Amino Acids (1995)9: 93–109

Amino
Acids

A mechanistic role for polypeptide hormone receptor lateral mobility in signal transduction

Review Article

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Accepted April 24, 1995

Summary. Lateral diffusion of membrane-integral receptors within the plane of the membrane has been postulated to be mechanistically important for signal transduction. Direct measurement of polypeptide hormone receptor lateral mobility using fluorescence photobleaching recovery techniques indicates that tyrosine kinase receptors are largely immobile at physiological temperatures. This is presumably due to their signal transduction mechanism which requires intermolecular autophosphorylation through receptor dimerization and thus immobilization for activation. In contrast, G-protein coupled receptors must interact with other membrane components to effect signal transduction, and consistent with this, the phospholipase C-activating vasopressin V_1- and adenylate cyclase activating V_2-receptors are highly laterally mobile at 37°C. Modulation of the V_2-receptor mobile fraction (f) has demonstrated a direct correlation between f and receptor-agonist-dependent maximal cAMP production *in vivo* at 37°C. This indicates that f is a key parameter in hormone signal transduction especially at physiological hormone concentrations, consistent with mobile receptors being required to effect V_2-agonist-dependent activation of G-proteins. Measurements using a V_2-specific antagonist show that antagonist-occupied receptors are highly mobile at 37°C, indicating that receptor immobilization is not the basis of antagonism. In contrast to agonist-occupied receptor however, antagonist-occupied receptors are not immobilized prior to endocytosis and down-regulation. Receptors may thus be freely mobile in the absence of agonistic ligand; stimulation by hormone agonist results in receptor association with other proteins, probably including cytoskeletal components, and immobilization. Receptor immobilization may be one of the important steps of desensitization subsequent to agonistic stimulation, through terminating receptor lateral movement which is instrumental in generating and amplifying the initial stimulatory signal within the plane of the membrane.

Keywords: Fluorescence photobleaching recovery – Vasopressin V_1- and V_2-receptor subtypes – GTP-binding proteins – Adenylate cyclase – Receptor agonist – Receptor antagonist

Abbreviations: FBR, fluorescence photobleaching recovery; EGF, epidermal growth factor; AC, adenylate cyclase; D, apparent lateral diffusion coefficient; f, mobile fraction; G-, GTP-binding protein; Gs, stimulatory G-protein; TKR, tyrosine kinase receptor; PDGF, platelet-derived growth factor; IL, interleukin

Introduction

Concepts such as the fluid mosaic model imply that membrane proteins float freely within the lipid bilayer. Direct measurements of membrane protein lateral diffusion using techniques such as fluorescence photobleaching recovery (FBR – Axelrod et al., 1976; Edidin et al., 1976; Jacobson et al., 1976), however, indicate that many integral membrane proteins are in fact largely immobile. The anion-exchanging erythrocyte band 3, for example, has an apparent lateral diffusion coefficient (D) of about 0.4×10^{-10} cm^2/sec at 37°C (see Peters, 1988), which is at least 20 times slower than erythrocyte plasma membrane lipid mobility (D value of 10–200 cm^2/sec). Many membrane integral proteins including hormone receptors exhibit a low mobile fraction (f – the fraction of proteins possessing a D value of $>10^{-12}$ cm^2/sec; a D value below 10^{-12} cm^2/sec indicates immobility) at physiological temperature. Since membrane receptor lateral mobility appears to be essential for signal transduction (see below), factors restricting lateral movement are of great relevance in a signalling context.

Membrane lipid fluidity has been shown to affect membrane protein lateral movement in experiments in which fluidity has been modulated by modifying the fatty acid composition of membranes through delipidation, alteration of the cellular growth medium or time in culture etc. (e.g. Yechiel et al., 1985; Zakharova et al., 1995). The cytoskeleton also influences membrane protein lateral mobility, as shown in a number of studies where perturbers of cytoskeletal components have been shown to affect lateral mobility parameters of membrane integral proteins (see Helmreich and Elson, 1984; Edelman, 1976); cytochalasin/colchicine treatment, for example, increases the lateral diffusion coefficient of the luteinizing hormone (LH) receptor (Roess et al., 1988). In the case of erythrocyte band 3, increasing the spectrin content of the red blood cell membrane decreases mobility, whereas increasing the ankyrin content increases band 3 mobility (Tsuji and Ohnishi, 1986), indicating that protein-mediated association with the cytoskeleton can modulate protein lateral mobility either negatively or positively.

The cytoplasmic domain of membrane proteins can also determine the lateral mobility properties of membrane proteins as shown in various studies. Tsuji and Ohnishi (1986) showed that proteolysis of erythrocyte band 3 increases its lateral mobility, probably through impairing interactions with spectrin and the cytoskeleton (see also Peters, 1988). A molecular biological

approach has similarly shown that the length of the cytoplasmic domain of the mouse class I major histocompatibility complex (MHC) molecule H-2L^d affects D by a factor of up to 3-fold (Edidin et al., 1994). In contrast, extensive deletions of the cytoplasmic domain, including the kinase domain, of the epidermal growth factor (EGF) receptor do not significantly affect EGF receptor mobility (Livneh et al., 1986). That transmembrane domains can also play a role in determining membrane protein lateral mobility has been demonstrated by mutagenesis of the insulin receptor, where structural optimisation of the transmembrane helix increases lateral mobility significantly (Goncalves et al., 1993).

Whilst receptor lateral mobility is important in the signalling context as will be expounded below, hormonal stimulation can also influence the lateral mobility of heterologous membrane proteins, this effect conceivably being a significant but largely ignored mechanistic aspect of signal transduction. Tumour necrosis factor and interferon-γ treatment, for example, affect the lateral mobility of the MHC class I molecule and glycoprotein gp96 in human endothelial cells (Stolpen et al., 1988). That membrane protein immobilization is integrally associated with endocytosis (see below) has been demonstrated by a point mutant of the influenza haemagglutinin molecule which, unlike wild type, is both able to be internalized through coated pits, and significantly slower in terms of D (Fire et al., 1991).

Restricted movement and domain structure

A number of proteins, and particularly receptors, display restricted mobility in certain areas or domains of the plasma membrane but not others. Restricted lateral movement appears to be a means of achieving precise localization of particular receptors in a small area of the membrane. One example is the glycine receptor which exhibits higher D and f values on the neuronal cell body than on neuronal processes (Srinivasan et al., 1990). Voltage dependent sodium channels display restricted mobility on axon hillocks where they appear to be aggregated, but are highly mobile on cell bodies (Angelides et al., 1989). Similar results have been reported for the acetyl choline and GABA receptors (see Srinivasan et al., 1990), and the fibronectin receptor, which is immobile in focal contacts and fibrillar streaks but highly mobile in embryonic locomoting cells (Duband et al., 1988).

The mechanism by which lateral mobility is restricted in particular domains of the membrane is probably a function of all of the parameters mentioned above – that protein aggregation effects protein immobility has been shown directly by Zakharova et al. (1995), who used poly-L-lysine treatment to bring about concomitant reduction of protein lateral movement (both D and f). Domain structure may be defined in part through cytoskeletal structures, such as the spectrin-actin meshwork structure of the red blood cell plasma membrane (Golan and Veatch, 1980). Consistent with this, cytoskeleton-less or -damaged membrane sub-areas (blebs) appear to exhibit unrestricted and high protein movement (Roess et al., 1988).

The mobile receptor hypothesis – a role for receptor lateral mobility in signal transduction

The "collision coupling" or "mobile receptor" hypothesis (Cuatrecasas, 1974; Kahn, 1976; Tolkovsky and Levitzki, 1978a) postulates an active role for the lateral diffusion of membrane-integral receptors within the plane of the membrane in effecting the protein-protein contacts necessary for signal transduction (see Jans, 1992, 1994). Lateral diffusion of receptors within the plane of the membrane appears to be required to bring about:

1) receptor dimerisation in the case of tyrosine kinase receptors (TKRs) such as those for insulin, EGF and platelet-derived growth factor (PDGF) (Ullrich and Schlessinger, 1990; Heldin, 1992), which is essential for signal transduction (Ullrich and Schlessinger, 1990; Jans, 1992);

2) the collisions between receptor and GTP-binding (G-)proteins which activate the latter, as shown by direct measurements for the vasopressin V_1- and V_2- receptors (Jans et al., 1989, 1990a, 1991, see Jans, 1992), and indirectly for the β-adrenergic receptor (e.g. Hanski et al., 1979; Tolkovsky et al., 1982); and

3) subunit association in the case of multiple subunit receptors (see Taga and Kishimoto, 1992), such as those for interleukins (IL)-3 and -5, and the related granulocyte-macrophage colony stimulating factor (all of which share a common receptor subunit), interferon-γ, IL-2 and -6, etc., which is necessary for signal transduction.

In the case of 1) and 2) above, *in vivo* FBR measurements have enabled direct analysis of the role of receptor lateral mobility in signal transduction (Jans, 1992), as will be discussed below.

Lateral mobility of tyrosine kinase receptors: a role in receptor dimerization

The receptors for insulin and EGF (see Table 1) diffuse about 20-times more slowly than membrane lipids in the plasma membrane (Axelrod et al., 1978; Schlessinger et al., 1977). As shown in Table 1, they are largely immobile in terms of f especially at 37°C (Schlessinger et al., 1978a). D is highest at 37°C and lowest at 4°C (Schlessinger et al., 1978a; Zidovetzki et al., 1981; Hillman and Schlessinger, 1982), this temperature dependence for D holding true for the nicotinic acetyl choline receptor (Axelrod et al., 1978), as well as for the G-protein coupled vasopressin V_1- and V_2-type receptors (see Table 1 – Jans et al., 1989, 1990a). That D is highest at 37°C implies a physiological role for receptor lateral mobility in cellular processes (Jans et al., 1989, 1990a).

The TKRs for EGF and insulin are distinct from G-protein-coupled receptors in requiring no interaction with heterologous membrane proteins for signal transduction. They seem, however, to require receptor oligomerization (receptor-receptor collision) to effect the activating inter- (not intra-) molecular autophosphorylation event necessary for signal transmission (Schlessinger, 1988, 1989). Only short-term, rapid receptor lateral diffusion would be required at physiological temperature to generate the

Table 1. Temperature dependence of lateral mobility of polypeptide hormone plasma membrane receptors, as measured by the technique of fluorescence recovery after photobleaching

Receptor		Parameter of lateral mobility*	
	Temperature	D (10^{-10} cm^2/sec)	f
G-protein coupled receptors			
V_1-receptor (Jans et al., 1990a)	37°C	5.13	0.36
	23°C	3.58	0.50
	13°C	2.85	0.44
V_2-receptor (agonist)	37°C#	2.75	0.91
(Jans et al., 1989;	23°C	1.50	0.65
Pavo et al., 1994)	10°C	UD[l]	0.10
Tyrosine-kinase receptors			
Insulin receptor	37°C	0.1–1.0	<0.10
(Schlessinger, 1978a)	23°C	4.0	0.4–0.8
EGF-receptor†	37°C	0.1–1.0	<0.10
(Schlessinger, 1978a;	23°C[x]	3.4, 5.0	0.5–0.9
Zidovetzki et al., 1981;	4°C	3.0	0.90
Hillman and Schlessinger, 1982)			

* Values are for the apparent lateral diffusion coefficient (D) and mobile fraction (f). # Compare values for the G-protein coupling luteinizing hormone receptor at 37°C ($D = 1.90 \times 10^{-10}$ cm^2/sec; $f = 0.38$) (Niswender et al., 1985). † It has been reported that the low density high-affinity EGF-receptor of A431 cells, in contrast to the high density low-affinity receptor, is essentially immobile at 7°C ($D < 10^{-12}$ cm^2/sec) (Rees et al., 1984). This has been interpreted as implying the lack of a need for lateral diffusion of EGF-receptor for signal transduction at low hormone concentrations (Rees et al., 1984). The high affinity receptor may however already be aggregated (immobilized) "ready" for autophosphorylation through weak association with the cytoskeleton, as implicated by measurements of rotational mobility (see Zidovetzki et al., 1981, 1991). [l] Unable to be determined. [x] These values are comparable to those for the PDGF-receptor at 23°C ($D = 3.2 \times 10^{-10}$ cm^2/sec; $f = 0.60$) (Ljungquist et al., 1989).

signal transducing receptor aggregates which have been observed both *in vivo*, in the case of the EGF-receptor (Zidovetzki et al., 1981; Schlessinger et al., 1978b), and *in vitro* after hormone addition in the case of the insulin receptor (Johnson et al., 1988). Thus, receptor lateral diffusion may play a mechanistically important role in signal transduction in the case of TKRs.

Lateral mobility of G-protein coupled receptors: receptor lateral movement is required to activate G-proteins

The original collision coupling theory predated the identification of G-proteins, which transduce the signal represented by hormone-binding to a specific membrane integral receptor on the external surface of the plasma membrane in many systems into intracellular signals such as production of second

messenger molecules, through the activation of effector enzymes such as adenylate cyclase (AC) or phospholipase C. In terms of collision coupling theory, G-protein activation occurs through collisionary contacts between the receptor-hormone complex (R-H) and G-proteins as a result of lateral movement within the plasma membrane. Detailed kinetic analyses indicates that AC activation is a diffusion-controlled process, independent of the concentrations of AC or stimulatory G-protein (Gs) as well as of GDP release from the latter, the rate determining step being the kinetics of interaction between the R-H and Gs components (Tolkovsky and Levitzki, 1978a,b; Hanski et al., 1979; Rimon et al., 1980; Tolkovsky et al., 1982; Bergman and Hechter, 1978; Orly and Schramm, 1975). Interestingly, *in vivo* analysis suggests that upon agonist-mediated G-protein activation, the Gsα-polypeptide component redistributes from the membrane, where it is anchored by the $\beta\gamma$-complex, to the cytosolic fraction (Stryer and Bourne, 1986; Lynch et al., 1986; McArdle et al., 1988; Ransnäs and Insel, 1988; Ransnäs et al., 1989; Negishi et al., 1992). Since protein lateral diffusion is much faster in the aqueous than in the membrane phase (e.g. ovalbumin – 45 kD – has a D value of c. 350 $\times$ 10^{-10} cm^2/sec at 25°C in the cytoplasm of J744.1 mouse macrophages – Wang et al., 1982; see Peters, 1986 – compare to the values in Table 1), this means that the rate-limiting step of AC activation is be likely to be that of the diffusion-driven collisionary contacts between R-H and G-protein complexes within the lipid bilayer which induce the liberation of Gsα into the aqueous phase (Chabre, 1987; Jans et al., 1991; Jans, 1992). Indirect evidence for G-protein-mediated signalling being limited by membrane protein lateral diffusion is provided by experiments in which modulation of membrane lipid fluidity (and hence receptor lateral mobility) markedly affects signal transduction kinetics (Hanski et al., 1979; Zakharova et al., 1995; Bakardjieva et al., 1979; Yechiel et al., 1985; Moscona-Amir et al. 1989; Gorospe and Conn, 1987; see Helmreich and Elson, 1984).

Direct FBR measurements indicate that, in contrast to TKRs, the G-protein and AC-stimulating V_2-receptor (Jans et al., 1989), and to a lesser extent the phospholipase C-activating V_1-receptor (Jans et al., 1990a) are largely mobile at 37°C (see Table 1 and above). The V_2-receptor mobile fraction (f) is highest at physiological temperature and lowest at 4°C, implying a mechanistic role for receptor lateral mobility in signal transduction (Jans et al., 1989). Interestingly, the V_2-receptor f can be reversibly reduced in LLC-PK_1 renal epithelial cells using pretreatments with either low temperature or NH_4Cl (Jans et al., 1990c, 1991), both of which have been shown to immobilize other membrane proteins such as a mutant influenza haemagglutinin derivative (Fire et al., 1991). Modulation of f using such pretreatments has enabled the role of receptor lateral mobility in signal transduction to be tested, whereby cells were pretreated to reduce f and then stimulated with hormone or the receptor-independent AC activator forskolin prior to the measurement of the kinetics of AC activation (Jans et al., 1991). Receptor-independent responses were unaffected by the pretreatments indicating that they do not affect AC directly, whereas responses to vasopressin were markedly reduced in terms of the maximal rates of cAMP production. The maximal rate of

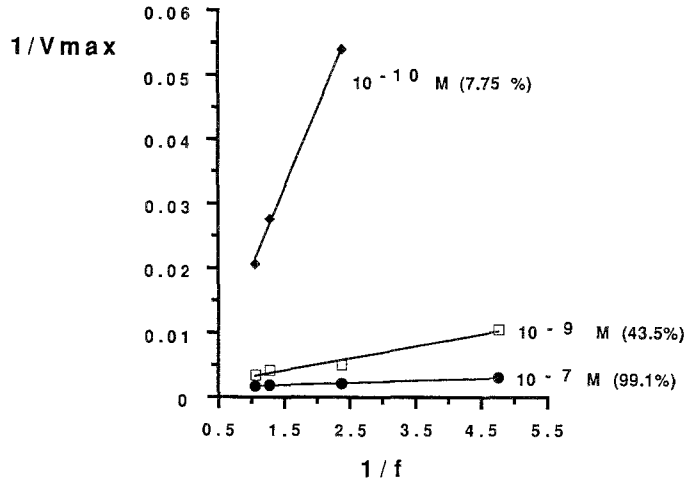

Fig. 1. Dependence of vasopressin-stimulated cAMP production on the V_2-receptor mobile fraction. The data for pretreatment at 4°C, 4/37°C, 37°C and 10 mM NH_4Cl (from Table 2) are plotted reciprocally to yield gradient values of 0.00035, 0.0019 and 0.025, and linear regression (r) values of 0.996, 0.985 and 0.999, for 10^{-7}, 10^{-9} M and 10^{-10} M vasopressin, respectively. V_2-receptor occupancies, indicated in parentheses, are from Luzius et al. (1991)

Table 2. Maximal velocities (Vmax) of cAMP production *in vivo* in response to vasopressin in LLC-PK_1 cells subsequent to pretreatments reducing the V_2-receptor mobile fraction

		Vasopressin concentration†		
	V_2-receptor mobile fraction (f)*	10^{-7}M	10^{-9}M	10^{-10}M
		Vmax (pmol/mg/min)*		
Pretreatment†				
37°C (1 h)	0.94	585	290	48.4
4°C (1 h)/37°C (1 h)	0.78	535	240	36.2
4°C (1 h)	0.42	470	200	18.5
10 mM NH_4Cl (2d)	0.21	330	95	ND‡

*From Jans et al. (1991).
†Incubation in the presence of the phosphodiesterase inhibitor iso-butyl-methyl-xanthine (500 uM). ‡Not determined.

vasopressin-stimulated cAMP production correlated directly with the magnitude of f (Jans et al., 1991), suggesting a direct role for V_2-receptor lateral mobility in hormone-mediated AC activation (Jans et al., 1989, 1990c, 1991). That low V_2-receptor f results in a more pronounced reduction of maximal ligand-stimulated AC activation at sub-K_D vasopressin concentrations, is consistent with f being particularly crucial under physiological conditions of low ligand concentrations and receptor occupancy (see Fig. 1; Jans et al., 1991).

The experimental data for cAMP production and V_2-receptor lateral mobility (see Table 2; Jans et al., 1991) is plotted in reciprocal form in Fig. 1 to reveal the linear relationship between AC activation and f. It seems likely that

only mobile receptors participate in signal transduction, with receptor lateral diffusion probably constituting the rate limiting step of AC activation. Interestingly, the temperature and NH_4Cl treatments affecting the V_2-receptor f also affect the actin cytoskeleton (Jans et al., 1990c, 1991). This implies that the cytoskeleton may directly modulate V_2-receptor mobility as has been shown for other membrane proteins (e.g. Fire et al., 1991), and thereby play a central regulatory role in signal transduction (see below).

Desensitization after hormonal activation through receptor immobilization: slow receptor immobilization brings about signal amplification in the AC system

One mechanism of desensitization of ligand-stimulated cells is accepted to be receptor-mediated endocytosis, which effectively reduces the number of ligand-specific cell surface receptors (see Lutz et al., 1991). Receptor aggregation and internalization have been visualized for EGF- (Zidovetzki et al., 1981; Schlessinger et al., 1978b), V_1- (Jans et al., 1990a) and V_2- (Jans et al., 1990b) receptors. The EGF and V_1-receptors are more rapidly internalized ($t_{1/2}$ = 6 and 2 min respectively at 37°C) than the V_2-receptor ($t_{1/2}$ = 14 min at 37°C) (Jans et al., 1989). The relatively low f values at 37°C measured for the V_1- and EGF-receptors may conceivably be a result of these rapid receptor internalization kinetics (Schlessinger et al., 1978a; Zidovetzki et al., 1981; Jans et al., 1990a). In the V_2-receptor system where the slower internalization kinetics permit such an analysis, internalization directly parallels V_2-receptor immobilization through reduction of f (Jans et al., 1990b; Jans, 1992). D is unaffected (Jans et al., 1990b), implying that the latter occurs through essentially irreversible receptor binding to immobile structures (see Fire et al., 1991). Similar qualitative results have been obtained for the nerve growth factor receptor where receptor aggregation and internalization parallels a reduction in receptor lateral mobility (Levi et al., 1980). The implication is that receptor immobilization precedes and is a prerequisite for endocytosis.

Interestingly in this regard, the EGF- (Zidovetzki et al., 1981; Schlessinger, 1989; Yarden and Schlessinger, 1987), insulin- (Kahn, 1985; Kahn and White, 1988) and V_1- (Jans et al., 1990a; Fishman et al., 1985; Doyle and Rüegg, 1985) receptors all display not only rapid activation of signal transduction, but also rapid desensitization of response concomitant with very rapid receptor internalization. The relatively slow activation and down-regulation kinetics of the V_2-receptor in comparison to the EGF-, insulin- and V_1-receptors, are concomitant with lower lateral diffusion rates (D) and slower internalization kinetics. This results in the persistence of a higher receptor mobile fraction, with the vasopressin-occupied V_2-receptor moving more slowly to ultimate aggregation/immobilization/internalization and desensitization of response than in the other systems mentioned. The well-established amplification property of the AC system, i.e. that one ligand-receptor complex can activate many Gs and subsequently AC molecules (Orly and Schramm, 1975; Brandt and Ross, 1986) may be understood in these terms.

Table 3. Comparison of the lateral mobility of the vasopressin V_2- and N-formyl-peptide receptors as measured using specific agonist and antagonists

Incubation		Parameter of lateral mobility			
Temperature	Time (min)	Agonist		Antagonist	
		D (10^{-10} cm^2/s)	f	D (10^{-10} cm^2/s)	f
V_2-receptor†					
10°C	10	UD[x]	0.10	UD[x]	0.10
23°C	10	1.5	0.65	1.9	0.62
37°C	10	2.5	0.92	2.6	0.77
37°C	30	2.8	0.68	2.8	0.76
37°C	60	2.0	0.43	2.3	0.67
N-formyl peptide receptor*					
14°C	60	5.5	0.40	5.4	0.63

† V_2-agonist: deamino-[Lys^8(tetramethylrhodamylaminothiocarbonyl)]vasopressin from Jans et al. (1989, 1991) and Pavo et al. (1994) and antagonist: [(β-mecapto-β,β-cyclopentamethylene propionic acid)^1,D-Tyr^2,Ile^4,Arg^8,Lys^9(N^6-tetramethylrhodamylaminothiocarbonyl)] vasopressin from Pavo et al. (1994). [x] Unable to be determined. * N-formyl-peptide agonist: formyl-Nle-Leu-Phe-Nle-Tyr-Lys and antagonist: tert-butyloxy-carbonyl-Phe(D)-Leu-Phe(D)-Leu-Phe-OH from Johansson et al. (1993).

Measurements with receptor antagonists: receptor immobilization is dependent on agonistic stimulation

FBR measurements using specific receptor antagonists indicate that the antagonist-occupied G-protein-coupling vasopressin V_2- and N-formyl peptide (chemotactic factor) receptors have lateral mobility properties very similar to those of agonist-occupied receptors (see Table 3; Pavo et al., 1994). The basis of antagonism is clearly not receptor immobilization; rather, lateral diffusion of the receptor in the cell membrane appears to be a constant process in the presence or absence of ligand, with the possibility of a collision with the membrane associated G-protein complex given all the time. Receptor occupation by an agonistic ligand, in contrast to that by an antagonist, is able to induce the conformational changes in the receptor which are necessary for G-protein activation (see Schmidt et al., 1991).

Significantly, measurements of antagonist-occupied V_2-receptor indicate that there is no reduction in f with time at 37°C, in stark contrast to agonist occupied receptors (see Fig. 2, Table 3 – Pavo et al., 1994; Jans et al., 1990b), which is consistent with results for the N-formyl peptide receptor (Johansson et al., 1993). This indicates that the receptor immobilization which precedes receptor internalization in the case of agonistic ligands is not induced by antagonists, implying that antagonist-occupied receptors may not be internalized. Qualitative observations for the vasopressin related ligand vasotocin and vasopressin V_1- and V_2-receptor antagonists in other systems (Lutz et al., 1992; Eggena and Buku, 1990) support this conclusion. Interestingly, Eggena and Buku (1990) demonstrated that vasotocin-antagonists could be internal-

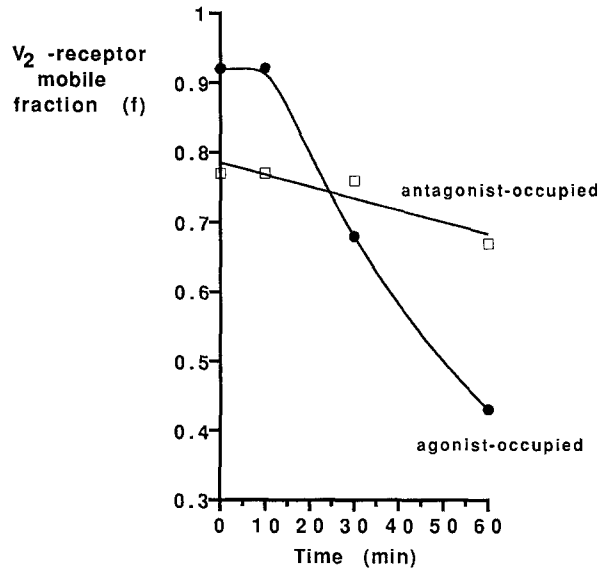

Fig. 2. Comparison of V_2-receptor agonist and antagonist with respect to time-dependent V_2-receptor immobilization. Results for f (from Jans et al., 1990b; Pavo et al., 1994) are plotted against time at 37°C

ized under conditions of receptor-independent stimulation of AC by forskolin, implying that endocytosis requires AC activation.

Uncoupling of agonist stimulation of AC through receptor phosphorylation prior to receptor sequestration has been described in detail by Lefkowitz and coworkers (see Hausdorff et al., 1990) for the β-adrenergic receptor system, where phosphorylation results in association (and concomitant immobilization?) of R-H with β-arrestin. Similar processes appear to be involved in the rhodopsin/transducin/β-arrestin visual system (Hausdorff et al., 1990; Wilden et al., 1986). Such detailed studies have yet to be performed with respect to the V_2-receptor, but there is strong evidence that phosphorylation, probably by the cAMP-dependent protein kinase (PK-A), plays a role in receptor endocytosis subsequent to AC activation (Jans and Hemmings, 1991). One can speculate in the light of the results for V_2-receptor lateral mobility that phosphorylation may effect receptor down-regulation through arresting lateral movement of R-H, whereby arrestin-like molecules, and possibly also the cytoskeleton, may be involved. This presumably occurs only upon AC and PK-A activation, and hence this phosphorylation/receptor immobilization prior to internalization does not occur in the case of antagonist-bound receptors. Signal transduction-mediated reduction of the number of mobile receptors may be central to the desensitization of response subsequent to hormonal stimulation.

Receptor diffusion rate-limited activation and desensitization of adenylate cyclase

It should not be overlooked in a signalling context that there is a 100- to 10,000-fold excess of G-proteins relative to the number of receptors (see Jans

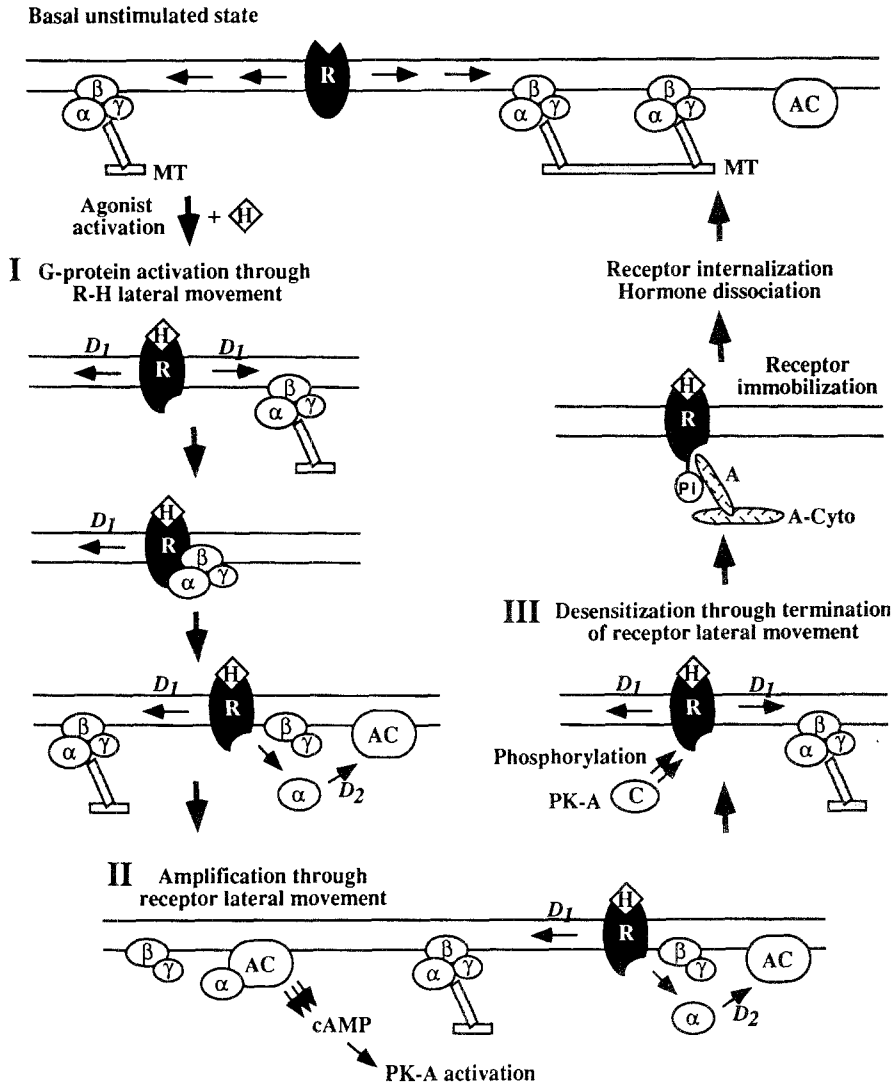

Fig. 3. A speculative scheme for diffusion-controlled receptor-mediated AC-activation (see Jans, 1992). In the basal unstimulated state, receptor (*R*) is freely mobile in the absence of agonistic hormone ligand (*H*) (Pavo et al., 1994), whilst the membrane-anchored trimeric G-protein complex (Chabre, 1987) may be essentially immobile through binding to microtubules (*MT* – Leiber et al., 1993). **I.** Upon agonist addition, H binds specifically to R and lateral diffusion of R-H results in collisionary activation of the G-protein complex and release of Gsα (GTP bound in the active state) into the aqueous phase. Rapid local diffusion in the cytosol of Gsα along the inner membrane surface leads to interaction with and activation of the AC catalytic subunit (*AC*) to stimulate cAMP production. Since the rate of receptor lateral movement (D_1) is much slower than Gsα movement in the aqueous phase (D_2), the former is rate-limiting. The concentration of H determines the absolute number of mobile receptors and thereby the rate of AC activation (see Table 2 and Fig. 1). **II.** Signal amplification is effected by continued diffusion of R-H to activate multiple G-protein complexes (Orly and Schramm, 1975; Ransnäs and Insel, 1988; Brandt and Ross, 1986). AC activation leads to activation of the cAMP-dependent protein kinase (*PK-A*). **III.** Termination of activation is initially effected by receptor immobilization, possibly through PK-A-catalytic subunit (*C*)-mediated receptor phosphorylation and association with arrestin-like molecules (*A*) and cytoskeletal components (probably of the actin cytoskeleton – *A-Cyto*) (Jans et al., 1990c; 1991). This precedes receptor internalization, dissociation of H from R etc. Abrogation of AC activity occurs through GTP hydrolysis by Gsα and the latter's dissociation from AC (Bourne et al., 1990; Birnbau-mer, 1990) prior to its ultimate reassociation with Gs$\beta\gamma$ in the membrane (Chabre, 1987; Bourne et al., 1990). Desensitization occurs at all levels of the intracellular activation cascade (not shown), including the stimulation of phosphodiesterase activity to break down cAMP, cAMP egression, and degradation of the PK-A catalytic subunit (see Jans and Hemmings, 1988, 1991), ultimately resulting in a return to the basal unstimulated state

et al., 1991; Alousi et al., 1991), which renders the exact basis of the need for mobile receptors for signal transduction unclear. If G-proteins were as mobile as receptors within the lipid bilayer, one could assume that receptor mobility of the latter would be largely superfluous since G-proteins are in such excess. On the contrary, however, the results for the vasopressin V_2-receptor show that mobile receptors are required to effect signal transduction, implying that trimeric G-protein complexes may in fact be immobile (see Neubig, 1994). Interestingly, the Gsα and Gnα subunits appear to be linked to the cytoskeleton in neutrophils in the absence of stimulation (Sarndahl et al., 1993), and there is evidence for G-protein association with microtubules in S49 lymphoma cells, in that the microtubule inhibitors colchicine/vinblastin increase GTP analog-stimulated AC activity (Leiber et al., 1993). Significantly, the membrane-linked cytoskeletal protein spectrin, among other proteins, has a "pleckstrin" homology domain, which has been clearly implicated in binding G-proteins (see Macias et al., 1994; Neubig, 1994). It thus appears not inconceivable that G proteins may indeed be closely linked to the cytoskeleton and hence immobile. If this were the case, movement of the R-H complex would of course be necessary for receptors to come into contact with and activate G-proteins. Direct measurement of the lateral mobility of the G-protein complex and subunits in membrane and aqueous phases *in vivo* is necessary to test the above hypothesis. Figure 3 shows a scheme depicting the role of receptor lateral diffusion in effecting and amplifying G-protein-mediated AC activation, as well as the inhibition of receptor lateral diffusion as one of the initial steps of desensitization subsequent to hormonal stimulation.

Conclusion

Direct FBR measurements imply that receptor lateral mobility in the plasma membrane is a determining factor in polypeptide hormone-mediated signal transduction. Lateral diffusion of TKRs brings about the receptor oligomerization necessary for the receptor-receptor autophos phorylation events which initiate signal transduction. In the case of G-protein-mediated signal transduction, receptor lateral diffusion is necessary to bring agonist-occupied receptors into contact with and activate G-proteins. Prior to desensitization, continued receptor movement results in amplification of the response. Signal transmission downstream of effector enzyme activation results in receptor immobilization, probably through phosphorylation and cytoskeletal association, prior to receptor internalization which ultimately abrogates the stimulatory signal at the level of the membrane. Receptor lateral movement is thus integral to hormonal stimulation and signal amplification, with receptor immobilization a key initial step in desensitization subsequent to agonistic activation.

Acknowledgments

Profs. R. Peters and F. Fahrenholz are acknowledged for past support.

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Received March 21, 1995