RECEPTOR MECHANISMS

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Introduction

Problems of receptor pharmacology have seemed stalemated for many years. Enormous quantities of data on drug potency have accumulated but it has proved singularly difficult to relate these to molecular events. Indeed it has become evident that structure activity studies involving a drug of uncertain conformation with a receptive system the nature of which we were almost totally ignorant was really not capable of yielding answers in molecular structural terms. The missing quantities are closer definition of the sites of drug action so that when comparing drugs we are certain that they are comparable, a knowledge of the structure of binding sites so that both components of the reaction should be known, and an understanding of the configuration of the drug in its bound state. Further, we must know what energy barriers must be surmounted in forming the drug receptor complex and what configurational changes result. Lastly the most pregnant question is how the receptor events are translated into physiological events. Advances in the biological sciences suggest that all these problems can now be solved with the available techniques, and in the period under review we are able to report a number of very encouraging advances. The problems are great but the task of isolating and characterizing a receptor now is surely no greater than was that of isolating a soluble enzyme forty years ago. This is not to suggest that with the characterization of even ten receptors pharmacology will become a simple science. Biological systems are intrinsically complicated and often devious, and pharmacological agents are often fearfully blunt weapons, but how helpful it would be to have at least a few problems solved at the molecular level to stiffen the foundations of our science.

In this review I have concentrated on the problems of the cholinoreceptor mainly because it is better studied than most other receptors and the problems it raises are therefore more continually before our eyes.

Structure and Conformation of Drugs

Until recently most attempts made by pharmacologists to characterize the spatial configurations of drugs were based on average values for bond lengths and angles derived from X-ray studies on other molecules together

with simple selection rules to derive the most probable conformation. It is heartening to see that a number of crystallographers are now taking an interest in drugs and we now have available reliable X-ray data on acetylcholine, methacholine, noradrenaline, bisoniums, histamine, and some related compounds (1–8). In the matter of bond lengths and angles there has so far been little novel revealed, but the question of conformation seems now to be yielding results consonant with other interpretations provided that reasonable precautions are taken. Few protein chemists still maintain that crystal structures are irrelevant to the behaviour of proteins in solution, but the retention of the dissolved conformation in the crystal depends on the massive character of the protein and the filling of the vacancies in the unit cell by solvent. In the case of small molecules the constraints of packing in the crystal lattice are more serious and more likely to be influenced by factors without biological significance such as the size of the anion. It is usually assumed that large anions are less likely to perturb the conformation of the drug cation than small anions. Nevertheless it is useful to have external supporting evidence of the preferred conformation.

Extensive use has been made of molecular orbital (MO) calculations (9–15) particularly by Kier. In this method the total energy of the molecule is evaluated by the linear combination of atomic orbitals. The calculation is carried through for all the combinations of torsion angles so that a plot of total molecular energy against torsion angle is obtained. The minimum energy corresponds to the preferred conformation. Other minima correspond to alternative less preferred conformations. In the case of muscaraine a sharp minimum is found at a torsion angle of 150° with respect to the ring. The torsion angle found agrees reasonably with the X-ray values (110°). The energy minimum is about 6 kcals/mol below the maximum, so that the conformation must be regarded as strongly preferred. In the case of acetylcholine, however, the α,β torsion angle shows a much smaller minimum of about 0.6 kcals/mol at 80°. This angle also agrees with the X-ray evidence. Less distinct minima are found for the ester linkage, but the preferred configuration for carbonyl-oxygen and oxygen-methylene links are both trans. The same technique has been applied to muscarone, nicotine, histamine, noradrenaline, ephedrine, and pralidoxime. The widely used MO method is recognized to be comparatively imprecise and is liable to overestimate the values of the energy barriers. An alternative theoretical approach in calculating peptide conformation (16–18) is to use van der Waal's interaction functions to compute the potential energy as a function of the torsion angles. For muscarine the torsional angle for minimum energy was 101° and the energy minimum 6.4 kcals/mol. This is in good agreement with the X-ray evidence. Application of this method to acetylcholine shows that there is little to choose between the conformations, the gauche conformation (107°) of $\alpha\beta$ bond being slightly preferred (0.4 kcal/mol). A gauche conformation of the oxygen-methylene bond was also slightly favoured. From the energies one can estimate the relative proportions of the four conformers as 0.40, 0.25, 0.21, 0.13.

These results suggest that acetylcholine in solution is a mixture of conformers undergoing inter-conversion and that it would be unwise to draw special pharmacological attention to the lowest energy state. Because of the existence of multiple conformations there is some importance in knowing how rapidly these are inter-convertible. Estimates of the stability of these conformations has been obtained by NMR relaxation measurements (19). The method measures the rate of rotation of molecular groups in solution and makes it possible to decide how much rotation of the group is caused by tumbling of molecules as a whole and how much by rotation around the bond axis. The energy barrier to rotation about C-C and C-N bonds in aliphatic molecules is sufficiently great that many rotations of the molecule occur for each bond rotation, but O-C bonds allow rotation around the bond nearly as easily as for rotation of the whole molecule. Applied to acetylcholine this means that the conformation of the acetyl group alters much more rapidly than that of the α-β bond, nevertheless the lifeline of the latter conformation is probably not more than a few microseconds. The conformations are thus very rapidly inter-converted. Nevertheless, considered in the time frame available for forming a complex with a receptor by collision the conformation of the α-β bond must be regarded as pre-existing and fixed whereas the acetyl conformation can change. In aromatic drugs it may be possible to determine the twist angle between the ring and a side chain by the ultraviolet spectrum (20). A study of the relationship between the conformation of choline aryl ethers and their nicotinic activity showed that activity was confined to those in which the ring and the side chain β-carbon were coplanar (21).

The latest attempt to avoid the uncertainties of multiple conformation by the use of rigid analogues of acetylcholine has been successful. The molecule was 1-acetoxy-cyclopropyl trimethylammonium. This molecule exists in four enantiomers, none of which is precisely the same as the acetylcholine conformers because of the distorted bond angles of the three membered ring. However, the trans (+) isomer was as active as acetylcholine on the ileum whereas the trans (−) isomer and the cis (±) were less than 1/200 as active (22, 23). A rather similar approach to the catecholamine receptor has been taken with the synthesis of stereospecific aminodecalols (24). The conformational similarities between muscarinic drugs and phospholipids, and betaine have been pointed out (25, 26) and a study of the binding of noradrenaline to phospholipid reported (27).

Attempts continue to put structure-activity studies on a more analytical basis. These consist mainly in correlating some chemical property of a drug series with activity. These may be Hammett substituent constituents, electronic densities derived from MO calculations, or oil-water partition coefficients or a combination of these (28–35). It appears that such measurements may have a considerable usefulness in planning synthetic programs, which is perhaps surprising in view of the omission of information on three-dimensional structures. For instance such studies fail to account for the pharma-

cological differences of enantiomers. Indeed the study of enantiomers remains a most sensitive test of drug specificity and has been especially of interest in β-adrenergic blocking agents (36–39). An excellent general review of stereospecificity in reactions of the cholinoreceptor and cholinesterase has also appeared (40).

A number of authors have seen the value of studying structure activity relationships against a well defined characteristic such as binding to a biological macromolecule. A readily available binder for parasympathomimetic molecules is acetylcholinesterase, and Belleau (41) has provided a thorough thermodynamic analysis of the binding of alkyltrimethylammoniums. He concludes that the binding of the ammonium head is mainly enthalpic but the binding of the lengthening alkyl chain is entropy controlled and hence primarily dependent on water structure. It is difficult to take this argument further because of the considerable uncertainties about the structures of liquid water; these are made clear in an excellent new book (42). The investigation of a distinct species of liquid water (polywater) may have relevance to water behaviour at interfaces and may well provide fuel for pharmacological imaginations (43). A related approach to the study of the structural correlates of activity is to generate binding macromolecules by making antibodies to an appropriate member of a drug series. Marlow et al. (44) have coupled the nicotinic drug cholinephenylether to protein and raised antibodies against it. The antibodies combined with a variety of drugs that react with cholinergic receptors, including both agonists and antagonists, both muscarinic and nicotinic drugs. While these results would appear to answer in the affirmative the question whether agonists and antagonists can really combine with the same site (45), the common feature of the drugs that combined was a basic group, so that the answer does not have the universality that is desirable. The same technique has been used to explore the binding of simple alkylamines (46) and to derive free energy components of binding. Again a low order of specificity is seen in the complex, and for the alkyl trimethylammonium group the results are in close accord with Belleau's results referred to above (41). The antibody technique has also been applied to the hallucinogenic methoxyphenyl alkylamines (47). Antigens were formed by coupling with polyglutamic acid by carbodiimide. Substantial differentiation between 2, 5 dimethoxy and 3, 4, 5 trimethoxy phenylalkylamines was achieved.

Since ideas of receptor activation are so strongly focused on conformation changes, it is important to keep under review the techniques that are useful in this field as well as the general results which are regularly reviewed in the companion volumes (48). Useful reviews on protein conformation (49), denaturation (50), and X-ray diffraction (51) have appeared. A technique that has come of age in biology this year is nuclear magnetic resonance (NMR). Jardetzky and his colleagues have completed an extensive series of studies on ribonuclease and staphylococcus nuclease and their reactions with inhibitors and have not only been able to identify binding

aminoacid residues but also to identity conformational conversions (52–55). With the technical development in the method a wide range of possibilities are opening up. Several direct applications to pharmacological problems of binding have appeared (56–59) and a comprehensive review is available (60).

Among the interesting conformational changes described in the period under review we may note that a large effect on the exchange of water with staphylococcus nuclease was found by the cooperative action of desoxythymidine diphosphate and Ca ions (61), that the kinetics of a fast allosteric process have been described (62), as well as an exceptionally slow conformational change ($t_{1/2} \sim 40$ min) produced by 4′-(4-aminophenylazo) phenylarsonic acid with subtilisins (63). Of particular interest is the demonstration by Mansour and his colleagues that serotonin and adenosine 3′ 5′ phosphate are able to alter the conformation of phosphofructokinase from the fluke Fasciola hepatica (64).

Discrimination of Receptor Sites

A continuing problem of studies in drug receptors is the difficulty of being sure that a set of drugs are all operating on the same system. One group have used a battery of ten tests to evaluate agonists on smooth muscle (65, 66) and have come to the conclusion that indirect action may be important. This may be the recognized method of ganglionic stimulation typified by nicotine or by some other mechanism of neural release of acetylcholine that may mediate the actions of acetylcarbocholine and acetylsilicocholine. It seems improbable that neural mechanisms are typical of the action of partial agonists (67). Evidence based mainly on agonist structure-activity relationships using muscle contraction and potassium efflux as criteria of drug action on guinea pig ileum, indicates that these are mediated by distinct but related receptors (68) which nevertheless are not distinguished by antagonists. A comparable differentiation of the nicotinic receptors has been found in leech muscle by measuring pA_2 values of gallamine (69) and in the electroplax by the finding of supramaximal addition (70).

Considerable doubt has been thrown on the use of β-haloalkylamines as means of determining spare receptor ratios by the finding that a nonalkylating but slowly reversible component of the antagonism is present which is rapidly reversible by thiosulphate (71–73). After removal of the reversible component, no spare receptors were detectable in the vas deferens treated with SY 28. Similarly in intestinal muscle when flux and contraction were studied a parallel shift occurred in the contraction, but no shift in the flux dose response curve, whereas both shifted with atropine and benzylcholine mustard (68). Nevertheless, this method continues to be used and often gives most plausible results (74).

Another very interesting development has been the finding that the two autonomic transmitters may affect the release of each other, presumably by neural mechanisms. In the heart, acetylcholine causes a small increase in

noradrenaline output; this increase is greatly potentiated by atropine. On the other hand the considerable increase in noradrenaline output caused by dimethylphenylpiperazine (DMPP) is not increased by atropine. The DMPP stimulated output, however, can be inhibited by acetylcholine, methacholine, or pilocarpine and this inhibition is prevented by atropine. There is evidently a muscarinic inhibition of noradrenaline output by the adrenergic neurons in the heart (75). In the guinea pig ileum, on the other hand, adrenaline and noradrenaline reduce the acetylcholine output both at rest and in the electrically stimulated preparation and this effect is annulled by α-blockers. Phenylephrine and amphetamine also produce these effects, but isoprenaline, dopamine, and methoxamine do not (76).

In the field of adrenergic β-receptors some important new agonists have appeared having selectivity for bronchodilator and vascular effects. Sotorenol (77) and salbutamol (78) are simple variants of catecholamines, but trimetoquinol (79) is related to papaverine. Two other new classes of β-stimulants have also been described (80, 81). There have also been further studies on β-receptor antagonists in which specificity has been emphasized (82, 83). An entirely new type of irreversible α-blocker is of particular interest because it is of low intrinsic chemical reactivity (84).

Studies on synapses in invertebrates continue to turn up interesting variants on drug receptors. In the pleural ganglia of Aplysia some neurons show two phases of depolarization in response to presynaptic stimulation. Both early and late phases are mimicked by acetylcholine and carbachol, but nicotine, DMPP, oxotremorine, propionylcholine, methacholine, and tetramethylammonium produced only the early phase. Tubocurarine blocked the early phase but had no effect on the late phase. Atropine and other muscarinic antagonists were without effect on either phase (85). Later experiments revealed that β-methylxylocholine could block the late phase and this is especially remarkable since xylocholine itself was ineffective (86). It was also found possible to block the late phase by the intracellular injection of TEA (87). These studies introduce two new kinds of acetylcholine receptors into our repertory and remind us that the variety of receptors may be large.

Uptake Processes

Accumulative processes for acetylcholine and other synaptic elements have continued to be studied and as they involve a binding site are of interest in the consideration of drug receptors. The uptake of acetylcholine into cerebral cortex slices is inhibited when energy metabolism is inhibited, by treatment with phospholipase A or C and also by drugs related to acetylcholine, such as hemicholinium, atropine, choline, and eserine (88, 89). Further studies have been made of the uptake of carbachol and decamethonium by the cerebral cortex, and inhibition by morphine has been reported (90–92). It is doubtful if this is connected with the pharmacodynamic action of morphine.

A systematic study of choline transport in erythrocytes has revealed a number of interesting features. Antagonism is shown by many drugs affecting cholinergic synapses. Uptake is confined to quaternaries with a side chain of three atoms or less. A striking change is seen in the effects on choline efflux with alkyltrimethylammoniums since the short chain members accelerate efflux by an exchange process, whereas the longer chain members inhibit efflux (93).

Relationship of Acetylcholinesterase to the Cholinoreceptors

The idea that acetylcholinesterase may either itself be the acetylcholine receptor or is at least associated with it in a multimeric assembly continues to have adherents. The first of these ideas is usually rejected on the basis that cholinesterase inhibitors do not block the receptors but indeed usually increase their responsiveness. However, it is now claimed that cholinesterase inhibited by alkylphosphates may actually have enhanced binding capacity (94, 95), but it is not clear that this can explain results with the edrophonium type of inhibitor. Another interpretation is based on the well known parallelism between agonist activity and substrate turnover (96) but the theory totally fails to account for the high potency of many nonhydrolysable cholinomimetics.

An interesting kinetic study has shown that quaternary ammonium ions can accelerate acylation of acetylcholinesterase by methylsulphonylfluoride if the quaternary is small in size, but inhibits it if the quaternary is larger (97). These effects can be accounted for on steric considerations, without the need to invoke control sites or conformation changes. On the other hand ORD studies have shown modifications in the Cotton effects at 197, and 205 nM when edrophonium reacts with acetylcholinesterase, and a Cotton effect at 210 nM when tetraethylpyrophosphate reacts (98). The idea of a regulation site has also been supported by the finding of co-operative inhibition by gallamine and 3-hydroxyphenyltrimethylammoniums, and protection by a number of quaternaries against labeling of acetylcholinesterase by 4-diazophenyltrimethylammonium. This is an agent which can also irreversibly block the acetylcholine receptors in the electroplax. A number of analogies are drawn between the two systems (99). Among the multitude of actions recorded for dibenamine may be added the ability to inhibit acetylcholinesterase (100).

Chemical Modification of Receptors

The most interesting results in receptor pharmacology in the period under consideration have come from the discovery that the behaviour of nicotinic receptors can be modified by the disulphide reducing agent dithiothreitol (101–103). The response of the electroplax to acetylcholine and carbachol is considerably reduced, the effects involving both affinity and maximum response. The effects can be reversed by an oxidizing agent 5, 5′dithiobis (2-nitrobenzoate) and can be made persistent by reacting one of

the thiol groups with N-ethylmaleimide. Maleimides with suitable aralkytrimethylammonium groups attached, react up to three orders of magnitude more rapidly than simple NEM. Two very interesting effects are that hexamethonium, normally a competitive antagonist of acetylcholine in the electroplax becomes an agonist after reduction of the receptor, and that decamethonium is potentiated by the reduction, but the response to decamethonium is almost totally abolished by NEM. Since the rate of reaction of the maleimides is only moderately depressed by hexamethonium and not at all affected by phenyltrimethylammonium, the disulphide group is believed to be adjoining but not within the receptor. The effects of reduction presumably result from some secondary conformational changes in the receptor areas. Two alkylating agonists, bromacetylcholine and the 4-nitrophenyl ester of 4-carboxphenyltrimethylammonium, also react with the reduced electroplax and lead to a persistent irreversible depolarization. In this situation a "rate" theory of drug action seems to be excluded. It is splendid to report that very comparable changes are found in the chick biventer muscle (104). Reduction grossly reduces the contractile response to carbachol and converts hexamethonium into an agonist; the effect is reversible on oxidation. Concurrent tubocurarine does not prevent the reduction but the potency of tubocurarine is itself doubled by reduction. Reduction moderately reduces responses to short chain alkyltrimethylammonium cations but does not affect the response to the longer chain ones.

Studies on the chemical nature of receptors are beginning to bear fruit. The results just described strongly suggest that a cystine group lies in association with the receptor, yet treatment with a range of proteolytic enzymes failed to alter the receptor while totally inactivating the synaptic cholinesterase (105). Nor did phospholipase C or uranyl ions affect the receptors although they did affect membrane properties (106–8). Studies of alkylators as selective site labels have been disappointing (71, 72, 109) in the dibenamine series but benzilylcholine mustard continues to give interesting results at the muscarinic receptor (110). A valuable new book on the design of site labels has appeared (111). Direct estimation of receptor occupation continues to give difficulties, an apparent saturable binding site for arecaidine ethyl ester is present in atria (112), but in smooth muscle the evidence for a binding site was equivocal (113). In the heart the kinetic constants of atropine binding are affected by the perfusion rate and by the concentration of the drug, and this suggests a large measure of diffusion limitation (114). A ribonucleoprotein has been isolated from skeletal muscle that has binding properties for tubocurarine, but its properties do not encourage the belief that it is a receptive substance (115). A useful review of the chondroitin sulphate binding substance for tubocurarine isolated by Chagas has appeared (116). An interesting new phenomenon has been called a metaphilic change (117, 118). When the chick biventer is treated with chloroethyl benzylaminodecyl-dimethylbenzylammonium, a substance which is an alkylating antagonist of acetylcholine, the extent of alkylation depends on how re-

cently the muscle has been exposed to an agonist. In the presence of an agonist the alkylation is considerably increased and this effect persists for many minutes after removal of the agonist. It is not produced by antagonists. It is suggested that a changed configuration of the receptor resulting from agonist interaction makes it more reactive with the alkylator.

The determination of receptor pattern in skeletal muscle by the innervation has been extended by the finding that the denervation pattern can be readily produced in a few days by injection of a local anaesthetic into the muscle (119), and that in crossing the nerves to soleus and extensor digitorum longus the characteristic sensitivity patterns in the muscle are also exchanged (120).

A further article has appeared on the intriguing receptor model system based on an interfacial layer composed of lipids and erythrocyte acetylcholinesterase (121). Acetylcholine induces an impedance change which can be blocked by hexamethonium or decamethonium. Nicotine also is highly effective but carbachol, methacholine, and pilocarpine are inactive. It is presumed that the active compounds produce conformation changes in the acetylcholinesterase.

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