A Molecular Theory of Drug Action Based on Induced Conformational Perturbations of Receptors[1]

B. BELLEAU

Department of Chemistry, University of Ottawa, Ottawa, Ontario

Received May 21, 1964

Recent modifications of Clark's classical theory of drug action are analyzed. Some basic deficiencies of the Ariens–Stephenson modification and the rate theory of Paton are pointed out. It is shown that these current concepts suffer from the absence of a biophysical basis and that they fail in providing a qualitative interpretation of drug properties at the receptor level. A molecular theory, defined as the macromolecular perturbation theory (MPT), is elaborated for the muscarinic cholinergic receptor. On the basis of the postulate that the receptor is a protein with acetylcholinesterase (AChE) like properties (with regard to Michaelis complex formation only), evidence is presented for the hydrophobic properties of the protein-binding surface which suggests that two modes of interaction of drugs is operative. The first, involving only the acetylcholine-binding portion of the surface would lead to a specific conformational perturbation (SCP) of the protein, the second requiring interations with the hydrophobic periphery would be accompanied by a nonspecific conformational perturbation (NSCCP) of the enzyme. Induction of a SCP by a molecule M_s (agonist) would produce a complex symbolized by P^*M_s; for the case of the involvement of a NSCP, the complex produced by a M_i molecule (antagonist) is denoted by $P^{\mp}M_i$ (eq. 1 and 2). The molecular basis for these rationalizations is discussed in detail. The structural requirements for the induction of a P^*M_s complex by an M_s molecule and a $P^{\mp}M_s$ complex by an M_i molecule are analyzed and it is shown that hydrophobic interactions (Fig. 1) largely condition the transition between the two types of complexes. A third class of molecules symbolized by M_{si} is shown to induce in the protein the formation of an equilibrium mixture of P^*M_{si} and $P^{\mp}M_{si}$ complexes, thus accounting for the occurrence of partial agonists (eq. 3). Also, the possibility for M_{si} molecules to combine with a P^*M_{si} complex is discussed. Ternary complex formation (eq. 4) accounts for bell-shaped dose-response curves. The MPT serves in the prediction of the general form of dose-response curves usually encountered in the study of drug effects on tissues. The expressions "intrinsic activity" and "efficacy" are shown to reflect the molecular mechanism described by eq. 3. The validity of the MPT was tested by evaluating its capacity to provide a basis for the interpretation of structure–activity relationships. With regard to muscarinic drugs, the theory was strikingly successful in accounting for the qualitative properties of various series of quaternary ammonium salts. A variety of parallelisms between the muscarinic receptor and AChE are summarized. Near identity of the two proteins is suggested. Discrepancies in their chemical reactivity such as towards the organophosphorus inhibitors are accounted for. On the basis of known kinetic evidence, it is suggested that the muscarinic receptor is acetylated AChE. In this form, the normal chemical reactivity of the esteratic sites of AChE would be masked but the enzyme would retain (as is now known) the ability to form complexes. The acetylated form of the enzyme would be produced in specific regions of sensitive membranes by the continuous quantal discharge of acetylcholine (ACh). The muscarinic receptor would therefore have its biochemical origin in the pool of AChE. This hypothesis may account for a variety of previously inexplicable observations.

Current theories of the mechanism of action of drugs at the receptor level rest primarily on the classical work of Clark[2] and Gaddum[3] who showed that drug receptor interactions closely approximate the relationships encompassed by the Langmuir adsorption isotherm. This subject has been reviewed in detail several times in the recent past[4-7] and it will suffice here to briefly summarize and evaluate some recent modifications of the basic concept as they pertain to the quantitiative interpretation of the anomalies that the classical theory fails to explain. Presently, among a number of modifications of the theory, two appear to have acquired momentum; the first may be referred to as the Ariens–Stephenson modification[5,8] and the other as the Paton theory.[9]

In a paper exposing the rate theory, Paton[9] presented some valid and pertinent criticism of the Ariens–Stephenson kinetic model and pointed out for instance that the concept of intrinsic activity or efficacy still fails to explain why drugs vary in their type of action (or their efficacy), "—so that when one drug occupies a receptor it stimulates, whereas another occupying the same receptor blocks?"[9] The question is a valid one indeed and clearly focuses attention on the fundamental deficiency of the Ariens–Stephenson theory, mainly that it does not afford an approach to mechanism at the molecular level in structural terms. Ariens attempted to answer this question[6,10] (after drawing heavily from the field of enzyme kinetics) by stating that "The intrinsic activity is analogous to the reaction velocity constant k_3 which determines the formation of the final product P in the case of an enzymological reaction as for instance"

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

Even though the concept of "efficacy" adequately accounts for the existence of so-called partial agonists, the implied analogy between the production of a physiological stimulus and the rate-limiting constant k_3 remains without foundation at the molecular level

(1) Published as Part IV of the series "The Chemical Basis for Cholinomimetic and Cholinolytic Activity." For part III see, B. Belleau and G. Lacasse, *J. Med. Chem.*, **7**, 768 (1964).

(2) A. J. Clark, *J. Physiol.*, **61**, 530, 547 (1926); A. J. Clark, "The Mode of Action of Drugs on Cells," Williams and Wilkins, Baltimore, Md., 1937.

(3) J. H. Gaddum, *J. Physiol.*, **61**, 141 (1926); *ibid.*, **89**, 7P (1937).

(4) E. J. Ariens, J. M. van Rossum, and A. M. Simonis, *Arzneimittel-Forsch.*, **6**, 282 (1956); E. J. Ariens, *Arch. Intern. Pharmacodyn.*, **99**, 32 (1954).

(5) Proceedings of a Symposium on Drug Antagonism at the XXIth International Physiological Congress, Brussels, 1956; E. J. Ariens, J. M. Van Rossum, and A. M. Simonis, *Pharmacol. Rev.*, **9**, 218 (1957).

(6) E. J. Ariens and A. M. Simonis, *J. Pharm. Pharmacol.*, **16**, 137 (1964).

(7) E. J. Ariens and A. M. Simonis, *ibid.*, **16**, 289 (1964).

(8) R. P. Stephenson, *Brit. J. Pharmacol.*, **11**, 379 (1956).

(9) W. D. M. Paton, *Proc. Roy. Soc.* (London), **B154**, 21 (1961).

(10) E. J. Ariens, J. M. van Rossum, and A. M. Simonis, *Pharmacol. Rev.*, **9**, 218 (1957).

for the simple reason that k_3 would be insensitive to structural variations in the case of pure agonists but sensitive to molecular structure in the case of partial agonists. There is no reason to believe that k_3 should not be sensitive to all types of structural variations, let it be in the agonist or partial-agonist series, since this is what corresponds to general experience in the field of enzyme chemistry. The postulated intervention of a rate-limiting change in an *initially inert drug–receptor complex* requires that a unique energy barrier for the formation of the transition state for rearrangement would apply to all agonists regardless of structure. The lack of physico–chemical precedents for such a hypothesis suggests that the fitting of dose–response curves to mathematical equations can hardly be used as evidence for the validity of a theory. With regard to Ariens' initial interpretation of the phenomenon of intrinsic activity (or efficacy), it would seem that the hypothesis of the rate-limiting change of an inactive to an active drug–receptor complex is probably distantly related to the true mechanism of drug action.

Interpretations of the physico–chemical significance of the parameters affinity and intrinsic activity are at best equivocal. Thus, Ariens[6] suggested that affinity could be determined initially by long-range electrostatic interactions between drug and receptor, interactions which would condition the subsequent operation of London dispersion forces culminating in complex formation. Such a stepwise mechanism of complex formation is without practical significance since the physico–chemical events occurring during the process of complex formation cannot in any way influence the ultimate response, the resulting drug–receptor complex supposedly becoming active after the binding process has occurred. With regard to the parameter intrinsic activity, the situation also appears ambiguous. The numerical values which reveal that some drugs are more or less intrinsically active were suggested recently by Ariens[6] to reflect the fraction of collisions between drug and receptor that are effective in producing a stimulus. This model which is now a statistical one, was first developed by Janku and Mandl[11] and constitutes a mechanistic transition which is incompatible with the primary postulate of the formation of an initially inactive drug–receptor complex. It seems clear that if the parameter intrinsic activity is to be relevant to the rate-limiting change of an inactive complex to an active one, all collisions must initially produce an inactive complex. On the other hand, if the statistical model is accepted, intrinsic activity can no longer be made analogous to the rate-limiting step k_3 of enzyme reactions. Inconsistencies of this kind emphasize the need for suitable molecular theories of drug action.

Partly because of the inadequacies of the Ariens–Stephenson modification and partly on the basis of Croxatto's postulate[12] that a drug would be effective only at the moment of encounter with its receptor, Paton[9] proposed his rate theory which states that drug activation of receptors would be proportional to the total number of encounters per unit of initial time. In other words, it would be the rate of complex formation with receptors that would determine the response rather than it constituting an obligatory step preceding the activating phase (as in the Ariens–Stephenson model). It is a requirement of this theory that high stimulant activity should reflect not only a high rate of combination with receptors but a still higher rate of dissociation of the drug. On that basis, the qualitative properties of drugs could be accounted for in terms of their characteristic rates of dissociation (k_2) from the receptors. Thus, agonists would be characterized by a high k_2, partial agonists by an intermediate, and antagonists by a low k_2. It was noted that antagonists are usually large molecules which because of their greater size would "stick" to the receptor substance. In addition, it was suggested that agonists would combine with the receptor at a unique rate but would dissociate at variable rates, thus allowing for a wide range of encounters per unit time. This rate theory of drug action has many attractive features but creates serious difficulties when analyzed at the molecular level. For instance, it is generally agreed that the action of acetylcholine (ACh) is catalytic at the effector level. It is clear that ACh produces its effects by inducing a change in a theoretical receptor and that this change is conditioned by the presence of the molecule on that receptor. Also, the longer a catalyst is present in any reaction medium, the longer its effect is felt; it seems normal therefore to expect that the ACh–receptor complex should persist if a strong effect is to be observed. According to Paton, however, this would not be so; the receptor acquires catalytic properties only during the act of combination but immediately loses this property if ACh should remain adsorbed. It follows that ACh will have to dissociate at a high rate in order that the receptor can again acquire the potential property of a catalyst. Now, if the rate of combination is not sensitive to structural variations in the substrate, the rate of dissociation has to be. Therefore, all strong stimulants (such as ACh) must be endowed with structural features that are characterized by a high degree of nuisance capacity towards complex formation since all must rapidly dissociate from the receptor. This conclusion contrasts with general experience in the field of enzyme chemistry, good substrates always possessing special structural features favoring an initially essential and specific affinity for binding sites rather than uniformly producing hindrance to adsorption. For instance, the ester methyl group of ACh which promotes affinity for acetylcholinesterase (AChE) would now be endowed with a considerable nuisance capacity with regard to adsorption on receptor sites, because when it is absent as in the analogous molecule of formylcholine, stimulating activity is sharply reduced. Hence, ACh would have a higher k_2 than formylcholine. But then, if instead of removing the methyl group of ACh which would result in a higher affinity for the receptor, we add two extra methylene groups (CH_2) to obtain butyrylcholine, a lower potency results, and now this has to be attributed to increased force of binding (leading to low k_2). Hence, both the removal and addition of CH_3 or CH_2 groups would have a similar influence on k_2. The nuisance capacity towards binding of the ACh methyl group would stand out as a unique phenomenon which is unlikely to be reproduced in the wide variety of known agonist molecules (such as carbachol

(11) I. Janku and P. Mandl, *Cesk. fysiol.*, **10**, 338 (1961).

(12) R. Croxatto and F. Huidobro, *Arch. Intern. Pharmacodyn.*, **106**, 207 (1956).

which includes a hydrophilic NH_2 group instead of a hydrophobic CH_3 in its molecule). Such deviations from commonly accepted physico-chemical knowledge serve to cast serious doubt on the practical significance of the rate theory, and a return to some form of Clark's original occupancy theory is indicated.

The Marcomolecular Perturbation Theory (MPT).—A molecular theory of drug action will now be described which is based on biophysical principles and which appears to account not only for drug-receptor kinetics but which also serves to explain structure-activity relationships which can be illustrated for drugs acting on the muscarinic cholinergic receptor. In addition, the theory allows some intriguing conclusions concerning the probable nature of the receptor and of its relationship with AChE. The theory, which may be conveniently referred to as the macromolecular perturbation theory (MPT), is based on the marked and unique conformational adaptability of enzymes. Because receptors are still of unknown constitution, it will be essential at first, if any progress is to be made, to accept the primary postulate that the muscarinic receptor is a macromolecule with protein-like properties and specifically designed for interaction with the natural substrate ACh. Circumstantial evidence for this hypothesis is available and can be summarized as follows. (a) The receptor substance can bind a wide variety of drug molecules, thus suggesting a degree of conformational adaptability characteristic of proteins. (b) Receptors display marked absolute and relative stereospecificity,[13,14] a property shared by enzymes. (c) Since both AChE and the cholinergic receptor are specific for ACh, it seems likely that they should at least share the common property of being proteins. The investigations of Waser[15] on the fixation of labeled curarine and muscarone in the end plates support the hypothesis of a "structural link" between AChE and cholinergic receptors.

Acceptance of the reasonable assumption that the receptor is a protein of specific structure and composition will now make it possible to attempt an interpretation of drug action. The system ACh–receptor can therefore be treated as an enzyme–substrate-like system and it will remain to analyze the basic consequences of complex formation in physico-chemical terms. Due to the elegant work of Koshland,[16] it seems now well established that enzymes are endowed to a high degree with conformational adaptability towards substrates and inhibitors, a phenomenon encompassed in the induced-fit theory of enzyme action. Evidence has been produced by Wilson and Cabib[17] that the presence of the trimethylammonium head in ACh induces a marked loss of entropy in AChE upon complex formation. This is suggestive of a profound conformational rearrangement of the enzyme accompanying complex formation, a phenomenon which is explained by the induced-fit theory of enzyme action.[16] On the basis of these considerations, Nachmansohn[18] proposed that the primary function of ACh at the receptor level would be to induce a similar conformational reorganization in a hypothetical protein, thereby inducing a sequence of physico-chemical changes ultimately reflected in the appearance of a stimulus. However, no elaboration of any utility could be made on this intriguing hypothesis which has thus remained somewhat gratuitous in the context of drug actions in general.

Dualism in the Mode of Drug Interactions with Receptors.—It is worthwhile noting that the induced-fit theory of enzyme action is not restricted only to enzymes but appears to be applicable also to proteins devoid of known catalytic properties. For instance, it was recently shown that serum albumin will undergo profound conformational reorganization when in contact with certain hydrocarbon-like molecules.[19] Therefore, protein occupancy (which may be a receptor) by a foreign molecule (which may be an enzyme inhibitor) appears to be conditioned by an accommodating perturbation of the tertiary structure. It is clear that such nonspecific perturbations will also be operative in the case of enzyme-inhibitor interactions and that the adsorption of an inhibitor molecule will not always be conditioned by an obligatory interaction involving only the specific catalytic sites. It is to be expected, therefore, that enzyme occupancy by foreign molecules will as well reflect the operation of nonspecific accommodating perturbations of the protein chains and it follows that such structural reorganizations may bear little relationship to the conformational rearrangement resulting from complex formation with a natural substrate. The classical symbolism used in enzymology does not take into account the structural fate of the protein component, an oversight which is no longer permissible in the light of recent advances. In the context of drug and inhibitor actions, it will be convenient to distinguish between two general types of perturbations in the protein component of the complex: the first may be referred to as a specific conformational perturbation (SCP) which conditions the adsorption of certain substrate-related molecules, and the second as a nonspecific conformational perturbation (NSCP) which may serve to accommodate other classes of foreign molecules (which may be inhibitors or even substrates). The following symbolism will be used: P for the protein in its resting state, P* for the protein transformed by a SCP, M_s for the substrate-like molecule, $P^{\pm}$ for the protein transformed by a NSCP, and M_i for an inhibitor molecule. Equations 1 and 2 illustrate these relationships. It now remains to search for a criterion

$$P + M_s \overset{K_1}{\rightleftharpoons} P^*M_s \qquad (1)$$

$$P + M_i \overset{K_2}{\rightleftharpoons} P^{\pm}M_i \qquad (2)$$

justifying especially the applicability of eq. 2, eq. 1 being valid because of the key substrate-like characteristics of M_s molecules. In an accompanying paper,[13] evidence was presented for the occurrence of two entirely distinct modes of inhibitor interactions with AChE. The first of these was shown to involve the operation of the highly distance-specific van der

(13) B. Belleau and G. Lacasse, *J. Med. Chem.*, **7**, 768 (1964).

(14) A. H. Beckett, *Proc. Intern. Pharmacol. Meeting, 1st, Stockholm, 1961*, **7**, 28, 31 (1962).

(15) P. Waser, ref. 14, p. 101.

(16) D. E. Koshland, Jr., ref. 12, p. 161; D. E. Koshland, Jr., in "The Enzymes," Vol. 1, P. D. Boyer, K. Myrback, and H. Lardy, Ed., 2nd Ed., Academic Press Inc., New York, N. Y., 1958, p. 305.

(17) I. B. Wilson and E. Cabib, *J. Am. Chem. Soc.*, **78**, 202 (1956).

(18) D. Nachmansohn, "Chemical and Molecular Basis of Nerve Activity," Academic Press Inc., New York, N. Y., 1959; E. Schoffeniels and D. Nachmansohn, *Biochem. Biophys. Acta*, **26**, 1 (1957).

(19) R. Lovrien, *J. Am. Chem. Soc.*, **85**, 3677 (1963).

Waals forces,[20] a phenomenon normally characteristic of enzyme–substrate interactions; the second mode of adsorption was shown to derive its driving force exclusively from hydrophobic interactions, thus necessitating the participation of an accommodative reorganization of nonpolar chains on the enzyme. Clearly, the protein structure must be disturbed nonspecifically in the second instance, a consequence of binding (defined as a NSCP) which validates eq. 2.

Molecular Requirements for Active and Inactive Complex Formation. A. Inactive Complexes.—The structural requirements for the formation of P^*M_s and $P^{\mp}M_i$ complexes must be defined in order that structure–activity relationships may be interpretated. The case of $P^{\mp}M_i$ complex formation will be examined first. It is essential initially to have knowledge of the over-all physico-chemical properties of the ACh-receptor surface. One must distinguish as in the case of AChE[13] between three possibilities: the binding surface may be either nonpolar in character, of intermediate polarity, or polar. Compelling evidence supporting the first possibility is available in the observations of Paton[9] and more recently of Barlow, *et al.*,[21] who evaluated the free-energy contribution to binding of added methylene groups in antagonist molecules. For the C_8 to C_{12} series of alkyltrimethylammonium inhibitors,[9] a free-energy contribution of 650 cal./CH_2 to binding was obtained (calculated by the present author using the available data[9]), whereas a value of 300–600 cal./CH_2 was estimated when the N-methyl groups of choline derivatives were replaced by ethyl groups. As was pointed out in an accompanying paper,[13] such effects of added CH_2 groups in drug molecules are entirely accountable on the basis of the contribution of hydrophobic interactions to binding and not to van der Waals forces as is generally believed.[21] It may be recalled that the free energy of transfer (ΔF_t) of CH_2 or CH_3 groups from an aqueous phase to a nonpolar environment amounts to 730 cal. under ideal conditions.[13] Such values as 650 cal. for the free energy of binding of a CH_2 on the receptor definitely establish the nonpolar character of the total binding surface. The molecular consequences of complex formation may then be anticipated on the basis of this knowledge. Similarly to AChE,[13] a portion of the receptor hydrophobic surface is specifically designed for the accommodation of ACh. However, no discontinuity in the nonpolar character of the surface is apparent since much larger molecules such as dodecyltrimethylammonium are firmly bound (with ΔF_t as the driving force). This diffusely compartmented hydrophobic binding area may be rationalized as in Fig. 1. On that basis, it is only necessary to postulate that those molecules possessing nonpolar substituents susceptible to accommodation (through the operation of ΔF_t as the driving force) by the hydrophobic periphery of the ACh compartment will induce a NSCP in the receptor protein. The mechanism of such nonspecific hydrophobic interactions has been discussed in detail.[13] The key consequence of such an intrusion of a substituent into the hydrophobic periphery consists in the disruption of a delicate network of nonpolar chains in the receptor. There would result an ineffective conformational perturbation (NSCP) designated by $P^{\mp}M_i$ (Fig. 1). It is of considerable interest that the chemical behavior of AChE towards ACh homologs can be rationalized using the above concept as a basis. Thus, butyrylcholine would not act as a substrate because of the peripheral hydrophobic interactions that it allows. Such intrusion of two CH_2 groups beyond the ACh compartment would result in a NSCP of the enzyme, a consequence which in this case is reflected in cancellation of enzymatic activity (Fig. 1). This observation suggests that AChE may be used as a reliable model in the evaluation of the concept of dual conformational perturbations (see below).

B. Active Complexes.—The case of the structural requirements for P^*M_s complex formation may now be considered. One of the key factors which becomes apparent from the above discussion is that M_s molecules may not include such substituents that are susceptible to accommodation into the hydrophobic periphery of the ACh compartment. Assuming all other factors (see below) to be satisfied, the receptor protein would assume a degree of structural organization designated by P^*M_s and endowed with catalytic properties ultimately reflected in the production of a stimulus. In contrast, the protein component of a $P^{\mp}M_i$ complex would be structurally disorganized or deformed. The next most important factor conditioning the formation of a P^*M_s complex would consist in the production by the M_s molecule of strong electrostatic interaction with a counter ion on the receptor surface. It is known that such interactions between enzymes and charged substrates frequently lead to profound conformational changes in the protein. A most pertinent example is found in the marked loss of entropy accompanying complex formation between ACh and AChE[17]; another example is the ATP–myosin system. This phenomenon can be ascribed to the presence of a quaternary head in ACh since tertiary and secondary amine analogs of the latter do not show the effect. It seems of special significance that strong muscarinic activity should also be generally associated with the presence of nonbulky quaternary onium groups in suitable drug molecules (thus emphasizing again the basic similarity of muscarinic receptors to AChE). The size of the cationic head of M_s drugs will be of critical importance since larger groups than methyl on the nitrogen will be likely to induce accommodative perturbations in the hydrophobic periphery of the counter anion and thus favor complex formation of the $P^{\mp}$ type. The investigations of Barlow, *et al.*,[21] serve to establish the nonpolar character of the environment of the receptor anionic site.

Our discussion[13] of the mechanism of interaction of quaternary ions with AChE would appear to deserve comment in relation to their effects at the receptor level. The recognition of the essentially hydrophobic nature of quaternary ammonium cations strongly suggest that the primary function of these ions may be to disrupt the structure of water at a critical point of either the AChE[13] or receptor surface. It would seem hardly fortuitous that nature should have selected cationic structures to activate cationic fluxes (Na^+ and K^+) across sensitive membranes. The

(20) L. Salem, *Nature*, **193**, 476 (1962); L. Salem, *Can. J. Biochem. Physiol.*, **40**, 1287 (1962).

(21) R. B. Barlow, K. A. Scott, and R. P. Stephenson, *Brit. J. Pharmacol.*, **21**, 509 (1963).

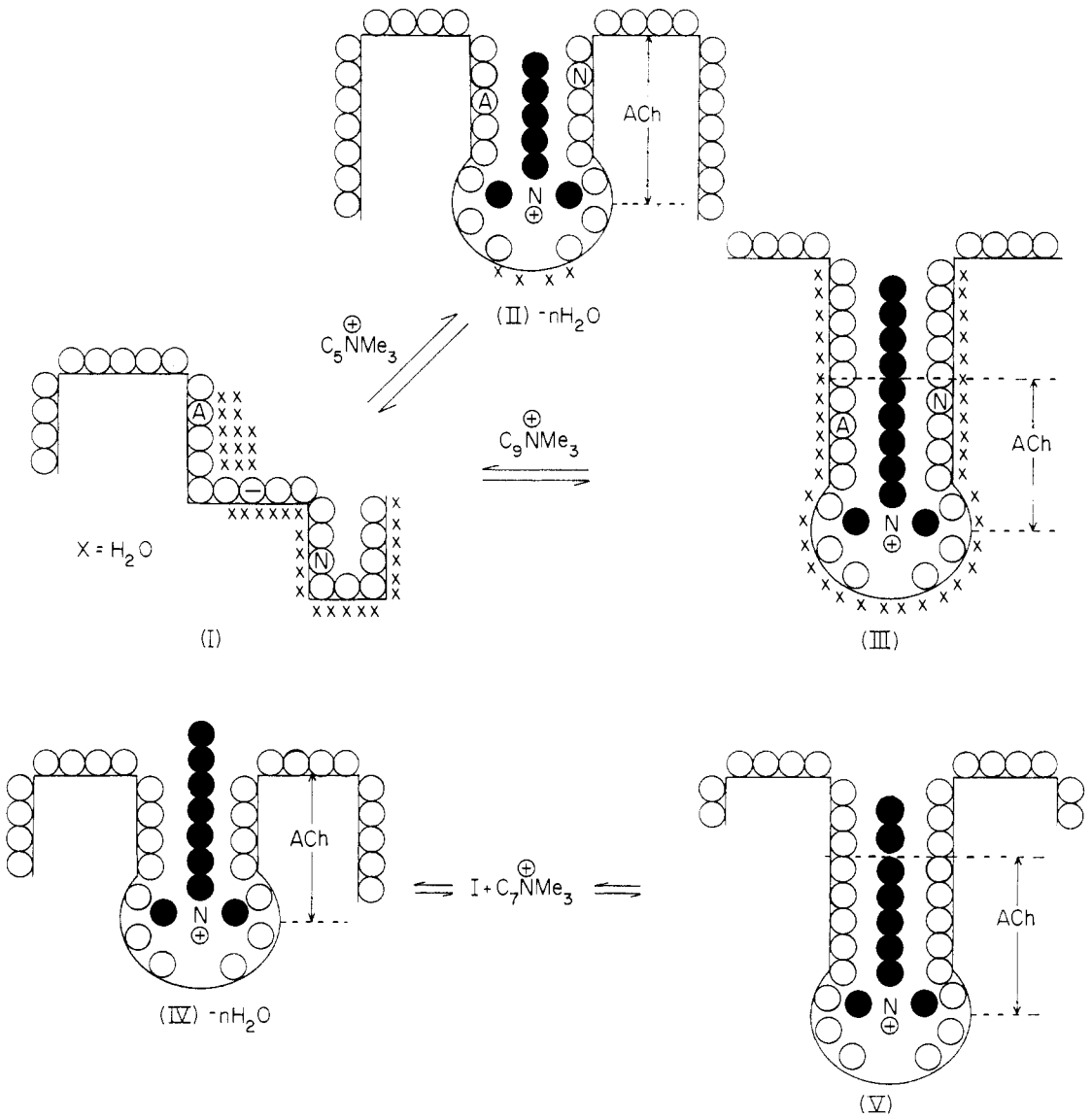


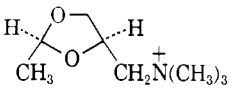
Fig. 1.—Diagram I represents protein chains in the resting state where drugs interact. The circles are hydrophobic residues. The symbol X denotes water molecules of hydration; A and N are reactive species representing specific binding sites for ACh and other strong stimulants. Diagram II represents the structure of the complex (P^*M_s) with the stimulant pentyltrimethylammonium ($C_5H_{11}N^+Me_3$) (darkened circles). The driving force for binding originates in hydrophobic interactions. Some bonds between nonpolar chains in I are broken and new ones are created with the substrate. The shape of the protein in II represents a SCP whose formation is accompanied by the expulsion of water molecules (X = H_2O). The hydrophobic character of the exposed protein surface would be increased in II. Diagram III illustrates the consequences of complex formation with the homologous nonyltrimethylammonium ion ($C_9H_{19}N^+Me_3$) (darkened circles). The four extra methylene groups induce a NSCP because hydrophobic interactions in excess of that created by ACh or ($C_5H_{11}N^+Me$) are operative. A different kind of complex results ($P^{\mp}M_i$) in which the outer surface would be less hydrophobic. Diagrams IV and V represent the consequences of complex formation with a molecule of the M_{si} type (heptyltrimethylammonium, $C_7H_{15}N^+Me_3$); an equilibrium mixture of P^*M_{si} and $P^{\mp}M_{si}$ complexes would result. The replacement of N-methyl by ethyl groups (not shown in the above diagrams) would also disturb the hydrophobic periphery of the anionic site and a complex of the $P^{\mp}$ variety would be favored.

point of greatest interest lies in the fact that whereas the ions of Na and K are tightly hydrated, quaternary cations are actually hydrophobic.[13] It may be, therefore, that *the effect of the latter is to disrupt the structure of bound water in the membrane, thus making it available for Na^+ and K^+ transport.* It may be possible to label the water in membranes and study its movements in relation to stimulation by quaternary ions. Conceivably, in a P^*M_s complex, the protein would assume that conformation which loosens a critical number of water molecules. This would not apply to $P^{\mp}M_i$ complexes.

The question of what factors will determine the affinity of M_s molecules for the receptor will be examined next. It will be useful initially the ascertain to over-all physico-chemical properties of the ACh-specific binding region of the receptor. The equally nonpolar character of this diffuse compartment is readily evidenced by the gradual increase in affinity for the receptor of alkyltrimethylammonium stimulants as the hydrocarbon chain is increased from C_1 to C_5.[22] Similar to the observations of Barlow, *et al.*,[21] on the effect of added CH_2 groups on the onium head of antagonists, the operation of ΔF_t in the stimulant series serves to establish the hydrophobic nature of the ACh compart-

(22) J. M. van Rossum and E. J. Ariens, *Arch. intern. Pharmacodyn.*, **110**, 349 (1957); J. M. van Rossum, Doctoral Dissertation, Roman Catholic University, Nijmegen, 1958.

ment. There is little doubt on that basis that Ing's rule of five[23] is relevant in a majority of cases to the phenomenon of hydrophobic interactions, the factor least exacting in its requirements. However, specific interactions between certain M_s molecules and the ACh compartment will be likely to occur as was previously demonstrated for the case of the muscarones and the dioxolane series of stimulants.[24] The presence of shielded reactive functional groups in the ACh compartment is required in order that the high potency of ACh can be accounted for. It is also conceivable that M_s molecules including π-electrons, such as the furfuryltrimethylammonium ion, may engage into charge-transfer binding with the receptor surface and thus display increased affinity. Ideally, however, maximum efficiency in the induction of a SCP will be reserved to M_s molecules producing true lock-and-key type of fits with the ACh compartment. Normally, such fits are a privilege of natural substrates (ACh in this case), but evidence has now been obtained that certain nonsubstrate molecules can interact in a substrate-like fashion with AChE.[13] Thus, the dioxolane inhibitor I of AChE was shown to allow the application of the distance-specific van der Waals attractions in its complex with AChE, a feature characteristic of ACh.[13] This finding serves to explain the high potency of I at the receptor level (an observation which once more points to the similarity of AChE to the muscarinic receptor).



I

C. Mixed Complexes.—A problem of key importance consists in deciding whether the transition from eq. 1 to eq. 2 will be a continuous or discontinuous one. In its present form, the MPT requires that only one conformation for P^*M_s complexes is productive with respect to the criterion of pharmacological activity. In contrast, an indefinite number of NSCP is encompassed by the symbol $P^{\pm}M_i$. It is conceivable that several related conformations may characterize P^*M_s complexes, but for the sake of preserving a minimum of rigor in this exposé, the tortuous paths of least resistance may profitably be avoided and P^*M_s complexes assigned unique structural features. The possibility therefore arises that certain molecules may induce the production of equilibrium mixtures of P^* and $P^{\pm}$ complexes. Obviously, energy barriers must be overcome in order that a P^*M_s or $P^{\pm}M_i$ complex can form and it is quite conceivable that for certain types of molecules, these energy barriers as well as the energies of the two types of complexes may be of comparable magnitudes. The molecular features most likely to allow this phenomenon would be incorporated in an M_s molecule carrying a substituent which does not protrude too deeply into the nonpolar periphery of the ACh compartment (Fig. 1). On a probability basis, such a molecule would be as susceptible to induce a NSCP as it would be to favor a SCP, there being two comparable energy paths for the reaction. This class of molecules will be designated by the symbol M_{si} and is typified by the stimulant heptyltrimethylammonium (Fig. 1) which includes two CH_2 groups susceptible to accommodation in the hydrophobic periphery. Equation 3 serves to rationalize this phenomenon of ambivalence. When $K_3 = 0$, eq. 2

$$P^{\pm}M_{si} \underset{K_4}{\rightleftharpoons} P + M_{si} \overset{K_3}{\rightleftharpoons} P^*M_{si} \qquad (3)$$

applies, whereas when $K_4 = 0$, eq. 1 describes the process.

It will be realized at this point that the corner stone of the MPT rests on the question of transition in the mechanism of complex formation accompanying the formal chemical conversion of an M_s molecule to the M_i type. The validity of this hypothesis was tested using AChE as a model and the C_1 to C_{12} series of alkyltrimethylammonium inhibitors as test substances. At the receptor level the transition from stimulant to antagonist in this series occurs with a chain length of 7–8 carbon atoms. In agreement with expectations based on the MPT, a sharp transition in the mechanism of interaction of these ions with AChE was observed with a chain of 8 carbon atoms.[25] These results constitute definitive evidence for the validity of the concepts forming the basis of the MPT.

D. Polynary Complexes.—It will be expected that the structural integrity of P^*M_s or P^*M_{si} complexes will be maintained principally through the operation of hydrophobic interactions. Evidence for this notion is available in the work of Tanford[26] on the key role of hydrophobic forces in the stabilization of the tertiary structure of proteins. The multiplicity of nonpolar regions (as entities distinct from the active surface) in proteins[26] suggests that an indefinite number of new binding sites may be created when additional molecules of nonpolar character are brought into contact with the protein. This will be especially true for charged substrate molecules which allow for initial contact with counter ions on the outer surface of the protein. Pertinent evidence supporting this view is available in the observations of Lovrie[19] on the nonspecific binding of nonpolar chains by serum albumin, a protein devoid of known catalytic properties. It is of interest that the latter will create new binding sites only if it is initially perturbed by alkali. Extrapolation of these observations to the case of a P^*M_s or P^*M_{si} complex leads to the conclusion that suitable quaternary ions carrying hydrophobic chains will likely create additional binding sites in such complexes and therefore induce conformational deformation (NSCP). The driving force for such reactions resides in the hydrophobic interactions exerted upon the substrate molecules. No specific preformed binding sites are required for the reaction to occur. As pointed out above, if the formation of a P^* complex implies the "loosening up" of water molecules, the nonpolar character of the entire protein surface would have to increase, thus facilitating the nonspecific accommodation of additional hydrophobic ions. These considerations suggest that eq. 4 should be applicable to active receptor complexes. The same ought to apply to

$$P^*M_s \text{ or } P^*M_{si} + nM_s \text{ or } nM_{si} \rightleftharpoons P^{\pm}(M_s)^n \text{ or } P^{\pm}(M_{si})^n \qquad (4)$$

(23) H. R. Ing, P. Kordik, and D. P. H. T. Williams, *Brit. J. Pharmacol.*, **7**, 103 (1952).

(24) B. Belleau and J. Puranen, *J. Med. Chem.*, **6**, 325 (1963).

(25) B. Belleau and F. Lie, *Pharmacol. Rev.*, in preparation.

(26) C. Tanford, *J. Am. Chem. Soc.*, **84**, 4240 (1962).

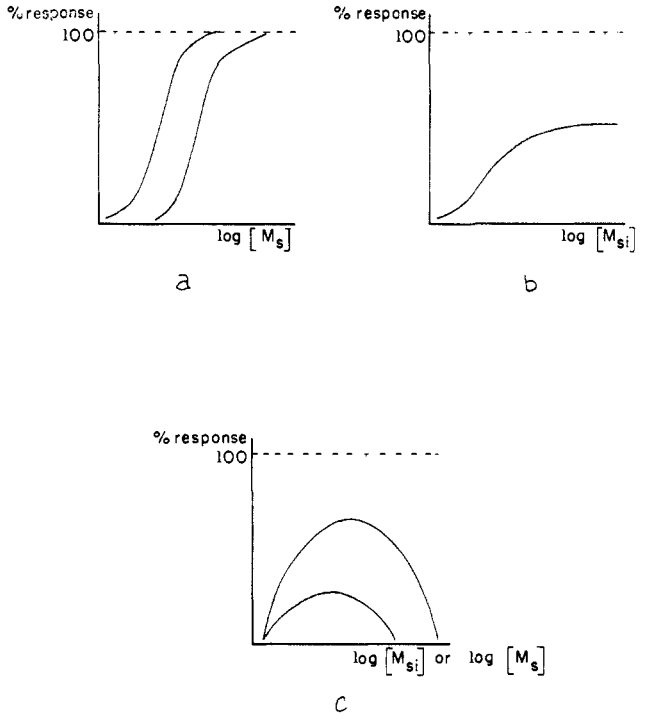


Fig. 2.—Dose–response curves for: a, agonists (eq. 1); b, partial agonists (eq. 3); c, ternary complex formation (eq. 4).

$P{\mp}M_i$ complexes, but this case need not be considered further since such complexes are already inactive as such. On a probability basis, a fairly large number of M_s molecules should exist out of which very few can be expected to produce a lock-and-key type of fit with the specific binding surface of the protein. Similarly, an equally large number of compounds will be of the M_i type (since the requirements for the induction of a NSCP are quite low) but only a restricted number will behave as M_{si} molecules because of the more exacting requirements for the applicablity of eq. 3.

Derivation of Dose–Response Relationships Using the Macromolecular Perturbation Theory as a Basis.—The application of an M_s molecule (such as the natural substrate ACh) to the receptor protein will result in the formation of a P^*M_s complex, the equilibrium concentration of which will depend, as discussed above, on the structural features of the molecule. A maximal response will always be obtainable with this class of molecules which therefore must belong to the category of pure agonists. The general form of the corresponding dose–response curve shown in Fig. 2a is the simple reflection of the application of the mass action law. The parallel shift of the curves obtained with different M_s molecules is an index of the relative efficiencies in the induction of a SCP in the protein. An M_i type of molecule will on the other hand produce a NSCP in the protein (eq. 2) and competitive antagonism will be observed. However, an M_{si} molecule will now induce the formation of an equilibrium mixture of $P{\mp}M_{si}$ and P^*M_{si} complexes (eq. 3) and only submaximal responses will obtain. This phenomenon accounts for the existence of partial agonists and the dose–response curve will have the general form shown in Fig. 2b. Finally, the operation of eq. 4 at the receptor protein level will be reflected in bell-shaped dose–response curves as illustrated in Fig. 2c. This phenomenon will tend to be observed with M_s and M_{si} molecules of hydrophobic character for reasons discussed above.

It can be seen without any necessity for further elaboration that the predicted dose–response curves shown in Fig. 2 are of a familiar and well-precedented form[5–8] in the field of drug–receptor interactions. It is significant that a purely molecular approach to the mechanism of such interactions should allow the prediction of dose–response curves which are in agreement with the general form of experimental curves. These observed curves have already been fitted mathematically to various equations[5–9] which, however, failed to reveal the biophysical basis of drug action. It is of special significance that the MPT should correspond at the molecular level to the statistical model of Jankn and Mandl.[11] The phenomenon of intrinsic activity or efficacy[5,6,8] can therefore be seen to reflect the operation of the molecular mechanism described by eq. 3. The key question of Paton[9] as to why drugs vary in their type of action "—so that when one drug occupies a receptor it stimulates, whereas another occupying the same receptor blocks," appears to find an answer in the MPT.

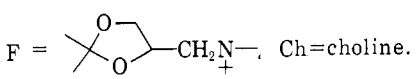
The Macromolecular Perturbation Theory as a Basis for the Interpretation of Structure–Activity Relationships.—The muscarinic cholinergic receptor was selected as the test model and its active surface assigned the diffusely compartmented hydrophobic structure depicted in Fig. 1. The total number of hydrophobic interactions favoring a NSCP of the receptor were computed for various series of quaternary salts using the extended conformation of ACh as the reference structure. Better correlations might be achieved if the conformation of receptor-bound ACh[24] were used as the reference model. Qualitative agreement between pharmacological properties and the total number of accommodative perturbations at the periphery of the ACh compartment should be achieved if the MPT is applicable. It is clear that only those drugs for which dose–response curves are available can be used in correlation studies of this kind. Using literature data[5–8,22] as a basis, the compilation shown in Table I could be constructed. The results are reliable only to the extent that the literature data are meaningful.

It is significant that a virtually perfect correlation between hydrophobic interactions in excess of that produced by ACh and qualitative pharmacological properties should be revealed. As would be expected on the basis of the MPT, the average number of disturbing hydrophobic interactions determines whether a molecule will act as an agonist, a partial agonist, or an antagonist. For M_s, M_{si}, and M_i molecules the respective average numbers of extra hydrophobic interactions are 0.15, 2.1, and 4.3; these numbers serve to characterize the respective application of eq. 1, 3, and 2 in that order. Since the most rigorous test for any theory of drug action has consisted thus far in appraising its suitability for the interpretation of structure–activity relationships, one may conclude that the MPT fulfills this criterion in a most satisfactory manner. It will also be noted that, as anticipated above, the observation of bell-shaped dose–response curves which would reflect the operation of eq. 4 is a characteristic of molecules carrying highly hydrophobic side chains.

TABLE I

Compd.[a]	Nature of complex[b]	Hydrophobic interactions favoring NSCP	Height of parasympathetic (muscarinic) stimulation[c]
$HC{\equiv}C{-}N^+Me_3$	P^*M_s	0	Maximal
$CH_2{=}CHN^+Me_3$	P^*M_s	0	Maximal
$BrCH_2CH_2N^+Me_3$	P^*M_s	0	Maximal
$Br(CH_2)_3N^+Me_3$	P^*M_s	0	Maximal
$CH_3OCCH_2N^+Me_3$ (‖ CH_2)	P^*M_s	0	Maximal
$AcO(CH_2)_2S^+Me_2$	P^*M_s	0	Maximal
$AcNH(CH_2)_2N^+Me_3$	P^*M_s	0	Maximal
(ACh)	P^*M_s	0	Maximal
Carbachol	P^*M_s	0	Maximal
(Ac- -Me-Ch)	P^*M_s	0	Maximal
(Muscarine)	P^*M_s	0	Maximal
$HFMe_3$	P^*M_s	0	Maximal
$HFMe_2Et$	P^*M_s	1	Maximal
dl-$MeFMe_3$	P^*M_s	0	Maximal
(L-*cis*-$MeFMe_3$)	P^*M_s	0	Maximal
$EtFMe_3$	P^*M_s	1	Maximal
$MeFMe_2Et$	P^*M_s	1	Maximal
Me_2FMe_3	P^*M_s	0	Maximal
Ch	P^*M_s	0	Maximal
MeN^+Me_3	P^*M_s	0	Maximal
EtN^+Me_3	P^*M_s	0	Maximal
PrN^+Me_3	P^*M_s	0	Maximal
BuN^+Me_3	P^*M_s	0	Maximal
n-$C_5H_{11}N^+Me_3$	P^*M_s	0	Maximal (BSC)[d]
n-$C_6H_{13}N^+Me_3$	P^*M_s	1	Maximal (BSC)[d]
		(av. = 0.15)	
$HFMeEt_2$	$P^*M_{si} \rightleftarrows P{\mp}M_{si}$	2	Submaximal
$EtFMe_2Et$	$P^*M_{si} \rightleftarrows P{\mp}M_{si}$	2	Submaximal
$PrFMe_3$	$P^*M_{si} \rightleftarrows P{\mp}M_{si}$	2	Submaximal
$HFEt_3$	$P^*M_{si} \rightleftarrows P{\mp}M_{si}$	3	Submaximal
$MeFMeEt_2$	$P^*M_{si} \rightleftarrows P{\mp}M_{si}$	2	Submaximal
Et_2FMe_3	$P^*M_{si} \rightleftarrows P{\mp}M_{si}$	2	Submaximal
$ChMe_2Et$	$P^*M_{si} \rightleftarrows P{\mp}M_{si}$	1	Submaximal
$ChMeEt_2$	$P^*M_{si} \rightleftarrows P{\mp}M_{si}$	2	Submaximal
n-$C_7H_{15}N^+Me_3$	$P^*M_{si} \rightleftarrows P{\mp}M_{si}$	2	Submaximal (BSC)[d]
BuN^+Me_2Et	$P^*M_{si} \rightleftarrows P{\mp}M_{si}$	2	Submaximal
n-$C_6H_{11}N^+Me_2Et$	$P^*M_{si} \rightleftarrows P{\mp}M_{si}$	1	Submaximal
		(av. = 2.1)	
$BuFMe_3$	$P{\mp}M_i$	3	Nil
n-$C_6H_{13}FMe_3$	$P{\mp}M_i$	5	Nil
$C_6H_5FMe_3$	$P{\mp}M_i$	5	Nil
$PrFMe_2Et$	$P{\mp}M_i$	3	Nil
$EtFMeEt_2$	$P{\mp}M_i$	3	Nil
Pr_2FMe_3	$P{\mp}M_i$	5	Nil
Bu_2FMe_3	$P{\mp}M_i$	7	Nil
$(C_6H_5)_2FMe_3$	$P{\mp}M_i$	11	Nil
$ChEt_3$	$P{\mp}M_i$	3	Nil
n-$C_8H_{17}N^+Me_3$	$P{\mp}M_i$ (weak P^*M_{si})	3	Nil (BSC)[d]
n-$C_9H_{19}N^+Me_3$	$P{\mp}M_i$ (weak P^*M_{si})	4	Nil (BSC)[d]
n-$C_{10}H_{21}N^+Me_3$	$P{\mp}M_i$	5	Nil
n-$C_6H_{13}N^+Me_2Et$	$P{\mp}M_i$	2	Nil
BuN^+MeEt_2	$P{\mp}M_i$	2	Nil
n-$C_5H_{11}N^+MeEt_2$	$P{\mp}M_i$	2	Nil
BuN^+Et_3	$P{\mp}M_i$	3	Nil
n-$C_{12}H_{25}N^+Me_3$	$P{\mp}M_i$	7	Nil
		(av. = 4.3)	

[a] Compounds enclosed in parentheses are taken as interacting ideally (or nearly so) with the protein;



Ch=choline.

[b] P^*M_s = addition complex resulting in a SCP, $P{\mp}M_i$ = addition complex characterized by a NSCP, P^*M_{si} and $P{\mp}M_{si}$ are complexes resulting from an equilibrium mixture of SCP and NSCP. [c] BSC = bell-shaped curve.

Some of the most efficient inducers of a P^*M_s complex are enclosed in parentheses in Table I. It seems reasonable to ascribe their high potency to their ability to produce lock-and-key type of fits with the protein's specific binding surface or to engage in highly specific binding other than by van der Waals forces with that surface. Thus far, evidence that specific van der Waals binding has a marked favorable influence on potency has been adduced only in the case of the L-*cis*-dioxolane quaternary salt (I) which is believed[13] to produce a true lock-and-key type of fit with the related protein AChE. The high potency of L-(+)-muscarine[27] suggests that specific van der Waals attractions may be operative. It would be presumptuous at this stage to attempt a detailed interpretation of all the other factors controlling the relative potencies of stimulants. Much more will have to be learned about the factors influencing affinity and conditioning the induction of a SCP. The recognition of the key role of hydrophobic interactions (accounting for the ΔF_t term) as a factor distinct from van der Waals attractions certainly constitutes an important step in this direction.

It will be noted that the special case of noncompetitive antagonism has not been considered in the treatment of the MPT. The reason for this lies in the fact that noncompetitive inhibition can usually be demonstrated only with substances bearing a distant structural analogy (such as papaverine, inorganic ions) to stimulants or competitive inhibitors. It is known that noncompetitive inhibitors of enzymes often do not interact with the same active sites that normally bind substrates or competitive inhibitors.[28]

The Nature of the Muscarinic Cholinergic Receptor.—It has long been noted by several investigators that many similarities exist between AChE and the cholinergic receptor. A number of new parallelisms between the two have been noted in this paper and elsewhere.[13] The most striking of them consist in that (a) both active surfaces are similarly hydrophobic in character and (b) both display identical patterns of absolute and relative stereospecificities towards the dioxolane and muscarine series[13] of stimulants. Other more revealing correlations will be discussed separately in the near future.[25] The hypothesis of the identity of AChE and the cholinergic receptor was formulated first by Roepke in 1937.[29] Since then, an impressive accumulation of conflicting data has cast considerable uncertainty on this hypothesis. Pertinent literature on this subject is available.[30] One of the key inconsistencies created by the AChE hypothesis for the receptor consists in that the latter is not inactivated by the organophosphorus drugs. However, some recent kinetic studies by Krupka and Laidler[31] have revealed that acetylated AChE, the intermediate which is formed during hydrolysis of ACh, interacts equally well with inhibitors as free AChE does. It is currently believed that the acetyl group is attached to the same serine hydroxyl that serves as the eventual acceptor of phosphoryl groups. If it is now postulated that the induction of the physiologically significant SCP results from interactions with acetylated AChE, an explana-

(27) P. Waser, *Experientia*, **17**, 300 (1961).

(28) K. J. Laidler, "The Chemical Kinetics of Enzyme Action," Clarendon Press, Oxford, 1958, p. 77.

(29) M. H. Roepke, *J. Pharmacol. Expil. Therap.*, **59**, 264 (1937); see also W. C. Wescoe and W. F. Riker, Jr., *Ann. N. Y. Acad. Sci.*, **54**, 438 (1951).

(30) "Handbuch der Experimentellen Pharmackologie," Vol. 15, G. B. Koelle, Sub-Ed., Springer-Verlag, Berlin, 1963, Chapters 9, 13.

(31) R. M. Krupka and K. J. Laidler, *J. Am. Chem. Soc.*, **83**, 1445, 1448, 1454 (1961).

tion for the receptor resistance to attack by organophosphorus drugs would lie in the unavailability of a free serine hydroxyl for phosphorylation. Some intriguing features of this hypothesis may be summarized as follows. The receptor would have its biochemical origin in the pool of AChE, a portion of which would be trapped in the membrane network and maintained in the acetylated form through a steady-state quantal discharge of ACh from the synaptic cleft. This would constitute a self-generating system, a phenomenon not uncommon in biochemistry (as is the case for instance for some of the catalytic intermediates of the tricarboxylic acid cycle). Assuming that some drugs could cause release of additional quantities of ACh[32] in addition to interacting directly with the receptors, more acetylated AChE could be made available with the result that steeper dose-response curves than expected would be observed as is often the case. Finally, the physico-chemical events following receptor stimulation might allow hydrolytic splitting of the acetyl group, thus accounting for desensitization. Reacetylation would be essential for sensitivity to reappear. It would be of considerable interest to attempt labeling of these receptors with radioactive acetyl groups and then study their turnover rate in the presence of various drugs.

It should be emphasized that the characteristics of the preceding speculation do not affect in any way the arguments forming the basis of the MPT.

(32) G. B. Koelle, *J. Pharm. Pharmacol.*, **14**, 65 (1962).

1-Aralkyl-4,4-dialkylpiperidines as Hypotensive Agents

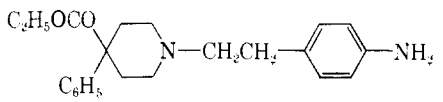
T. C. SOMERS AND G. J. HANDLEY[1]

Nicholas Institute for Medical and Veterinary Research, Sherbrooke, Victoria, Australia

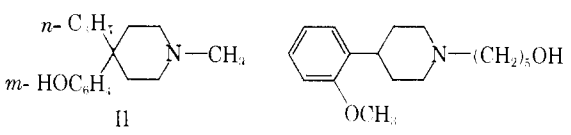
Received June 1, 1964

A large number of 1,4,4-trisubstituted piperidines have been synthesized by lithium aluminum hydride reduction of the corresponding glutarimides. Many of the piperidines are highly active as hypotensives when administered intraperitoneally to intact conscious rabbits. The most active compounds were 1-(3,4-diethoxyphenethyl)-4-methyl-4-*n*-hexylpiperidine hydrochloride and 1-(*p*-methoxyphenethyl)-4-spirocyclohexanepiperidine hydrochloride. Structure–activity relationships are discussed.

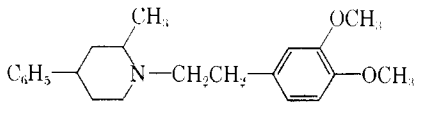
In recent years variations on the piperidine structure have been the subject of many investigations in addition to the earlier work which led to the introduction of 4-carboethoxy-1-methyl-4-phenylpiperidine (pethidine) as an analgesic. Other such compounds having potent analgesic activity have since been reported, *e.g.*, I[2] and II.[3] Hypotensive activity has also been demonstrated in this class, 1,2,2,6,6-pentamethylpiperidine (Pempidine) being the most prominent example. Other piperidines having hypotensive activity are III[4] and related compounds, while IV[5] has been reported to have neurosedative as well as hypotensive and antiemetic actions.



I

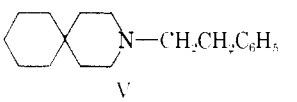


II III

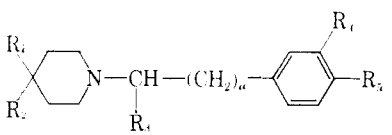


IV

It appeared that, with the exception of the pempidine category, a phenyl substituent at the 4-position was necessary for useful pharmacological activity. We had been engaged in a study of β,β-dialkylglutarimides[6] from which piperidines are easily obtained by lithium aluminum hydride reduction. The availability of a large number of glutaric acids, therefore, prompted our investigation of the effects of 4,4-dialkyl substitution in the piperidine ring with a variety of substituents on the nitrogen. Among the first compounds synthesized was 1-phenethyl-4-spirocyclohexanepiperidine (V). This was found to have negligible analgesic activity, but further screening showed interesting hypotensive action. Increased hypotensive activity resulted on introduction of a 1-methyl substituent into the side chain and encouraged further investigation of such compounds.



V



VI

This publication deals principally with the preparation and evaluation as hypotensives of piperidines

(1) To whom all inquiries should be addressed.

(2) J. Weijlard, P. Orahovats, A. Sullivan, G. Purdue, F. Heath, and K. Pfister, *J. Am. Chem. Soc.*, **78**, 2342 (1956).

(3) S. M. McElvain and D. H. Clemens, *ibid.*, **80**, 3915 (1958).

(4) U. S. Patent 2,891,066 (1959); J. Owen and T. Verhave, *J. Pharmacol. Exptl. Therap.*, **122**, 59 (1958).

(5) Eli Lilly and Co., Australian Patent 225,975 (1959).

(6) G. J. Handley, E. R. Nelson, and T. C. Somers, *Australian J. Chem.*, **13**, 129 (1960).