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COMMENTARY

THE RECEPTOR CONCEPT IN EVOLUTION

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A proper understanding of drug action requires a molecular approach, since a bioactive agent can only induce a pharmacological effect in a biological object as the result of an interaction between its molecules and certain counterparts in the biological object. Consequently the chemical properties of a drug are determinant for its action and activity, and a relationship between chemical properties and action must exist [1]. Based on experiments with nicotine Langley [2] introduced more than 50 years ago already the concept of a receptor substance present in the biological object with which a drug has to interact in order to exert its biological effect. Since then the receptor concept and especially the views on drug-receptor interaction as a basis for drug action have evolved. Starting from a purely conceptual receptor idea its full materialization has been realized now with the isolation of the nicotinic acetylcholine receptor in several laboratories [3–13]. The existence of β-adrenergic receptors [14–17], muscarinic acetylcholine receptors [18–26], insulin receptors [27–29], estrogen receptors [30–33] and many others not to be mentioned separately have been firmly established on basis of direct binding studies with radioactively labeled ligands (see review articles [81–83]).

The molecular sites of action of drugs, i.e. those molecules with which the active agent must interact in order to induce the effect considered, are called the specific receptors. They are located in or on target cells, which are not necessarily the cells in which the effect is generated. The parameter, considered as effect, is to a certain degree arbitrary. The receptors for the convulsant agent strychnine, for instance, are located in the central nervous system, but the convulsions are generated in the striated muscle. Instead of the convulsions, as a matter of fact also the changes in the electroencephalogram may be measured as the effect.

The sequence of processes at the basis of drug action can be divided into three main phases: the pharmaceutical phase comprising the release processes of the active drug from its dosage form, thus determining the concentration available for absorption (the pharmaceutical availability); the pharmacokinetic phase comprising the processes that play a part in the absorption, distribution, metabolic conversion and excretion of the drug, determining the concentration of the active agent in the plasma and thus at the site of action (the biological availability); the pharmacodynamic phase comprising the interaction between the active agent and its molecular sites of action, initiating a stimulus, leading via a sequence of processes covering the transduction, amplification and modulation of the stimulus to the change in the parameter measured and considered as the effect [34, 35].

Sometimes the receptors are the active sites on enzymes, like for the MAO-inhibitors and acetylcholinesterase-inhibitors. In other cases receptors appear to be closely associated with enzyme action, possibly by allosteric changes in the enzyme molecule. Receptors further may be associated with functional macromolecules or macromolecular complexes, for instance particular lipoproteins, determinant for the properties of membranes. The various types of binding sites for bioactive agents that are not directly involved in the induction of the effect, such as those on plasma albumin, are often indicated as silent receptors or sites of loss. They play a role in the pharmacokinetic phase of drug action. The same holds true for the active sites on enzymes involved in the bio-activation or -inactivation of the drug.

Drug-receptor interaction as a rule is reversible and is much more dynamic than the classical key-and-lock model suggests. It implies a mutual moulding of drug and receptor by intermolecular forces. In the receptor molecule conformational changes are induced constituting the stimulus that triggers the sequence of events leading to the effect [36, 37]. In the case of enzyme-substrate interaction the changes induced in the substrate molecule are most prominent and determinant for the effect. Although highly dynamic, the receptors can yet be regarded as pre-existing specific structural entities since, for instance, the activities of optical isomers often differ to a large extent. However, the structural requirements for action are not always highly specific. For the various gaseous anaesthetics, for instance, a certain lipophilicity appears to be the only requirement. Their anaesthetic action is mainly based on a change in the cell membrane properties due to a diffuse accumulation in the lipid bilayer of the membrane. For plasma extenders and osmotic diuretics the term receptor is hardly applicable since they mainly act by binding of water [1].

Analogously to the differentiation between the active site on an enzyme and the enzyme, the molecule as a whole, it makes sense to distinguish between the receptor site and the receptor molecule—the receptor—bearing the receptor site. Enzymes can be isolated but not their active sites; similarly receptor molecules or macromolecular complexes may be isolated but not the receptor sites. Since structure-action relationship is based on the interaction between the drug molecule and the receptor site it may give information on the properties of the site but not on the receptor molecule as a whole.

Bioactive agents can be extremely potent. This implies that apparently only a few molecules of the

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active agent have to interact with the receptors to induce a massive response in which tremendous numbers of molecules are involved. This requires amplifier mechanisms. The simplest unit for assembling strong amplifier systems is the step in which one drug molecule activates one enzyme molecule which then converts hundreds of substrate molecules to product molecules [34]. Such an amplifier unit can be coupled to a second one if the product molecules in their turn can activate a second enzyme etc. Examples are the various agents which act by activation of adenylate cyclase and generation of cyclic AMP [38].

The activation process may imply an action of the bioactive agent as a co-factor. Examples are the vitamins and vitamin analogues that serve as co-enzymes for apoenzymes. The binding of steroid hormones such as estrogens to their cytosol receptor protein followed by migration of the active complex into the nucleus where it finally acts as a derepressor, too can be regarded as the combination of a "coderepressor", the steroid hormone, with an "apoderepressor", the cytosol receptor, under formation of the active derepressor.

In efforts to interpret the characteristics of dose–response curves and time–response curves of drugs on basis of the mass action law, a variety of partially overlapping theoretical models have been worked out part of which can be found in various reviews [39–55, 90–92]. The following model presents a condensate, covering essential aspects of various models.

$$[D] + [R] \underset{k_2}{\overset{k_1}{\rightleftarrows}} [DR] \xrightarrow{k_3} [DR^*] \xrightarrow{k_4} [D] + [\underline{R}]\,;\ [\underline{R}] \xrightarrow{k_5} [R] \qquad \text{(case I)}$$

$$[D] + [R] \underset{k_2}{\overset{k_1}{\rightleftarrows}} [DR] \xrightarrow{k_3} [D] + [R^*]\,;\ [R^*] \xrightarrow{k_4} [\underline{R}] \xrightarrow{k_5} [R] \qquad \text{(case II)}$$

The symbols represent: D drug; R receptive receptors; R^* activated receptors; $\underline{R}$ non-receptive receptors.

Case I "occupation" model, case II "Hit and run" model related to the "rate" model (47). The affinity is mainly determined by k_1/k_2, the intrinsic activity by k_3/k_2, high for full, intermediate for partial agonists and very low for competitive antagonists. The rate of receptor regeneration, (k_5) is mainly determinant for the degree of "fade" in the dose–response curves or for the degree of specific desensitization of the receptors and thus for tachyphylaxis if k_5 is low. The stimulus is defined as proportional to the fraction of the receptors in the activated state. The receptor reserve phenomenon is interpreted on basis of the processes, especially the amplifier systems, relating the stimulus to the effect [34–37].

Receptor activation results in the induction of a stimulus which by definition is proportional to the fraction of the receptors present in the activated state. The effect as a rule will not be proportional to the stimulus although a certain stimulus is postulated to result always in the same effect. The amplification processes in the transduction of the stimulus to the effect imply that possibly only a fraction of the receptors has to be activated to obtain the maximal effect attainable with the effector system. This is illustrated by the action for, for instance, ACTH and ACTH analogues with regard to the enhancement of the cortisol synthesis in the adrenal cortex. The various analogues give clearly different rates of cyclic AMP generation. Only those analogues which have a very low capacity in this respect, in other words with a very low intrinsic activity with regard to cyclic AMP generation, behave as partial agonists with regard to the rate of cortisol production. Analogues with an intermediate intrinsic activity as far as the cyclic AMP generation is concerned still act as full agonists for the cortisol production [56]. There is a spare capacity as far as the receptor system is concerned. It illustrates that in situations where an effector system is more closely connected with the receptor system more direct information on drug-receptor-interaction is provided. Direct binding studies of drugs to receptors are advantageous in the sense that they give more direct information on receptor occupation and therewith on affinity, but on the other hand other aspects of drug action like receptor activation are largely obscured.

As shown by binding studies the receptors for various drugs, hormones and hormonoids are located in plasma membranes [57]. The receptors for acetylcholine (the cholinergic receptors) could be proven to be located on the outside of the plasma membrane since application of cholinergic agents intracellularly appears to be ineffective [58]. For other receptor types, viz. those for noradrenaline, histamine and serotonin, a location at the outside of the cell membrane is highly probable since these receptors are easily accessible for the quaternized form of the respective competitive blocking agents, compounds known to penetrate intracellularly only with great difficulty or not at all [1, 59]. Also for the receptors of various peptide hormones and noradrenaline involved in the activation of adenylate cyclase a location on the cell membrane has been confirmed by binding studies [60, 61, 82].

With regard to receptor isolation and identification there is an essential difference between soluble receptors, e.g. the cytoplasmic steroid receptor proteins and membrane-bound receptors. There is a tight interrelation of the receptor molecule and surrounding molecules in the latter case. Separation of the receptor molecule from its surroundings may well disturb its conformation, and thus its specific characteristics. Undoubtedly the isolation techniques, especially those in which detergents [62] are used as solubilizing agents, may influence the conformation of the isolated protein drastically. The variation in binding constants reported for the interaction of drugs with "isolated receptors" therefore does not warrant the existence of various receptor states or conformations physiologically. A special problem which holds for the soluble as well as the membrane-bound receptors, is that with isolated receptors no measurable "pharmacological" effect can be induced anymore, making their identification extremely difficult in certain cases.

Since structure-action relationships (SAR) are based on an interaction between the drug molecule and the receptor site, it may give information on the properties of this site. Thereby a certain degree of chemical complementarity between the drug and the specific receptor site with which it interacts is assumed. Structure-binding relationships, therefore, can serve as a tool for the identification of isolated

receptors. However, only the receptor molecules, not the sites can be isolated. One may expect that structure-binding relationships for isolated receptors, as far as these are not to a certain degree denaturated in the isolated form, will parallel the SAR, with this distinction that on the isolated receptors as a rule there will be no differentiation between agonists, partial agonists and competitive antagonists, because only affinity and binding capacity are measured and not intrinsic activity. Opiate receptors are an exception in this sense because agonist binding is dependent on the presence of Na^+ or Li^+ ions, while antagonist binding is not, making a clear differentiation possible [63]. In efforts to isolate and identify receptors also use is made of agents which irreversibly bind in a selective way to the receptors or better to the specific receptor sites. However, such agents are only exceptionally available [64].

Based on the postulated complementarity for the drug molecules and the specific receptor site, SAR-studies can be used for receptor-site-mapping. Interesting results are thus obtained with regard to various membrane-active agents such as acetylcholine, histamine and noradrenaline and their respective competitive antagonists. Agonist and antagonist both have an affinity to "common" receptors, only the agonists have an intrinsic activity. The interaction of the agonist molecule with the receptor results in a change to the activated (R^*) state or conformation. The competitive antagonist keeps the receptor in the non-activated (R) state. With partial agonists only a fraction of the drug-receptor interactions result in an activation; the intrinsic activity depends on the size of this fraction [65].

Taken into account the postulated complementarity of drugs and their receptor sites agonists and antagonists should be chemically related. For the membrane-active agents just mentioned this appears hardly or not to be the case [34, 55, 59, 66, 67]. The various agonists, like acetylcholine, histamine and noradrenaline are highly polar molecules with clear-cut differences in, for instance, charge distribution. There is little or no chemical relationship between agonists and corresponding antagonists. There is, however, much similarity in the chemical structure of the various types of antagonists, anticholinergics, antihistaminics and α-adrenergic blockers, predominantly hydrophobic, non-polar in nature. As a rule they have hydrophobic double ring systems located at a certain distance (3–5 atoms) of an amino or a quaternary ammonium group in common. The hydrophobic groups essentially contribute to the high affinity for the receptors and impossibly can be bound to the receptor site complementary to the highly polar agonistic molecules. Introduction of centers of asymmetry into various moieties of the drugs, for instance in cholinergic and anticholinergic agents, indicates that in the binding of the agonists and the corresponding antagonists essentially different chemical groups and different receptor sites are involved [34, 55, 59].

The anticholinergics apparently bind through their hydrophobic moieties to accessory binding areas topically or functionally related to the receptor site for cholinergic agonists, in some way blocking the activation of the receptor by agonists. They interfere with the binding of the agonist to its receptor site, without occupying this receptor site itself in a strict sense. There appears to be a "dualism" in the receptor sites for agonists and corresponding antagonists. On basis of this concept the lack of structural similarity between agonists and their corresponding competitive antagonists, the existence of close structural relationships between competitive antagonists blocking different types of receptors, the existence of competitive antagonists blocking receptors for different agonists, and the dependence of the selectivity in action of the competitive antagonists on the sterical configuration of the hydrophobic moieties in the molecule [68, 69] are understandable [59]. The receptor sites for agonists and competitive antagonists are not as common as suggested by the original "one receptor site concept".

The receptor sites for the drugs not necessarily have to be located on the surface of receptor proteins. The accessory receptor sites for the hydrophobic moieties in the various competitive antagonists of membrane-active agents may well be constituted by an interface between a hydrophobic surface of the receptor protein and lipid groups in the membrane. The extremely high affinity constants, 10^9 and 10^{10}, for competitive antagonists (e.g. anticholinergics and antihistaminics) which are mainly based on the contribution of the hydrophobic groups to the affinity can hardly be realized on a mere protein surface. Lipoproteins would be more suitable in this respect. The lipid molecules in a membrane facing the hydrophobic surface of the protein, are fixated to a large extent in a quasi crystalline form, making the high degree of stereospecificity of the antagonists, especially with regard to centers of asymmetry in their hydrophobic moiety, understandable. Drug-receptor interaction then results in changes in the interface characteristics. It is even possible that the relatively polar sites for agonists are also located at or constituted by an interface, e.g. there where the polar groups of a membrane protein are close to polar groups of membrane lipids like the phosphate and choline groups in phospatidic acids, phosphatidylcholines and sphingomyelins. The isolation of receptors as molecular entities would become an impossible task, if this turns out to be the case. Studies on purified receptors of such a nature would only be possible after isolation of the constituting parts and reassembly into artificial membrane-like structures [57].

The receptor molecule or macromolecular complex must be assumed to occur in different states, an activated and a non-activated form dependent on the presence of an agonist or a competitive antagonist. Kasai and Changeux [70] postulated that even in the absence of drugs there is a dynamic equilibrium between different functional states of the cholinergic receptor. The simplest concept in this respect is the "dual receptor concept", supposing the occurrence in the cell membrane of an equilibrium between the receptors in the R^*- and those in the R-form. The R^*-form contributes to the effect, the R-form does not. An agonist shifts the equilibrium towards the R^*-conformation and a competitive antagonist to the R-conformation; the agonist has a relatively high affinity to the receptors in the R^*-conformation, the competitive antagonist to those in the R-conforma-

tion, thus in a way fixating the receptors in their respective states [65, 93, 94]. Partial agonists have an affinity for both states, the ratio of the affinities being determinant for the intrinsic activity of the compound. As long as the rate constants for the equilibrium between R and R^* are large enough, this model will predict phenomena similar to those expected for a competition between drugs based on the "one receptor site concept". The receptor sites for agonist and "competitive" antagonist—although one receptor protein is involved—are different now [65, 93]. This is in accordance with the dualism in receptor sites outlined before on basis of structure-action relationships [65]. In case that the receptor molecules are embedded in the lipid membrane structure, one may expect that the behaviour of such protein molecules, for instance their tendency to aggregate or segregate, strongly depends on the hydrophobic and hydrophilic properties of the protein surface, especially of that part of the receptor molecule in contact with the lipids in the membrane. The receptor proteins, although hydrophobic, in their overall nature [71], have an amphiphilic character. Binding of agonists, usually strongly polar agents, could stabilize a relatively polar conformation and thus shift the equilibrium towards the more hydrophilic R^*-form. Binding of the hydrophobic antagonist would then stabilize a hydrophobic R-state. The different character of the R^*- and R-form will imply differences in the quaternary structure of the proteins, the degree and type of aggregation among receptor molecules or among receptor molecules and other proteins (e.g. effector proteins) present in the membrane.

This activation-aggregation concept postulates the presence of a receptor as well as an effector molecule in the membrane. The degree of aggregation between the two, influenced by the presence of a hormone is supposed to be determinant for the effect. The model fits quite well with the fluid mosaic concept for membrane structure [74].

Various membrane-active drugs clearly change the degree and type of receptor protein aggregation in membrane preparations. The proteins in the luminal cell membrane of the toad bladder aggregate to a mosaic pattern under the influence of the antidiuretic hormone [75]. The protein-walled pores thus formed may well account for the increased water permeability induced by this hormone. Specific receptor proteins for the different hormones activating adenyl cyclase are supposed to aggregate with the enzyme molecule under influence of the hormones, thus activating the enzyme in an allosteric way [72, 73]. Also existing modes of aggregation of receptor proteins may be changed under influence of drugs.

The amphiphilic character of receptor proteins, shifting towards a more hydrophilic or hydrophobic state by agonists and antagonists respectively, with as a consequence a change in intermolecular relationships between receptor and other membrane constituents, may well imply that the extraction of receptor proteins from membrane fragments could be either facilitated or impeded by the presence of certain drugs. Moreover, such drugs might protect the receptor from becoming denaturated once outside the membrane by preventing conformational changes to occur.

Receptor sites for membrane-active drugs also may be located on proteins or protein complexes constituting the wall of pores [76, 77]. The passage by ions of such protein-walled pores in a membrane may well change under the influence of drugs as a result of an altered charge distribution in the receptor molecules constituting the pores. The proteins may switch from an open to a closed conformation. Again an equilibrium between both conformations, shifted by agonists to an apparently open conformation and by competitive antagonists to a closed conformation can be postulated. In the dual receptor site model, although only one type receptor protein or protein complex is postulated, the receptor sites for agonists and for the corresponding antagonists are functionally distinct interdependent entities. The observation that affinity constants of agonists derived from displacement experiments depend on whether agonists or antagonists are displaced [78, 79] does not find an explanation in such a model.

One should not expect that one universal receptor concept or theory of drug action will account for all the modes of action of the nearly unlimited variability of bioactive agents. Undoubtedly the one receptor site concept and the classical theories for drug action explain a variety of experimental findings. The dual receptor concept opens intriguing perspectives for the interpretation of the action of drugs and hormones, there where the classical concepts do not provide a satisfactory explanation. An extensive discussion of both theoretical and experimental aspects of the dual receptor model is in press [80].

Although the receptor concept was mainly of theoretical significance in the past, it has evolved into concrete reality and receptors can now be studied as tangible molecular entities. The design of new drugs can be based on relationship between structure and receptor affinities measured by direct binding studies [84–86]; receptor density measurements in malignant tissue can tell whether hormonal treatment is indicated or not [87] and alterations in receptor properties and density distributions enhance insight in certain pathological conditions like myasthenia gravis and diabetes [88, 89].

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