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M. STERIC EFFECTS IN CATECHOLAMINE INTERACTIONS WITH ENZYMES AND RECEPTORS

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The specific receptors for epinephrine (E), norepinephrine (NE) and closely related compounds constitute a field where there exists no deficiency of speculations, a state of affairs to which the present author has contributed more than his normal share. The feeling of uneasiness imposed by this situation has recently been alleviated by Bloom and Goldman (11), who elaborated extensively on some of our primitive ideas on the molecular basis of catecholamine actions. Concomitantly, some novel general views on the mechanism of drug-receptor interactions have been formulated (6) and a biophysical basis for the pharmacological transitions from agonist to antagonist attending the molecular modification of drugs has become available (10). A taste of this concoction of analyses and concepts readily reveals that steric effects (taken in the broadest sense) may constitute the chief factor controlling the qualitative properties of drugs. Some of the most striking (as well as confusing) examples of the key role that steric effects assume at the receptor level are found in the field of the catecholamines. A brief exposé of the state of our comprehension (or lack of it) of these puzzling steric factors as they pertain to modern receptor theory may be in order at this time.

GENERAL STERIC REQUIREMENTS FOR α- AND β-EFFECTS

A clue to the general structural requirements of α- and β-receptors was given by us some time ago (4) and amplified by Ariëns and Simonis (1). A first-order analysis of structure-action relationships revealed that for active α-complex formation, a small cationic head (such as ammonium or methylammonium) was essential in a catecholamine, but that the presence of bulkier N-substituents (as in isopropyl NE, isoproterenol) would hinder ion-pair formation with an α-anionic site while promoting β-receptor activation. It was reasoned at that time that ion-pair formation with an α-anionic site would condition α-stimulation. Since β-effects manifest themselves when steric hindrance to ion-pair formation occurs, the conclusion was reached that the catechol ring would be the key moiety contributing to β-receptor activation. This general trend has been corroborated by Ariëns and Simonis (1). It was emphasized also that strong α-effects are usually characteristic of the presence of catechol hydroxyls (an exception is phenylephrine, which has only one *meta*-hydroxyl). It remains to comprehend the biochemical basis of these specific requirements.

STEREOELECTRONIC EFFECTS ON COMPLEX FORMATION BETWEEN β-RECEPTORS AND CATECHOL RINGS

Consideration should be given initially to recent findings in the general field of the elementary processes governing complex formation between small mole-

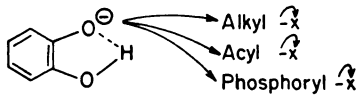
cules and proteins, and by inference between drugs and receptors. Concrete evidence was produced (10) that there exist two divergent biophysical consequences for a receptor protein when it participates in the formation of a complex with structurally related drugs: with agonist drugs, an *ordering effect* (or specific conformational perturbation, SCP) would be induced in the protein whereas a *disordering effect* (or nonspecific conformational perturbation, NSCP) on the macromolecule would characterize interactions with antagonists (10). In the simplest cases, disordering effects would result from the accommodation of substituent groups into the peripheral nonpolar regions of the receptor active surface (6). The driving force (expressed as free energy of transfer ΔF_t from an aqueous biophase) for such nonspecific accommodations originates in hydrophobic repulsions, which attain a maximal value when the substituent group on the drug is essentially nonpolar. It is a prerequisite also that the acceptor surface be hydrophobic in character for the transfer process to be effective.

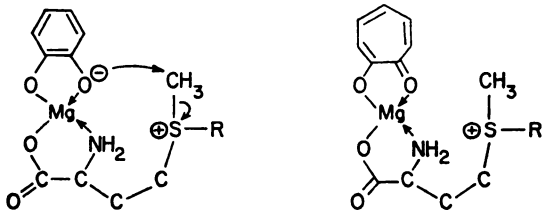
Since the β-receptor surface can accommodate the dichlorophenyl ring (ΔF_t = several kilocalories) of dichloroisoproterenol (DCI) (or the naphthalene ring of pronethalol), it follows that the region of binding of the catechol ring must be largely nonpolar. The consequence of binding of such a group as dichlorophenyl is a NSCP or disordering effect, reflected in antagonism. On the other hand, catechol itself is water-soluble even though the phenyl ring as such is highly hydrophobic. Its transfer to the receptor surface will occur only at the expense of breaking strong hydrogen bonds with water. If an additional driving force such as chelate formation with the nonpolar area of the receptor surface intervenes, more ready transfer should occur, which would result in an ordering effect (SCP) in the protein. The question is to interpret this ordering effect in structural terms. This may be tentatively achieved by using the suggested identity (22) of β-receptors with adenylcyclase as a starting point. Because of the total structural dissimilarity of the substrate ATP with catecholamines, it would seem clear that adenylcyclase belongs to the category of regulatory enzymes whose catalytic activity towards substrates is controlled by the interaction of specific regulators with regulatory (or allosteric) sites (7). Whether activation or inhibition of adenylcyclase will occur, ought to depend on whether the drug molecule induces a favorable (SCP) or an unfavorable (NSCP) conformational change. The regulatory site for catecholamines may either be adjacent to the ATP binding surface or completely removed from it. For *strictly chemical reasons*, the first possibility has been tentatively given priority (4). This point is discussed below. In any event, structure-action relationships indicate that the catechol binding site has a predilection for aromatic rings carrying electron-rich substituents; this relationship suggests that complex formation may be largely dependent on π-bonding. Whether the catechol hydroxyls serve only as anchoring groups or participate actively in the ring closure of ATP to cyclic 3′,5′-AMP will be decided only after careful studies with the purified enzyme. In the meantime, the answer may be tentatively anticipated on the basis of presently available information.

SOME PERTINENT BIOCHEMICAL PROPERTIES OF CATECHOL RINGS IN MODEL SYSTEMS

The dramatic effect of catechol ring compounds at the receptor level suggests that one of its known outstanding catalytic properties may be involved. In particular, the association of catecholamines with the adenylcyclase-catalyzed conversion of ATP to cyclic 3′,5′-AMP suggests a general role for catechol rings in phosphoryl group transfer reactions. It is an interesting coincidence, albeit irrelevant perhaps, that ATP and catecholamines should also be associated in the specific storage granules (17).

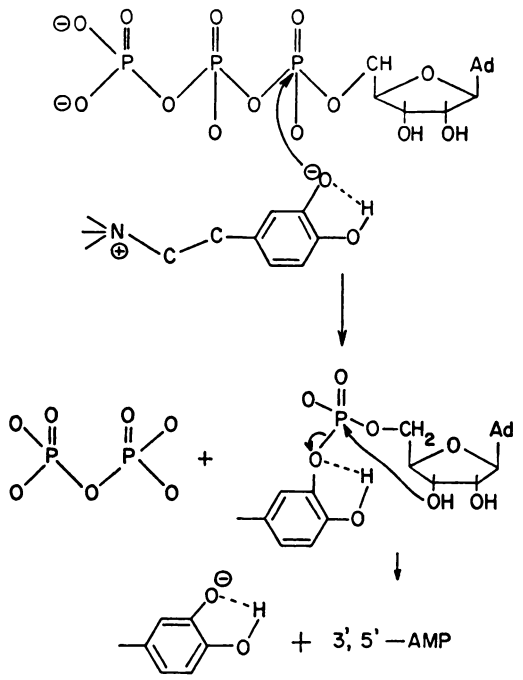
The most conspicuous chemical properties of catechols consist in their ready participation in redox reactions (16), their ability to form chelates (2) and their obvious ability to engage in hydrogen binding. Another less known property which may be of considerable biochemical significance, is their marked reactivity in the monoanionic form in acyl (13), alkyl (21), and phosphoryl group transfer reactions (15) (fig. 1). This latter property can be markedly enhanced if cationic substituents are attached to the ring (15). In the case of alkyl group transfer reactions, the participation of a metal ion has been demonstrated both with model organic chemical systems (21) and with the enzyme preparation catechol-O-methyl transferase (COMT) (20). With the latter enzyme, the catechol ring has been visualized as forming a quaternary 1:1:1:1 complex with magnesium, S-adenosylmethionine and enzyme (21) (fig. 1). The resulting steric and electronic configuration of the Michaelis complex would allow for easy nucleophilic attack of the active methyl group of the cofactor and would result in the transfer of the methyl group to one of the catechol hydroxyl groups. It would seem clear therefore that chelation would condition the formation of a productive Michaelis complex with COMT, and, in agreement with this view, the isosteric





Chemical and Enzymatic capabilities of catechols

FIG. 1



Hypothetical participation of a catechol monoanion in 3', 5' -AMP formation

FIG. 2

ring of the tropolones [which have strong chelating properties (12)] was shown (8) to act as a relatively potent competitive inhibitor of the enzyme (fig. 1). These observations support the hypothesis that chelation may be a key factor controlling complex formation between catechol rings and natural bioreceptors. This hypothesis may be elaborated further by postulating that in the metal-bound monoanionic form, the role of catechol rings might be to participate in nucleophilic displacement reactions (as in the case of COMT). Theoretically the nucleophilic displacement reaction leading from ATP to cyclic 3′,5′-AMP might also involve a catechol monoanion as a participant (fig. 2). However, a serious difficulty with this visualization is that β-agonist activity is currently believed to be associated with certain noncatechol analogs of the catecholamines (23). Assuming that these effects are genuine and not mediated through some unknown endogenous release mechanisms, only the restricted hypothesis of simple anchoring by way of chelation would be worthwhile retaining at the receptor level. It must be emphasized however that while chelation may be required for strong β-effects, it cannot be a general prerequisite for complex formation since DCI or pronethalol can act as competitive inhibitors of adenyl-cyclase (18) and β-receptors. An interesting analogous situation is found in the observation that 3,5-diodo-*p*-hydroxybenzoic acid, a nonchelating substance,

will competitively inhibit COMT (14) presumably because the large hydrophobic repulsions created by the iodine atoms supply a sufficient driving force for transfer of the molecule to the enzyme surface.

STERIC FACTORS AT THE α-RECEPTOR *VERSUS* THE β-RECEPTOR LEVELS

A first-order analysis of the effect of nonpolar substituents on the nitrogen of NE brings to light some remarkable facts: the formation of an active (ordered by virtue of SCP of the protein) β-receptor complex is virtually independent of the presence or size of the N-substituents. Since there can be no question that hydrophobic repulsions are principally concerned in the nonspecific transfer of nonpolar N-substituents to the β-receptor protein (a β-effect being triggered by such drugs as N-(phenylbutyl)-NE), it follows that this receptor would tolerate to a remarkable degree the induction of disordering effects (NSCP) without any hindrance to the activating effect of the molecule. In marked contrast, but in line with theoretical expectations, the induction of disordering effects by the same type of nonpolar N-substituents at the α-receptor level leads to antagonism. This remarkable biophysical paradox has not been explained, but it can be resolved by assuming different chemical environments for the α- and β-sites of binding of the N-aralkyl substituents. Considerations of this kind illustrate best the divergent structural and steric requirements of the two types of receptors at the anionic site level and cast considerable doubt on the author's tentative and primitive single receptor hypothesis of a few years ago (5).

It would seem clear that whereas the *immediate* environment of the α-anionic site would be largely *polar* in character, that of the β-anionic site would be essentially *nonpolar*. This is evidenced by the fact that the N-isopropyl group of isoproterenol NE largely precludes either activation or blockade of α-receptors while promoting β-agonistic activity. Only with overwhelming hydrophobic repulsions such as are created by a N-phenylbutyl group can transfer of the substituent to the peripheral protein chains of the α-anionic site occur and thus induce a NSCP, ultimately reflected in blockade. Transfers of this kind would shield the α-anionic site as required by the theory of ion-pair formation (4), but in view of some more recent observations on the mechanism of drug-receptor interactions (6), competitive antagonism at the α-receptor level may best be ascribed to the induction by large N-substituents of disordering effects (NSCP). The basic question as to why the same N-aralkyl substituents do not produce inhibition of β-receptors may be taken as an indication that they are not accommodated by the protein chains at the periphery of the β-anionic site (thus precluding the induction of a NSCP) but rather by some other moiety such as the hydrophobic adenine ring of the substrate ATP. In this manner, catalysis of the ring closure of ATP to cyclic 3′,5′-AMP would remain feasible, the conformational integrity of the protein chains being preserved (in contrast to the effect produced by changing the catechol ring for a dichlorophenyl ring).

This brings us to the recent provocative contribution of Bloom and Goldman (11) to the molecular mechanisms underlying α- and β-receptor activation and blockade. Using an earlier construct of the present author (4) as a starting point

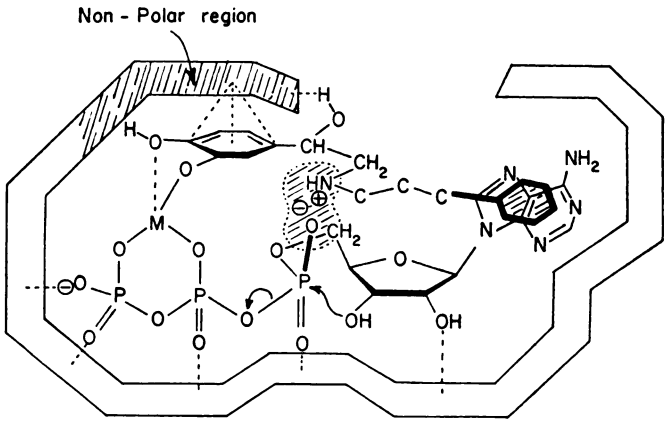


FIG. 3

for the chemical mechanism of catecholamine activation of adenylcyclase, these authors remarked that large nonpolar N-substituents can produce optimal overlap with the adenine ring of ATP as judged from the use of molecular models. Also, as was originally suggested (4), these authors retained the idea that anchoring of the catechol ring through chelation and charge neutralization of the 5′-phosphate moiety of ATP would favor displacement of pyrophosphate by the 3′-hydroxyl of the ribose residue. The N-alkyl substituent would project into the enzyme surface; however, for the reasons given above, such substituents should preferably be bound to the adenine ring. This leads to the scheme shown in figure 3. These speculations serve to account for the diverging effects of N-aralkyl groups at the α- and β-receptor levels. It must be emphasized that the suggested representation (fig. 3) may be applicable only as long as the regulatory site of adenylcyclase for catecholamines makes an integral part of the ATP binding surface. No direct evidence that this is so is available.

With regard to α-receptor activation, the effect of N-substituents has been tentatively interpreted as involving steric control of ion-pair formation between the cationic head of agonists and the α-anionic site (4). Sufficient charge neutralization of an anionic site may be visualized as bringing about momentary cancellation of repulsive forces between two like charges, thus allowing for an ordering effect (SCP) in the receptor and conceivably permitting a charged substrate to approach some specific catalytic sites (fig. 4). This latter effect might precede and condition a nucleophilic attack on the substrate and might serve to convert it to "something" intimately associated with contractile processes. In other words, the α-receptor would be just another regulatory enzyme of some kind and would possess a regulatory site designed to accommodate primarily NE and E. It is tempting to speculate that ATP (or some other suit-

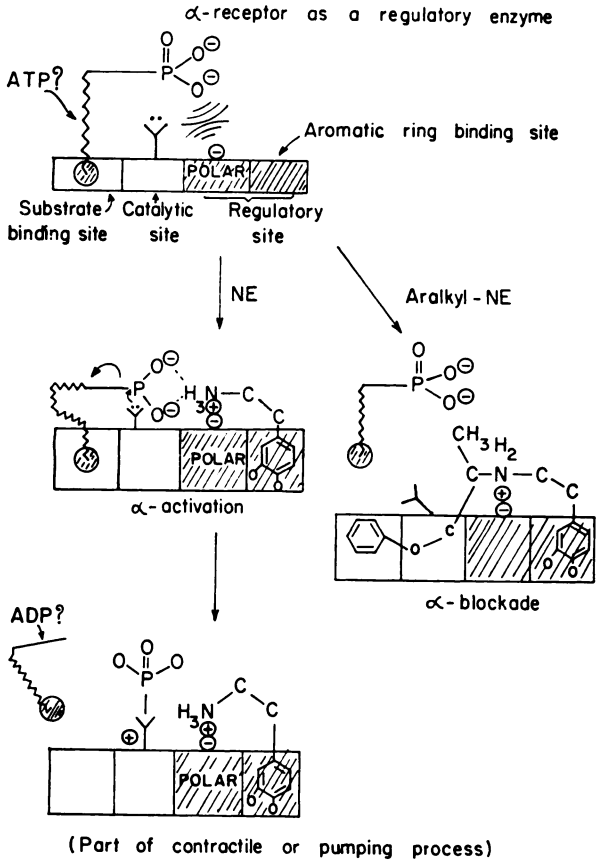
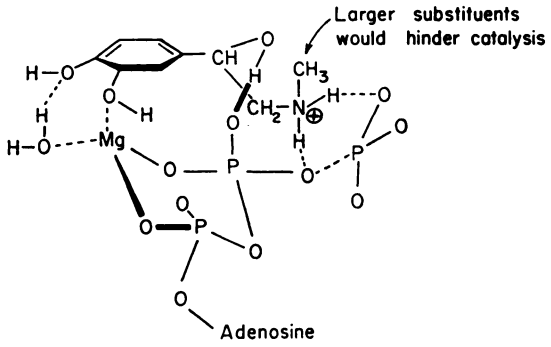


FIG. 4

able phosphoryl group donor) may again act as the substrate; in this case, the charged group to be transferred might be the terminal phosphate group (fig. 4). A highly ingenious alternative interpretation of α-receptor activation was recently advanced by Bloom and Goldman (11). These authors suggested that the cationic heads of E and NE would actually catalyze an ATPase-like reaction (fig. 5). Strong mechanistic arguments based on suitable chemical precedents were presented, and these account nicely in a large measure for steric effects about the cationic head as well as the effects of cations on adrenergic responses. Increased ATPase activity would be linked to the release of Ca^{++}, which in turn would activate a contractile process. According to Bloom and Goldman (11), the effect of large N-alkyl substituents would be to hinder catalysis of the hydrolysis of the terminal phosphate group of ATP rather than hindering ion-pairing processes as was initially envisaged by us (4). Of course anyone adventurous enough to attempt the extraction of biochemical mechanisms from the molecular properties of the catecholamine probes, runs the risks normally associated with basing conclusions on hearsay testimony. This con-



Catecholamine - catalysed ATP - ase (after Bloom and Goldman)

FIG. 5

stitutes one of the major reasons why the visualizations of Bloom and Goldman will fail for instance to explain the α-agonistic activity of N,N-dimethyl-N (19). Because of steric hinderance and because only one proton can be bound to the nitrogen of this latter compound, no catalysis of the ATPase reaction would seem feasible. Conceivably, the charge-neutralization hypothesis (fig. 4) may be reconciled better with observations of this kind since the N-substituents are not so bulky. In any event, the analysis of Bloom and Goldman (11) is a most penetrating and provocative one. A constructive general criticism that one may offer at this time is that these authors may have paid too little attention to the probable role of the protein component acting as the primary catalyst of any postulated transformaion of ATP or other potential substrates. When this is possible, catecholamine catalysis at the α-receptor level may prove to be generally interpretable in terms of the regulation of an enzyme-catalyzed transformation of ATP as appears to be the case for adenylcyclase or β-receptors.

STEREOELECTRONIC EFFECTS IN RELATION TO IRREVERSIBLE ADRENERGIC BLOCKADE. MODEL STUDIES

Two outstanding factors are associated with the irreversible blocking activity of 2-haloalkylamines of the Dibenamine series: a) chemical reactivity and b) steric requirements (3). Within a homogeneous series, the relative potencies and the onset of blockade are a function of the rate of formation of quaternary aziridinium ions (QAI) (5). The fact that the stability of the α-receptor alkyl derivative (or the blocked receptor) is markedly sensitive to the chemical structure of the drug and that it also roughly parallels the relative chemical reactivity of the initial 2-haloalkylamine precursor (5) was used as an argument in favor of the view that the mechanism of regeneration of the receptor would involve the spontaneous hydrolytic splitting, at variable rates, of an ester-like bond (4, 5). This interpretation coupled with the suggested steric and electronic analogy between adrenergic amines and QAI led to the characterization of the alkylatable α-site as a carboxylate or a phosphate anion (3). Other chemical

arguments were advanced which would favor the latter possibility (5). The α-receptor was tentatively defined as a phosphoprotein or a protein-bound nucleotide (4, 5) (fig. 4). The recent elaborations of Bloom and Goldman (11) would serve to further characterize the postulated phosphate anion as the terminal phosphate of ATP (fig. 5). It was noted by these authors that alkylation of this group to produce an ester would make ATP unhydrolyzable by the hypothetical receptor ATPase, and that the inability to hydrolyze ATP would account for the irreversible blockade produced by Dibenamine. Clearly, the alkylation of a protein anionic site at the periphery of ATP would induce an irreversible NSCP and cancel the ATPase activity just as well.

The basic postulate that an ester-like bond is formed between a suitable QAI and the α-receptor has its origin in the peculiar steric and electronic factors which govern the stability of the receptor alkyl derivatives (5). The question of the behavior of known catalytic anionic sites on enzyme surfaces towards potentially positive carbons (as in QAI) has not been explored experimentally. We have recently investigated this problem using the anionic site of acetylcholinesterase (AChE) as the target and N,N-dimethyl-2-phenylaziridinium (DPA) (a short acting adrenergic blocker of high potency) as a stereoelectronic analog of the well known competitive inhibitor phenyltrimethylammonium. It was found (9) that DPA specifically and irreversibly alkylates some functional group in the vicinity of the anionic site. Since catalytic activity cannot be regenerated once the enzyme has been treated with DPA, it seems clear that a nonhydrolyzable covalent bond must be formed between the enzyme and DPA. Therefore the covalent bond which is formed when DPA reacts with α-receptors must be of an entirely different kind since the α-receptor surface regenerates spontaneously, as would be the case if the drug is held by an ester linkage. Recent measurements by Triggle (24) of the rate of regeneration of α-receptors after DPA treatment substantiate this conclusion. That the site of alkylation of α-receptors by 2-haloalkylamines is anionic in character would seem to be more firmly established; this adds weight to the proposed interpretations of steric effects at this level.

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