillens, 1982; Sobolev et al., 1988) propose that these interactions occur through transient collisionary contacts between protein components as the result of lateral diffusion of receptors and G-proteins within the plasma membrane. This is presumably then sufficient to activate the G-protein complex via liberation of Gs into the aqueous phase.

We have reported direct lateral mobility measurements, using the technique of fluorescence microphotolysis (fluorescence photobleaching recovery or FRAP), for the G-protein-coupled vasopressin V1- and V2-type receptors (Jans et al., 1989, 1990a–c). The V2-receptor of LLC-PK1 renal epithelial cells shows a marked temperature dependence of receptor-mobile fraction (f), whereby f is highest (almost 1.0) at physiological temperature, and lowest (<0.1) at 10°C (Jans et al., 1989). We have also observed (Jans et al., 1990c) that prolonged treatment with the acidotrophic agent ammonium chloride results in almost total immobilization of the V2-receptor (f = 0.2) concomitant with changes in the actin cytoskeleton.

The present study furthers the above observations, showing in particular that receptor immobilization by a low-temperature pretreatment is reversible. This effect, which was...
accompanied by reversible changes within the cytoskeleton as revealed by F-actin staining, provided a unique opportunity to investigate the role of F in signal transduction. A linear correlation was observed between F and maximal cAMP production in response to AVP, providing strong evidence for a mechanistic role for V2-receptor lateral mobility in AC activation in vivo.

Materials and Methods

Materials were as described previously (Jans et al., 1989, 1990a,c). The rhodamine-labeled analogue of vasopressin, 1-deamino-[8-lysine (N-betamethylrhodamylaminothiocarbonyl)] vasopressin (TR-LVP) was prepared as described (Jans et al., 1989). Cells of the LLC-PK1 pig kidney epithelial cell line (Hull et al., 1976) were cultured in DMEM supplemented with 10% (vol/vol) fetal calf serum, 0.2 mg/ml streptomycin, and 50 U/ml penicillin (Jans et al., 1986, 1989).

Vasopressin Binding

Vasopressin binding was measured on whole cell monolayers using tritium-labeled [Arg8]vasopressin (AVP) as described (Jans et al., 1989). Internalized hormone was determined using a 30-min treatment with 200 nM Gly-HCl, pH 3, 200 mM NaCl subsequent to the binding incubation (Jans et al., 1989, 1990a).

In Vivo cAMP Production

Cell monolayers were grown in 12-well microtiter plates to 90-95% confluence. With or without prior pretreatment either at 4°C (1 h) or 4°C (1 h) followed by 37°C (1 h) in serum-free DMEM containing 2 mg/ml BSA, or with prior pretreatment at 37°C for 2 d with 10 mM NH4Cl in normal medium (Jans et al., 1990c), cells were treated with agonists in the presence of 500 μM isobutylmethylxanthine (IBMX) (in the absence of NH4Cl) in serum-free DMEM/BSA, and the incubation stopped by washing at 4°C with NaCl/Pi, and the addition of bovine 2 mM IBMX/0.05 M Na+ acetate, pH 6.2 (Steinberg et al., 1979; Jans et al., 1987). Extracts were then boiled for 3 min, and kept at -20°C before the determination of cAMP in serum-free DMEM/BSA, and the incubation stopped by washing at 4°C with BSA (fatty-acid-free) as standard.

Fluorescence Measurements

Cells to be used for fluorescence and lateral diffusion measurements were grown on coverslips (15 x 15 mm) for 3-4 d to ~50% confluence (Jans et al., 1989). After incubation with ligand, cells were washed with NaCl/Pi (containing 0.5 mg/ml BSA), and mounted in the incubation medium in the absence of ligand. The methods used in measurements of fluorescence intensity and the fluorescence microphotolysis apparatus used have been described previously in detail (Peters, 1986) and in particular for LLC-PK1 cells (Jans et al., 1989, 1990b,c).

F-actin Staining

Cells were grown on coverslips, and fixed with 4% paraformaldehyde in NaCl/Pi subsequent to various pretreatments. Cells were then stained with 1 μg/ml rhodamine-labeled phalloidin (R-PH) as described (Wulf et al., 1979; Jans et al., 1990c). Staining could be completely competed with 50 μg/ml nonlabeled phalloidin (Jans et al., 1990c).

Results

V2-receptor Lateral Mobility

We were interested in further investigating the marked temperature dependence of the V2-receptor mobile fraction (f), and in particular the low F (~0.1) measured at 10°C (Jans et al., 1989). As previously, the technique of fluorescence microphotolysis (photobleaching) (Axelrod et al., 1976; Peters, 1986), together with the rhodamine-labeled vasopressin analogue TR-LVP (Buku et al., 1985; Jans et al., 1989), was used, with fluorescence monitored at 20-s intervals, rather than in the usual continuous fashion, to avoid (unintentional) bleaching during the course of the measurements. A series of measurements under identical conditions were performed for photobleaching recovery in LLC-PK1; cells subsequent to treatment with 10−7 M TR-LVP in the absence (total binding) or presence (nonspecific binding) of 10−8 M AVP. Measurements for each time interval were pooled and averaged. Subtraction of the measurements for nonspecifically bound from those for total bound fluorescence enabled estimation of the recovery of specifically bound fluorescence after photobleaching. Experimental data were then fitted by theoretical curves (Soumpasis, 1983) for two components: a mobile fraction (f) with apparent lateral diffusion coefficient (D) and an immobile fraction (D < 10−12 cm2/s) (Axelrod et al., 1976).

We initially tested whether the low V2-receptor mobile fraction previously determined at 10°C was due to the fact that the incubation with ligand was performed at 4°C (1 h) before the measurements (Jans et al., 1989). It seemed possible that low temperature itself may induce receptor immobilization. To address this question, cells were incubated at 37°C with ligand (10 min), and then washed and lateral mobility measurements performed at 10°C. The mobile fraction was 0.23 (Table I) indicating that the V2-receptor is largely immobile at 10°C. This value is significantly higher than that for 4°C-preincubated cells, however (f of ~0.1) (Jans et al., 1989), implying that treatment at 4°C may have an immobilizing effect on the V2-receptor. This observation was further examined by pretreating cells for 1 h either at 37°C or 4°C in the absence of ligand, prior to incubation with ligand, washing and measurement of V2-receptor lateral mobility, all at 37°C (Table I, Fig. 1). Interestingly, a marked reduction of V2-receptor lateral mobility was evident in the 4°C-pre-

| Parameter of mobility† | Pretreatment (without ligand)‡ | 10−12 cm2/s | f | n |
|------------------------|-------------------------------|-------------|---|---|
| A) Measurements performed at 10°C (10-30 min)§ | 1) 37°C | 2.70 ± 0.12 | 0.94 ± 0.02 | 27 |
| | 2) 4°C | 2.19 ± 0.21 | 0.42 ± 0.02 | 10 |
| | 3) 4°C/37°C | 2.37 ± 0.17 | 0.78 ± 0.04 | 10 |
| B) Measurements performed at 37°C (10-30 min)§ | i) 37°C | 3.01 ± 0.45 | 0.72 ± 0.05 | 8 |
| | ii) 4°C | 2.64 ± 0.17 | 0.62 ± 0.03 | 11 |

† Values for the apparent lateral diffusion coefficient (D) and mobile fraction (f) represent the mean ± SEM, where n is the number of experiments. Each experiment comprises at least five separate measurements on different cells for total and nonspecifically bound fluorescence (see text and legend to Fig. 1).

‡ Pretreatment involved incubation at the indicated temperature in serum-free DMEM (without ligand) for 1 h. Treatment B3 involved 1 h at 4°C followed by 1 h at 37°C.

§ In all cases, cells were washed at 37°C, subsequent to the appropriate pretreatment, and then incubated with ligand for 10 min at 37°C, washed at 37°C, and mounted in serum-free, ligand-free DMEM. Measurements were then performed at the temperatures and for the times after ligand addition, as indicated.

¶ Unable to be determined.
Figure 1. Lateral mobility of the V2-receptor in the basal plasma membranes of LLC-PK1 cells with or without pretreatment at 4°C. Pretreatments were for 1 h at 4°C or 37°C or at 4°C followed by 37°C (1 h) without ligand in serum-free DMEM. Photobleaching measurements were subsequently performed on cells incubated with ligand for 10 min at 37°C, washed, and mounted in serum-free medium at 37°C. The curves represent the least square best fits to the experimental data for specific fluorescence, calculated from at least five individual measurements for each of total and nonspecifically bound fluorescence (see Results).

Treated cells, whereby both D (2.2 \times 10^{-10} \text{ cm}^2/\text{s} compared with 2.7 \times 10^{-10} \text{ cm}^2/\text{s} for 37°C-pretreated cells) and in particular f (0.42 compared with 0.94 for 37°C-pretreated cells) were reduced (Table I, B2; Fig. 1). This effect of 4°C pretreatment appeared to be transient in that longer incubation at 37°C resulted in an increase in f (0.62) and D (2.6 \times 10^{-10} \text{ cm}^2/\text{s}) (Table I, Cii), values almost comparable to those of 37°C-pretreated cells (Table I, Ci). As shown previously (Jans et al., 1990b) internalization is associated with V2-receptor immobilization with time at 37°C (f = 0.72, Table I, Ci) where internalized ligand accounts for \sim 40% of total bound hormone after \sim 45 min at 37°C (Jans et al., 1989). The reduction of f by 4°C pretreatment could be largely reversed by a 1-h treatment at 37°C subsequent to the 4°C treatment (Table I, B3; Fig. 1). In this case the values for D and f were intermediate between those for exclusively 4°C pretreated or 37°C pretreated cells. It thus appeared that pretreatment of LLC-PK1 cells at 4°C affected a reduction in V2-receptor lateral mobility, which could be reversed by subsequent incubation at 37°C.

Some experiments (not shown), performed using longer incubation times at 4°C, indicated that 4°C pretreatment for up to 3 h had essentially the same maximal effect (60% reduction of f). The reversibility of this effect by subsequent 37°C pretreatment, however, seemed to be influenced by the duration of 4°C pretreatment. Longer times at 37°C were required in the case of cells treated for longer at 4°C to bring about reversal of the "immobile" phenotype, whereas 30 min 4°C pretreatment reduced f to a somewhat lesser extent (f \sim 0.6). The time-consuming nature of the diffusion measurements (25–30 min for a series of five to six measurements for a single experimental condition) prevented closer examination of the kinetics of this phenomenon.

[H]AVP Binding

To ensure that the temperature effects on V2-receptor lateral mobility did not result from altered ligand binding properties, maximal specific [H]AVP binding and internalization at both 4°C and 37°C were measured on cells pretreated for 1 h at 4°C or 37°C, or 4°C followed by 37°C as above. No differences were observed in maximal binding or internalization at either temperature for cells with or without 4°C or 4°C/37°C pretreatment over the time intervals used in the lateral diffusion measurements (Fig. 2), and no differences were detected in the binding affinity for AVP (results not shown). The effects of 4°C pretreatment on V2-receptor lateral mobility were accordingly not the result of altered ligand binding or internalization.

F-actin Staining

A discernible effect of the 4°C pretreatment of LLC-PK1 cells was a morphological change, whereby cells became rounder and cell–cell contact regions more distinct. We investigated this in more detail by staining the actin cytoskeleton of non- and pretreated cells with R-PH. Cells were fixed with p-formaldehyde subsequent to the temperature pretreatment.

Figure 2. Specific [H]AVP binding and internalization by LLC-PK1 cells with or without temperature pretreatments. Cell monolayers in 12-well plates (Costar Corp., Cambridge, MA) were pretreated as in Fig. 1, and then washed and incubated with 10^(-4) M [H]AVP in the absence or presence (nonspecific) of 10^(-4) M AVP at the indicated temperature for the indicated times. Duplicate cultures were then analyzed for total binding (C) or internalized ligand (B), determined as described in Materials and Methods, using a pH3 treatment (Jans et al., 1989, 1990a). Results represent the means from a single typical experiment performed in triplicate (SEM < 12% the value of the mean).
ments described above, stained with R-PH at room temperature (30 min), washed and photographed using fluorescence microscopy (Fig. 3). LLC-PK₁ cells revealed the typical actin filament or stress fiber staining previously observed (Jans et al., 1990c) (Fig. 3 B). Interestingly, 4°C-pretreated cells exhibited a quite different staining pattern, in that fibers were markedly reduced in both prevalence and distinctness (Fig. 3 F) resulting in a cloudy or fuzzy appearance. Treatments as short as 15 min at 4°C (not shown) were sufficient to bring about an unequivocal declarification of actin microfilament structure as visualized with R-PH here. This effect could be almost completely reversed in a time-dependent manner by subsequent treatment at 37°C before actin staining (not shown), in similar fashion to the effect on /f above. Specificity of the staining for the actin cytoskeleton was demonstrated by almost total competition of the labeling by 50-fold higher concentrations of unlabeled R-PH (Fig. 3 D). It could accordingly be concluded that the treatment of cells at 4°C led to alterations in the actin cytoskeleton, presumably as a result of the inhibition of actin polymerization (Howard and
Oresajo, 1985). 4°C treatment thus appeared to concomitantly affect both V2-receptor lateral mobility and the actin cytoskeleton of LLC-PK1 cells, thereby resembling prolonged treatment of LLC-PK1 cells with 10 mM NH4Cl (Jans et al., 1990c). The latter treatment also reduces f (to 0.2) and concomitantly alters the actin cytoskeleton, such that stress fibers are more pronounced and thicker than in untreated cells (shown for comparison in Fig. 3 H). These results show that alteration of cytoskeletal structure, either through primary or secondary effects, can strongly influence lateral mobility of the V2-receptor.

In Vivo cAMP Production in LLC-PK1 Cells Pretreated to Reduce V2-Receptor Mobile Fraction

That treatments could be used in the absence of ligand to effect different V2-receptor f values at 37°C presented a unique possibility to test for the role of f in signal transduction in vivo. Cells were pretreated as above at 37°C, 4°C, 4°C then 37°C, or 10 mM NH4Cl (2 d), before washing at 37°C, and treatment without or with different concentrations of either AVP or forskolin (nonreceptor-mediated AC activator) in the presence of IBMX. The latter amplifies cAMP production through inhibiting phosphodiesterase activity. Representative results are presented in Fig. 4, and summarized in Table II and Fig. 5. A quite striking result was obtained for AVP-mediated stimulation of cAMP production in that, without exception, the maximal rate of cAMP production positively correlated with V2-receptor mobile fraction (Fig. 4, Table II; see Fig. 5). The treatments reducing f resulted in lower maximal rates of cAMP production, with the effects being more pronounced at AVP concentrations below the Kp of ligand binding (3.2 × 10^-9 M; Jans et al., 1986), in terms of the percent reduction compared with untreated cells. For example, the maximal cAMP responses of 4°C-pretreated cells to 10^-7, 10^-9, and 10^-10 M AVP were 20, 31, and 62% reduced respectively, compared with untreated cells (Fig. 4 a; Table II). The AVP concentrations inducing half-maximal response (~1.5 × 10^-9 M) were the same in all cases (not shown). In contrast, forskolin-induced (nonreceptor mediated) cAMP production was unaffected by the various treatments, with the possible exception of NH4Cl treatment (Fig. 4 b, Table II), implying that the effects of the treatments on AVP-induced cAMP production were not attributable to effects on AC itself. It was concluded that the reduced AVP-stimulated AC activation was the direct result of reduction of f induced by the various temperature and NH4Cl pretreatments. The direct correlation between the Vmax of AVP-stimulated cAMP production at the three AVP concentrations tested is shown in Fig. 5. The linear re-

![Figure 4(a)](image-url)  
**Figure 4 (a).** cAMP production in response to AVP in LLC-PK1 cells variously pretreated to reduce the V2-receptor mobile fraction. Cells were pretreated (●) no addition, 37°C; (□) 4°C, 1 h; (▲), 4°C, 1 h then 37°C, 1 h; or (×) 10 mM NH4Cl (37°C, 2 d) before incubation with different concentrations of AVP in the presence of 500 μM IBMX for the times indicated. Intracellular cAMP was then determined as described in Materials and Methods. Results represent the means for at least three separate determinations performed in duplicate (SEM < 22% the value of the mean).

![Figure 4(b)](image-url)  
**Figure 4 (b).** cAMP production in response to forskolin in LLC-PK1 cells variously pretreated to reduce the V2-receptor mobile fraction. Incubations, methods and symbols for the various pretreatments are as per Fig. 4 a. Results represent the mean for at least two separate determinations performed in duplicate (SEM < 26% the value of the mean).
of at least two separated determinations performed in duplicate, for which the
4°C 0.42 470 200 18.5 285 188
4°037°C 0.78 535 240 36.2 280
37°C 0.94 585 290 48.4 285 170
Pretreatment§ fß 10-7M 10-9 M 10-10M 10~ M 10-5M

In Vivo in Response to AVP or Forskolin in LLC-PK,
Meyts et al., 1976; Kahn, 1976; Bergman and Hechter,
pothesisthatthe processof interactionbetween recep-
tors and hormone is controlled by the rate of receptor lateral mobility,
allowing for a possible role for AVP-stimulated cAMP production.

Discussion

This study represents the first direct investigation of the relationship between receptor lateral diffusion and signal tran-
duction in vivo. The surprising observation that treatment of LLC-PK1 cells without AVP at 4°C reversibly immobilizes V2-receptor provided the means to test for the role of receptor lateral mobility, and, in particular, of the receptor mobile fraction, in stimulation of cAMP production by AC. Briefly, cells could be pretreated to reduce f as measured at 37°C, and then treated with hormone or forskolin at 37°C to examine activation kinetics. Importantly, forsko-
lin (nonreceptor mediated) response was unaffected, indicating no influence of the pretreatments on AC itself. Ac-
tive responses were markedly reduced in contrast, in terms of the maximal rates of cAMP production. The extent of response to AVP correlated directly with the magnitude of f, the effects of reducing f being more pronounced in terms of cAMP re-
sponse at lower (sub-K0) AVP concentrations. This close correlation of f with AVP-stimulated AC response (see Fig. 5) suggests a possible role for V2-receptor lateral mobility in AVP-mediated AC activation, in support of previous data from this laboratory (Jans et al., 1989, 1990c) and of the hypothesis that the process of interaction between receptor–hormone complex, G-proteins, and the AC catalytic subunit are diffusion controlled (Cuatrecasas, 1974; De Meyts et al., 1976; Kahn, 1976; Bergman and Hechter, 1978; Tolkovsky and Levitzki, 1978; Hanski et al., 1979; Tolkovskiy et al., 1982). From the recent data suggesting that the AC-stimulating Gs polypeptide is redistributed from the membrane to the cytosolic (aqueous) fraction upon agonist binding to receptor (Stryer and Bourne, 1986; Lynch et al., 1986; McArrol et al., 1988; Ransnäs and Insel, 1988; Ransnäs et al., 1989), it can be concluded that the rate-limiting step of AC activation may be lateral diffusion within

The plasma membrane. That the interactions between the receptor–hormone complex and Gs will occur through diffusion-controlled collision also explains the well-established amplifying property of the AC system, i.e., that one receptor–
hormone complex can activate many Gs and subsequently AC molecules (Orly and Schramm, 1975; Brandt and Ross, 1986; Ransnäs and Insel, 1988). In this respect, one would expect the receptor mobile fraction to be a more important factor at low (physiological) hormone concentrations, where only a minor fraction of receptors are occupied. Our results, that low V2-receptor f results in a more pronounced reduction of maximal ligand-stimulated AC activation at sub-K0 AVP concentrations compared with 10-7 M (100% receptor occupancy), are consistent with this. It should be stressed that the conclusions here concerning a role for receptor mobility in signal transduction are not necessarily applicable to in vitro/membrane vesicle systems where local concentrations of all membrane components as well as of GTP, GDP, ATP, Pi, kinase, phosphatase, etcetera, are unlikely to reflect the dynamic in vivo situation. Our results are all the more rele-
vant because they reflect physiological conditions, including the complex membrane and cytosolic activation/downregu-
alation apparatus. In this complex in vivo system, receptor lateral mobility clearly is a very important factor. Interest-
ingly, the only other direct measurement of lateral mobility of an AC-coupled receptor indicated that whilst a β-receptor
agonist may induce receptor mobility, the receptor--agonist complex is largely immobile (Henis et al., 1982). The intriguing possibility that antagonists of the AC system may function by receptor immobilization also argues for the physiological importance of receptor lateral mobility. The role of receptor lateral mobility in signal transduction by the polypeptide hormone receptors for insulin and epidermal growth factor has been discussed elsewhere (Jans et al., 1989, 1990a.; and see Schlessinger, 1988, 1989).

The mechanism whereby the V2-receptor is immobilized by treatment at 4°C remains unclear. As mentioned above, forskolin responses are not affected, indicating that the results are not explicable in terms of effects on AC itself. AVP binding is also completely unaffected, but a destabilization of the actin stress fibers is clearly effected, as revealed by staining of the actin cytoskeleton. The exact explanation of the effect is unclear, but probably is the result of the inhibition of actin polymerization, which has been observed at low temperature (Howard and Oresajo, 1985). The concomitant effects of 4°C treatment on both the actin cytoskeleton and V2-receptor mobility in LLC-PK1 cells is comparable to that of prolonged treatment of the same cells with NH4Cl or cytochalasin B (see Jans et al., 1990c). It can be concluded that the cytoskeleton has an important influence on V2-receptor mobility, as has been reported for a number of other integral membrane proteins (Edelman, 1976; Schlessinger et al., 1976; Koppel et al., 1981; Tank et al., 1982). It should be remembered that the alterations to the cytoskeleton induced by low temperature or NH4Cl treatment probably have pleiotropic effects on the cell, possibly including membrane structural integrity.

It is accordingly unlikely that the actin cytoskeleton is the sole mediator of the effects on V2-receptor mobile fraction. A role for lipid bilayer fluidity in structure and function has been documented for many membrane proteins and receptors, including the β-adrenergic receptor (Orly and Schramm, 1975; Axelrod et al., 1978; Hanski et al., 1979; Bakardjieva et al., 1979; Helmreich and Elson, 1984).

The results here imply that V2-receptor lateral mobility may be a determining factor for AVP-dependent AC activation. Since this activation is known to be mediated by the G-protein components, which are in a 100- to 150-fold excess (Pfeuffer, 1977; Jans, D. A., unpublished results) with respect to the V2-receptor, our results imply either (a) that the G complex is essentially immobile, and that activation of the latter is solely brought about by lateral diffusion of the mobile hormone-receptor complex; or (b) that both G and the V2-receptor are mobile and that the domain of the receptor that interacts with/activates G is masked under conditions of low mobility, e.g., at low temperature. Since the cytoskeleton appears to play an active role in the system, based on the results here, it is possible that the receptor is in some way linked to cytoskeletal or cytoskeleton-attached components, such as actin, spectrin, ankyrin, et cetera (see Peters, 1988). At 37°C, the G-protein interaction domains of the receptor are/become accessible, so that lateral diffusion of and collateral contacts between both mobile G-protein and hormone--receptor can result in activation of G. A variant of hypothesis b is that G and receptor are freely mobile, but in separate microcompartments of the plasma membrane, and only at 37°C are the constraints to movement between the microcompartments less strict. Again, an active role of the cytoskeleton in regulating these processes could be envisaged, and evidence indeed exists for a spectrin--actin meshwork structure of the red blood cell plasma membrane skeleton whose rigidity as a lateral diffusion barrier is reduced at 37°C (Golan and Veatch, 1980; Lin et al., 1987). Experimental differentiation between hypotheses a and b above will be achieved via direct in vivo measurement of the lateral mobility of the G-protein complex and polypeptide components in both membrane and aqueous phases.

The authors thank Profs. H. Faulstich and Th. Wieland for the gift of rhodamine-phalloidin.

This work was supported by grants of the Deutsche Forschungsgemeinschaft to R. Peters and F. Fahrenholz, respectively.

Received for publication 22 January 1991 and in revised form 13 March 1991.

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