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Mechanisms of receptor-mediated transmembrane signalling

by M.D. Hollenberg

Endocrine Research Group, Department of Pharmacology and Therapeutics, University of Calgary, Faculty of Medicine, 3330 Hospital Drive N.W., Calgary (Alberta T2N 4N1, Canada)

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Receptors, acceptors, channels and the problem of transmembrane signalling

Fundamental to the successful function of any multicellular organism is an efficient communication system that can convey information from one cell to another. Although the overall function of the cell membrane is to maintain an effective barrier between the intracellular and extracellular milieu, highly specialized membrane structures (e.g. ion channels, nutrient transporters, histocompatability determinants) can be singled out as playing particularly pivotal roles in terms of selectively transmitting information from the external to the internal cell environment (and in some cases, vice versa). Over the

past decade there has been much progress in the biochemical and pharmacologic characterization of the membrane constituents that participate in the transmembrane signalling process. This chapter will deal in general with selected aspects of transmembrane signalling and will focus in particular on the plasma membrane-localized processes used by pharmacologic receptors.

In some cases, information is transferred simply by the selective uptake of specific ions or nutrients via transmembrane channels. The energy for this transfer process may come either from a chemical concentration gradient of the transported ligand or from a coupled process in which cellular energy (e.g. in the form of ATP) is used to 'drive' the ion or metabolite across the membrane. In such cases, the ion or metabolite *is* the message that is being communicated to the intracellular space, and it is up to the intracellular milieu to respond to increased (or decreased) concentrations of the transported substance and to regulate the rate (or amount) of transport that may occur. It is now evident that the membrane constituents that act as selective transport molecules are highly complex proteins that may be subject to the same kind of allosteric regulation as are cellular enzymes. The biochemical and pharmacologic characterization of the voltage-sensitive sodium channel, with its complex toxin binding sites[7, 34a], may serve as a useful model for the characterization of channels for other ions and metabolites.

In a number of cases, substances that convey information for the regulation of intracellular processes (for example, vitamin B_{12} or cholesterol) are associated with carrier proteins in the blood stream (e.g. transcobalamin-II for B_{12}; low density lipoprotein, or LDL for cholesterol). The blood-borne carrier proteins can be recognized in a very specific high-affinity manner by specialized transporters responsible for the selective adsorptive pinocytosis of the regulatory ligands (the examples being used here are B_{12} and cholesterol). Here again, as in the case of the sodium ion, the ligand transported (i.e. cholesterol or B_{12}) *is* the message being taken into the cell. The binding constituents (either the carrier proteins or the cell-surface uptake proteins) perform a message-carrying function and do not, of themselves, generate a transmembrane signal. Thus, the binding constituents for substances like the LDL-cholesterol complex[3, 4] or the transcobalamin-II-B_{12} complex[32, 48] while commonly referred to as 'receptors', do not truly function as receptors in the pharmacologic sense. It has proved convenient to refer to such highly specific transporters either as 'acceptors'[17] or receptors of the class II type[25].

In contrast to acceptors, pharmacologic receptors located in the plasma membrane exhibit not only the ability to recognize a ligand with high affinity and selectivity, but also the capacity, once combined with the specific ligand, to participate in the process of cell activation. It is this dual recognition-activation property, by which the receptor per se acts in part as a message-generating system, that distinguishes receptors from other cell surface recognition/transport constituents. The remainder of this article will focus on the general mechanisms whereby cell surface receptors generate a transmembrane signal. Receptors for steroid hormones will not be discussed.

General mechanisms of transmembrane signalling

1. Basic mechanisms. In order to fulfill their recognition/activation function, membrane receptors must be able to generate an intracellular signal that can be greatly amplified. Based on observations made over the past decade, it is possible to generalize somewhat concerning the mechanisms employed by receptors for transmembrane signalling. Structural data obtained to date indicate that those constituents capable of generating a transmembrane signal actually have transmembrane domains that can either communicate with (e.g. via an ion channel) or interact with the intracellular/submembraneous environment. Thus, a physical contiguity between an external and internal receptor domain may turn out to be a prerequisite for a transmembrane signalling molecule. Constituents that exist solely on the external aspect of the plasma membrane may be able to generate intracellular signals only via an interaction with a second membrane-localized component that possesses a transmembrane domain.

The mechanisms whereby the transmembrane receptor domain generates the intracellular signal may turn out to be few in number. Based on data accumulated to date, one can single out three basic processes, as illustrated in figure 1: a) ligand-modulated ion channel activity, b) ligand-regulated enzymatic activity, with the catalytic domain situated in the intracellular portion of the receptor and c) ligand-regulated liberation of cryptic mediators via interactions of intracellular receptor domains with other submembraneous constituents like the so-called G- or N-regulatory components of the adenylate cyclase system (see below). The process of receptor internalization, whereby the receptor-bound ligand finds its way into intracellular organelles[36, 37], provides an intriguing mechanism whereby one of the three basic reactions performed by receptors can be translocated to a targeted intracellular environment. A key element of receptor function, irrespective of which of the above cited mechanism is used, is the basic requirement of the ligand (or its surrogate) to act as an allosteric regulator of the receptor mechanism.

2. Signal amplification. A fundamental question to answer is: how does the cell amplify the signal generated by each of the three basic processes outlined above? Fortunately, information acquired to date provides a number of answers to this question for each basic mechanism. For instance, a change in membrane potential, resulting from a ligand-induced change in ion flux (panel A, fig. 1) would, as elaborated upon by Zierler and colleagues[56, 57], have a profound effect on the orientation of many membrane proteins, thereby rapidly generating an overall intracellular signal. The voltage-sensitive sodium channel[7, 34a] provides an interesting example of the effect of membrane potential on membrane protein function, with consequent signal amplication. Alternatively, a ligand-regulated channel for an enzyme-regulatory cation like calcium could rapidly control intracellular calcium-modulated enzymes that participate in cascade reactions vital to cell regulation. In principle, ligand-modulated channels for either anions or cations could lead to such amplified intracellular signals. The distinction between the receptor channel activity and the activities of other

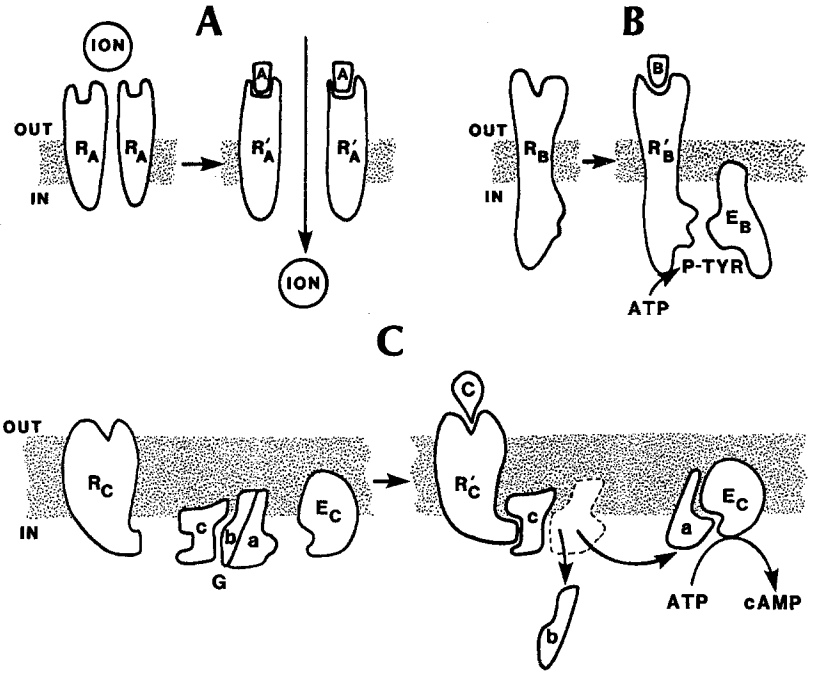

Figure 1. Hypothetical models of ligand-activated transmembrane signalling using three basic mechanisms. *A* Ligand-regulated ion channel, like the nicotinic cholinergic receptor. *B* Ligand-regulated enzyme activity. Receptor tyrosine kinase is used as the example, wherein phosphorylation of a membrane effector, E_B plays a role in signalling. *C* Ligand-modulated release of cryptic mediators. Upon interaction with a ligand-occupied receptor, a hypothetical guanine nucleotide regulatory complex (G) is shown to release an effector-stimulatory component (a) and at least one other regulatory protein (b). In this instance, adenylate cyclase is depicted as the effector (E_C). In principle, any other membrane process (ion channel, phosphodiesterase, phospholipase) could be similarly regulated. Models are shown for receptors in either the absence (R_A, R_B, R_C) or presence (R_A', R_B', R_C') of their regulatory ligands (A, B, C.). None of the components are drawn to scale. The extracellular (out) domains of the receptors face the top of the page; the intracellular (in) receptor domains, anchored in the membrane (stippled areas) face the bottom of the page.

ion/metabolite channels rests entirely on the ligand-regulated properties of the receptor channels. The nicotinic cholinergic receptor represents the best understood ligand-regulated channel of this kind[9, 30, 31, 34, 52].

It is comparatively easy to visualize how the second basic receptor process, ligand-regulated enzymatic activity could yield an amplified receptor signal. The discovery that receptors for epidermal growth factor-urogastrone (EGF-URO)[5, 6], insulin[26, 40, 41, 55] and platelet-derived growth factor (PDGF) are ligand-regulated tyrosine kinases (for a brief review, see ref. 19), indicates that phosphorylation/dephosphorylation cascade reactions, known to play a vital role in many metabolic pathways, probably also play a central role in receptor function. Such cascade reactions, initiated by ligand-triggered tyrosine kinase activity (panel B, fig. 1) could readily amplify the initial receptor stimulus. Apart from kinase activities, any other enzymatic activity (e.g. proteolytic activity; phospholipase activity) could, in principle, be used by a receptor system to initiate a cascade signal amplification process. To date, there is not yet evidence for or against the existence of receptors with such intrinsic enzymatic activities. Such ligand driven enzymatic processes could readily generate compounds (e.g. peptides; diacylglycerol; archidonic acid) that might serve as intracellular 'second messengers'. It will be of great interest in the future to look for receptors with intrinsic enzymatic activities other than that of the kinase class.

Those receptors that regulate the production of the second messenger, cyclic AMP, represent well-known examples of receptors that utilize the third basic process outlined above. Only recently has it been appreciated that the catalytic activity of the adenylate cyclase system is not controlled directly by the receptor itself, but rather by an indirect process, whereby the receptor liberates a separate cyclase-regulatory polypeptide (so-called α_s[15, 35]). The liberation of such cryptic regulatory polypeptides from the so-called guanine nucleotide binding regulatory proteins (G-proteins or N-proteins[15, 39]) can provide for the concerted regulation of a variety of cell activities apart from adenylate cyclase. A generalized case for this mechanism is illustrated in panel C of figure 1. The oligomeric structure of the G-proteins (known to contain α, β and γ subunits) and the discovery of several distinct -substituents[35] provides for enormous flexibility in the use of such a transmembrane signalling process. In terms of the cyclic AMP system, the amplification reactions involving cyclic AMP-dependent protein kinases have been widely studied. This represents perhaps the best-understood (biochemically) receptor-mediated signalling system. However, because the common β-substituent can combine with (and presumably regulate) a variety of the family of α-substituents, and because the metabolic functions of the α-subunits other than the cyclase-regulatory α_s-substituent have yet to be determined, it appears likely that the cyclase system may represent only the 'tip of the iceberg' in terms of the regulation of cell function by this third distinct basic receptor-signalling process. One looks forward eagerly to interesting developments in this area of study.

3. Messengers. As pointed out in an earlier section, in the situation where information transfer is mediated via ion/metabolite channels, the ion itself represents 'the message'. In contrast, receptor-mediated information transfer involves the generation of at least one, and perhaps multiple diffusible messengers. In one sense, for the action of hormones like insulin, the receptor per se can be called the 'second messenger' (the hormone itself may be thought of as the 'first messenger' in the communication chain leading from hormone binding to cellular activation). For some time, cyclic AMP was designated as a 'second messenger' for the action of hormones like glucagon or epinephrine. Now, however, it can be appreciated that the so-called α_s-stimulatory subunit of the cyclase-regulatory guanine nucleotide binding complex[15,35] also participates as a messenger in the course of the action of either glucagon or epinephrine. Further, it is not unlikely, as elaborated upon in a subsequent section, that a single hormone-receptor combination may initiate not one, but perhaps many membrane-localized reactions. Thus, it may be inappropriate to single out any particular diffusable low-molecular-weight compound as a primary messenger for any hormone; rather, it may be more fruitful to think in terms of a characteristic matrix of diffusable messengers generated by the combination of a single hormone with its receptor. Compounds that have been singled out for particular attention in terms of transmembrane signalling processes are: 1) Sodium ion (nicotinic cholinergic receptor); 2) Cyclic AMP (cyclase-related hormones); 3) Cyclic GMP (several neurotransmitter receptors; including the muscarinic cholinergic receptor); 4) Calcium (for neurotransmitter receptors, including muscarinic cholinergic as well as for other peptide receptors like those for angiotensin and pancreozymin); 5) diacylglycerol; 6) inositol trisphosphate; 7) α-substituents of the G-protein complex[15,35]; and 8) as yet unidentified peptide regulators related to the action of insulin[23,28,43,47]. It is important to note that in the case of many neurotransmitters and hormones, not one, but three of the above identified 'messengers', namely calcium, diacylglycerol and inositol trisposphate may be involved in a complex bifurcating signal pathway, involving the hydrolysis of membrane phosphoinositides[1]. A critical factor in understanding the chain of information transfer via the receptor to the cell relates to the identification of the key targets of the messengers, viz. the protein kinases regulated by cyclic AMP; kinase C regulated by diacylglycerol[33]; intracellular calcium-binding proteins from which calcium is released in the presence of inositiol triphosphate[1]; and calcium-modulated regulatory enzymes or proteins like calmodulin that may have a widespread effect on overall cell regulation. Possibly, only a handful of such messengers, generated via membrane-localized reactions will be involved in the action of most neurotransmitters and hormones.

Receptor dynamics and transmembrane signalling

1. *Receptor mobility and transmembrane signalling.* In the not-too-distant past, possibly due to the focus of pharmacological studies on nerve-muscle preparations, receptors were thought of as specifically localized entities (e.g. at the neuromuscular junction) that were tacitly assumed to be in a more or less static state as a consequence of cell differentiation. Now, however, it is realized that in perhaps the majority of cases, receptors for agents such as insulin and epidermal growth factor-urogastrone (EGF-URO) are dynamic cell surface constituents that can migrate in the plane of the cell membrane. Both pharmacologic receptors and acceptors (like the one for LDL-cholesterol) appear to share this mobile property. In studies with cultured cells (e.g. mouse fibroblasts) it has been observed that subsequent to the binding of a ligand-like insulin, a receptor (or acceptor) can undergo a complex series of protein-protein interactions leading to the cellular internalization and intracellular processing (e.g. degradation; or possibly recycling to the cell surface) of both the receptor and the bound ligand[27,36,37]. The concept of a hormone receptor as a 'mobile' or 'floating' membrane constituent evolved along with the development of understanding of the general properties of cell surface proteins. In terms of hormone-triggered transmembrane signalling, receptor mobility is viewed as a most important property that can enable the receptor to interact with a variety of membrane constituents in the course of cell activation. It is a basic tenet of the 'mobile' or 'floating' receptor paradigm of hormone action[2,10-12,17,22] that the binding of a ligand dramatically alters the ability of a receptor to migrate in the plane of the membrane and to interact with other membrane components. Thus, the entity, [HR], resulting from the combination of a hormone with its receptor:

$$[H]+[R]\rightleftharpoons[HR]$$

can go on to form ternary complexes, of the kind, [HRE]:

$$[HR]+[E]\rightleftharpoons[HRE]$$

wherein E represents an effector molecule involved in the process of cell activation. The hormone-receptor complex may also undergo an isomerization reaction ($[HR]\rightleftharpoons[HR^*]$) as has been suggested for the muscarinic receptor[50a]. A number of variations of this model have been developed[22]. For instance, the above equations illustrate an 'association' model, wherein the formation of hormone-receptor complex promotes receptor-effector coupling. An alternative possibility is a 'dissociation' model, wherein an inactive effector, held in a receptor-effector complex, RE is dissociated when the ligand binds, to yield a free active effector, E*:

$$H+RE\rightleftharpoons HR+E^*$$

In principle, the mobile receptor model does not restrict the number of distinct effector moieties with which the ligand receptor complex might interact. In this manner, by interacting with multiple effectors, a single hormone receptor complex could liberate simultaneously a variety of intracellular mediators.

2. Receptor microclustering, patching and internalization. Largely stimulated by the 'floating' or 'mobile' receptor model, summarized in the previous section and heralded by the work of Edidin and collaborators[14], much work has focussed on measurements of receptor mobility. With the use of fluorescent ligand probes (insulin, EGF-URO) lateral diffusion coefficients in the range of 5×10^{-10} cm^2/s have been observed[27,36,44]. Studies using radiolabeled ligands or ligands tagged with heavy metals (gold; ferritin) have also visualized either preclustered or ligand-induced receptor clusters in the cell membrane. The speed of re-

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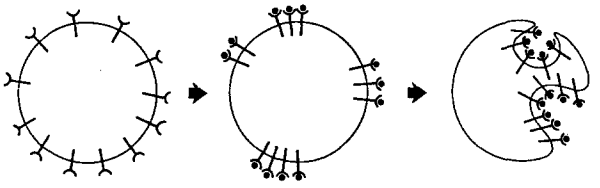

Figure 2. Microclustering and patching of receptors: early events related to cell activation. The scheme illustrates the ligand-mediated microclustering and patch formation processes described in the text. Upon going from the predominantly diffuse distribution (hormone-free state) to the ligand-occupied microclustered state, it is thought that in many cells, such as fibroblasts, ligand-receptor complexes coalesce in coated pit regions prior to cellular internalization. It is important to note that in some cell types receptors may exist in a preclustered state and that in certain cells, receptor internalization can occur predominantly at sites distinct from the coated pit region[50].

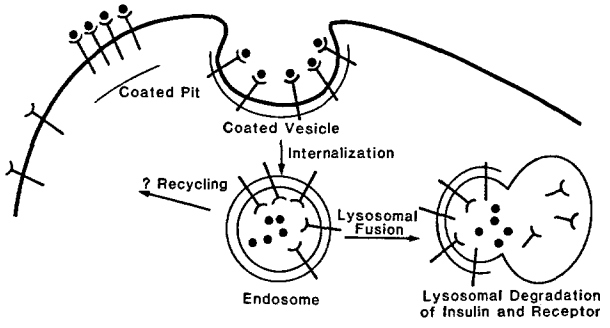

Figure 3. Formation and migration of receptor-bearing vesicles. The aggregated receptors are thought to become trapped in an endocytic vesicle that buds inward, so as to form an intracellular vesicle, the endosome (or receptosome). Evidence suggests that the internal environment of the endosome becomes acidified, favoring the dissociation of many ligands, e.g., insulin, from their receptors. After the initial internalization event, the receptor-bearing vesicles are thought to change their shape (possibly by fusing with other nonlysosomal intracellular constituents) and to migrate to a variety of cellular locations. Two further possibilities are also depicted: fusion with lysosomal structures and recycling of the receptor to the cell surface.

ceptor diffusion is sufficient to allow the ligand-occupied receptor to collide with many other membrane components in a very short time period (e.g. tens of milliseconds).

From observations with a number of ligands, it appears that many receptors follow a common sequence of mobile reactions subsequent to the ligand binding event (fig. 2). In the absence of their specific ligands, receptors can be diffusely distributed over the cell surface. However, as illustrated in figure 2, at physiological temperatures, the binding of a ligand can lead to a rapid microclustering (receptor microclusters, containing perhaps 2 to 10 receptors) and a reduction in receptor mobility, accompanied by the progressive aggregation of ligand receptor complexes into immobile patches (aggregates containing 10's to 100's of receptors) that can be visualized by fluorescence photomicrography. In cultured fibroblasts, the microclustering event is thought to preceed the formation of patches that can be seen in the fluorescence microscope. In terms of the process of transmembrane signalling, to be discussed below, the microclustering event (formation of n-mers containing groups of 2 to 10 receptors) is viewed as a phenomenon related to, but separate from the aggregation process (formation of patches, containing 10's to 100's of receptors). Subsequent to the formation of the comparatively large receptor aggregates, the ligand-receptor complexes can be either shed into the medium or taken into the cell (internalized). Receptor internalization appears to be an ongoing process that is accelerated when a ligand such as insulin binds to its receptor. It is not clear whether or not receptor occupation is a prerequisite for forming small receptor clusters in all cell types. For instance in adipocytes[50], there are data to indicate that insulin receptors exist as clusters prior to the addition of insulin. The mechanism(s) that lead to microclustering, aggregation and internalization of receptors (or acceptors) are poorly understood. In many cells, such as fibroblasts, internalization appears to occur at specific sites on the cell surface – the so-called 'bristle-coated pit'[36, 37]. In some cell types (e.g. adipocytes) receptors may be localized and internalized at sites other than the 'coated pit' regions[50]. Subsequent to aggregation, the receptor can be internalized via an endocytic process into a cellular compartment that appears to be distinct from the lysosome (fig. 3). The intracellular receptor-bearing vesicles, which in contrast with lysosomes are not phase-dense in the electron microscope and are acid phosphatase negative, have been termed 'endosomes' or 'receptosomes'[36]; the latter term emphasizes the role of these specialized endocytic vesicles in the process of receptor-mediated endocytosis. One possible fate of such receptor-bearing endosomes is fusion with lysosomes, followed by the lysosomal degradation of the receptor (so-called receptor processing) and of the bound ligand. Several studies suggest that limited receptor processing may also occur at a prelysosomal site (possibly, endosome-associated). An alternative route that the receptosome may follow leads back to the cell surface via a recycling process that reintegrates the receptor into the plasma membrane. A possible fusion of the receptosome with other intracellular organelles (e.g. nuclear envelope) cannot be ruled out, but has yet to be documented. At present, little is known about the factors that control either the internalization process or the trafficking process that may lead on the one hand to lysosomal receptor processing or, on the other hand, to a recycling of the receptor back to the cell surface (fig. 3). The intracellular receptor domains may play an important role in this trafficking process. There is also little known about the possible role(s) for the degradation products (ligand or receptor fragments) that may be released into the cytoplasma as a result of the endosomal and lysosomal degradation (processing) events. In view of the paripatetic nature of the hormone-receptor complex, migrating from the cell surface to the cytoplasmic space, a key question to answer is: What role (if any) do these receptor-migratory pathways play in the process of transmembrane signalling? The following sections will deal with this question.

Receptor microclustering and cell activation

It has been appreciated for some time that ligand bivalency (or multivalency) is critical for the patching and capping of surface macromolecules[38]. Only comparati-

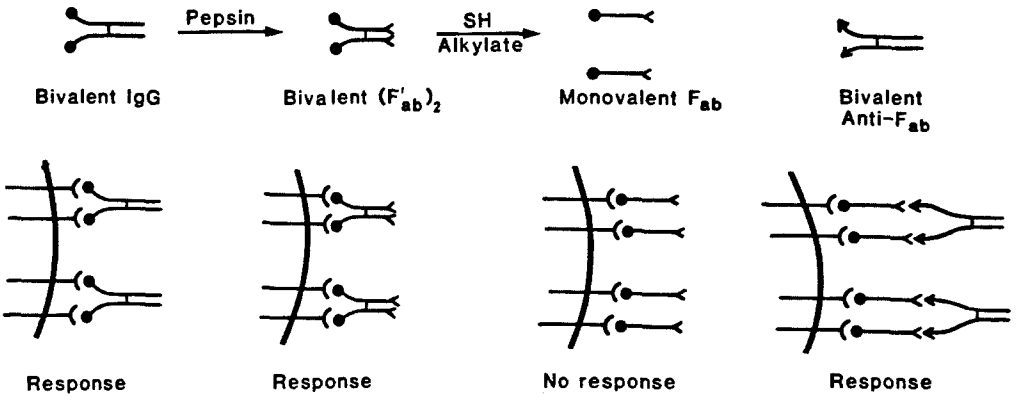

Figure 4. Effects of divalent and monovalent antireceptor antibodies. These results were obtained with polyclonal anti-insulin receptor antibodies[24]. Intact antibody (IgG) and the bivalent antibody derivative generated by pepsin cleavage $(F'_{ab})_2$ were capable of stimulating cells. However, the monovalent antibody derivative (F_{ab}) produced by reduction (SH) and alkylation of the $(F'_{ab})_2$ species did not generate a cellular response. Nonetheless, crosslinking of the receptor-bound F_{ab} fragments by bivalent anti-F_{ab} molecules yield a cellular response. Results akin to these have been obtained via a totally different approach using derivatives of EGF-URO and of LHRH (see text). A role for receptor microclustering in generating a cell response has thus been postulated[24].

vely recently, however, has it become apparent that pharmacologic receptors can patch and cap, and that crosslinking (or microclustering) may be a key event in the process of transmembrane signalling. Some of the most impressive data implicating receptor crosslinking as a key event have come from experiments using polyclonal anti-insulin receptor antibodies (derived either from insulin-resistant patients[24]; or from rabbits immunized with purified insulin receptor[27]).

In brief, the results with the polyclonal anti-receptor antibodies are summarized in figure 4. Both intact antibody (IgG) and the bivalent $(Fab)_2$ derivative were capable of stimulating cells. However, the monovalent Fab derivative was not biologically active even though it acted as a competitive inhibitor of insulin binding[24]. Strikingly, the biological activity of the monovalent Fab fragment was restored by the addition of a second bivalent antibody directed against the Fab fragment (fig. 4). In essence, the crosslinking of the receptor-associated antibody Fab binding domain appears to be required for cell activation. The anti-insulin receptor antibodies are capable of mimicking many of the actions of insulin in target cells[24]. Thus, the work with the antireceptor antibodies led to two conclusions: 1) the receptor alone and not the ligand (e.g. insulin) possesses the information required for transmembrane signalling and 2) receptor microaggregation appears to be a key event related to transmembrane signalling. As mentioned above, the mechanism(s) whereby the binding of an agent like insulin leads to receptor crosslinking are as yet poorly understood.

Evidence implicating receptor microclustering as an important event for cell activation has now been obtained for other peptide hormones (EGF-URO and luteinizing hormone-releasing-hormone: LHRH or GnRH). In one series of experiments, it was observed that a chemically modified derivative of epidermal growth factor-urogastrone (CNBr-EGF-URO, see below), that of itself bound to the EGF-URO receptor but was not mitogenic, became mitogenic when aggregated by anti-EGF-URO antibody[49]. In other work, studying the action of luteinizing hormone releasing hormone (LHRH, also called gonadotropin-releasing hormone or GnRH), two independent laboratories[8, 16, 20] have observed that LHRH antagonists (biologically nonstimulatory derivatives that bind to the receptor and that block the LH-releasing activity of intact LHRH) can be caused to stimulate LH release from pituitary cells in the presence of crosslinking antibodies. The ability of ferritin derivatives of LHRH to cause the microclustering and internalization of LHRH receptors has also been observed at the electron microscopic level[20]. Thus, the microclustering event, associated with cell activation, is probably involved in the activation of a variety of cells by a number of hormones, including insulin.

In the adipocyte, the exact relationship between insulin-mediated receptor microclustering and cell activation is not entirely clear. Since the initial work with antiinsulin receptor antibodies[21, 24] monoclonal antibodies directed against the human insulin receptor have been developed[42]. Because of their nature, the monoclonal bivalent IgG antibodies are thought to react with a single receptor locus, whereas the polyclonal IgG antibody preparations previously used (derived both from insulin-resistant patients and from receptor-injected rabbits) would presumably contain IgG molecules capable of interacting at several receptor loci. In constrast to the polyclonal antibodies for which the results were described above, one of the monoclonal anti-insulin receptor antibody preparations that has been described[42] blocks insulin receptor binding, but does not itself possess intrinsic insulin-like activity in human adipocytes. The lack of response to the monoclonal antibody, which can crosslink the insulin receptors into dimers, is not well understood. Possibly, receptor aggregates containing more than two insulin receptors are required for signalling. Further work will be required to resolve the apparent discrepancies between the observations with the polyclonal and monoclonal antibodies, in terms of the requirement of insulin receptor microclustering for adipocyte activation. Other observations that relate to the question of receptor microclustering and adipocyte activation have been summarized[50]. In brief, studies with insulin-ferritin reveal that a substantial proportion of adipocyte insulin receptors appear to be present in groups of two or more prior to

insulin binding. Furthermore, the presence of insulin-ferritin does not lead to an increase in the degree of receptor clustering. These results can be contrasted with the effect of ferritin-LHRH, which clearly caused the microaggregation of previously dispersed LHRH receptors in anterior pituitary cells[20]. Thus, although receptor microaggregation may be a general phenomenon associated with cell activation, the exact mechanism whereby the preclustered insulin receptors in adipocytes are triggered upon occupation by either polyclonal anti-receptor antibody or by insulin (or the insulin-ferritin derivative) remains to be determined. Taken together, the data suggest that the receptor may not only have to be clustered but that in addition, the receptor may also require a special conformational perturbation (e.g., caused only be insulin or by a subset of antireceptor antibodies) to generate the reaction that leads to cell activation. In summary, receptor clustering may be necessary but not necessarily sufficient to initiate a response in target cells for insulin and other hormones.

Internalized receptor and cell activation

It is recognized that agents like insulin or EGF-URO can cause a wide spectrum of cellular responses, ranging in time course from the immediate (seconds to minutes) stimulation of membrane transport (e.g. glucose, amino acids) to the much delayed (hours to tens of hours) effects on DNA synthesis and cell division. How, one may ask, might the receptor dynamics discussed in this article bear on the varied time courses of the multiple actions of insulin, EGF-URO and other hormones? In part, the answer to this question comes from work in fibroblast cell culture systems with the mitogenic/acid-inhibitory polypeptide, epidermal growth factor-urogastrone (EGF-URO)[5,6,18,45,46]. Under normal circumstances, EGF-URO can, like insulin, activate a large number of cell responses that occur over periods of seconds to minutes (e.g., stimulation of membrane transport and inhibition of acid secretion), up to tens of hours (e.g., stimulation of RNA and DNA synthesis and cell division). A chemical derivative of mouse EGF-URO has been prepared in which the molecule has been cleaved at methionine residue 21 using cyanogen bromide (CNBr). This cleavage results in an opening of one of the disulfide-maintained loops of the EGF-URO molecule. The derivative, denoted CNBr-EGF, still binds to the receptor, but is unable to stimulate DNA synthesis and cell division[49]. Some of the results obtained using CNBr-EGF have already been discussed above. It has also been observed that although microclustering presumably still occurs when CNBr-EGF binds to the receptor, the CNBr-EGF derivative is unable to cause gross aggregation of the EGF-URO receptor. Nonetheless, the nonmitogenic CNBr-EGF derivative is still able to simulate a number of the rapid cellular responses caused by intact EGF-URO (e.g., stimulation of ion flux and induction of morphological changes). The data obtained with CNBr-EGF have been considerably amplified by work with monoclonal antibodies directed against the EGF-URO receptor[45,46]. In brief, those antibodies capable of causing receptor aggregation (and, consequently, internalization) were able to mimic both the short-term and well as the long-term (mitogenesis) effects of EGF-URO. However, monovalent Fab antibody derivatives that were not capable of inducing receptor aggregation and that were unable to stimulate DNA synthesis, were nonetheless capable of stimulating an early event (membrane phosphorylation) associated with EGF-URO action. A number of hypotheses have thus been put forward concerning the possible role(s) of aggregated receptor and of internalized receptor or internalized receptor fragments in the long-term processes of cell growth or cell differentiation[27]. The short-term membrane-localized reactions may be related to the receptor microclustering process.

As discussed above, it is now realized that in many cell types appreciable amounts of both a ligand-like insulin and its receptor can be taken up and degraded by insulin-responsive cells. Furthermore, there is evidence that binding sites for insulin can be found on Golgi elements, on smooth and rough endoplasmic reticulum[37], as well as on the nuclei of selected cells. Thus, it has been suggested that an internalized degradation product either of insulin or of the insulin receptor may act at an intracellular site to cause at least some of the biological responses triggered by insulin. A role for internalized insulin or insulin fragments appears to be ruled out, in view of the remarkable effects of the anti-receptor antibodies and in view of the stimulatory actions of macromolecular insulin derivatives (e.g., insulin-Sepharose) that cannot enter the cell. Nonetheless, it is quite possible that there is a role for internalized receptor in directing some of the delayed actions of insulin (e.g., gene regulation and stimulation of cell division). Similarly, a role for internalized receptors (or receptor fragments) must be considered for agents like EGF-URO.

In keeping with the data that have been described in connection with EGF-URO action, it is possible to speculate on a series of events that may occur during the course of insulin-mediated cell activation. Although very hypothetical, the scheme proposed here (fig. 5), derived largely from experiments done with cultured fibroblasts, places in a feasible context both the receptor dynamics described in this article and the intrinsic kinase activities of the insulin and EGF-URO receptors that have recently been described in work from a number of laboratories. (The insulin and EGF-URO receptors can become phosphorylated and may also phosphorylate other cellular substrates.) It must also be kept in mind that the scheme presented is a generalized picture that synthesizes information obtained from a number of cultured cell systems using several peptide hormone probes. For a specific ligand such as insulin acting on a particular cell type (e.g., the adipocyte), some but not all of the processes outlined in the generalized scheme may in fact occur.

It is quite likely that the rapid actions (seconds to minutes) of insulin, EGF-URO and other agents are caused by biochemical reactions in which the receptor participates within the plane of the plasma membrane. The initial receptor microclustering event probably represents a critical process that is directly related to these membrane-localized reactions. One exciting possibility is that the intrinsic tyrosine kinase activity of the receptors may initiate some of these key reactions. As indicated above, the rapid modulation of cellular activity may require a special receptor conformation that would permit

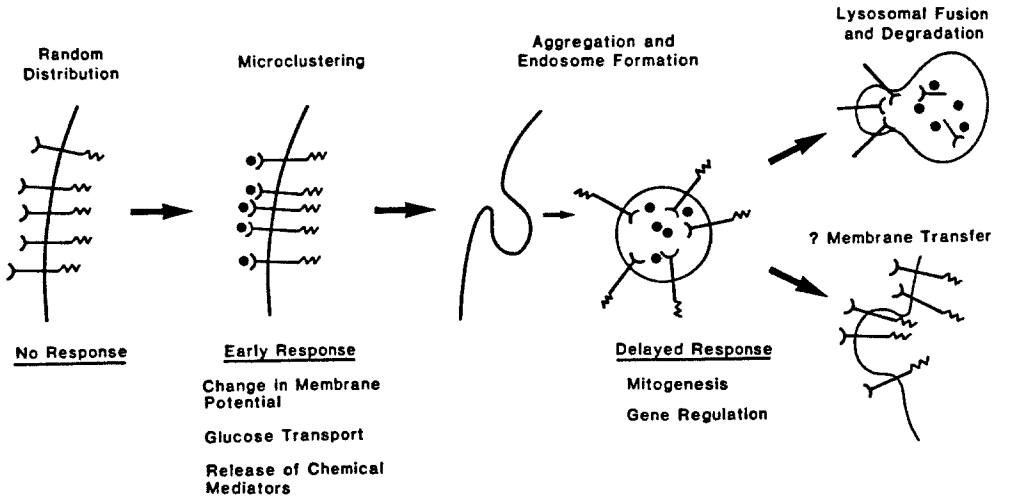

Figure 5. Receptor dynamics and cell activation. As discussed in the text, cell respones that are rapidly regulated probably involve the initial microclustering event. Delayed effects may be caused by a receptor that is internalized in the endosomal organelle. The topography of the endosome would permit the intracellular portion of the receptor (zigzag line) to interact with a variety of intracellular constituents located at considerable distances from the plasma membrane. In the course of its intracellular migration, the receptor-bearing endosome could ultimately fuse either with the lysosomes or with other membrane structures (e.g., Golgi elements or nuclear membranes), resulting in a further relocation of the receptor.

effective interactions between the receptors that associate in microclusters. In this context, the ligands (insulin, EGF-URO) can be viewed as essential allosteric regulators that can modulate membrane-localized reactions (and thereby cell response) on a minute-to-minute basis. Cells would thus be very responsive to extracellular changes in hormone concentrations.

Once internalized, however, the receptor would no longer be exposed to the variations in ligand concentrations that might occur in the extracellular milieu. Thus, the time course of reactions in which the internalized receptor might participate could differ considerably from the time frame of those reactions occurring in the plasma membrane. One role for the internalized receptor may be to regulate some of the delayed effects (hours to tens of hours) that a ligand may have on its target cells.

As hypothesized in figure 5, the internalized receptor-bearing endosome or 'receptosome'[36] may do more than simply function as a way station for the receptor, en route either to the lysosome or, via recycling, back to the cell surface. Rather, the endosome may function as a site-directed receptor-kinase-bearing vesicle that may regulate enzyme activity by phosphorylation reactions that could occur in regions quite distant from the cell surface. In addition, by membrane fusion, the endosome may even transfer the receptor to a new membrane environment, such as the nuclear membrane. Since the endosome-associated receptors are subject to inactivation by lysosomal degradation, a constant influx of fresh receptor-bearing endosomes sustained over a prolonged time period may be required to bring about some of the delayed cellular effects of a ligand like EGF-URO or insulin. The essence of the above discussion is that the temporally distinct actions of certain ligands may relate directly to the topographically distinct receptor dynamic events that, subsequent to ligand binding, occur over quite different time frames. In this context, the continued internalization of receptor may play a key role in the generation of the delayed effects of insulin, EGF-URO and other active ligands. Thus, transmembrane signalling would have two 'tiers' and two associated time frames.

Summary

Although this paper has dealt with general mechanisms whereby a hormonal signal is transmitted across the cell membrane, advances in work with a number of receptors should permit a precision of description of these mechanisms that would have delighted both Langley and Ehrlich. For instance, the detailed sequences now known for the separate subunits of the nicotinic cholinergic receptor[9, 30, 34, 52] and the cellular manipulations made possible by the cloning of the separate subunit genes[31, 51] will make it possible to determine the precise receptor sequence involved either in acetylcholine binding or in ion channel function.The complete sequences and biochemical properties now known for the insulin and EGF-URO receptors[13, 53, 54] to be dealt with in part by a subsequent article (van Obberghen and Gammeltoft, this series) should lay the groundwork for elucidating the transmembrane signalling mechanisms used by the kinase family of growth factor receptors. Continuing work on the structure of the β-adrenergic receptor[29], and on the interaction of such receptors with guanine nucleotide regulatory complexes and on the detailed properties of the family of so-called G-proteins and their associated regulatory subunits[15, 35, 39] should unravel the details for a variety of transmembrane signalling reactions. Thus, at least for three basic transmembrane signalling mechanisms: ligand modulated ion transport; ligand-modulated receptor enzyme activity (e.g. tyrosine kinase); and ligand-modulated liberation of cryptic mediators (like the α- and β-subunits of the guanine nucleotide regulatory complexes) one can look forward with excitement to the elucidation in the not-too-distant future of a number of specific biochemical reaction pathways that lead to cell activation.

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Insulin receptors: Structure and function

E. Van Obberghen and S. Gammeltoft

INSERM U 145, Faculté de Médecine (Pasteur), Université de Nice, Avenue de Vallombrose, F-06034 Nice Cedex (France), and Dept Clinical Chemistry, Rigshospitalet, University Hospital, Blegdamsvej 9, DK-2100 Copenhagen (Denmark)

Key words. Insulin receptors; subunits; phosphorylation; serine kinase; tyrosine kinase.

Introduction

More than fifty years after the discovery of insulin, its cellular mechanism of action still remains one of the major obstacles in cell biology. Recent progress in the molecular characterization of the insulin receptor itself due to concerted efforts in several laboratories have led to important discoveries. One of these, the kinase activity and autophosphorylation of the insulin receptor, will be reviewed and its putative role in insulin action discussed. Furthermore, conditions with cellular insulin resistance are coupled with decreased phosphorylation of the insulin receptor, giving a clue to a molecular defect in the disease states.

The molecular mechanism of insulin action

Regulation of cellular metabolism and growth by insulin is a result of a series of events initiated by the interaction of the hormone with specific cell surface receptors (fig. 1). In the past, insulin receptors on a large number of cell types have been characterized in detail by their structure and function[33, 38, 75]. This achievement is based on the development and application of a variety of biochemical methods including kinetic analysis for description of the receptor binding[16]; affinity labeling technique for identification of the receptor subunits[10]; and recently, recombinant DNA technology for the elucidation of receptor amino-acid sequences[13, 73]. In spite of this progress, the

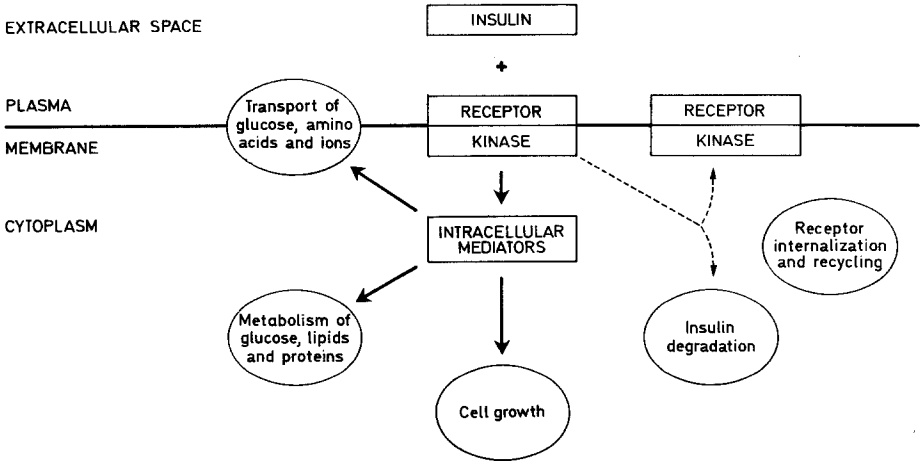

Figure 1. Cellular mechanism of insulin action. The receptor-kinase complex in the plasma membrane transmits the intracellular insulin signal to intracellular mediators e.g. phosphoproteins, which stimulate transport of glucose, amino acids and ions, metabolism of glucose, lipids and proteins and cell growth. The receptor-bound insulin is internalized and degraded whereas the receptor is recycled to the plasma membrane.