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Molecular Geometry and Adrenergic Drug Activity*

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I. Introduction

PHARMACOLOGICAL actions of drugs can be divided into two major categories, structurally specific and structurally nonspecific. The physicochemical properties of a drug appear to play a profound role in determining actions of structurally nonspecific drugs. Actions of drugs such as ether and nitrous oxide obviously belong to the latter type. However many other drugs such as sympathomimetic amines, neurotransmitters, and hormones act on specific target cell receptors in a structurally specific fashion to elicit pharmacological effects. These effects are not only structurally specific but also "stereoselective." Thus, like other receptors, stereoselectivity is a property of the adrenoreceptors.

Studies with optical isomers furnish information about the space and its geometry at and near the active binding regions of the receptor macromolecule. Such studies provide some information on essential structural elements of the receptor. This approach is perhaps the best available and most useful until the three-dimensional structure of an isolated receptor can be defined.

There is a good analogy between interactions of drugs with pharmacological receptors and those of enzymes and substrates. For example, stereoisomers of various substrates of α-chymotrypsin have been utilized to map the active site of the enzyme (11). Although earlier workers had thought it impossible to utilize symmetrical intermediates in biochemical reactions to explain their observed results from radiolabeled substances, Ogston (281) illustrated that molecules composed of two chemically like groups (a and a′) and two dissimilar groups (b and c), but which do not possess an asymmetric center, could undergo stereospecific reactions with an enzyme (fig. 1A). Thus assuming a three-point attachment of a substrate such as aminomalonic acid with groups a(CO_2H), a′(CO_2H), b(NH_2), and c(H) to an en-

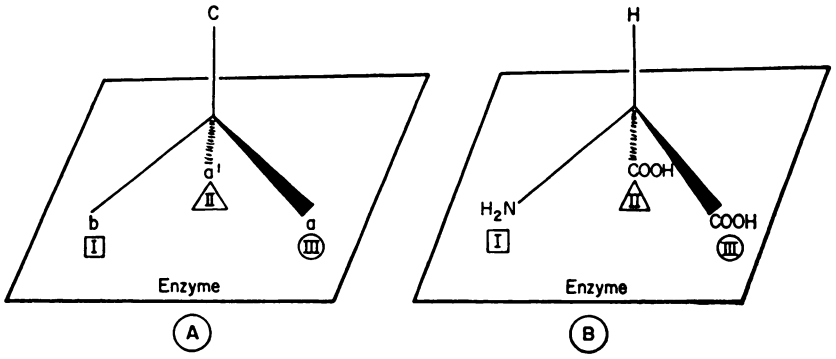


FIG. 1. A, A general representation of Ogston's concept (281) regarding interaction of a substrate with similar groups (a and a') with catalytically different points (II and III) on the enzyme. B, Specific example of aminomalonic acid binding with an enzyme at these different points. Note that two "like groups," carboxylic groups, bind two catalytically different points. Hence only one carboxyl group is decarboxylated.

zyme with points I, II and III (fig. 1B) Ogston (281) first showed that if decarboxylation could take place at only point II on the enzyme when all three groups were bound then the enzyme was in fact able to distinguish between chemically like groups attached to a carbon with two additional dissimilar groups. Other examples (11, 308a), lending additional proof to his concept, were the distinction made by enzymes between chemically like groups in citric acid and glycerol.

Important enzymes in the adrenergic nervous system are capable of converting symmetrical substrates to optically active products. Dopamine-β-hydroxylase, for example, is capable of distinguishing between the chemically like benzylic hydrogens of dopamine and removing one of the hydrogens and replacing it with a hydroxyl group giving rise, stereospecifically, to 1R-(−)-norepinephrine. The enzyme monoamine oxidase has also been shown to remove specifically one of the chemically identical α-hydrogens of tyramine in its conversion to an oxidation product. It has also been shown that there is steric preference in the chemical reaction (308a) between β-phenylglutamic acid anhydride, a symmetrical molecule, and (−)-α-phenylethylamine, an asymmetrical reagent. Chemical reactions of this nature, however, give poor stereospecificity compared to substrate-enzyme reactions.

Soon after the presence of epinephrine in the adrenal medulla was demonstrated, Cushny (92) in 1908 tested the pressor effects of naturally occurring (−)-epinephrine and synthetic (±)-epinephrine in the anesthetized dog. The (±)-epinephrine was one half as active as the (−)-form. Subsequently, Cushny (93) compared the effects of (−)- and (+)-epinephrine and found that the (+)-form was 1/12 as active as the (−)-form in raising blood pressure. Chen *et al.* (79) not only introduced ephedrines to therapeutics but reported on the pharmacology of the stereoisomers of these drugs. In 1948, Euler (122) showed that the major neurotransmitter of the mammalian sympathetic nervous system was (−)-norepinephrine. By the mid-fifties the stereoisomers of many catecholamines and other nonphenolic amines were resolved, absolute configurations established (314), and pharmacological effects studied (223, 240).

Along with the development of the field of adrenergic mechanisms (54), it appeared essential to study the stereoisomers of various adrenergic drugs to clarify the selectivity in the mechanisms. Hence, in the last five years there has been a renewed interest in the use of stereoisomers

as tools to prove adrenergic mechanisms. Except when essential, the review will not discuss early literature on the stereoisomers if it has been reviewed previously (293). The main emphasis will be on the literature from the early sixties to the present date. Also, where necessary, work on stereoisomers of other substances is discussed briefly to emphasize a given point. Sastry (336) has reviewed recently the work on stereoisomers of cholinergic drugs.

II. Nomenclature

A. Selected Definitions

chirality—pertains to handedness and is used to indicate the nonsuperimposability of one's right and left hands and is the condition which exists with enantiomers.

enantiomers—stereoisomers which possess a mirror image relationship. A synonym is antipodes.

stereoisomers—substances with the same molecular formula but differ in their arrangement of atoms in space.

configuration—the arrangement of atoms that characterizes a particular stereoisomer. When discussing the absolute configuration of a carbon atom, we are talking about the absolute order of the arrangement of atoms about an asymmetric carbon atom.

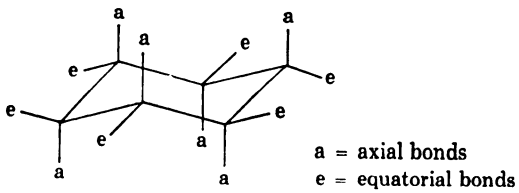
conformation—term used to describe any one of an infinite number of arrangements of atoms in space that result from rotation about any of the single bonds in a molecule. The possible individual momentary forms may be called conformers, rotamers, or conformational isomers.

geometrical isomers—compounds in which rotation about a certain bond is hindered resulting in different spatial arrangements. When dealing with a carbon-carbon double bond and if there is more than one possible structure we often differentiate the two possible arrangements as *cis* and *trans*. These isomers differ in physical and chemical properties.

epimer—diastereoisomers that vary in the configuration about only one asymmetric center.

axial—bonds which are parallel to one another and are pointed along an axis perpendicular to the plane of a cyclohexane ring (see illustration).

equatorial—bonds which lie roughly in the plane of the cyclohexane ring in a belt about the equator of the ring (see illustration).



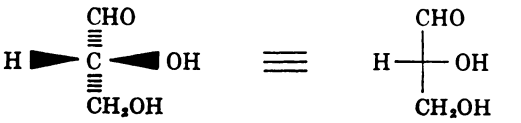
stereospecific—pharmacological activity found in only one isomer.

stereoselective—pharmacological activity found predominantly in one isomer.

B. Stereochemical Notations

A number of notations have been used in the past to describe the three-dimensional shape of molecules. A chiral molecule is one which can be resolved into enantiomers. The use of (+) and (−) or *d* and *l* with respect to enantiomers has been used to specify the sign of rotation of plane-polarized light either to the right (dextrorotatory) or left (levorotatory). The absolute configuration is defined according to the relative arrangement of an atom or group of atoms in the space about a dissymmetric or rigid part of a molecule. The absolute configuration of optical antipodes can be designated by the Fischer-Rosanoff method as either D or L (11). This system uses as an arbitrary standard reference dextrorotatory glyceraldehyde which has the designation D used for its absolute configuration. There is no simple relationship between the sign of rotation and the configurational assignment. In a specific series of compounds it may be pos-

D-(+)-Glyceraldehyde

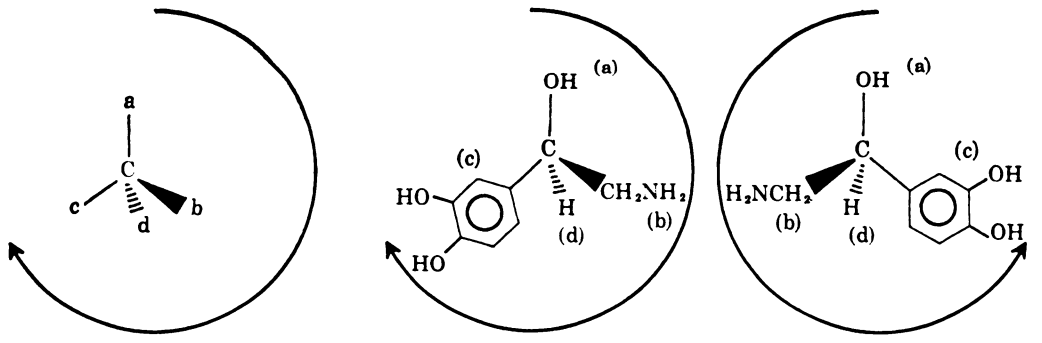


Perspective Formula Fischer Projection

sible to assign the absolute configuration from optical rotatory dispersion (ORD) curves (9).

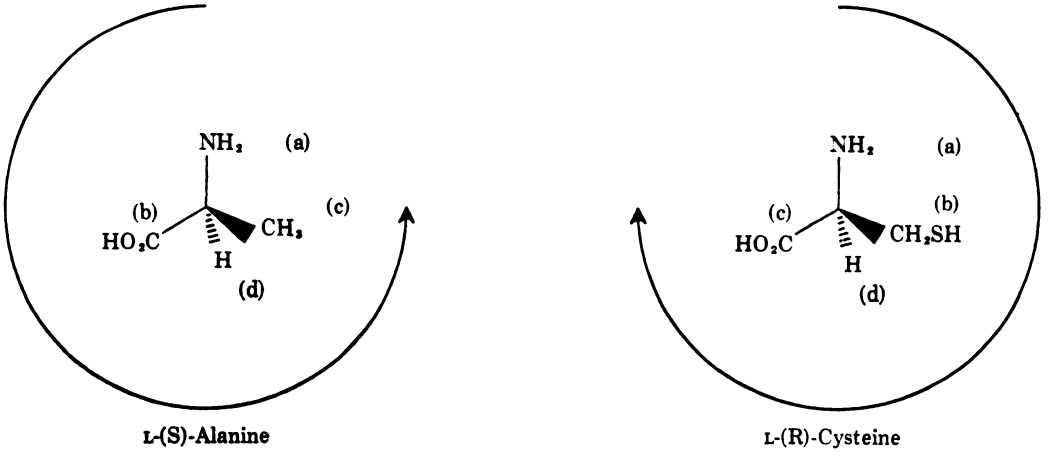
Complications may arise when lower case *d* and *l* are interchanged with upper case D and L. Other ambiguities with the D and L notation method have been discussed by Slocum *et al.* (359). However, the use of the Fischer-Rosanoff nomenclature has continued extensively in the areas of carbohydrates and amino acids. This method of using an arbitrary standard has largely been superseded in most other areas by the Cahn-Ingold-Prelog sequence rule for designating a particular configuration (70, 71). A priority is assigned to all possible functional groups (a, b, c, d) and the group which has the lowest priority (d) is directed away from the viewer. Then we proceed by moving from highest priority (a) to the second (b) and third (c) priority groups and if the one travels in a clockwise direction the configuration is described as being R, and if one travels in a counterclockwise direction, S. The sequence rule assigns the highest priority to the functional groups with the highest atomic number or, if isotopes are involved, to the highest mass number and then proceeds to the lower. Scheme I illustrates (R) and (S) nomenclature for norepinephrine isomers. Since the sequence method is self-consistent for each molecule, it cannot be used to relate a series of compounds like the Fischer-Rosanoff method. This can be illustrated with the amino acids L-alanine and L-cysteine which have the (S)-configuration and (R)-configuration, respectively (see Scheme II).

When a molecule is composed of more than one asymmetric center, diastereoisomeric forms of the molecule exist. Diastereoisomers are stereoisomers which are not mirror images of one another and thus *they have different chemical and physical properties.* When just two asymmetric centers are present one may use the *erythro* and *threo* nomenclature prefix to refer to the diastereoisomeric forms. Erythrose and threose serve as the basis for this nomenclature (121). The prefixes do not specify a single stereoisomer. In general, the pair of isomers that have two similar, but not necessarily identical groups on the same side are called the *erythro* form; the set of isomers which have like groups on the opposite side of the Fischer projection formula are called *threo.* Of the four possible 1-phenyl-2-aminomethylpropanols, the two ephedrine enantiomers have the *erythro* configuration and the two ψ-ephedrine stereoisomers have the *threo* configuration (see Scheme III). A Newman projection of (1R,2S)-(−)-ephedrine illustrates the simultaneous eclipsing of like or similar



(R) Clockwise Direction (R)-(−)-Norepinephrine (S)-(+)-Norepinephrine

SCHEME I.



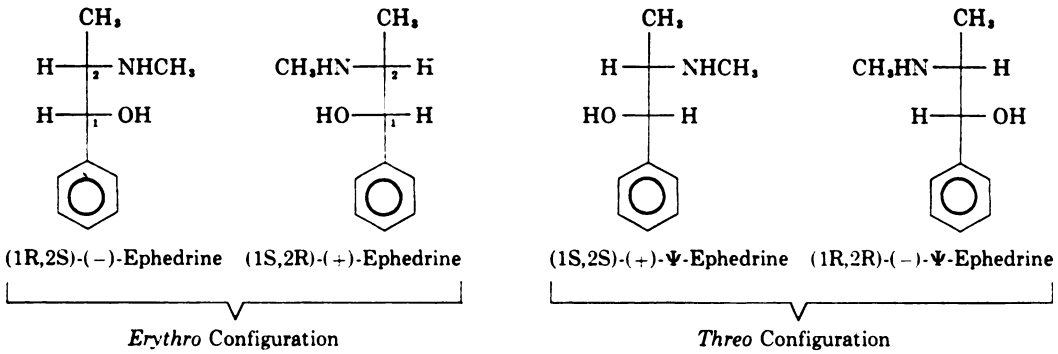
SCHEME II.

groups (H, H; CH_3, ◎; -OH, $NHCH_3$) in the *erythro* series, whereas this is not possible with (1R,2R)-(−)-ψ-ephedrine in the *threo* series (see Scheme IV).

To illustrate how confusing it is to designate the configuration of (−)-ephedrine when using the Fischer-Rosanoff D and L nomenclature, one can describe the molecule as being either L-(−)-ephedrine or D-(−)-ephedrine, depending upon what reference compound is used (300). The symbol D_m represents a compound which has the D configuration when related to D-(−)-mandelic acid and the symbol L_a denotes a compound that can be related to L-(+)-alanine. It can be seen that with the ambiguity which might arise with the Fischer-Rosanoff nomenclature, the Cahn-Ingold-Prelog sequence rule is to be preferred in the case of the ephedrines. Throughout the review, except in the case of amino acids, isomers are identified by signs (−) and (+) (see Scheme V).

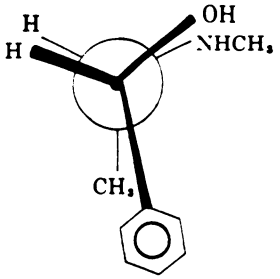
C. Conformational Notations

When molecules differ from one another only by rotation about a single bond (C—C), we call them conformational isomers (rotational isomers, rotamers and conformers are terms also used). The conformational flexibility of phenethylamine derivatives, dopamine, epinephrine, and norepinephrine, *etc.*, may permit these compounds to have different biological actions dependent upon the conformational requirements of the various biological receptors. Rotation about acyclic C—C bonds is sometimes referred to as being "free"; however, energy is required for the rotation about the bond and this is called torsional energy. The strain which results from bond-eclipsing is called Pitzer strain.

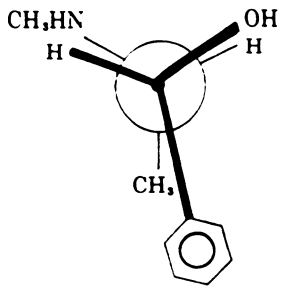


SCHEME III.

Newman Projections



(1R,2S)-(−)-Ephedrine



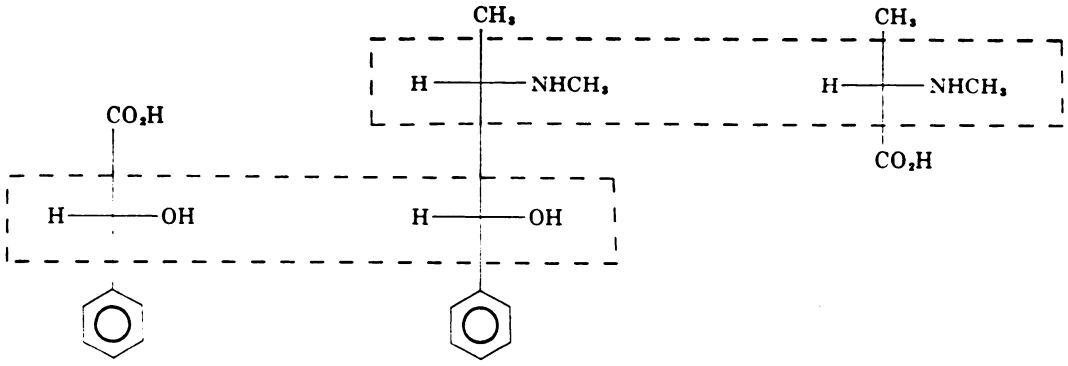
(1R,2R)-(−)-Ψ-Ephedrine

SCHEME IV.

To illustrate the conformational isomers of a molecule, we often use the saw-horse and/or Newman projections on the two dimensional page (see Scheme VI). The dihedral angle denotes the angle between the two planes described by:
1) catechol-C_1 bond along the C_1—C_2 bond; and 2) H_2N—C_2 bond along the C_1—C_2 bond. This angle is also sometimes called the torsion angle. There is an infinite number of possible conformational isomers which are capable of a finite existence with a molecule such as dopamine. Illustrated are six distinguishable conformations with Newman projections which arise from rotation about the C—C bond of dopamine (fig. 2). Instead of using the number of degrees for the various dihedral angles, words are often substituted to designate specific conformations such as fully eclipsed, skew, gauche, *etc.*, described in table 1. In some instances the exact dihedral angle may not be known with a great deal of certainty. For these cases, Klyne and Prelog (210) proposed a nomenclature in which the dihedral angle is assumed to be ±30°. Thus, one can use this terminology when discussing an approximate dihedral angle. Because of the "free" rotation about the C—C bond of phenethylamine acyclic derivatives like dopamine, it is difficult to say which conformation is preferred by receptors. However, studies have been carried out recently on molecules in which atoms are added to or deleted from the parent molecule in order to provide a "conformationally rigid" or "conformationally restricted" drug molecule. These analogs should provide a better insight into the position functional groups must occupy in order to interact with receptors to produce a biological effect.

D. Configurational Assignments

The absolute configuration of a large number of stereoisomers of adrenergic

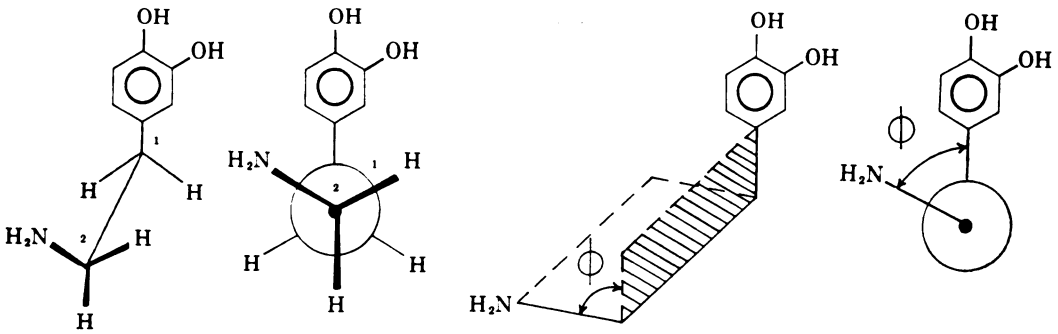


D-(−)-Mandelic acid

D_m or L_a-(−)-Ephedrine

L-(+)-Alanine

SCHEME V.



SCHEME VI.

drugs, possessing a β-hydroxy-β-phenethylamine system, has been reviewed by Pratesi (314). The absolute configuration of (−)-epinephrine, (−)-norepinephrine, (−)-isoproterenol, (−)-synephrine and (−)-phenylephrine was determined by relating the configuration of the asymmetric center to that of D(−)-mandelic acid which possesses the R absolute configuration. (−)-Tertiarybutylnoradrenaline has been assigned the D-configuration (R) by comparison of the rotatory dispersion curves of this substance with the previously mentioned β-hydroxy-β-phenethylamine derivatives. Analogously, the R absolute configuration was assigned to (−)salbutamol by comparison of the circular dichroism spectra of (−)-salbutamol with those of R(−)-octopamine (165). (+)-Phendimetrazine (3,4-dimethyl-2-phenylmorpholine) was shown to have the 1S:2S absolute configuration by chemically converting (−)-ephedrine to (+)-phendimetrazine and utilizing the nuclear magnetic resonance (NMR) spectrum of the product. Shafi'ee and Hite (346) have also determined the absolute configurations of the isomeric methylphenidates and pipradols. The absolute configuration of the β-adrenergic blocking agent (+)-propranolol was found to be R through a chemical correlation with S-(+)-lactic acid. The absolute configurations of a number of other aryloxypropanolamines and arylethanolamines were also determined (116).

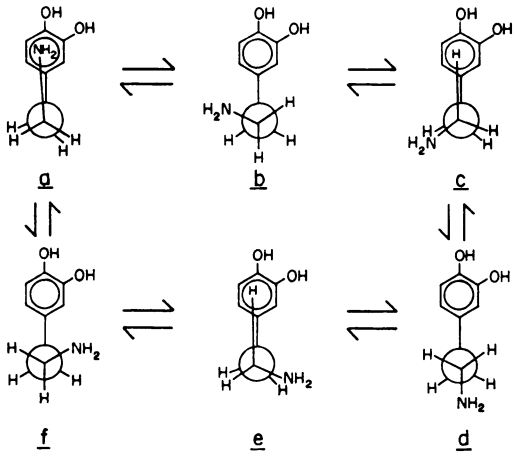


FIG. 2. Various conformations of dopamine are illustrated. The preferred conformation of the amine in solution (69) and the crystal (46, 47) appears to be shown in "d."

III. General Considerations

A. Stereoselective Processes at the Neuroeffector Junction

For a meaningful presentation of the subject material, it is essential that various stereoselective sites at the neuroeffector junction be schematically illustrated (fig. 3). It is not the purpose of this review to describe in detail the pharmacology of adrenergic drugs which has been well documented (54, 401a). The review will discuss the steric substrate and inhibitor specificity of tyrosine hydroxylase (site 1), dopa decarboxylase (site 2), and dopamine-β-hydroxylase (site 3), which are in-

TABLE 1

Designation and terminology used to describe various conformations that are presented in figure 2

Figure	Dihedral Angle ϕ	Designation	Klyne-Prelog Terminology ($\phi \pm 30$)	Abbreviation
a	0°	Fully eclipsed, cis	Synperiplanar	(SP)
b	+60°	Skew, gauche	+Synclinal	(+SC)
c	+120°	Partially eclipsed	+Anticlinal	(+AC)
d	180°	Anti, trans, fully staggered	Antiperiplanar	(AP)
e	−120°	Partially eclipsed	−Anticlinal	(−AC)
f	−60°	Skew, gauche	−Synclinal	(−SC)

volved in the biosynthesis of the transmitter, (−)-norepinephrine. Since dopamine is transported into the storage vesicle, the vesicular membrane (site 4) will exhibit selectivity in the transport of drugs. In the storage vesicles, adenosine triphosphate (ATP)-Mg^{++} dependent, site 5, is essential for the storage of (−)-norepinephrine and other related amines, the latter often functioning as false neurochemical transmitters. The importance of the steric configuration of amines for the retention of amines by the storage vesicles will be emphasized. The steric structure activity for the retention of amines by the dopaminergic neurons is yet to be thoroughly studied, hence, the topic will not be discussed.

When the sympathetic nerve is stimulated it depolarizes the nerve terminal and the transmitter is released by the process of exocytosis. The release of the transmitter appears to be regulated by the prejunctional α-adrenoceptors (site 9). The inhibitory control of the receptor on the release appears to be stereoselective for norepinephrine isomers (373a). More work is necessary to describe the details of the prejunctional receptor specificity for other stereoisomers. Similarly, the muscarinic inhibitory receptor also appears to control the release of the transmitter; it will be rewarding to study the stereoselectivity of the process. The transmitter which is released into the junctional cleft is reabsorbed in the nerve terminal through the transport or the uptake site (site 8). The transport of exogenously administered iso-

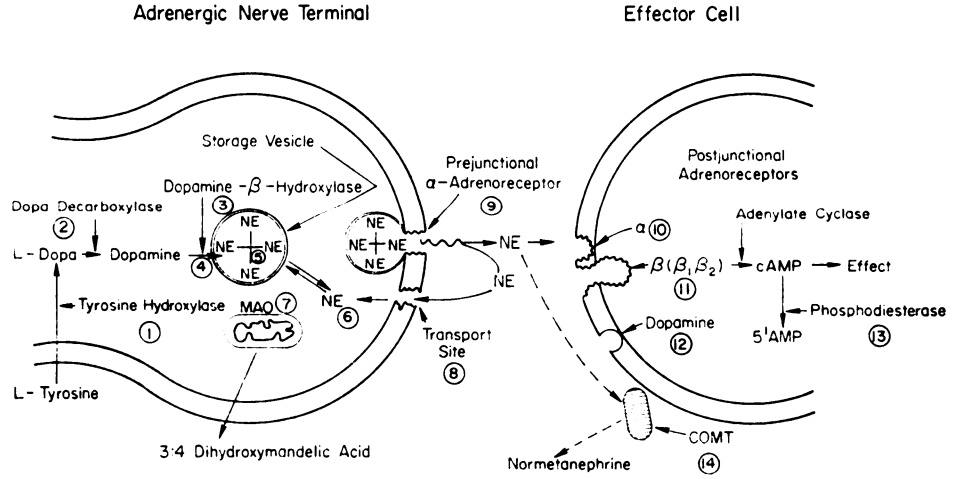


FIG. 3. Various stereoselective processes (sites 1–14) at the adrenergic neuroeffector junction are illustrated schematically. Except for a few sites, the steric structure activity relationship is well defined.

mers of norepinephrine through site 8 remains a controversial issue (109, 188, 217, 218), and the methodological problems related to these studies will be critically analyzed. The stereoisomers of drugs which block the uptake of the transmitter and its functional significance will be reviewed in detail. The reabsorbed transmitter presumably is in the cytoplasm, but the site for its temporary binding (site 6) has not been identified. So-called free or cytoplasmic norepinephrine is partly incorporated in the storage vesicle and in part it is metabolized by the enzyme monoamine oxidase (MAO) (site 7). Another enzyme, catechol-O-methyltransferase (COMT) (site 14), is also involved in the metabolism of the transmitter. The substrate and inhibitor selectivity for the enzymes will be discussed.

Endogenously released transmitter or exogenously administered drugs interact with the postjunctional α-adrenoreceptor, β-adrenoreceptor, and dopamine receptor (sites 10, 11, and 12, respectively). The latter receptor is recognized in the caudate nucleus and the renal artery. The details of the steric structure activity for the activation or the blockade of receptors will be presented. The activation of β-adrenoreceptors or dopamine receptors stimulates adenylate cyclase which catalyzes the conversion of ATP to 3′,5′-cyclic adenosine monophosphate (AMP). The nucleotide is responsible for many observed pharmacological effects. Cyclic AMP is converted to 5′-AMP by the enzyme phosphodiesterase (site 13). The details of the steric structure activity relationships needed for the activation or inhibition of the enzyme are not well documented.

At the neuroeffector junction, a conservative estimate recognizes 14 different stereoselective sites. In an isolated subcellular fraction containing a given site, the steric structure activity could be precisely studied. However, in the whole animal or even in the isolated tissue, due to the different degrees of stereoselective interaction of isomers at each site, pharmacological effects could be obscured. Hence, it is not surprising that various agents and procedures must be used for the accurate quantitation of the effects of isomers.

B. Purity, Impurity, Racemization, and Related Problems with Use of Isomers

Whenever optical isomers are obtained through the resolution of the racemate, the presence of the active isomer in the "totally inactive or inert" isomer may present a subject for argument. When isoproterenol was first resolved, the ratio of relative potencies of the isomers was reported to be 11.8 for the cat blood pressure. After repeated fractional crystallization, the ratio was increased to 1000 (223). It was pointed out that the constant optical rotation or melting point was not the most sensitive criterion for the purity of isomers, but that constant biological activity was (24a, 223). Optical rotation values of many samples of (+)-isoproterenol were nearly identical, but when tested on rat uterus the pharmacological activity among the samples varied as much as 50-fold (342). Alternatively, pure isomers can be obtained from optically pure precursors by chemical synthesis which does not affect the asymmetric centers. Barlow *et al.* (25) and McLean *et al.* (258) obtained (−)- and (+)-isomers of drugs by synthetic procedures. If starting materials are absolutely pure the chances for obtaining optically pure end products are very high (see also section X C for further discussion).

No other procedure guarantees near 100% purity of isomers, except for isomers obtained from biological sources or from methods involving enzymatic treatment. The enzyme atropinesterase found in rabbits is absolutely specific for the degradation of (−)-hyoscyamine (411). Hence, absolutely pure (+)-hyoscyamine can be obtained from the racemate subjected to the enzymatic treatment. Conversely, presence of one isomer in the other can be ex-

amined by means of specific enzymes. With the aid of D-amino acid oxidase, L-amino acid oxidase and bacterial L-specific decarboxylases, less than one molecule of an amino acid isomer in the presence of even 10,000 molecules of its enantiomorph can be detected (260).

Although only the (−)-isomers of norepinephrine and epinephrine are found in tissues, Kilbinger *et al.* (209) observed an excretion of a high amount of (±)-isoproterenol in two patients. The puzzling occurrence of the racemate could not be attributed to exogenous medication or related factors. The significance of the finding can be assessed only when additional information is available. Since the catecholamines from natural sources are (−)-isomers, racemization in the blood or during the excretion process is not likely. If the urinary pH is acidic, the body may excrete a single form and the racemization may occur during storage of the sample. As an assurance, it would be valuable to establish the absolute configuration and/or to determine the optical rotation of normetanephrine and 3:4 dihydroxymandelic acid which are the major metabolites of the endogenous catecholamines. If no racemization occurs during metabolism, the absolute configuration of hydroxyl function around the β-carbon should be the same in the parent amine and its metabolites.

Under selected conditions racemization of the isomer could occur in solution. The routine procedure for the resolution of the isomers of catecholamines includes the racemization of the (−)-isomer with the aid of a strong acid and subsequent formation of the diastereoisomers with other optically asymmetric molecules, such as (+)- or (−)-mandelates. Because diastereoisomers differ in solubility characteristics, such substances are separated by fractional crystallization. It is quite common to obtain (−)-norepinephrine-(+)-bitartrate and (+)-norepinephrine-(+)-bitartrate. Can a given isomer undergo racemization during the period of testing procedures? A solution of (+)-epinephrine when tested over a period of two years, was found to increase in its ability to stimulate adenylate cyclase activity (268). Presumably the (+)-form is racemized to the active (±)-form. However, such a racemization is less likely in a weak acid solution (398). When pharmacological activity of fresh and 4-day-old solutions of (+)-norepinephrine-(+)-bitartrate was tested on rabbit aorta, the activity of the fresh and the old solution appeared to be identical, indicating that racemization did not take place (77). A small quantity of sodium-metabisulfite as antioxidant was present in both solutions.

Since chemical reactivity of (−)- and (+)-isomers toward nonasymmetric substances is the same, any fluorometric analysis which involves nonasymmetric substances would not detect the differences between the isomers after the injection of the racemate. Hence, the fate of isomers in the body after the injection of (±)-isomers is studied through the formation of diastereoisomers (34, 153, 307). It should be pointed out that availability of radiolabeled substances simplifies the analytical problem, but simultaneously it has added another complication. Many radiolabeled sympathomimetic amines are available only as racemic substances, hence the fate of a single molecular species cannot be studied precisely. Labeled neurotransmitter is available as a single molecular species, however.

C. Actions and Interactions of Isomers

On the basis of previously established absolute configurations of a series of catecholamines and other adrenergic drugs, the active form of the isomer of a new drug can be predicted. However, relative differences in pharmacological effect between the isomers cannot be predicted at the present time. Depending upon the pair of isomers and test parameter used, various possibilities can occur. It is important that when isomers of adrenergic drugs are

to be tested on tissues, other factors which modify the pharmacological effects should be controlled (138).

1) One isomer can be relatively more active than the other isomer. On the rabbit aorta (+)-norepinephrine has 1/300 the activity of the (−)-isomer. The effect is stereoselective (294).

2) Although in many test situations, one isomer may be consistently less active than the corresponding isomer, the activity values may be reversed in a different test situation. When tested for heart rate, blood pressure and tracheal relaxation, (−)-isoproterenol is consistently more potent than the (+)-form, but for lowering the intraocular pressure of the rabbit eye, (+)-isoproterenol is more potent than the (−)-form (343). This finding is not only of theoretical interest, but it could be of considerable therapeutic value. As compared to the systemic chronotropic effect of (−)-isoproterenol, the effect of the (+)-form is negligible.

3) A given isomer may not produce any apparent effect, but it may influence the pharmacological activity of the other isomer. The apparently inactive isomer may be a competitive and/or noncompetitive antagonist to the active isomer. Luduena (239) reported that (+)-isoproterenol antagonizes the effect of (−)-isoproterenol. Detailed analysis indicates that the interaction between the isomers on rabbit aorta is competitive as well as noncompetitive. In other words, relative to the (−)-forms, a 10-fold higher concentration of (+)-isoproterenol shifts the dose response curve to the right with a depression in the maximum response (78). The affinity of a given isomer measured as a blocker (pA_2 or K_i) may be higher than the affinity of the other isomer measured as a stimulant or a substrate (pD_2 or K_m). The K_i value for chymotrypsin of acetyl-D-tyrosinamide is higher than the K_m value of acetyl-L-tyrosinamide (106).

4) Both optical isomers can be equipotent. On the isolated rat vas deferens (−)- and (+)-isomers of the indirectly-acting *p*-hydroxyamphetamine produce equal effects which do not differ from that of the racemic form (290, 292). Similarly, the stimulant effects of (+)- and (−)-α-methyldopamine are identical on the α-adrenoreceptor of the rabbit aorta (76, 78).

5) Only one isomer can be active while the other isomer may be practically inactive; for example, (−)-isopropylmethoxamine is a good β-receptor antagonist in the trachea, while (+)-isopropylmethoxamine is practically inactive (284). The effect is stereospecific. Since most of the biological effects discussed here are stereoselective and not stereospecific, the former term is the preferred one.

6) A very unusual finding reported by Porter *et al.* (310) is the greater depletion of norepinephrine from tissues by racemic α-methyl-*p*-tyrosine than is expected after the physical combination of isomers. In other words, the racemic form can behave as the third molecular species. In the normal cat nictitating membrane the ED50 for (−)-norepinephrine is more than half the ED50 for the racemate. The (+)-isomer from the racemate may partially saturate the neuronal transport, and as a result more (−)-norepinephrine will be available at the pharmacological receptors. Graefe and Eckert (155) determined the K_m for uptake into nictitating membrane and found it to be a) identical for (−)- and (+)-norepinephrine, and b) below the ED50 for the dose-response curve for (−)-norepinephrine. Thus, there is now quantitative evidence for the view that the (+)-isomer at least partially saturates uptake when the ED50 of the racemic mixture is used. However, in denervated tissues the (−)-norepinephrine/(±)-norepinephrine activity ratio is 2:1 as expected (396). These observations suggest that even if two isomers are equipotent, a single isomer does not necessarily represent ½ the activity of a racemate.

Occasionally, the relationship between

activity of (+)- and (−)-isomers may be different when different tests are used. (+)-β-Hydroxyphenethylguanidine has about one quarter the norepinephrine-depleting activity of the (−)-isomer. However, as compared to the (−)-form, the (+)-isomer is more effective in preventing ptosis caused by an adrenergic neuron-blocking agent. Such findings might be very useful in analyzing the mechanism of drug action. These observations clearly show that the norepinephrine depletion on the one hand, and the antagonism of adrenergic neuron blockade on the other hand, must involve two different mechanisms (133).

IV. Substrate and Inhibitor Selectivity of the Catecholamine Synthesizing Enzymes

The biosynthetic pathway of norepinephrine or epinephrine from tyrosine is well established. The optically active substrates L(−)-tyrosine and L-(−)-dopa give rise to the optically inactive or symmetrical molecule dopamine which gives rise to an optically active (−)-norepinephrine. The substrate specificity of most of the enzymes involved in the biosynthesis is reasonably well worked out. In many studies the stereoisomers were also included along with various substrates.

A. Tyrosine Hydroxylase

The stereoisomers of tyrosine proved to be a valuable tool to characterize the enzyme which was undetected for some time. Nagatsu *et al.* (276) stated that boiled tissues which were used as a control in the study of the enzymatic activity contained auto-oxidizable substances. The artifact was presumably produced by the heat treatment. However, such a system oxidized both L(−)- and D(+)-tyrosine to produce equal amounts of dopa. Normal tissue slices or the carefully isolated enzyme showed a high degree of stereoselectivity for L-(−)-tyrosine. In tests *in vitro*, small amounts of D(+)-tyrosine appeared to be converted to dopa which could have been due to a small contamination of dopa in the D(+)-tyrosine. Another enzyme, tyrosinase, which was originally thought to be involved in the biosynthesis of catecholamines, shows a lesser degree of stereoselectivity in the conversion of L(−)- or D(+)-tryrosine to dopa (308). Substances which are structurally similar to tyrosine were expected to act as inhibitors of the enzyme. When tested on the purified enzyme from adrenals and brain tissues, L(−)-phenylalanine, L(−)-tryptophan, L(−)-dopa, and L(−)-α-methyldopa were better inhibitors than the corresponding D-isomers. The competitive inhibitory nature of L(−)α-methyl-*p*-tyrosine was also established and the substance was found to be more active than the racemate (276).

B. Dopa Decarboxylase (Aromatic-L-Amino Acid Decarboxylase)

In guinea pigs absolute stereoselectivity (*i.e.*, stereospecificity) of this enzyme was observed by Holtz *et al.* (178). Thereafter, Blaschko (52) commented on the stereochemistry of the functional groups of the substrate and its interactions with the enzyme. Although mammalian decarboxylases are stereospecific, in rats exogenous administration of D(+)-dopa increases the urinary output of dopamine (177). This conversion is localized in the kidney (350, 351). It appears that, as presented in figure 4, D(+)-dopa is converted to L(−)-dopa as follows: D(+)-dopa is changed to 3,4 dihydroxyphenyl pyruvic acid by the enzyme D-amino oxidase. Then, L-aminotransferase converts 3,4 dihydroxyphenyl pyruvic acid to L(−)-dopa. L(−)-dopa is then decarboxylated by dopa decarboxylase (370).

The utilization of the D(+)-isomer depends on the rate of conversion to the L(−)-isomer. In rats, after intravenous administration of both isomers, the unnatural isomer was not deposited in many tissues to the same extent as the natural isomer. The low tissue deposition of the

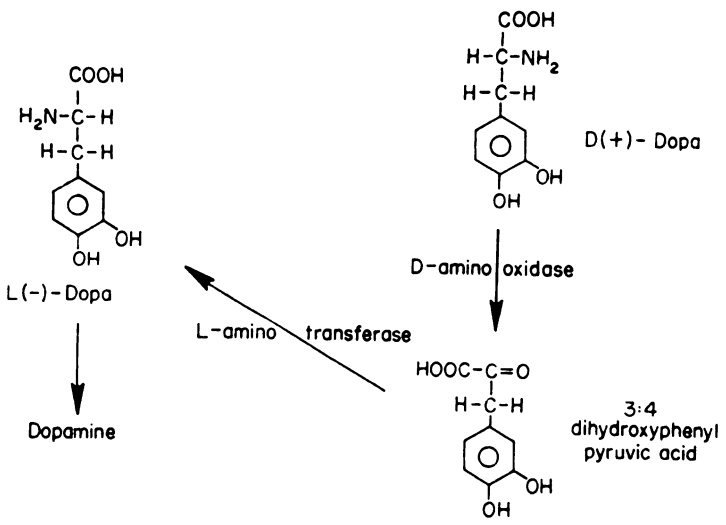


FIG. 4. The biotransformation of D(+)-dopa to L(−)-dopa in rats is illustrated [after Sourkes *et al.* (370)].

unnatural isomer was reflected in high urinary excretion of the drug. In the pancreas, however, both isomers were deposited to the same extent (350, 351).

Dairman and Christenson (94) found that lysed human red blood cells decarboxylated both D(+)- and L(−)-dopa in contrast to the human liver enzyme which was stereospecific for L(−)-dopa. The stereochemistry of the enzymatic decarboxylation of amino acids was reported by Belleau and Burba (38). The authors indicated that during decarboxylation of the substrate, the absolute configuration around the asymmetric carbon was retained. The reaction is of considerable importance in the decarboxylation of α-methyldopa where the decarboxylated product, (+)-α-methyldopamine, retains the same configuration around the α-carbon as it had in the parent molecule. D(+)-α-Methyldopa does not appear to be decarboxylated. The substrate 3,4 dihydroxyphenylserine provides an interesting tool to study the selectivity of the enzyme. Two asymmetric centers in the molecule provide four stereoisomers. With the aid of combined biochemical and bioassay techniques, Blaschko *et al.* (53) concluded that (−)-norepinephrine was formed only when L-*threo*-3,4 dihydroxyphenylserine was incubated with guinea pig kidney extract. Hartman *et al.* (166) examined DL-*threo* and DL-*erythro* forms of the compound and found that DL-*erythro* 3,4 dihydroxyphenylserine can be converted to (+)-norepinephrine (fig. 5). Only the

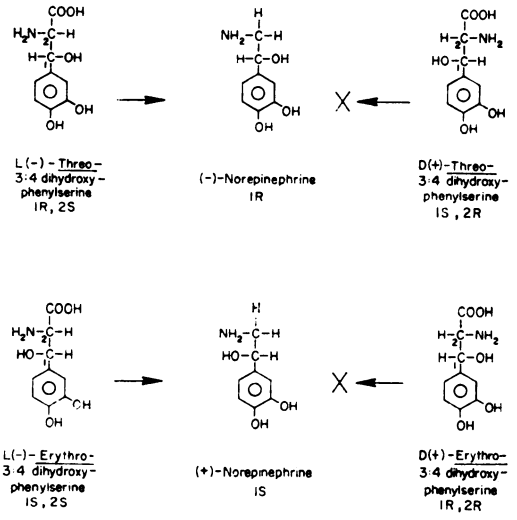


FIG. 5. The decarboxylation of isomers of 3:4 dihydroxyphenylserine to yield (−)- or (+)-norepinephrine is illustrated. Note that only 2S isomers are decarboxylated. After Blaschko *et al.* (53), Hartman *et al.* (166), Porter *et al.* (309), and Puig *et al.* (315a).

L-*erythro* form is decarboxylated to the unnatural form of norepinephrine (315a). The relative rates of decarboxylation of (±)-*erythro* and (±)-*threo* forms differ; the former racemate was decarboxylated about three times as rapidly as the *threo* form (309). Thus, in the *threo* form the β-OH must sterically interfere with the decarboxylation by the enzyme.

Some information is available on the structure activity relationship for inhibition of dopa decarboxylase (265). Indirect lines of evidence based on pharmacological experiments indicate that the (+)-isomer of α-methyl-α-hydrazino-3,4 dihydroxyphenylpropionic acid is inactive while the (−)-isomer appears to be a potent inhibitor (237). The pharmacological significance of the study is unclear.

C. Dopamine-β-Hydroxylase

The conversion of the symmetrical molecule dopamine to a single asymmetric (−)-norepinephrine is another good example of stereospecificity by enzymes. It also serves as a proof for the statement that although the substrate is symmetrical, the interaction between the substrate and the enzyme is asymmetric. Obviously, out of two, only one hydrogen at the β-carbon is involved in the enzymatic action. It would be interesting to prepare deuterium-labeled optical isomers of dopamine and to test the selectivity of the enzyme for them. The enzyme shows remarkable specificity toward other agents. (+)-α-Methyldopamine and (+)-*p*-hydroxyamphetamine were good substrates while their (−)-isomers were not β-hydroxylated (152, 389). When equal amounts of (+)-α-methyldopamine or (−)-α-methyldopamine were injected, the depletion of endogenous catecholamine by the (−)-isomer was of greater intensity (389). Considering mole to mole displacement of endogenous norephinephrine as the mode of action of these drugs, the depleting effects of two isomers should either be stereospecific or at least equal. Thus, it is likely that (−)-α-methyldopamine may affect the endogenous level of catecholamines by an additional mechanism which is not shared by the (+)-form. The drug presumably inhibits the dopamine-β-hydroxylase.

D. Phenethanolamine-N-Methyl Transferase

Both isomers of norepinephrine are N-methylated but the (−)-isomer is a better substrate (20). Amphetamine isomers are not N-methylated but these substances act as reversible competitive inhibitors of the enzyme. Dichloroamphetamine is more active as an inhibitor than amphetamine. In both cases the (+)-form is more potent than the (−)-form of the drug (136).

V. Substrate and Inhibitor Selectivity of Metabolizing Enzymes

A. Monoamine Oxidase

At the adrenergic neuroeffector junction, MAO and COMT are involved in the metabolic degradation of many sympathomimetic amines. As early as 1937 Blaschko *et al.* (55) observed that the relative rate of oxidation of (−)-epinephrine was greater than that of (+)-epinephrine when these isomers were tested on amine oxidase from guinea pig liver. The two optical isomers of β-hydroxyphenethylamine were oxidized at the same rate by rabbit liver, but the guinea pig liver extracts oxidized the (+)-form more rapidly than the (−)-form (315). In tissue slices or homogenates the stereoselective oxidation can be a function of access of substrate to the enzymatic site. The preferential oxidation was retained by the solubilized enzyme. The enzymatic preparations obtained from rat liver, brain or rabbit liver and heart oxidized both isomers of norepinephrine but the selectivity for (−)-norepinephrine was distinct (146). Recent studies show that there are different types of MAO. Hence, it is not surprising that they differ from each other with respect to stereoselectivity.

The mechanism of enzymatic deamina-

tion of the well known substrate, tyramine, was analyzed by the deuterium labeling of the drug at the α-carbon atom. The resulting (−)- and (+)-isomers of tyramine were oxidized by the enzyme at different rates. The findings indicated that the two hydrogens at the α-carbon of tyramine are not equivalent. During deamination of tyramine the enzyme attacks only one, or prefers only one, hydrogen atom (41). The deuterium-labeled isomer, which is a poor substrate for the enzyme, should produce a prolonged pharmacological effect. Results from the testing of both deuterium-labeled isomer *in vivo* appears to complement the biochemical findings (39).

Several types of drugs inhibit the activity of MAO (211). The two stereoisomers of amphetamine were equipotent as inhibitors of the rabbit liver oxidase, but with guinea pig liver extracts (+)-amphetamine was more potent as an inhibitor (315). On the rat liver preparation (+)-isomers of 2,4-dichloro- and 3,4-dichloroamphetamine were also better inhibitors of the enzyme than the corresponding (−)-isomers (137). However, a negative correlation between MAO inhibition and the central stimulant effect of the stereoisomers was obtained (157). Cyclized amphetamine analogs, cyclopropylamines, appear to be more potent inhibitors of the enzyme (423). Highly purified beef liver mitochondrial MAO showed little or no specificity towards the inhibition by (±)-*cis*- or (±)-*trans*-2-phenylcyclopropylamine. However, the (+)-form was found to be the more effective inhibitor when (±)- and (−)-*trans*-2-phenylcyclopropylamines were compared (68). Clinically, (±)-*trans*-2-phenylcyclopropylamine (tranylcypromine) was introduced as an antidepressant. Recently Horn and Snyder (181) compared the catecholamine uptake inhibitor effects of the isomers. In the hypothalamus, (−)-*trans*-2-phenylcyclopropylamine is more potent as an inhibitor than the (+)-form. If the antidepressant effect is related to the inhibition of uptake of norepinephrine and not to the inhibition of MAO, the (−)-form of the drug should be a more effective antidepressant than the (+)-form.

B. Catechol-O-Methyl Transferase

Both (−)- and (+)-isomers of norepinephrine, epinephrine, trimetoquinol, and dopa appear to be "O" methylated by the enzyme COMT (21, 215, 261, 350). *In vivo*, however, when equal doses of the norepinephrine isomers are injected, more "O" methylated metabolites are obtained from the unnatural isomer (141, 188). The reversal of stereoselectivity may in part be related to the selective rapid uptake of the (−)-isomer by the nerve endings, so that only a small fraction of the total injected dose is available for "O" methylation by the enzyme. In addition, the potent vasoconstrictor effect of the (−)-isomer may be a limiting factor for "O" methylation. Creveling *et al.* (89) indicated that under proper conditions both *para* and *meta* "O" methylated metabolites are formed from a given substrate of the enzyme. When optical isomers of conformationally flexible catecholamines were incubated with the enzyme, for each isomer different *meta*:*para* ratios of the "O" methylated products were obtained. In all pairs of (−)- and (+)-isomers of norepinephrine, epinephrine, isoproterenol, and *erythro*-α-methylnorepinephrine, the (+)-isomers gave the higher *meta*:*para* ratio. Since the absolute configurations of all four (+)-isomers are the same, it was possible to suggest the most probable conformation of the substrate at the active site of the enzyme. The dihedral angle between the β-OH group and the -NH_2 group appears to be 60° (90). To obtain more information on the active site of the enzyme, conformationally rigid decalin analogs of α-methyl norepinephrine and α-methyldopamine were synthesized and some of the newly synthesized substances were found to be good substrates for the enzyme (360, 361). The results, however, indicate

a different conformation of the functional groups. (a) In α-methylnorepinephrine analogs, the conformation in which the -NH_2 group and the β-OH group have a dihedral angle of 180° best fits the active site. (b) In the α-methyldopamine analog, the conformation in which the -NH_2 group and the aryl group are completely staggered best fits the active site. Since two different conformations of the same functional groups of conformationally flexible and rigid molecules are involved for the same enzyme, it appears highly probable that the enzyme itself can undergo conformational change.

VI. Stereoselectivity of Storage Vesicles for Amines

Most of the studies on "uptake" were done by measuring "accumulation" which involves *two* transport systems (sites 4 and 8 in fig. 3) plus vesicular binding. Caution is necessary with regard to conclusions about the uptake across the membrane since any observed stereoselectivity may reside in the second step (154, 217).

From the standpoint of methodology, it was relatively simple to define stereoselectivity of uptake and/or retention by the storage granule. The spontaneous loss of endogenous norepinephrine from bovine splenic nerve granules is prevented to a greater extent by (−)-norepinephrine than by the (+)-isomer. The amine uptake is enhanced by ATP. It is concluded that uptake is stereoselective at low concentrations for naturally-occurring isomers of both norepinephrine and epinephrine (123). The reuptake of spontaneously released norepinephrine from the surrounding medium can distort the calculation of the "affinity ratio" for norepinephrine isomers. Euler and Lishajko (125) overcame the difficulty by studying the ratio in the presence of potassium ferricyanide, an agent which continuously removes norepinephrine from the incubation medium of adrenergic nerve granules. The values for the "affinity ratio" found by the new method is 9.4:1.

The inhibition of uptake and the release of (−)-^{3}H-norepinephrine were studied in isolated bovine splenic nerve granules after incubation with close structural analogs of norepinephrine. (+)-*Erythro*-α-methylnorepinephrine, at 3×10^{-4}M, failed to influence the uptake of (−)-^{3}H-norepinephrine. However, at the same concentration, (+)-α-methyldopamine inhibited the uptake by 40%. In other experiments, when partially depleted storage vesicles were filled with (−)-^{3}H-norepinephrine, incubation with (+)-*erythro*-α-methylnorepinephrine failed to increase the efflux of the tritium, but (+)-α-methyldopamine increased it by approximately 40%. The data suggest that (+)-*erythro* isomer is not transported by the vesicular membrane. Secondly, equal values for the inhibition of uptake and release after (+)-α-methyldopamine indicate that the same mechanism operates for influx and efflux of the amine (326). The release will obscure the inhibition of uptake of the eoxgenous (−)-^{3}H-norepinephrine by the drug.

The retention of (+)- and (−)-^{14}C-norepinephrine, presumably by storage vesicles, was examined in the mouse heart (141). *In vivo* selectivity in the vesicular retention, as evidenced by different rates of loss of the isomers, was clearly seen. Half-times of 7.6 hours and 2.5 hours for (−)- and (+)-norepinephrine, respectively, were obtained. The manipulation of the vesicular accumulation by pharmacological tools further suggested that retention site favors the (−)-isomer. a) Reserpine, which is known to inhibit the uptake by the vesicles, abolishes the stereoselectivity. b) Pretreatment of mice with α-methyl-*p*-tyrosine, which depletes the vesicular stores by inhibiting the enzyme tyrosine hydroxylase, favors the accumulation of the (−)-isomer only. c) Chemical sympathectomy with 6-hydroxydopamine drasti-

cally reduces the accumulation. d) Almost all of the (+)-isomer is lost from the heart after 24 hours, while a considerable amount of the (−)-form can be found in the tissue. The results favor the view that the stereochemically "correct" orientation of the β-hydroxyl group is important for the ATP-magnesium-dependent storage process in the adrenergic nerve granules. As compared to the (−)- or (+)-isomer, racemic ^{14}C-norepinephrine disappeared from the heart in a multiphasic fashion. In many early studies racemic ^{3}H-norepinephrine was used as a valid tracer for investigating neural processes. It may be that both isomers are released from the storage sites at different rates and, thus, manifest multiphasic disappearance (141). The catecholamine storage vesicles, isolated as high speed sediment from hearts of rat, rabbit, and cat, and rat vas deferens, also showed preference for the (−)-isomer of norepinephrine or epinephrine (124, 217, 244, 267, 413).

VII. Concerning the Stereoselectivity of the Neuronal Uptake of Norepinephrine

Differences in the neuronal uptake, storage and metabolism of the stereoisomers of norepinephrine were first reported in 1963 (185, 212, 246). Although the problem of stereoselectivity of neuronal uptake (uptake$_1$) was studied by many groups during the following eight years, a general consensus of opinion has not been reached. In fact, the available evidence is contradictory. Nevertheless, it seems possible to agree with the conclusion of Iversen *et al.* (188) who stated that: ". . . various differences exist in the uptake, storage, and metabolism of (+)- and (−)-noradrenaline. These differences, however, are not very great. It would seem that the biochemical mechanisms involved in the handling of noradrenaline are less able to discriminate between the optical isomers than is the case for adrenoreceptors, where differences in the potency of (+)- and (−)-noradrenaline of 100-fold and more are frequently found." This summary appeared at the end of a report which included evidence that neuronal uptake in the guinea pig heart lacked stereoselectivity. Hence, the sentence "These differences, however, are not very great." includes the absence of stereoselectivity. Since very little can be added to this summary at the present time, the following account is an attempt to delineate the factors which make it so very difficult to provide clear and convincing answers to the simple question of whether or not neuronal uptake is stereoselective.

Neuronal uptake of norepinephrine is followed by deamination by MAO or by uptake into and binding in the storage vesicles. In addition, the possibility of a neuronal COMT has also to be entertained (193). Of these mechanisms at least two exhibit stereoselectivity: storage vesicles (see section VI) and MAO (see section V).

Any study of neuronal uptake is complicated by the fact that there is also a mechanism for extraneuronal uptake which lacks stereoselectivity (uptake$_2$; 149, 171, 186, 231).

From these considerations it follows that the stereoselectivity of neuronal uptake should be studied by experimental designs which avoid distortion of the results by a) the stereoselective mechanisms located inside the neuron, b) metabolism of the amine, and c) uptake$_2$.

In order to stress the importance of separating the stereoselective system under study from other systems lacking stereoselectivity, an example from studies of aortic strips is presented, although the stereoselective system of this example resides in the storage vesicles rather than in "neuronal uptake." After inhibition of both metabolizing enzymes rabbit aortic strips were incubated with 1.18 μM labeled (−)- or (+)-norepinephrine for 30 minutes;

thereafter they were washed out, and the distribution of the amine at the end of the incubation period was determined from compartmental analysis of the efflux curves. Without this compartmental analysis, strips exhibited some stereoselectivity with respect to "accumulation of radioactivity in the tissue during 30 min of incubation", the degree of stereoselectivity being rather small (10.7 and 8.1 nmoles/g for the accumulation of (−)- and (+)-norepinephrine, respectively). However, compartmental analysis revealed that about 3.5 nmoles/g were accumulated in the extraneuronal stores and about 4.0 nmoles/g in the axoplasm of the neuron, both compartments lacking stereoselectivity of accumulation. Thus, if the nonstereoselective compartments are subtracted from total accumulation, a very pronounced degree of stereoselectivity for vesicular accumulation becomes apparent (173a).

Stereoselectivity of neuronal uptake can be determined either by measurements of the inhibitory potency of unlabeled isomers of norepinephrine in antagonizing the neuronal uptake of labeled amine or by measurements of the neuronal uptake of (−)- or (+)-norepinephrine. As discussed by Iversen (187) the two methods yield identical results (if certain experimental conditions are met; see below). Two methods are available for the measurement of the neuronal uptake of an amine: either the accumulation of the amine in the tissue is measured or the removal of the amine from the incubation medium or perfusion fluid is determined (232). All methods have their advantages and disadvantages, some of which will be discussed in the following.

1) The stereoselective mechanisms residing in the neuron. When removal is measured, the interference by the stereoselective vesicular uptake can be avoided by pretreatment with reserpine, since such pretreatment does not affect uptake$_1$ (154, 188, 232). However, when accumulation is measured, the pretreatment with reserpine must be combined with inhibition of MAO in order to ensure a measurable accumulation of the amine in the nerve endings. A very pronounced axoplasmatic accumulation can then be achieved (154).

2) Metabolism of norepinephrine. Neuronal uptake equals the amount of norepinephrine accumulated in the neurons plus the amount metabolized intraneuronally. Thus, except after block of both metabolizing enzymes, the accumulation of unchanged norepinephrine in the neuron is but part of neuronal uptake. Many studies of the accumulation (or retention) of unchanged norepinephrine in the tissue are open to the criticism that neuronal uptake was underestimated to some degree because of neglect of neuronal metabolism. The use of radioactively labeled norepinephrine and the measurement of the total radioactivity of the tissue (*i.e.*, of labeled norepinephrine and metabolites) can reduce this error, but it rarely eliminates it. The reason for this complication lies in the fact that normetanephrine and the neutral metabolites are not retained by the tissue; some retention is observed only for the acid metabolites (*i.e.*, dihydroxymandelic acid and vanillylmandelic acid) (173, 228a). How quickly the neutral metabolites leave the neuron is illustrated by the observation that, on perfusion of the isolated cat heart with a constant concentration of ^{3}H-(−)-norepinephrine, the most important metabolite of neuronal origin (*i.e.*, dihydroxyphenylglycol) is detected in the venous effluent after 1 minute of perfusion (Graefe, personal communication). Hence, even when radioactively labeled norepinephrine is used, measurements of "accumulation in the tissue" (of ^{3}H activity, in this case) can be equated with "uptake" only for perfusions of not more than 1 minute. For incubated rather than perfused tissues it is likely that more time will be required for the neutral metabolites to diffuse into the incubation medium, but even under these conditions one

would have to be careful not to underestimate "uptake" by using too long an incubation period.

A complete and reliable picture of neuronal uptake is obtained only if the measurements of the accumulation of the amine (or of total radioactivity) in the tissue are complemented by determinations of the metabolites in the tissue and in the incubation medium (or venous effluent, in the case of a perfused organ). Unfortunately, one then faces the problem of the admixture of metabolites of extraneuronal origin (see below). A study of "removal," on the other hand, offers the considerable advantage of being largely independent of the intraneuronal fate of the amine (154). In this context, "removal" means "net removal." Therefore, any efflux or release of norepinephrine from the neuron must interfere with measurements of "removal," but this also applies to measurements of "accumulation." See below for the need to determine initial rates of removal or accumulation, if unidirectional fluxes have to be determined.

3) Extraneuronal uptake. Lightman and Iversen (231) found that an extraneuronal accumulation of norepinephrine does not occur when the rat heart is perfused with concentrations of the amine which are below the K_m for uptake$_1$. Apparently, there is a complete metabolism of the amine transported by uptake$_2$ under these conditions. Much higher concentrations of norepinephrine are required for an extraneuronal accumulation of unchanged amine. For any study that is based on the measurement of accumulation of unchanged amine in the tissue, the lack of extraneuronal accumulation of norepinephrine is a clear advantage. However, it is by no means certain that the observations by Lightman and Iversen with rat hearts can be applied to other organs or species. If the extraneuronal metabolizing enzymes of other organs or species were less effective than those of the rat heart, an extraneuronal accumulation of the amine might occur. Hence, independent evidence concerning this point should be obtained.

Of the numerous differences between neuronal and extraneuronal uptake mechanisms, one should be mentioned here, since its relevance to the problems discussed here has become evident recently. In the hearts of various species, the cat nictitating membrane and the rabbit aorta (228a, and unpublished observations from the Würzburg laboratories) extraneuronal metabolism and accumulation of catecholamines equilibrate much more quickly with incubation medium or perfusion fluid than do neuronal metabolism and accumulation of catecholamines (when the tissue is exposed to a constant concentration of the catecholamine). For instance, in the perfused cat hearts mentioned above, the most important metabolite of extraneuronal origin (*i.e.*, normetanephrine) appears in the venous effluent *before* the most important metabolite of neuronal origin (*i.e.*, dihydroxyphenylglycol) is detected. Moreover, the concentration of normetanephrine in the venous effluent approaches a steady-state level much more quickly than does the concentration of dihydroxyphenylglycol (Graefe, unpublished observations). Because of these pronounced differences in the half-time of the approach of the two systems to equilibrium, the relative contribution of each system to "total removal" (*i.e.*, neuronal plus extraneuronal removal) varies with the duration of the perfusion. The quickly equilibrating extraneuronal removal is responsible for a certain percentage of "total removal" soon after the beginning of the perfusion. The slowly equilibrating neuronal removal, on the other hand, accounts for a gradually increasing percentage of "total removal" when the perfusion is continued. These considerations probably explain the following observation. When isolated nictitating membranes (obtained from reserpine-pretreated cats) were exposed to a low concentration of norepinephrine for a short period (7.5 minutes),

about 50% of the "total removal obtained during 7.5 min" was due to extraneuronal mechanisms (155). However, a lower value was obtained when "total removal obtained during 60 min" was determined under similar conditions (156).

Thus, while short periods of exposure to norepinephrine are desirable for a variety of reasons (see above), they have the disadvantage of increasing the chance for extraneuronal mechanisms to interfere with measurements of neuronal uptake.

The problem of extraneuronal removal by the isolated nictitating membrane (see above) was minimized by taking the production of deaminated metabolites (COMT being blocked) as a measure of neuronal uptake (155). The justification for regarding $uptake_1$ as the rate-limiting step of intraneuronal deamination is presented by these authors.

Any correction of neuronal accumulation or removal for extraneuronal accumulation or removal presents the following problem. If it is correct that $uptake_1$ generates a concentration gradient from the medium (or perfusion fluid) to the nerve endings, its magnitude depends on the homogeneity and density of the adrenergic innervation (393, 394, 403). If $uptake_1$ is impaired by either cocaine or surgical or chemical denervation, the concentration gradient is greatly diminished and the concentration of the amine at the receptors is increased (for more detailed discussion, see 392, 393, 395). It is possible (but not certain) that the concentration of the amine is also increased in that part of the extracellular space from which $uptake_2$ occurs. If this is the case, cocaine or denervation might increase $uptake_2$.

It would be ideal, if it were possible, to use an inhibitor of $uptake_2$ which does not affect $uptake_1$. Various inhibitors of varying selectivity are known, but their usefulness has not yet been tested in attempts to measure the stereoselectivity of $uptake_1$. Because of their relative selectivity for $uptake_2$, either the corticosteroids (189) or the O-methylated catecholamines (187, 263) might be useful for this purpose.

4) Extracellular, vascular and other spaces. In all studies of accumulation or removal allowance must be made for the amine lost into extracellular and vascular spaces. In addition, the perfused heart has an additional space into which amine is lost from the perfusion fluid: the cavity of the right ventricle (59). Special care should be taken to subtract this "apparent removal" from the removal due to $uptake_1$ and $uptake_2$.

The problems enumerated under 1) and 2) are avoided if *initial* rates of accumulation or removal are measured. Intraneuronal storage and metabolism can then be disregarded. However, the experimental difficulties encountered when attempting to measure initial rates are considerable.

The use of very short exposure times entails not only the need for very high specific activities of the labeled amine, measurements of the extracellular space become very important when the exposure time is too short for equilibration of the amine in the extracellular space. The problem can be avoided at least partly by the simultaneous determination of the distribution volume of a marker of extracellular space of similar molecular weight (*e.g.*, by the combination of ^{3}H-norepinephrine with ^{14}C-sorbitol). Other problems that become increasingly troublesome for decreasing periods of incubation have been described under 3) and 4).

By far the most satisfactory method for obtaining accurate values for initial rates consists of a painstaking determination of the time course of rates of uptake. Such measurements are much more easily obtained with determinations of removal than of accumulation. Such values should be subjected to compartmental analysis (185), and accurate initial rates obtained by extrapolation to zero time. However, if rates of accumulation or removal decline

multiphasically, the neuronal nature of each compartment has to be assessed in separate experiments.

There should be no doubt that reliable statements concerning the stereoselectivity (or lack of it) of uptake$_1$ should be based on determinations of initial rates of uptake$_1$. If this criterion is not met, the intraneuronal fate of the amine is bound to affect the results. Graefe *et al.* (154) demonstrated how the intraneuronal fate of norepinephrine affects rates of neuronal uptake that are *not* initial rates. Moreover, initial rates should be determined for several concentrations of norepinephrine (lying below the K_m for uptake$_1$), and the K_m should be determined by kinetic analysis.

However, even when the criterion of reliability of measurements of initial rates of uptake$_1$ is met, the result may well be affected by an additional factor, the mass of tissue in relation to the density and homogeneity of innervation. Experimental evidence for this view was provided recently by Hendley and Snyder (169) who found stereoselectivity of neuronal uptake in thin slices of rabbit iris and vas deferens, while no stereoselectivity was obtained with thick slices.

No doubt, if the primary interest of the experimenter lies in the determination of the stereoselectivity of "the adrenergic neuron," tissues of small mass should be used. However, it is equally pertinent to determine stereoselectivity of uptake$_1$ for preparations as they are used in many pharmacological experiments. For instance, cocaine is known to potentiate the effects of (−)- but not of (+)-norepinephrine on the nictitating membrane (344, 391). The lack of stereoselectivity of uptake$_1$ (whole muscle) shows that the stereoselectivity of the effect of cocaine is unrelated to the stereoselectivity of uptake$_1$. The explanation for the stereoselectivity of the effect of cocaine is found in the fact that uptake$_1$ is saturable [for further discussion, see Draskóczy and Trendelenburg (109)].

Discussion of the concentration gradient generated by uptake$_1$ leads to another consideration. K_m values for uptake$_1$ relate to the concentration of the amine in the incubation medium (or perfusion fluid), they do *not* relate to the concentration outside the nerve endings. Hence, the true affinity of norepinephrine to the sites of uptake$_1$ may be considerably higher than indicated by the apparent K_m values obtained experimentally. Adrenoreceptors may serve as an example. The ED50 serves as a measure of the affinity of the amine to the receptors. Experiments with innervated nictitating membranes yield values which seriously underestimate affinity. The true affinity for (−)-norepinephrine is revealed after the combination of denervation (of short duration) and block of COMT, which together increase sensitivity about 100-fold (396). It is quite possible that the same argument has to be applied to the apparent K_m for neuronal uptake.

VIII. False Neurochemical Transmitters

A. α-Methylnorepinephrine Isomers

While the fate of the therapeutically ineffective (+)-α-methyldopa (7) is unknown, it is suggested that enzymatic decarboxylation and subsequent β-hydroxylation of (−)-α-methyldopa probably operates in an orderly fashion to yield a single amine metabolite, (−)-*erythro*-α-methylnorepinephrine (271). The biotransformation of (−)-α-methyldopa and the structure of related isomers are presented in figure 6. If the enzymes in the biotransformation were not stereoselective (+)-α-methyldopa should have been metabolized to (−)-*threo*-α-methylnorepinephrine or racemic amine metabolites.

Except for the preparation of (−)-*threo*-α-methylnorepinephrine by the resolution of (±)-*threo*-α-methylnorepinephrine, all other optical isomers that are pre-

sented in figure 6 have been synthesized and resolved (128, 389). Exogenous administration of these isomers provides a good tool to investigate various pharmacological effects of the false transmitters. Henning and Van Zwieten (172) claim that the hypotensive effect of α-methyldopa is mediated *via* the central nervous system (CNS). This claim is based on the observation that in cats, intravertebral infusion of 20 mg/kg of (−)-α-methyldopa lowered the blood pressure. The (+)-isomer was

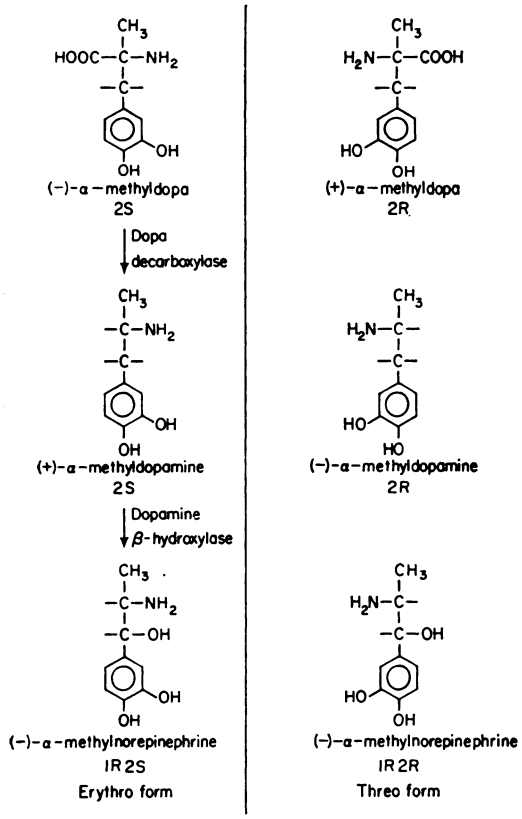


FIG. 6. The stereospecific biotransformation of (−)-α-methyldopa. (+)-α-methyldopa is not decarboxylated and (−)-α-methyldopamine is not β-hydroxylated. Although after the decarboxylation of (−)-α-methyldopa, the optical rotation for α-methyldopamine is (+)-rotatory, the absolute configuration at C_2 is unchanged. The steric structure of other isomers is shown for comparative purposes. [Data condensed from Muscholl (271), Patil and Jacobowitz (289), Kilbinger *et al.* (208), Torchiana *et al.* (389), and Marshall and Castagnoli (251).]

ineffective. The reduced effect of tyramine on catecholamine-depleted tissues is restored readily by (−)-α-methyldopa or (+)-α-methyldopamine. This restoration is presumably caused by the formation of (−)-α-methylnorepinephrine from its precursors. The other isomer, namely (+)-α-methyldopa, does not restore the effects of tyramine (303). Furthermore, after the injection of (+)-α-methyldopamine, the formation of (−)-*erythro*-α-methylnorepinephrine in tissues is detected, while no such amine metabolite is obtained from (−)-α-methyldopamine (208). Even at high concentrations (+)-α-methylnorepinephrine is not taken up by the nerve granules (326). Recently, the absolute configuration of the α-methyldopamine excreted in the urine of man treated with (−)-α-methyldopa has been established as 2S (251). All these observations suggest that biotransformation of (−)-α-methyldopa must proceed as illustrated in figure 6.

Like (−)-*erythro*-α-methylnorepinephrine, (±)-*threo*-α-methylnorepinephrine was readily taken up and retained by the rabbit heart with concomitant equivalent loss of endogenous norepinephrine. While the initial uptake for (−)-*erythro*-α-methylnorepinephrine appeared to be the same, their release rates were different (272). Similarly, in the mouse heart there was essentially equal uptake of both (±)-*erythro*-H^3- and *threo*-α-methylnorepinephrine, but the (±)-*threo* form appeared to leave faster than the (±)-*erythro* form. The half-life after 20 μg/kg of (±)-*threo* and (±)-*erythro* form was 20 hours and 72 hours, respectively (73, 406). The potency of the α-adrenoreceptor-activating effect of (±)-*threo*-α-methylnorepinephrine is approximately 1/100 that of the (−)-*erythro* form. In agreement with the above lines of evidence, Drews *et al.* (110) observed in experiments with renal hypertensive rats a long-lasting antihypertensive effect of the false neurochemical transmitter (±)-*threo*-α-methylnorepinephrine. The N-methyl ana-

log of (±)-*erythro*-α-methylnorepinephrine was also investigated to determine uptake, binding, and release of catecholamine from rabbit hearts. Like (±)-*threo*-α-methylnorepinephrine, the N-methyl analog appears to function as a false neurochemical transmitter (233, 248, 249, 273). Since all the isomers of α-methylnorepinephrine have the essential chemical structure for histofluorescence, these amines were investigated for uptake by the rat iris. In reserpine-pretreated animals, only (+)- and (−)-*erythro*-α-methylnorepinephrine were able to reconstitute the fluorescence. *In vivo* and in higher doses, (±)-*threo*-α-methylnorepinephrine and its N-methyl analog were unable to do so (194, 289). If the *threo* form functions as a false neurochemical transmitter, uptake by the neuron should be a first prerequisite. High concentrations of the *threo* form were able to reconstitute the fluorescence *in vitro* only. *In vivo*, the amine is probably lost rapidly.

B. α-Methyldopamine Isomers

A study of the isomers of (+)- and (−)-α-methyldopamine presents an interesting case. In the heart, after equimolar doses, the catecholamine-depleting activity of (+)-α-methyldopamine is only one-third that of (−)-α-methyldopamine (208, 389, 407). On the isolated rabbit heart, doses of the isomers which cause similar norepinephrine depletion affect sympathetic nerve stimulation differently. The response to nerve stimulation is inhibited after (−)-α-methyldopamine, while it is unchanged after the (+)-isomer. The differential effect is attributed to a higher cardiac β-adrenoreceptor-activating effect of the (+)-isomer and/or its metabolite (208).

In rabbit atria treated with phenoxybenzamine the chronotropic effect of (−)-α-methyldopamine was inhibited more than that of the (+)-form. Results indicate that the (+)-α-methyldopamine is a better β-adrenoreceptor activator. After incubation of reserpine-pretreated rabbit atria with either (−)- or (+)-α-methyldopamine, the effects of tyramine were restored by both isomers, but the (+)-form was more effective (420). This indicates that in addition to the release of the (+)-form by tyramine, the active metabolite (−)-α-methylnorepinephrine might also be displaced by the drug. These results are consistent with the suggestions made by Kilbinger *et al.* (208).

C. Metaraminol Isomers

Although no systematic study is reported on the biotransformation of the optical isomers of α-methyl-*m*-tyrosine, it appears quite logical that (−)-α-methyl-*m*-tyrosine is first decarboxylated to (+)-α-methyl-*m*-tyramine which is selectively β-hydroxylated to yield (−)-metaraminol (152, 323). Three lines of evidence indicate that the biotransformation might be similar to that illustrated in figure 6. Firstly, the absolute configuration of the biosynthetic intermediate (+)-α-methyl-*m*-tyramine is 2S, which is the same as that assigned for (+)-α-methyldopamine (30, 329). Secondly, as compared with (−)-α-methyl-*m*-tyramine, the (+)-isomer is a potent norepinephrine depleter. This depleting effect is blocked by a dopamine β-hydroxylase inhibitor (353). Presumaably, formation of (−)-metaraminol occurs. Thirdly, administration of the four isomers of metaraminol shows that the potent catecholamine-depleting effect is associated only with (−)-metaraminol (6, 95, 241). In other words, the (+)-*erythro*, (+)- or (−)-*threo* isomers of metaraminol are probably not formed from the precursor substance. Shore *et al.* (354) observed that after intravenous injection of 50 μg/kg of either (−)-metaraminol, (+)-metaraminol or (±)-α-methyl-*m*-tyramine, only (−)-metaraminol was retained in the rat heart for up to 24 hours. The tissue concentration of (+)-metaraminol and (±)-α-methyl-*m*-tyramine falls sharply 10 to 15 minutes after their administration.

Lack of accumulation of (−)-metaraminol in immunosympathectomized animals indicated that accumulation of (−)-metaraminol in normal animals was in sympathetic nerves only. The intracellular distribution of these agents in different fractions from the heart revealed that (−)-metaraminol displaces heart norepinephrine and is significantly associated with the particulate cell fraction, whereas (±)-α-methyl-*m*-tyramine neither depletes norepinephrine nor is associated significantly with cell particles. Although (+)-metaraminol does not deplete norepinephrine, it shows some association with cell particles (145). *In vitro*, rabbit heart slices also exhibit selective accumulation of (−)-metaraminol over (+)-metaraminol or (±)-α-methyl-*m*-tyramine. Pretreatment of these slices with imipramine or ouabain markedly inhibited accumulation of both isomers of metaraminol. Pretreatment with reserpine does not prevent the accumulation of (−)- and (+)-metaraminol. Combined treatment with reserpine and ouabain, however, decreases the accumulation of (−)-metaraminol to a greater extent than does ouabain alone. A similar combination is not synergistic for the prevention of accumulation of (+)-metaraminol (48). On the basis of these findings together with additional studies, Sugrue and Shore (375, 376) postulated the presence of a sodium-dependent, optically-specific and reserpine-sensitive amine carrier mechanism at the adrenergic neuron membrane.

D. Other Amines

Allen *et al.* (8) demonstrated that exogenously administered (+)-^{14}C-norepinephrine could be released from the rabbit ear artery by nerve stimulation. The possibility that the drug may become a useful "false neurochemical transmitter" is suggested. The indirectly-acting amine, tyramine, is capable of displacing the isomer (219). The large dose of (±)-*erythro*-3:4-dihydroxyphenylserine, which is capable of forming (+)-norepinephrine, reduces the endogenous norepinephrine (309). As compared to the (−)-isomer, the receptor-activating effect of (+)-norepinephrine is much inferior. In terms of intravenous toxicity in rats, (+)-norepinephrine has $\frac{1}{14}$ the toxicity of the (−)-isomer (179). All these properties can be advantageous for a drug to be an effective "false transmitter" like (−)-metaraminol; however, the retention of (+)-norepinephrine is short lived (141). Hence, the advantages and disadvantages of various properties of (+)-norepinephrine in promoting this drug in therapeutics remain to be assessed. Drugs which are effective false transmitters are also effective in depleting endogenous catecholamine. The depletion presumably occurs due to the occupancy of the vesicular stores by the amine.

Daly *et al.* (95) investigated the norepinephrine-releasing potencies of a wide variety of sympathomimetic amines and related compounds. It is clear that monophenolic or diphenolic amines with 1R sterochemistry are better at depleting cardiac norepinephrine than are corresponding isomers with 1S sterochemistry. *In vivo* tyramine is converted rapidly to its β-hydroxylated product, octopamine. In all probability only the (−)-isomer is formed and stored as a false neurochemical transmitter in the vesicles. Again, monophenolic or diphenolic amines with 1R stereochemistry are better at depleting octopamine than are the corresponding isomers with 1S stereochemistry (74).

In relation to the chronic and catecholamine-depleting effects of amphetamine isomers, various investigators considered the formation of a false neurochemical transmitter (63, 230, 264, 317). Although both isomers of amphetamine are *para*-hydroxylated (111, 112), only (+)-*p*-hydroxyamphetamine (2S) is further β-hydroxylated to form (−)-*p*-hydroxynorephedrine (1R, 2S). This amine metabolite then depletes catecholamines from the neuron (fig. 7). (−)-*p*-Hydroxyamphetamine is not β-hydroxylated (152), which explains the

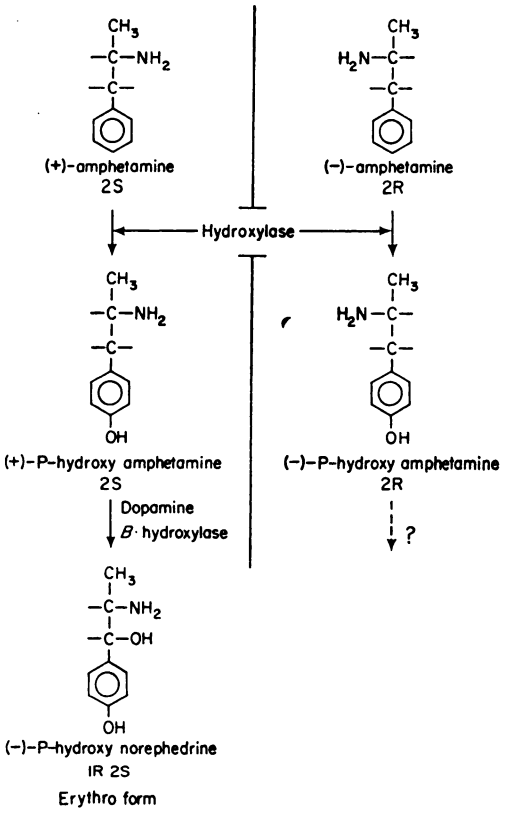


FIG. 7. The formation of a "false" neurotransmitter, (−)-*p*-hydroxynorephedrine from (+)-amphetamine is illustrated. (−)-*p*-Hydroxyamphetamine is not β-hydroxylated [after Goldstein and Anagnoste (152), Brodie *et al.* (63), and Lewander (230)].

weaker catecholamine-depleting effect of (−)-amphetamine. Vidrio *et al.* (404) studied the antihypertensive effects of methamphetamine isomers in dogs with experimentally induced hypertension. Only (+)-methamphetamine lowered the blood pressure, presumably *via* formation of a false neurotransmitter. Like (−)-amphetamine, (−)-methamphetamine may not undergo the biotransformation.

It has been suggested that *in vivo* dopamine can condense with dopamine aldehyde to form tetrahydroisoquinolines. Many isoquinoline alkaloids have an asymmetric center at the carbon (C_1) adjacent to the nitrogen. This should result in optical isomers; however, *in vivo* the enzyme which catalyzes the reaction is not clearly defined. Knowing the specificity of the enzymatic reaction, one would expect it to yield a single isomer (fig. 8). In view of the suggested role of the tetrahydroisoquinolines as a false neurochemical transmitter (334), the comparison of the specificity in the enzymatic action or in the formation of the false neurotransmitter should be rewarding. Chemical synthesis of (+)- and (−)-1 substituted 6, 7-dihydroxy-1,2,3,4-tetrahydroisoquinolines (383) and some pharmacological activities of (+)- and (−)-salsoline are reported. The isomers were found to be devoid of antiparkinson activity in mice (384). If these substances do not pass the blood brain barrier, the administration of drugs by the intravenous route may not give a clue regarding their effects in the CNS.

IX. Inhibition of Uptake

A. Configurational Requirements for Inhibition of Uptake

Sympathomimetic amines not only release norepinephrine, but also block the uptake of catecholamines. Affinities of several amines for the neuronal transport were studied on the rat heart (67), rabbit heart (274), and the mouse cerebral cortex (322). Regarding the steric structure activity for the inhibition of uptake, the following conclusion can be drawn. (a) In phenolic or nonphenolic amines desoxy derivatives (without β-OH group) are better inhibitors of uptake than are the corresponding β-hydroxylated (−)-isomers. (b) In phenolic amines β-hydroxylated (−)-isomers are more potent than the corresponding (+)-isomers. (c) (+)-Amphetamine is 20 times more potent than (−)-amphetamine. (d) (+)-*Norpseudo*-ephedrine is more potent than either (−)-norephedrine or (+)-amphetamine. However, experiments in the above studies were conducted on tissues in which endogenous stores of norepinephrine were intact. The amine in question can liberate

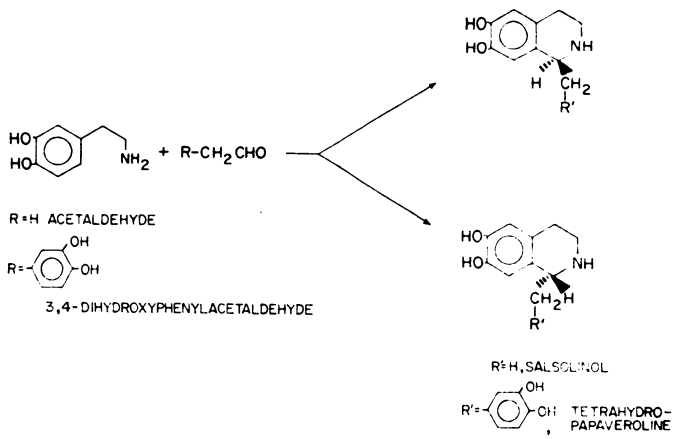


FIG. 8. Hypothetical formation of stereoisomeric isoquinoline alkaloids from dopamine. The enzyme involved in the biosynthesis is not clearly defined. *In vivo*, if the reaction between the aldehyde and dopamine is nonenzymatic, a racemic product is expected.

the endogenous norepinephrine, which can compete with the exogenous labeled norepinephrine for uptake sites. The affinity of an amine for the transport mechanism is obtained by measuring the net accumulation of the exogenous norepinephrine. The amine which liberates more endogenous norepinephrine or labeled norepinephrine which has been taken up will appear as a drug with higher affinity for the neuronal transport than is actually the case. In other words, the reported structural characteristics of amines for the affinity at the neuronal transport may in part be a reflection of their catecholamine-releasing potencies. Thus, liberation of endogenous amine can distort the calculation of true affinity of the amine. The capacity of the amine to release must be determined independently, or experiments must be done after the depletion of endogenous amines with a suitable drug which does not modify the properties of the neuronal membrane. Furthermore, all indirectly-acting amines do not appear to release the endogenous catecholamines by the same mechanisms because a) the physical and chemical properties of phenolic and nonphenolic amines differ, and b) when uptake is saturated by high doses of indirectly-acting nonphenolic amine or amines, the phenolic amines can release the endogenous amine (295). The dose-response curves of phenolic and nonphenolic amines for the inhibition of uptake were not parallel (322). Paton (298) has re-examined the problem. The potency ratio of amphetamine isomers for inhibition of uptake was reported to be 20 (67). However, both isomers were found to be equipotent when endogenous norepinephrine was depleted by reserpine (298).

Patil *et al.* (295) observed that in the normal vas deferens, indirectly-acting desoxy derivatives always produced a greater magnitude of pharmacological effects than their corresponding β-hydroxylated (+)-isomers. For example, desoxy α-methyldopamine produced greater effects than (+)-α-methylnorepinephrine. These results were explained on the basis that desoxy derivatives enter the intraneuronal stores at a faster rate and displace the stored norepinephrine at a faster rate than the corresponding β-hydroxylated (+)-isomers. An incorrectly oriented alcoholic hydroxyl group in the (+)-isomer was believed to cause hindrance in uptake of these agents. However, it can be argued that both desoxy derivative and (+)-isomer

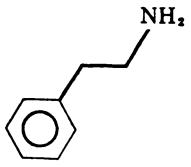
are taken up to an equal degree and displace equal amounts of norepinephrine. The higher activity of desoxy derivatives then can be explained by greater inhibition of re-uptake by the agent. The lower activity of the (+)-isomer is caused by its inability to inhibit the re-uptake of released norepinephrine.

A series of experiments was done to test the cocaine-like effect of indirectly acting agents in the reserpine-pretreated rat vas deferens. The concentrations of the desoxy derivative and (+)-isomer were the same as those at which they exhibit unequal pharmacological effects on the normal tissue. The desoxy derivative and corresponding (+)-isomer were tested on the contralateral vas deferens of the same reserpine-pretreated rat. It was observed that the desoxy derivative and corresponding (+)-isomer caused equal potentiation of the effects of exogenous norepinephrine. Again, not in the normal but in catecholamine-depleted tissues, the inhibition of uptake of ^{3}H-norepinephrine by L(+)-isomer or desoxy derivative was equal (327). Thus, the unequal pharmacological effects of desoxy and L(+)-isomers may be related to their unequal catecholamine-releasing effects.

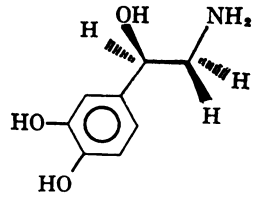
B. Conformational Requirements for Inhibition of Uptake

Maxwell *et al.* (253–256) have proposed that norepinephrine and the other amine derivatives interact with the "amine pump receptor" in a planar, *trans* conformation. Phenethylamines closely related to norepinephrine act as competitive substrates for the amine pump transport site. The authors have also suggested that several classes of potent drugs such as methylphenidate, cocaine, tricyclic antidepressants, and pipradols are capable of superimposing portions of each of the respective molecules upon the extended phenethylamine structure (see below).

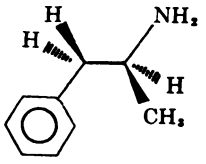
One can easily see the *trans*-β-phenethylamine segment of molecules such as R-(−)-norepinephrine, S-(+)-amphetamine and R-(−)-desoxypipradol. Other potent inhibitors of (−)-norepinephrine uptake such as desmethylimipramine and cocaine do not possess this segment, and it has been proposed that the latter molecules are capable of positioning a phenyl ring and nitrogen atom in approximately the same position thus mimicking the essential portions of the *trans*-β-phenethylamine model required for interaction with the amine pump receptor. Since all of these agents



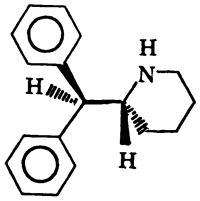
β-Phenethylamine
trans conformation



R-(−)-Norepinephrine



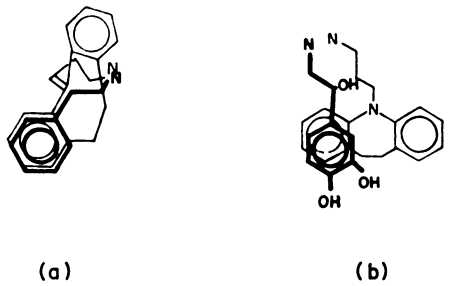
S-(+)-Amphetamine



R-(−)-Desoxypipradol

are amines with pK_a values greater than 8.5, they would be more than 90% protonated at physiological pH. It has been postulated that the cationic portions of these molecules could be interacting with an anionic center on the amine pump receptor (254, 255). Some quaternary amine compounds were found to be moderately potent. With the tricyclic compounds, *e.g.*, desmethylimipramine, these investigators have found the optimal activity in compounds which do not have the phenyl rings coplanar as with the carbazole derivatives, but which have the rings at a fixed angle to one another. It has been suggested that tricyclic derivatives in which the phenyl rings are conformationally restricted and coplanar have the second phenyl ring, *e.g.*, "in the carbazole series" extending into the region in which the cationic nitrogen should be and thus reducing the effectiveness of the binding (256, 332). Maxwell, *et al.* (255) attribute the higher potency of tricyclic antidepressants to the fact that the two phenyl rings are not coplanar and the second phenyl ring is raised out of the plane of the "phenethylamine portion" of the molecule while binding to the amine pump receptor. Horn has proposed a modification of this proposal (see fig. 9). Although the phenethylamine pump may be quite similar in the periphery, the cortex and hypothalamus, there appears to be a difference in the accessibility to, or a difference in, the amine pump in these tissues and the amine pump in the striatum (132, 180, 365). The carbazole derivatives are more potent than the imipramine-like drugs in preventing uptake into the striatal dopamine neurons.

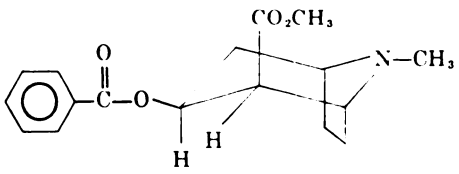
It can be seen easily that cocaine does not possess a phenethylamine segment as an integral part of the molecule. Maxwell *et al.* (254, 255) rationalized the lower potency of cocaine relative to other potent inhibitors of uptake such as desoxypiperidol, methylphenidate, and desmethylimipramine in several ways. Among these rationalizations was the belief that the



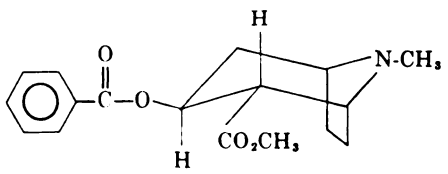
(a) (b)

FIG. 9. a, Tricyclic antidepressant (amitriptyline) with the phenethylamine segment superimposed as proposed by Maxwell *et al.* (256). b, Tricyclic antidepressant (imipramine) with norepinephrine superimposed as viewed by Horn (179a). In both proposals one can note the *trans* extended side chain of the phenethylamine molecule with the difference being in the manner in which folding of the side chain of the tricyclic antidepressant is carried out in order that the nitrogen and aromatic rings will be superimposable.

positioning the tropane ring system and the phenyl ring system giving rise to the model phenethylamine structure required a conformation of cocaine which is thermodynamically unfavorable. In connection with the conformation of cocaine, Gabe and Barnes (139) showed the piperidine ring of the tropane nucleus to have the chair form by means of X-ray diffraction analysis. This work shows the benzoyloxy substituent at C-3 to be in the equatorial position and in an extended form away from the tropane ring system and that the carbomethoxy side chain at C-2 is in the axial position. This analysis confirmed earlier work in which the absolute configuration of (−)-cocaine was determined by correlation with L-glutamic acid (163). Nuclear magnetic resonance studies of cocaine also indicate a preference for the chair conformation. The position of the carbomethoxy group appears to be important and must be *cis* with the pyrrolidine (218) nitrogen for maximum potency (see the chemical structure). (−)-Cocaine is 14 and 19 times more potent than (+)-*pseudo*cocaine as an inhibitor of the uptake of (−)-norepinephrine in ventricle and vas deferens slices. A study of the effect of all optical isomers of cocaine on



(−)-Cocaine



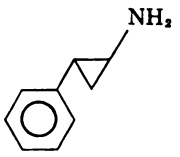
(+)-Pseudococaine

inhibition of uptake and the relationship between uptake inhibition and CNS stimulation should be rewarding.

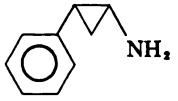
The use of conformationally restricted molecules appears to lend some credence to the proposal that molecules show optimal binding to the "amine pump receptor" when they possess a certain spatial relationship between the aromatic ring and the amine function. Horn and Snyder (181) found in a study in the CNS that *trans*-2-phenylcyclopropylamine, a conformationally rigid analog of amphetamine, was 600 times more potent than the *cis*-isomer in inhibiting norepinephrine uptake in the hypothalamus and the *trans*-isomer was more than 320 times as potent as the *cis*-isomer in inhibiting dopamine uptake in the striatum. In confirming this work Tuomisto *et al.* (399) found *trans*-2-phenylcyclopropylamine to be almost 10^3 times as potent as the *cis*-isomer in inhibiting norepinephrine uptake in the hypothalamus. The latter workers also point out that these results should not be taken to suggest that the active conformation of amphetamine would be the *trans* form. This is understandable since the *trans*-2-phenylcyclopropylamine molecule is not directly analogous to a phenethylamine molecule in the *trans* conformation. One can visualize the *trans*-2-phenylcyclopropylamine molecule as being a partially eclipsed form rather than a representation of a *trans* coformation of a phenethylamine derivative.

Miller *et al.* (262) have also recently shown *trans*-2-phenylcyclopropylamine to be more than 600 times as active as the *cis*-isomer in inhibiting uptake of norepinephrine in the peripheral tissue. When studies were carried out with the optical isomers of *trans*-2-phenylcyclopropylamine, the (−)-form was found to be three times as potent as the (+)-form in inhibiting uptake by hypothalamic synaptosomes and twice as potent in the corpus striatum (366). Riley and Brier (320) have since shown the absolute configuration of (−)-*trans*-2-phenylcyclopropylamine to be 1R, 2S. It has been suggested by Snyder *et al.* (366) that *trans*-2-phenylcyclopropylamine might in part owe its antidepressant clinical effects to inhibition of reuptake of catecholamines released from synaptosomes.

Tuomisto *et al.* (400) have studied the four racemic diastereoisomers of 2-amino-3-(3,4-dihydroxyphenyl)-3-*trans*-decalol (see table 2) in order to gain a better understanding of the conformational requirements for the inhibition of norepinephrine and dopamine uptake. These norepinephrine analogs incorporate the functional groups of norepinephrine on the decalin ring systems to give rise to one *anti* and three *gauche* conformations if one considers the relationship between the aromatic



trans-2-phenylcyclopropylamine



cis-2-phenylcyclopropylamine

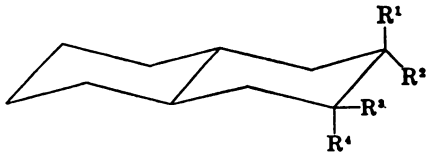
ring and amino functions. All four diastereoisomers had less than ½ the activity of (−)-norepinephrine in the inhibition of norepinephrine uptake in the synaptosomes of a medulla-midbrain homogenate. It was found that 2(a)-amino-3(e)-(3,4-dihydroxyphenyl)-3-*trans*-decalol, a *gauche* form (isomer d in table 2), was the most potent compound in inhibiting norepinephrine uptake in this series of compounds. Differences were small among the isomers and this did not allow the authors to draw any definite conclusions as to the preferred conformation for a substrate or an inhibitor of norepinephrine uptake (also see section XIII). However, Tuomisto *et al.* (400) found the *gauche* form to be the most potent inhibitor of norepinephrine uptake and to be a most potent inhibitor of dopamine uptake in the striatum and hypothalamus. It is interesting to note that 3(e)-amino-*trans*-decalin (a compound lacking a dihydroxyphenyl group) was more potent than the least active norepinephrine analogs indicating that inhibition of dopamine uptake can be achieved with compounds not even closely related to the catecholamines.

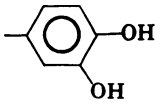
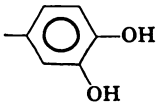
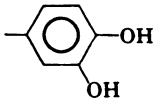
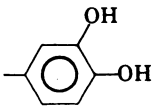
In the periphery it has been shown (262) that *trans*-3-phenyl-2-methylazetidin-3-ol was more than seven times as potent as its geometrical isomer and 3-phenylazetidin-3-ol in inhibiting uptake of norepinephrine. Thus, the stereochemistry of the α-methyl group in this series plays an effective role in the inhibition of norepinephrine uptake in the periphery. The information gained from *trans*-2-phenylcyclopropylamine and the 3-phenyl-2-methylazetidin-3-ol compounds indicates that compounds with an anti-clinal (partially eclipsed) conformation do possess significant ability to block the uptake of catecholamines in the CNS and the periphery.

Tetrahydoisoquinolines have been shown to inhibit the uptake of catecholamines

TABLE 2

Conformationally restricted analogs of norepinephrine [after Tuomisto et al. (400)]



R¹	R²	R³	R⁴	Conformation	
	OH	H	NH_2	anti	(a)
OH		NH_2	H	gauche	(b)
	OH	NH_2	H	gauche	(c)
OH		H	NH_2	gauche	(d)

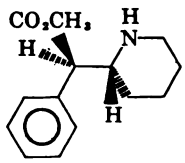
in tissue homogenates (82, 168, 235). Both 6,7-dihydroxy- and 4,6,7-trihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline are taken up by rat brain synaptosomes. Studies carried out with 6,7-dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline also showed these compounds to be capable of inhibiting the uptake of norepinephrine into brain and peripheral nervous tissue. It was shown that uptake of 6,7-dihydroxyl-1-methyl-1,2,3,4-tetrahydroisoquinoline into peripheral sympathetic tissues can be blocked by a) pretreatment with known catecholamine uptake blockers, cocaine, and desemthylimipramine, b) chemical denervation, 6-hydroxydopamine-treatment, and c) surgical denervation (235). If one is to consider the conformational aspects of the phenethylamine portion of the molecule in the tetrahydroisoquinoline, the maximum dihedral angle that could be formed between the amine and aromatic function would be around 60° or a *gauche* conformation. It appears that the tetrahydroisoquinolines with their restricted possible conformations are capable of inhibiting the uptake of norepinephrine and that they can act as substrates for the norepinephrine uptake pump.

It is interesting to note that with the diastereomeric methylphenidates the *threo* isomers are more potent than the *erythro* isomers. The preferred conformation of *threo* methylphenidate would have the phenyl ring and nitrogen atom in a *trans* conformation while the phenyl ring and nitrogen would be *gauche* in the preferred conformation of the *erythro* form of methylphenidate (66, 255).

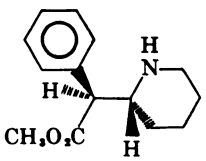
It has been proposed that many drug molecules will engage their receptors, in this instance the "amine pump receptor," in an efficacious manner while in a preferred conformation. Molecular orbital calculations (205) indicate that norephinephrine should exist in a *trans* conformation while similar calculations show dopamine to exist in a *gauche* conformation (207). However, dopamine has been calculated by Bustard and Egan (69) to prefer the *trans* conformation. X-ray crystal analysis of both norepinephrine and dopamine indicate the *trans* conformation is the preferred solid state conformation (46, 47). Studies of solutions of amphetamine as the free base and as the hydrochloride salt have shown that the preferred conformation in both instances was the *trans* conformation (278). Although the preferred conformation may be known in the solid or solution states, the induced-fit theory (148, 213, 214) of drug-receptor interaction could allow for a specific conformation to be the biologically active form of the drug even though at any one period of time this conformation would be found only to a small extent in solution or solid states.

C. Inhibition of Uptake of Catecholamines by Amphetamine Isomers and Proposed Pharmacological Implications

Although the involvement of catecholamines in the unequal central stimulant effects of amphetamine isomers under various experimental conditions has been known for some time (127, 222, 247, 264, 386, 417, 418, 421, 422), it was not until the report of Coyle and Snyder (88) that a



Threo-methylphenidate



Erythro-methylphenidate

dissociation between various central effects of amphetamines was possible. They observed that (+)-amphetamine was 10 times as effective as (−)-amphetamine in blocking the uptake of norepinephrine in the synaptosomes prepared from rat cortex where norepinephrine is the major neurotransmitter. However, when synaptosomes were prepared from the corpus striatum in which dopamine is the major neurotransmitter, there was no difference in blocking of the uptake by the two isomers. Subsequently, under carefully selected dose ranges, (+)-amphetamine was found to be 10 times as effective as the (−)-amphetamine in eliciting locomotor stimulation. For gnawing, the potency ratio was less than two. On the basis of these observations the central stimulation and the stereotype (gnawing) elicited by amphetamine isomers were correlated with noradrenergic and dopaminergic mechanisms, respectively (381). After these studies, various researchers used amphetamines as a tool to study the involvement of norepinephrine and dopamine in various pharmacological effects of centrally acting drugs (12, 13, 17, 18, 80, 83–85, 195, 221, 305, 339, 340, 377, 405, 408, 409). The amphetamine psychosis, after large doses, which produced similar behavioral disturbances at approximately equal doses of amphetamine isomers, suggested a possible involvement of dopaminergic mechanism in the psychosis (12, 13). The assumption was that the animal stereotypy was a valid model for the stimulant psychosis in man. Withdrawal of rats from morphine causes aggression which includes attacks, vocalization, and rearing. The effects were enhanced by amphetamine isomers. Depending upon the type of parameters used, the relative potency of the potent (+)-amphetamine and (−)-amphetamine varied between 2- and 4-fold. A dopaminergic involvement in the aggression was suggested (221). In cats, stereotyped behavior is characterized by continuous head turning, back and forth, in an apparently fearful "paranoid type" behavior and a second stereotype may include standing and sniffing, usually near the cage door. Equal doses of amphetamine isomers were required to produce these effects (279a, 409). Amphetamines exert facilitatory effects on the stimulation elicited by intracranial electrodes either placed in the lateral hypothalamus or substantia nigra. The (+)-isomer was 7 to 10 times more effective than the (−)-isomer, at the hypothalamic placement, whereas both isomers were equipotent for stimulation with the substantia nigra electrodes (305). Rats with unilateral lesion of the nigro-neostriatal pathway show a marked rotation toward the side of the lesion when treated with indirectly acting drugs such as amphetamines. Experimentally, results can be accurately quantified. Both stereoisomers of amphetamine were equipotent in their effects on turning behavior (80). The relative potency for the turning behavior of the selected isomers of ephedrine were (−)-ephedrine > (+)-ephedrine > pseudoephedrine. The relative potency for inhibiting the uptake by synaptosomes prepared from corpus striatum was the same (170). Corson *et al.* (83–85) discovered a breed of hyperkinetic dogs ("mad dogs") which mimic the hyperkinetic child syndrome in many ways, including paradoxical calming with amphetamines. In the control of hyperkinesis and in the production of stereotypy, the potency ratio for the isomers in each case was 3 to 4, the (+)-amphetamine being more active. From the comparative studies in hyperkinetic children and dogs and based on the potency ratio of the isomers, these author concluded that, in the hyperkinetic syndrome, aggression and hostility may be counteracted equally by the two stereoisomers of amphetamine *via* a possible dopaminergic mechanism (17, 18), while anxiety and overactivity may be benefited only by the (+)-isomer *via* a noradrenergic mechanism. The suggestions are interesting, but obviously more data are needed to substantiate the findings. All

these findings support the original postulation regarding noradrenergic and dopaminergic involvement in the central effects of amphetamines.

Other researchers were unable to observe the large, namely 10-fold, differences in potencies of amphetamine isomers for blocking the uptake of norepinephrine by synaptosomes prepared from the brain areas which are rich in noradrenergic neurons (132, 164, 387). In these studies, (+)-amphetamine was reported to be four times as potent as the (−)-isomer in blocking the uptake of dopamine by rat striatal homogenates (132, 164). Efflux of labeled dopamine from cat caudate nucleus was increased by amphetamines, in which the (+)-isomer was four times as potent as the (−)-form (408). All these observations differ to some extent from that of the report of Coyle and Snyder (88). Although the investigators used similar preparations, the experimental protocol in each case was somewhat different. Hence, a strict comparison of reports is not possible. There is one more important aspect of the central effects of amphetamines which has received some attention (382) and it deserves some comment. It is well known that the enzyme dopamine β-hydroxylase is present in noradrenergic and not in dopaminergic neurons. Coupled with this is the fact that only (+)-amphetamine will form an amine metabolite, (−)-*p*-hydroxy norephedrine (see section VIII). In other words, in noradrenergic neurons (+)-amphetamine will be rapidly converted to the amine metabolite which is also known to exert central stimulant effects comparable to those of the parent amine (382), whereas (−)-amphetamine may not form similar amine metabolites. Thus, after the injection of isomers, the comparison of the observed effects could be between the combined catecholamine releasing effects of (+)-amphetamine and *p*-hydroxyamphetamine and those of (−)-amphetamine. The comparisons of the true potency of the isomers could be valid only in dopaminergic areas, as no biotransformation of the amphetamines is possible in those areas. With the aid of the optical isomers of anticholinergic and antihistaminic drugs, the cholinergic and/or adrenergic mechanism in parkinsonism should be explored. If (−)- and (+)-isomers of atropine and related drugs have equal inhibitory effects on dopamine uptake and, indeed, if this is the therapeutic rationale for the use of these drugs in parkinsonian patients, the isomer which is most potent as an anticholinergic in the peripheral nervous system should be avoided to minimize expected side effects. Alternatively, if anticholinergic agents are used to block central cholinergic mechanisms, then only the isomer which is a potent muscarinic blocker should be therapeutically effective. Resolution and pharmacological experiments with the isomers of anticholinergic and antihistaminic drugs were previously carried out (25, 371).

In the isolated rat vas deferens the indirect pharmacological effects of (+)-amphetamine were slightly more pronounced than those of the (−)-form (290, 292). When catecholamines were depleted, the potentiation of the effects of exogenous norepinephrine by 10^{-7} to 10^{-5}M concentrations of amphetamine isomers was identical (378). In the presence of amphetamine, responses to the effects of endogenous norepinephrine released by the nerve stimulation can be potentiated. Both isomers produce identical potentiation (100). Thus, even in noradrenergic neurons smaller differences in the potency of amphetamine isomers can be observed.

X. Effects of Isomers at Postjunctional Sites

A. Easson-Stedman Hypothesis

The most important contribution to an explanation of the behavior of optical isomers toward the specific pharmacological receptors came from Easson and Stedman (119). The theory proposed was that in an

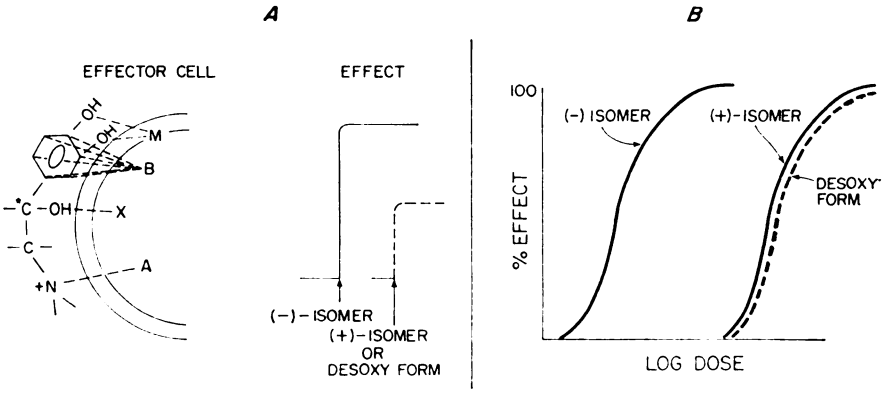


FIG. 10. A, Illustrated are the interactions of various functional groups of (−)-norepinephrine with the adrenergic receptors. As proposed originally by Easson and Stedman (119) the (−)-isomer will interact with at least three sites. In (+)-form, since OH-group will be oriented away from the site X, hence only two point interaction is expected. Thus, the correct orientation of β-hydroxyl of (−)-isomer results in higher activity while lack of interaction of β-OH group in (+)-form with site X, or the absence of the group in desoxy derivative results in lesser but equal effect. Belleau (36) postulated that the anionic site may be $-CO_2$ or $-O-PO_3$ group and hydroxyl groups may chelate with a metal (M). B, Illustrated are the log dose-response curves for (−)-isomer, (+)-isomer, and the desoxy derivative of catecholamines.

asymmetric molecule such as (−)-epinephrine, three of the four groups linked to the asymmetric carbon are concerned in the attachment to the receptor. These groups are a) the basic nitrogen, b) the aromatic group (with *m*- and *p*-hydroxyl groups which determine the high intensity of attachment), c) the alcoholic hydroxyl group. In the (+)-isomer, since the alcoholic hydroxyl group is oriented in the wrong position, only a two-point interaction is expected. This view was strengthened by the fact that desoxyepinephrine (epinine), which lacks the alcoholic hydroxyl group, is equiactive with (+)-epinephrine. The above theory was further elaborated by Blaschko (52), and Beckett (28). Subsequently, Belleau (36, 37) developed a concept of ion-pair formation and chelation as prerequisites for the initial interactions of catecholamines with the receptor. Thus, a somewhat modified version of the Easson-Stedman hypothesis can be illustrated as shown in figure 10. Since stereoselectivity is observed for both receptors, the simple illustration provides a good working model for interactions of stereoisomers and desoxy derivatives with both α- and β-adrenergic receptors. The theory has an additional attraction: it can be experimentally tested. Many tissues have been examined either for α- or β-adrenoreceptor activity; under optimal experimental conditions, the relative potency of the (−)-isomers of the catecholamine was greater than the (+)-isomer which, in turn, was equipotent with the desoxyderivative. The theory holds true for either isomers of epinephrine, norepinephrine, or isoproterenol and the corresponding desoxy derivatives, epinine, dopamine, and N-isopropyl dopamine, respectively (14, 56, 225, 287, 290, 348, 352). The question emerges, when there are two asymmetric carbons in a given amine, should the theory hold true? The stereoisomers of α-methylnorepinephrine and its desoxy analogs provide a good tool to test the case. There are four stereoisomers of α-methylnorepinephrine; however, the two *erythro* forms [(−) and (+)] are resolved. The desoxy (+) and (−) corresponding isomers are also resolved. However, only (+)-α-methyldopamine, whose configura-

tion at the α-carbon is identical with that of (−)-α-methylnorepinephrine, could be utilized for the study. The data are summarized in table 3.

For either α- or β-adrenoreceptor activation, the desoxy derivative appears to be more active than the (+)-*erythro*-α-methylnorepinephrine. The low pharmacological activity of (+)-*erythro*-α-methylnorepinephrine, coupled with the possibility that there may be some contamination of the active isomer, suggests that the (+)-isomer may not interact at all with the receptor. The conformation of the β-OH and α-methyl group may be such that it completely prevents the effective interaction of the isomer with the receptor. Thus, the validity of the Easson-Stedman theory may not necessarily extend to molecules with two asymmetric centers. In β-phenethylamines, in the absence of the β-OH group, the α-methyl substitution creates asymmetry. However, differences between the receptor activating properties of (+)- and (−)-isomers of α-methyldopamine, *p*-OH-amphetamine, amphetamine, and methamphetamine are small or absent (78, 225, 289, 292). Perhaps there is only a two-point interaction between the drug and the receptor; in such a case either the R or S configuration at the α-carbon of the methyl group should have little bearing on the pharmacological activity.

B. Criteria for Investigating Dopamine Receptors

There has been considerable interest in finding a new, potent and specific dopamine-receptor activator or blocker for elucidating the details of central dopaminergic mechanisms. A search was made for these specific receptors in peripheral tissues. The criterion utilized was as follows: If the configuration of dopamine receptors is different from that of α- or β-adrenergic receptors, there should be marked differences in activity between (+)-norepinephrine and its desoxy derivative, dopamine. If the configuration of the dopamine-receptors is the same as that of α- or β-adrenoreceptors, then, according to the Easson-Stedman hypothesis, the activity of (+)-norepinephrine and dopamine should be the same. The data are summarized in table 4. According to this criterion, the presence of the dopamine receptor could not be detected in 10 different tissues, but while studying the renal blood flow in dogs, Goldberg *et al.* (150) were able to dissociate the renal vasodilator activity of (+)-norepinephrine and dopamine. The effect of the former but not the latter drug was blocked by propranolol. Dopamine is very effective in inhibiting the spontaneous activity of garden snail neurons. The relative activities for this effect of dopamine, (+)-norepine-

TABLE 3

The relative pharmacological activities of erythro *isomers of α-methyl norepinephrine and the desoxy derivative (+)-α-methyl dopamine presented as negative log molar ED50 values*

Test Tissue[a]	Receptor Type	(−)-*Erythro*-α-methyl-norepinephrine	(+)-*Erythro*-α-methyl-norepinephrine	(+)-α-Methyl-dopamine
Rat vas def (R)	α	4.8	Indirectly acting	Indirectly acting
Rabbit aorta (C)	α	7.1	≤3.5	5.0
Guinea pig trachea (R)	β	5.52	0	4.5
Fat cell homogenate	β	6.5	<3	4.3

Data collected from Patil *et al.* (290); Chai and Patil (77, 78); and Lee *et al.* (225).

[a] The abbreviations used are: R, Reserpine pretreated; C cocaine pretreated.

TABLE 4

Summarized are the negative log ED50 values for (+)-norepinephrine and dopamine (desoxynorepinephrine) obtained from various isolated tissue

Tissue [a]	Receptor Type Examined	Negative log Molar ED50 with 95% C.I.	
		(+)-Norepinephrine [b]	Dopamine [b]
Rat aorta (R) [c]	α	6.70 (6.34–7.06) n = 9	6.58 (6.39–6.77) n = 8
Rat vas def. (R)	α	4.49 (4.29–4.69) n = 8	4.31 (4.11–4.51) n = 8
Rat adipose tissue (lypolysis)	β	3.8 [d] (3.6–4.0) n = 4	3.5 [d] (3.1–3.9) n = 4
Rabbit aorta	α	5.71 (5.45–5.97) n = 8	5.58 (5.41–5.75) n = 8
Rabbit ileum	α	3.68 (3.42–3.94) n = 10	4.08 (3.60–4.40) n = 7
Rabbit spleen (R)	α	4.16 (3.82–4.50) n = 8	3.94 (3.84–4.04) n = 12
Rabbit vena cava	α	3.96 (3.67–4.25) n = 6	4.31 (3.92–4.70) n = 5
Cat aorta	α	5.42 (5.23–5.61) n = 8	5.44 (5.18–5.70) n = 11
Guinea pig aorta	α	3.94 (3.69–4.19) n = 7	4.20 (4.01–4.39) n = 8
Guinea pig atria (rate or force)	β	4.9	4.9

Data condensed from Blinks (56), Patil *et al.* (288–294) and Shonk *et al.* (352).

[a] All tissues except rat adipose tissue and guinea pig atria were treated with cocaine (10^{-5} M), tropolone (3×10^{-5}) and sotalol (10^{-5}–10^{-4} M).

[b] Except for lipolysis, maximal effects of both drugs were the same.

[c] The abbreviations used are: R, Reserpine pretreated (16 to 24 hours); n, number of observations.

[d] Maximum response to dopamine was approximately ½ that obtained with (+) norepinephrine.

phrine, and (−)-norepinephrine are 1, $\frac{1}{30}$, and $\frac{1}{60}$, respectively. According to the above criterion, it is very likely that the central neurons or renal blood vessels contain dopamine receptors which are different from α- or β-adrenoreceptors (374a, 419). After α-adrenoreceptor blockade, the tone induced by potassium chloride in canine mesenteric arteries can be reduced by dopamine. Since β-receptor antagonists do not block the relaxation, the presence of specific dopamine receptors in the tissue is suggested (151). Rat caudate nucleus is known to contain dopamine-sensitive adenylate cyclase (200). In this tissue it should be interesting to test (+)-norepinephrine and see whether a dissociation between the activities of dopamine and (+)-norepinephrine is possible. Other substances such as (−)-apomorphine, epinine, and (+)-bulbocapnine which resemble dopamine are considered to be drugs interacting with

specific dopamine receptors. It was observed that (−)-apomorphine, like dopamine although less potent, elicited sympathomimetic effects on rabbit aorta and rat vas deferens. In both tissues bulbocapnine, or a low concentration of (−)-apomorphine, equally antagonized the effects of phenylephrine or dopamine (286). It is possible that these specific dopaminergic drugs also interact with the α-adrenoreceptor and since norepinephrine is the major neurotransmitter in these tissues, the effector organ may only contain α-adrenoreceptors. Recently, optical isomers of apomorphine have been resolved and tested biologically. It appears that (+)-apormorphine is totally inactive in its interaction with the dopamine receptors (328). So far, very little effort has been directed toward dissociating the emetic and stereotypical effects of apomorphine. From the studies conducted by Burkman (68a) with apomorphine analogs, it appears that these two effects could be dissociated.

C. Isomeric Activity Ratios and Pharmacological Receptors

In addition to drug receptors being selectively blocked by specific antagonists, stereoselectivity appears to be the only characteristic of many pharmacological receptors. Pfeiffer (304) pointed out that high isomeric activity ratios are found for highly active drugs and low ratios for weakly active drugs. The statement can be examined in terms of the pharmacological activity of catecholamine isomers on the α- or β-adrenoreceptors. When tested on β-adrenoreceptors of guinea pig atria the relative negative log molar ED50 of the catecholamines (−)-isoproterenol (8.6) > (−)-norepinephrine (7.5) > (−)-epinephrine (7.1). The isomeric ratios follow the same pattern (140). However, if asymmetry in the molecule is located at the nonessential part, the Pfeiffer rule may not apply.

In order to investigate various types of adrenoreceptors, it was postulated that from one tissue to another similar types of receptors should generate similar isomeric-activity ratios, and the converse was expected if the receptors were different (285, 294). In normal tissues the activity difference between the isomers is obscured by several factors such as stereoselective uptake and/or degradation by the enzymes. Hence, it is necessary that various routes of drug disposition are reduced to a minimum. Optical isomers of norepinephrine were tested in various tissues containing α- or β-adrenoreceptors. In normal tissues the isomeric activity ratios vary greatly; however, in the presence of cocaine, tropolone, and sotalol which reduce the drug disposition in many tissues containing α-adrenoreceptors, equal isomeric activity ratios were obtained. The results indicate that α-adrenoreceptors of rabbit aorta, vena cava, ileum, and spleen, rat vas deferens and seminal vesicle are of a single type (fig. 11). This approach was extended to investigate the nature of α-adrenoreceptors of frog aorta, rat aorta, guinea pig aorta, cat aorta, and cat spleen. In all these tissues, under proper experimental conditions, the isomeric activity difference between (−)- and (+)-norepinephrine was the same, 2.2 to 2.4 log units (287, 288). Recently, Hattan and Wolf (167) investigated thermoregulatory receptors in the preoptic anterior hypothalamic region of the cat. After chemical sympathectomy the isomeric ratio for hypothermia is approximately 10. This small ratio suggests this α-adrenoreceptor differs from those described above. On the basis of the relative potency of agonists, others have subclassified the β-adrenoreceptors. As expected, the isomeric activity ratios for tissues containing β-adrenoreceptors were different (fig. 12). However, heart and trachea β-receptors previously classified as β_1 and β_2, respectively, produced statistically similar isomeric ratios for isomers of various agonists and antagonists (64, 65). A remote possibility exists that the β-adrenoreceptor macromolecules may

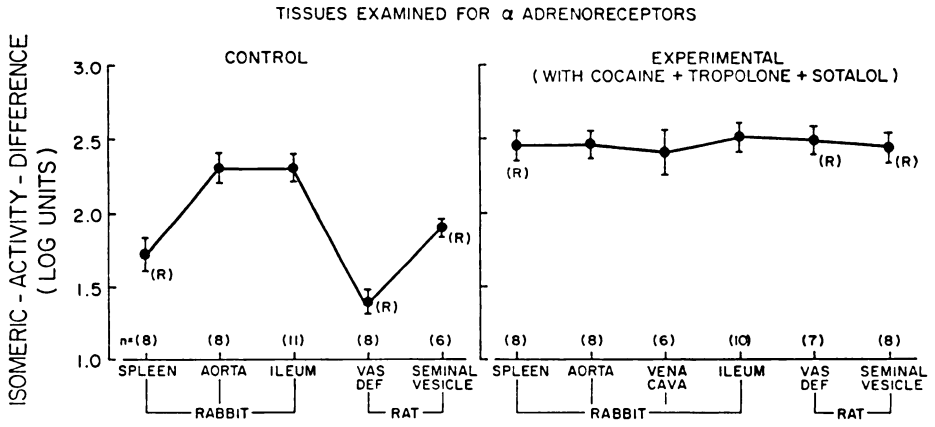


Fig. 11. Isomeric activity differences for (−)- and (+)-norepinephrine from normal or reserpine-pretreated (R) tissues, referred to as untreated preparations, and those after various routes of drug disposition are blocked, referred to as treated preparations. Note that in treated preparations, the activity difference between optical isomers is the same (2.5 log units) in all six tissues examined for α-adrenoreceptor activity. [After Patil *et al.* (294). Reproduced with permission from Williams & Wilkins, Baltimore.]

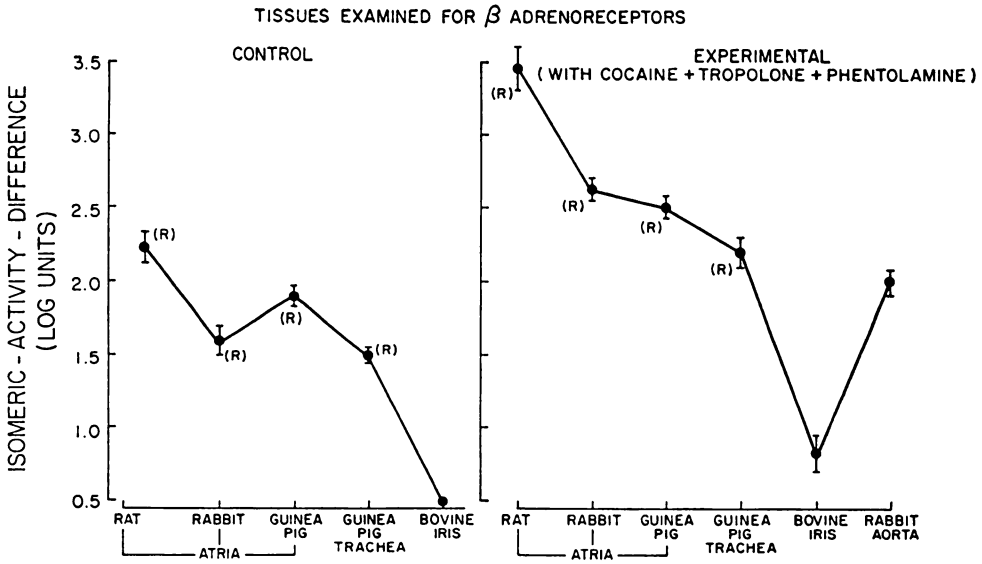


Fig. 12. Isomeric activity differences for (−)- and (+)-norepinephrine in untreated and treated preparations (*i.e.*, after various routes of drug disposition were blocked). From both treated and untreated preparations, the isomeric activity differences were markedly different. It may be that β-adrenoreceptors in various tissues are not a single type. [After Patil *et al.* (294). Reproduced with permission from Williams & Wilkins, Baltimore.]

be slightly different from one tissue to another but still maintain similar reactivity of the active site toward the optical isomers. Then some other additional criteria will be needed to subclassify the receptor.

The isomeric activity ratio of the β-receptor antagonist n-isopropyl-*p*-nitrophenylethylamine (INPEA) against norepinephrine-induced lipolysis was 100, while the ratio against adrenocorticotropic hor-

mone (ACTH)-induced lipolysis was only 1 (374). The isomers of β-receptor antagonists are expected to exhibit a high degree of stereoselectivity against the corresponding agonist, but little or no stereoselectivity against nonadrenergic agonists. Conversely, the isomers of nonadrenoreceptor antagonists are expected to exhibit a high degree of steroselectivity against the corresponding agonist, but little or no stereoselectivity against the adrenoreceptor agonist. In the rabbit aorta against histamine, the antihistamine (+)-chlorpheniramine is more potent than the (−) form by a factor of 100; however, against phenylephrine both isomers are weak but equieffective antagonists (282). When specific drug receptors are involved, such results are expected. An interesting analogy exists between the active sites of enzymes and pharmacological receptors. Boter and Van Dijk (62) carried out the inhibition of two types of cholinesterases by optical isomers of sarin. (−)-Sarin reacts at least 4200 times faster than (+)-sarin with acetylcholinesterase from bovine erythrocytes, whereas both isomers inhibit butyrylcholinesterase at virtually equal rates. Thus, the use of optical isomers enables us to distinguish between subtypes of receptor sites or active sites of the enzymes.

The question of optical purity of isomers and the interaction of the so-called lesser active isomer with the receptor is of crucial importance in selecting the isomers to be used in a study of receptors. If, in a given pair of optical isomers, the observed effects of the inert isomer are due *solely* to contamination of the active form, constant ratios will be obtained for different tissues. Although the amount of the small contamination of (−)-norepinephrine in the (+)-form has not been accurately determined, two lines of reasoning indicate that the (+)-form must also interact with the receptor. a) The pharmacological activity of dopamine and (+)-norepinephrine is identical, hence these drugs may, according to the Easson-Stedman theory, interact with the adrenergic receptor. b) If the pharmacological activity of (+)-norepinephrine was solely due to the (−)-form, and if these isomers are mixed in various known proportions, an activity equal to the known proportion of the (−)-norepinephrine should be obtained. For example, a mixture of 1:1000, (−):(+) norepinephrine should have an ED50 value 3 log units away from the ED50 of the (−)-norepinephrine. However, the dose-response curve of such a mixture is only 2·0 log units away from that of the (−)-isomer. The dose response curve of the (+)-isomer alone is 2·2 log units away from the (−)-form. Results indicate that irrespective of proportions of the more active (−)- in the (+)-form, it is impossible to shift the dose response curve of a given mixture of isomers beyond 2·2 log units from that of the more active form. It appears that a high proportion of the (+)-isomers can effectively compete with the insignificantly small added proportions of the active (−)-isomer; presumably both isomers interact at the same site. Moreover, (+)-norepinephrine which was obtained from three different batches and was less likely to contain identical impurities of the (−)-isomer left during resolution, produced identical pharmacological activity on aortic smooth muscle. When a similar study was conducted with (−) and (+) *erythro*-α-methylnorepinephrine, it appeared that the (+) form did not interact at all with the adrenoreceptors (78).

Various agents and procedures were used to modify the isomeric activity ratios for norepinephrine isomers. The logic behind the approach was that, if there is a qualitative change of the receptor, *i.e.*, a steric alteration of the receptor site by a given drug, then this change might be reflected in the isomeric activity ratios. Postganglionic sympathetic denervation of the rat vas deferens or α-chymotrypsin treatment or cold storage of tissue at 5°C

for seven days did not alter the isomeric activity ratios for norepinephrine. Some of the procedures affected the maximum response but the change was equal for both isomers, which indicates that some contraction-coupling process may be altered and not the receptor (217). Selective reagents for sulfhydryl groups such as N-ethylmaleimide and dithioerythritol, 3×10^{-4} M, administered for 20 minutes to isolated rabbit aorta, shifted the dose-response curve of both isomers to the right. The former depressed the maximum while the latter did not. Again, the isomeric activity ratio for norepinephrine was unaltered; perhaps the SH groups of the active site are unaltered or such an alteration affects both isomers similarly (76).

D. Use of Stereoisomers for the Characterization of Isolated Pharmacological Receptors

There are several criteria to be fulfilled before a given isolated macromolecule can be labeled as a pharmacological receptor. One such criterion deals with the comparison of the drug affinities on the isolated tissue and the isolated macromolecule from the same tissue. If the affinities in two situations are identical there will be little argument against the acceptance of a given macromolecule as the pharmacological receptor. However, the chances for obtaining identical affinities in the two situations are small. The affinity of the drug in the isolated tissues is very much dependent on the diffusion coefficient, hence the reported affinities should be considered as *apparent* affinities as suggested by Furchgott (138). When the drug affinity is tested in terms of the binding constant on the isolated material there will be a minimum of diffusion to influence the affinity. The affinity value obtained for the isolated tissue may be lower than the one obtained on the isolated receptor molecule. Hence, the stereoisomers of drugs provide good tools to characterize the isolated material as a pharmacological receptor. Physical chemical properties of the optical isomers are the same, so the interacting molecular species of the drug will be the same. In other words, the lesser active isomer should provide a good internal control for studying the receptors in tissues and in the isolated macromolecule. Ideally, if one can obtain the same values for the isomeric activity ratios in tissues and ratios for the isomeric affinity constants in the isolated macromolecule, the proof that isolated material represents a receptor molecule will be accepted more readily. However, if the conformation of the active site changes during drastic isolation procedures, then the ratios in the two situations will not be the same.

Since the pharmacological effects of adrenergic drugs are stereoselective, binding to the receptor molecule is expected to exhibit the *same degree* of stereoselectivity (294). Several investigators studied the binding of (−)- and (+)-isomers of catecholamines or propranolol to fat, liver, myocardium, aortic cell membranes and turkey erythrocytes but failed to obtain a stereoselective binding (51, 91, 102, 117, 219a, 246a, 402a, 418a). Although several methodological problems are obvious, it may be that these fractions do not contain the adrenoreceptor. The macromolecule which strongly binds the drug may not necessarily be the receptor, but the one which binds it stereoselectively might be the one. The pigment cell constituent macromolecule, melanin, has a high capacity and affinity for binding many drugs but like the binding to serum albumin it lacks stereoselectivity (98, 349). Obviously, it will not be a candidate for the pharmacological receptor. Recently with the aid of radiolabeled β-adrenoreceptor antagonists of high specific activity, it was possible to show stereoselectivity in binding of the isomers of antagonists to the erythrocyte membrane (18a, 226a). The macromolecule isolated from bovine spleen, when incorporated into an artificial membrane, showed conductance changes in response to

(−)-norepinephrine but not to (+)-norepinephrine. Although the concentrations of the isomers used were very high, the proposal that the macromolecule represents an α-adrenoreceptor is an attractive one (280). Pert and Snyder (302) isolated a fraction from rat brain and ileum and this material shows a high degree of stereoselectivity in binding of narcotic drugs. The chances are good that the fraction contains or represents the opiate receptor (151a, 384a). Similarly, labeled optical isomers of the anticholinergic drug dexetimide were used to characterize the muscarinic receptor material from the bovine brain (369). Stereoselective binding was evident.

Some site on the macromolecule which is coupled with the enzyme adenylate cyclase, is considered to be identical with the β-adrenoreceptor. Recently, Birnbaum *et al.* (50) correlated the biochemical and pharmacological effects of (−)- and (+)-isoproterenol on rat atria. Under proper experimental conditions, the potency differences between isomers for cAMP formation was 3.50 log units. The potency difference for the chronotropic effect was the same. A similar correlation of norepinephrine isomers was examined in rat epididymal fat cells (101). Both isomers caused identical maximum responses. In the presence of theophylline, the ED50 for lipolysis by the (−)-isomer was 2.5×10^{-8}M and for the (+)-isomer it was 8.5×10^{-6}M. An isomeric activity difference of 2.54 log units (95% C.I.:2.43 to 2.83) for lipolysis during a 30-minute period in the presence of theophylline was obtained. A similar value of 2.52 log units (2.14 and 2.89) was found for the isomer-induced rise in cAMP during 10 minutes incubation under the same conditions. Thus, equality of values represents clear-cut evidence for a stereoselective receptor-mediated physiological effect. If these events are correlated with the binding constants of the isomers, our understanding of the drug receptors and receptor-mediated physiological processes may be much simplified.

XI. Isomers and α-Adrenoreceptor Blockade

Very little work has been reported on the stereoisomers of reversible α-adrenoreceptor antagonists. The imidazoline derivatives appear to be a series of compounds in which no optically active compounds have been studied pharmacologically. It would appear that one could prepare derivatives of tolazoline possessing an asymmetric center and study the relative blocking activity of these optical isomers. Possibly even more interesting might be a study of optically active forms of imidazolines having agonist activity. These studies could add to our knowledge of the mode of action of imidazolines and also to our understanding of the prerequisites for agonist or antagonist activity in this series of compounds. Only a few benzodioxan series of substances have been resolved and tested for pharmacological activity. Stenlake *et al.* (372) compared the α-adrenoreceptor blocking effects of (+)- and (−)-guanoxan. Although their adrenergic neuron blocking effects were the same, only the (+)-isomer possessed a weak α-adrenoreceptor blocking effect in cats. Stereoisomers of tetrahydroisoquinolines were tested for their α- and β-receptor activity. In contrast to the marked stereoselective agonist activity in a tissue containing the β-adrenoreceptor, no significant differences in blockade of norepinephrine-induced contractions were observed in a tissue containing α-adrenoreceptor. In guinea pig aorta, the pA_2 values for (+)- and (−)-trimetoquinol were identical, 4.7 (129). Hence asymmetry at the 1 position of tetrahydroisoquinoline is of little importance in blocking the α-adrenoreceptor.

When compared as stimulants in rabbit aortae, epinephrine isomers show a difference of 1.7 log units, the (−)-isomer being more active. Experiments were designed to

test the ability of these isomers to protect the same α-adrenoreceptor against the block by the irreversible α-adrenoreceptor antagonist, N-ethoxycarbonyl-2-ethoxy-1,2 dihydroquinoline (EEDQ). At equal concentrations of isomers, (−)-epinephrine gave significantly more protection than the (+)-form (72). Thus, as an α-adrenoreceptor stimulant or protector, the (−)-isomer is more potent than the (+)-form.

The optical isomers of the irreversible α-adrenoreceptor antagonists, N,N-dimethyl-β-chlorophenethylamine and *cis* and *trans* isomers of N-methyl-N-(2′-phenoxycyclopentyl)-2-chlorethylamines, were tested on cat blood pressure. Although these drugs are potent antagonists, any differences between the stereoisomers or geometric isomers could not be detected. A possibility that both isomeric forms generate a common reactive symmetric molecular species at the α-adrenoreceptor was suggested (40, 42). The optical isomers of the other well known irreversible α-adrenergic blocker, phenoxybenzamine, were resolved and carefully tested. In the isolated rat vas deferens the rate of development of α-adrenoreceptor blockade by (+)-phenoxybenzamine was 15 times that of the (−)-form. Since the intrinsic alkylating ability of the isomers is the same, Portoghese *et al.* (313) implied that the potency differences between isomers was caused by an affinity difference at the receptor. At present, (±)-phenoxybenzamine is used extensively for investigating a) presynaptic receptors, b) postsynaptic α-adrenoreceptors, and c) neuronal and extraneuronal uptake. The optical isomers of the drug should be a useful tool in dissociating or accurately quantifying the above mechanisms. McLean *et al.* (258) investigated the (+)- and (−)-isomers of N- 2-chloroethyl)-N-methly-2-hydroxy-2-phenylethylamine. In the rat vas deferens both isomers were significantly less effective as α-adrenoreceptor antagonist than (±)-phenoxybenzamine. The S(−)-isomer was more effective than the R(+)-isomer by a factor of 6. This apparent stereoselectivity is opposite to that of the well known directly acting α-adrenergic stimulants where R(−)-isomers are more active. However, the dose-response curves of two isomers for inhibitory effects were not parallel. It is possible that the lesser active isomer might be acting at some additional site. At the α-adrenoreceptor, irreversible blockers are postulated to act at the norepinephrine recognition and Ca^{++} mobilizing site (192).

XII. β-Adrenoreceptor Antagonists

A. Steric Structure Activity for the β-Adrenoreceptor Antagonism

It is well recognized that chemically the β-adrenoreceptor antagonists studied so far markedly resemble the agonist (−)-isoproterenol. Hence, like the optical isomers of the agonist, the isomers of the antagonist are expected to exhibit stereoselectivity in blockade. These antagonists possess at least one center of asymmetry at the β-carbon and occasionally one more at the α-carbon. The optical isomers of dichloroisoproterenol, pronethalol (182), propranolol (120, 183, 390), methoxamine and its derivatives (284, 333), INPEA (10), α-methyl-INPEA (367), sotalol (234), butedrine (131), alprenolol (4, 115), bunolol (196, 197, 341), and practolol (96, 202, 203) have been synthesized, resolved, and tested for pharmacological activity. The chemical structure and β-adrenorecptor blocking properties are summarized in table 5. Examination of the chemical structure reveals that all potent antagonists markedly resemble the agonist (−)-isoproterenol. The points of similarities are: a) substitution on the phenyl ring; b) alkyl substitution on the nitrogen; and c) alcoholic group in correct stereochemistry with the receptor (1R or D-configuration). The stereochemistry of the alcoholic hydroxyl is the same as that in (−)-isoproterenol. This structural requirement implies that these antagonists may be

TABLE 5

pA_2 values for the optical isomers of various reversible β-adrenoreceptor antagonists obtained from guinea pig trachea and atria

Isomer	pA_2 (Slope)	
	Trachea	Atria (rate)
(−)-Methoxamine [a]	6.3 (0.5)	
(+)-Methoxamine	4.3 (0.5)	
(−)-Isopropylmethoxamine [a]	6.5 (0.4)	
(+)-Isopropylmethoxamine	<3.5	
(−)-Butoxamine [a]	7.2 (0.5)	
(+)-Butoxamine	<4.0	
(±)-*Threo* butoxamine	<4.0	
(−)-INPEA [b*]	7.1 (0.6)	6.8 (1.0)
(+)-INPEA	4.7 (0.8)	5.4 (0.6)
(−)-Sotalol [b]	7.7 (0.6)	6.8 (1.0)
(+)-Sotalol	6.1 (0.6)	5.1 (1.0)
(±)-Pronethalol [a]	7.3 (0.6)	
(+)-Pronethalol	5.2 (0.7)	
(±)-Propranolol [c]		8.5 (?)
(+)-Propranolol		6.5 (?)
(−)-Alprenolol [b]	9.6 (0.6)	9.4 (0.9)
(+)-Alprenolol	7.8 (0.7)	7.1 (0.9)
Butedrine [d], 1R, 3R		7.8 (0.74)
Butedrine 1S, 3R		<6.0
Butedrine 1R, 3S		6.7 (1.1)
Butedrine 1S, 3S		<5.5

Data condensed from: [a] Patil (284); [b] Buckner and Patil (65); [c] Kaumann and Blinks (199); and [d] Krell *et al.* (216).

* INPEA, n-isopropyl-*p*-nitrophenylethylamine.

relatively more specific in their attachment to the β-adrenoreceptor than α-adrenoreceptor antagonists to the α-receptors. Substitution of the methyl group adjacent to the carbon-carrying alcoholic hydroxyl group can hinder the effective interaction of agonists or antagonists with the receptor. For example, (−)-*pseudo*butoxamine fulfils all the structural requirements for a β-adrenoreceptor blockade, but it does not appear to block receptors. An interesting study on the conformational aspects of the ephedrine isomers has been carried out by Portoghese (311). He states that: "It appears significant that (−)-ephedrine is the only isomer which possesses both 1R configuration and the C-methyl group which projects above the plane of phenethylamine moiety. The (−)-*pseudo*ephedrine also possesses the 1R stereochemistry necessary for direct action, but the C-methyl group is oriented below the plane. It is conceivable that the methyl group in the latter isomer hinders effective interaction with the receptor." In terms of this conformational analysis of ephedrine isomers, it becomes apparent why (−)-ephedrine blocks the β-adrenoreceptor and the (−)-*pseudo*isomer does not. Similar explanations may hold true for the isomers of methoxamine, isopropylmethoxamine, butoxamine, and α-methyl-INPEA in which there are two asymmetric centers like those in the ephedrine molecule. The actual conformations of these molecules at the receptors are not yet known. Butedrine also has two asymmetric centers, but the methyl substitution is very remote from that of the important

functional alcoholic hydroxyl group. As a consequence, it does not appear to influence the interaction of the alcoholic hydroxyl group with the β-adrenergic receptor. Butedrine 1R, 3R and 1R, 3S are almost equipotent (216).

(−)-Propranolol is more potent in antagonizing isoproterenol-induced tachycardia than the (+)-isomer. The desoxy analog of propranolol is approximately as active as (+)-propranolol (183). Thus, results are in line with suggestions by Easson and Stedman regarding interactions of asymmetric molecules with the receptors. However, many exceptions appear to have emerged, particularly when a given molecule has more than one asymmetric center in which the nonfunctional group may hinder the attachment of functional groups to receptors. The anatomical location of adrenoreceptors in the tissue is not known. If β-adrenoreceptors are inside the cell and if there is another transport system at the sites of direct action, then it becomes of primary importance to establish a structural requirement for such a system before the Easson-Stedman theory can be rejected. In the light of this suggestion the work of Reuter and Wollert (319) is particularly thought-provoking. They studied the effects of the isomers of some sympathomimetic amines on contractility and ^{45}Ca-uptake in isolated guinea pig atria. As compared to (+)-isomers, (−)-isomers of epinephrine and synephrine were more potent in producing an increase in force of contraction and influx of ^{45}Ca. It was concluded that an increase of Ca-influx during excitation is responsible for the positive inotropic effects of these agents. Since (−)-isomers are more effective than (+)-isomers, it seems possible that calcium and a correctly oriented alcoholic hydroxyl group together with basic nitrogen can form a chelate that is to be transported more effectively. The β-adrenoreceptor antagonist may simply block the transport system. The influence of β-adrenergic blockers and their isomers on the influx of ^{45}Ca needs to be investigated carefully.

Based on chemical grounds only, without solid experimental support, the notion that β-adrenoreceptor agonists and antagonists interact at the same points on the receptor may be misleading. Serrano and Hardman (345) suggested that a nonionized form of the drug might be essential for the production of β-adrenoreceptor blockade. These investigators have postulated that the agonist and the antagonist may not act at the same site. Antagonists perhaps act at a site more distal to the receptor. Although the hypothesis will need more experimental evidence, an interesting series of experiments can be made. The pK_a values of (−)- and (+)-isomers are identical, and thus, regardless of change in pH the relative number of nonionized forms of both isomers should be the same. If the postulate is correct the difference in pharmacological activity between (−)- and (+)-isomers of β-adrenergic blockers should remain the same at different pH values.

B. Use of Stereoisomers of β-Adrenoreceptor Antagonists in the Study of Cardiac Arrhythmia

Due to the potential clinical usefulness of these antagonists there has been considerable interest in studying cardiac actions of the drugs. The fact that one isomer is a more potent antagonist than the other form provided a good tool to dissociate the mechanism of cardiac arrhythmias induced by various procedures. The data are summarized in table 6. It can be seen that adrenergically-induced arrhythmias can be promptly terminated by a small dose of the (−)-isomer, whereas much larger and approximately equivalent doses of the stereoisomers are required to suppress the arrhythmias which result from ouabain or the ligation of coronary arteries (26, 114, 115, 131, 197, 198, 236, 238, 245, 337, 368). Although the relevance of β-adrenoreceptor blockade by (−)-isomers in the termi-

TABLE 6

Effects of stereoisomers of β-adrenoreceptor antagonists against the cardiac arrhythmias induced by various procedures

Drug	Isomer	Procedure for Induction of Arrhythmia	Criteria	Dose Required	Reference
				mg/kg	
Alprenolol	(−)-	Halothane + dopamine	Prevention of arrhythmia in cats	0.5	Katz *et al.* (198)
	(+)-	Halothane + dopamine		>7.5	
	(−)-	Ligation of coronary artery	Prevention of ventricular arrhythmia	7.5	Duce *et al.* (115)
	(+)-	Ligation of coronary artery		15.5	
Butedrine	1R,3S	$CaCl_2$	Prevention of ventricular fibrillation in 50% of rats	10	Ferrini *et al.* (131)
	1S,3S	$CaCl_2$		0.5	
	1R,3R	$CaCl_2$		2.5	
	1S,3R	$CaCl_2$		5.0	
Propranolol	(−)-	Halothane + epinephrine	Prevention of arrhythmia in cats	0.09	Barrett and Cullum (26)
	(+)-	Halothane + epinephrine		4.2	
	(−)-	Oubain	Conversion of ventricular tachycardia in cats	2	Barrett and Cullum (26)
	(+)-	Oubain		6.5	
Pronethalol	(±)-	COMT[a]-inhibitor + isoproterenol	Protection against ventricular fibrillation in dogs	5	Lucchesi (238)
	(+)-	COMT[a]-inhibitor + isoproterenol		>10	
Sotalol	(−)-	Large dose of epinephrine	Reduction of ventricular tachycardia in dogs	1	Somani and Watson (368)
	(+)-	Large dose of epinephrine		20	
	(−)-	Ouabain	To suppress ventricular tachycardia in dogs	35	
	(+)-	Ouabain		≥35	

[a] The abbreviation used is: COMT, catechol-O-methyl transferase.

nation of the adrenergically-induced arrhythmias is quite clear, the mechanism by which nonadrenergically-induced arrhythmias are suppressed by the blockers is unclear. Myocardial depressant effects and local anesthetic effects are generally similar for both isomers (15, 229). In some species the prevention of ouabain-induced arrhythmia by the drugs cannot be entirely attributed to these effects. Recently, Kelliher and Roberts (202) compared the effects of (−)- and (+)-practolol on ouabain-induced arrhythmia. (−)-Practolol but not (+)-practolol increased the dose of ouabain necessary to produce arrhythmias and death. In other experiments (203) investigators found that (−)-practolol suppressed the spontaneous neural activity recorded from postganglionic cardioaccelerator fibers and much higher doses of the (+)-isomer were required for such effects. Thus, protective effects of (−)-practolol against ouabain toxicity were correlated with the suppression of the neuronal activity (203). The studies by Dohadwalla *et al.* (107) indicate that (+)-propranolol, which is slightly more potent as a local anesthetic

than the (±)-compound, is significantly less active than (±)-propranolol in protecting against ouabain-induced arrhythmias. The authors excluded the relevance of a local anesthetic action in the protective effect. The influence of the isomers on the sympathetic nervous activity or β-adrenoreceptor blocking effects may explain the observed selectivity of isomers of β-adrenoreceptor antagonists. In dogs, intraventricular administration of acetylstrophanthidine elicited ventricular arrhythmia. Reserpine pretreatment or intraventricular administration of (±)-propranolol, but not administration of the (+)-form, prevented the arrhythmia. Probably catecholamines are involved in the production of the arrhythmia. Hence, it is not surprising that only a potent antagonist reduced the effect (373). Parmley and Braunwald (283) compared myocardial depressant and antiarrhythmic properties of (±)-propranolol, (+)-propranolol, and quinidine. The study suggests that (+)-propranolol might be a very useful agent in the treatment of certain arrhythmias where β-adrenoreceptor blockade is not desired. Quinidine lowers the blood pressure, while (+)-propranolol is without such a clinically undesired effect. The antiarrhythmic effects of (+)-alprenolol were studied in man. In patients with paroxysmal ventricular and supraventricular tachycardias, the arrhythmic attacks were prevented by this isomer (337).

Biochemical and electrophysiological correlates were studied to explain the cardiac actions of the isomers of these antagonists. β-Adrenoreceptor antagonists inhibit the Ca^{++} uptake by the cardiac sarcoplasma reticulum, a property which correlates reasonably well with the suppression of catecholamine-induced arrhythmias and the direct inotropic effects of the isomers (338). However, when transmembrane calcium exchange was studied in guinea pig atria and in resting left auricles, none of the β-adrenoreceptor antagonists (including the optical isomers of antagonists) influenced the Ca^{++} influx either during excitation or rest. Thus, changes in Ca^{++} permeability of the membrane cannot explain the negative inotropic properties of the β-adrenoreceptor antagonist (357). Injection of norepinephrine into the arterial supply of the isolated A-V node preparation of the dog decreases the A-V conduction time. The β-adrenoreceptor antagonists blocked the positive dromotropic (nerve conductivity) effect of (−)-norepinephrine, and the (+)-isomers of alprenolol and propranolol had 1/100 to 1/300 the potency of the corresponding (−)-isomers. The negative dromotropic effect of the two isomers were the same for both (184).

So far, the optical isomers of β-adrenoreceptor antagonists have been found useful in analyzing the mechanism of various types of arrythmias. This type of approach should be extended with the aid of isomers of local anesthetics (3, 43) and quinidine. Although the stereochemistry of the latter drug is well worked out (243), little has been done with isomers in relation to their pharmacological effects.

C. Vascular Effects of β-Adrenoreceptor Antagonists

With the aid of isomers the mechanism of the peripheral vasodilatory effects of some β-adrenoreceptor antagonists has been clarified. In dogs, femoral blood flow is increased equally by (−)- or (+)-isomers of propranolol. Obviously, β-adrenoreceptor blockade cannot be involved. The effects were attributed to the local anesthetic effects of the drugs (347). The effect on the coronary vasculature of a systemic injection of the drug is complicated by simultaneous effects on contractile force, heart rate, and the blood pressure. Intracoronary injections of (−)-propranolol increase coronary vascular resistance, and (+)-propranolol increases resistance after an initial transitory coronary dilation

(414). Both isomers of propranolol inhibit the response to electrical stimulation of sympathetic nerves to the rabbit ear artery; but again, the effect can be attributed to their local anesthetic effect (27). However, in cats Åblad *et al.* (5) were able to show selective block of the response to sympathetic nerve stimulation by (±)-propranolol. The (+)-isomer did not influence the nerve response. They attributed the blockade to a selective β-adrenoreceptor mechanism. In patients, some β-adrenoreceptor antagonists are known to produce hypotensive effects. Since both (−)- and (+)-INPEA are equally effective in lowering the blood pressure in hypertensive patients, the effects appear to be unrelated to the blockade (147). The intraventricular administration of (±)-propranolol or (+)-propranolol to cats produced a hypotensive response which was not statistically different after each isomer. Reserpine pretreatment abolished the hypotensive effect. Thus, the mechanism is independent of a β-adrenoreceptor mechanism, but appears to be dependent on the central adrenergic neurons (201). A similar study was carried out by Dollery *et al.* (108) in rabbits; intracerebroventricular injection of (±)-propranolol produces a biphasic effect. An initial pressor effect is followed by a prolonged fall. The (+)-isomer produces the pressor effect only. The pressor effects of both (+)-propranolol and procaine are abolished by barbiturate anesthesia. Thus, the initial rise in blood pressure in response to (±)-propranolol and (+)-propranolol may be related to the local anesthetic or membrane stabilizing effects of the isomers. Chemical sympathectomy abolished the long-lasting hypotensive effects of (−)-propranolol. Further studies by Reid *et al.* (317a) indicate the involvement of central adrenergic receptors for the observed hypotensive effect. In DOCA-hypertensive rats, however, propranolol was without this effect but a long-lasting paradoxical increase in blood pressure was observed after the (+)-isomer (242). More work is needed to clarify these discrepancies.

D. Interactions of β-Adrenoreceptor Antagonists with α-Adrenoreceptors

Any adrenergic drug with a basic chemical structure like that of ethylamine exhibits a variety of effects at the adrenergic neuroeffector junction. β-Adrenoreceptor antagonists are basically derivatives of phenethylamines. In higher concentrations many of these drugs produce a competitive reversible blockade of α-adrenoreceptors. Either in rabbit aorta or in rat seminal vesicles both (−)- and (+)-isomers of INPEA produced identical pA_2 values of 4.7 (159, 160). Two conclusions are apparent: 1) stereochemistry around the β-carbon of antagonists is not important for the α-adrenoreceptor blockade; 2) the α-adrenoreceptor of rabbit aorta and seminal vesicles may be of a single type. Protection of α-adrenoreceptors against the block by irreversible agents provides a method for studying the α-adrenoreceptive effects of drugs. On the isolated rat vas deferens both isomers of alprenolol and INPEA had about equal ability to protect against the block by dibenamine (297). Not all antagonists will produce competitive α-adrenorceptive blockade. In the isolated rat vas deferens all four stereoisomers of butedrine produce equal degrees of noncompetitive antagonism. The pD_2 values varied only between 3.7 to 4.2 (216). On the densely innervated tissues of the rat vas deferens, the effects of exogenously administered norepinephrine are potentiated by either (−)- and (+)-INPEA. This paradoxical observation can be explained by differential interplay between two main factors operative at the neuroeffector junction: 1) inhibition of uptake; and 2) competition at α-adrenorceptors. Whenever effects of norepinephrine are potentiated by an antagonist, the optical isomers do not differ in this respect. This indicates that inhibition of uptake of norepine-

phrine by (−)- and (+)-isomers of blocking agents must be similar. Biochemical studies tend to support this notion. Either in adrenergic nerve granules or in the perfused heart, inhibition of uptake of exogenous catecholamine by (−)- and (+)-isomers of blocker is similar (81, 126, 134, 190, 412).

E. Miscellaneous Effects of Stereoisomers of β-adrenoreceptor Antagonists

These blockers are known to enhance the respiratory difficulty in histamine-sensitive asthmatic patients. It was shown that (+)-isomers of propranolol and alprenolol have no influence on airway resistance, while (−)-isomers were responsible for the increase in resistance (49). Results from animal studies indicate that enhanced airway responsiveness which attends β-adrenoreceptor blockade is not totally dependent upon the existence of excitatory α-adrenoreceptors subserving broncho-constriction in the respiratory tract (105, 269). In dogs, intrarenal infusion of isoproterenol stimulated renin secretion. Both (−)- and (+)-propranolol were equally effective in blocking the isoproterenol-induced secretion. Since the local anesthetic lidocaine had no inhibitory effect, the observed block by propranolol isomers could not be attributed to their local anesthetic effect (415). In man, a rise in plasma renin was demonstrated after an acute postural stimulus; (±)-propranolol, but not (+)-propranolol, blocked the rise. Hence, the effect was correlated to the β-adrenoreceptor blockade and not to the local anesthetic effect (388). These experiments in animals needs to be repeated with the isomers of other β-receptor blocking agents which lack local anesthetic effects.

A variety of drugs lower intraocular pressure, and in rabbits and man (±)-propranolol and (+)-propranolol are equally effective in this regard (402). The optical isomers of agonist and antagonist should provide a good tool to dissociate receptor mechanisms involved in lowering the intraocular pressure. An unusual finding related to the influence of optical isomers of INPEA on the adenylcyclase activity in rat brain is reported (191). The increase in cAMP after epinephrine was not affected by either (−)- or (+)-isomers of INPEA. If the observation is correct, it may be that β-adrenoreceptor antagonists produce the blockade distal to the adenylcyclase where the agonists appears to act. Bonn and Turner (60), who investigated the antianxiety effects of (±)-propranolol, reported some beneficial effect presumably through peripheral autonomic blockade. Pendleton *et al.* (301) pointed out that these drugs may have an influence on the hemoglobin dissociation curve. Both isomers of propranolol shifted the curve to the right, the (+)-isomer being more effective, and such a finding may have some bearing on the clinical usefulness of the drug. Antioxytocin activity of optical isomers of propranolol and INPEA has been studied on rat uterus (330). Both isomers of propranolol shifted the dose response curve of oxytocin to the right. On the other hand, (−)-INPEA had a weak antioxytocin activity; the (+)-form potentiated the effects of oxytocin. Usefulness of the (+)-isomer in the bioassay of oxytocin was explored (331). In order to clarify the mechanisms of central effects of β-adrenoreceptor blockade, the influence of various isomers of β-adrenergic blockers on different enzymes was investigated (228). (−)-Propranolol, but not the (+)-form, significantly decreased glucose 6-phosphate levels. The other effects, such as CNS stimulation and potentiation of the hexobarbital anesthesia, were reported to be the same for both (−)- and (+)-INPEA (174, 270).

It has been known for some time that the depressor effect of epinephrine observed after irreversible blockade of α-adrenoreceptors is reversed by β-adrenoreceptor antagonists. (+)-Propranolol is ineffective in restoring the pressor effect

while racemic propranolol was highly effective (99, 364). Since the α-adrenoreceptor blocking effects of both (+)-propranolol and (±)-propranolol are the same, the reversal could be attributed to the block of β-receptors which unmasked residual α-effects.

XIII. Conformational Requirements at Adrenoreceptive Sites

One of the areas in which there is considerable speculation today is the conformation of drugs when they interact with the active sites of adrenoreceptors. One of the important problems of the future will be to see if effector, metabolic, storage, and transport sites prefer the same or different conformations of a drug molecule (363). Similarly, do the α- and β-adrenoreceptors require different conformations of a drug in order to produce a biological response? Most of the theoretical models in which postulations have been presented for adrenergic drug-receptor interaction have given little consideration to conformational aspects (37, 57). Theoretical calculations have been carried out for the preferred conformations of a number of drugs affecting the adrenoreceptors. Kier (204–206) and Kier and Truitt (207) employing the Extended Hückel Theory have indicated that the preferred conformation of (−)-norepinephrine and 1R, 2S-(−)-ephedrine occurs when the amine and aromatic portions have a dihedral angle of 180° (*trans* conformation). These calculations were utilized to describe a topology of the α-adrenoreceptor (204). Other investigators calculated the preferred conformation of a number of phenethylamines, naphazoline, and sympatholytic compounds and they (87, 316) have postulated α-adrenoreceptor requirements very similar to those of Kier (204). Pedersen *et al.* (299) also calculated norepinephrine to have a preferred *trans* conformation but in solution with an interaction with a metal, *e.g.*, as proposed for β-receptor, they concluded the preferred conformation would be *gauche*. Dopamine was calculated to prefer the *gauche* conformation by Kier and Truitt (207), but this work has been challenged by Bustard and Egan (69) and Rekker *et al.* (318) in which they indicate the preferred conformation to be *trans*. Any change of the drug molecule and of the medium (in which the molecule is present) can affect dramatically the preferred conformation in theoretical calculations (410).

Nuclear magnetic resonance (NMR) studies indicate that the *trans* conformation (180° between phenyl ring and amino function) is favored by both 1R, 2S-(−)-ephedrine and 1R,2R-(−)-*pseudo*ephedrine (311). The preferred conformation of 1R,2R-(−)-*pseudo*ephedrine in solution did not agree with the calculated conformation by Kier (204) as pointed out in a previous review (312). Dopamine has been shown by NMR studies to prefer the *trans* conformation and experimental data also indicates that there is an increase in the *trans* population with an increase in the solution temperature (69). In solid-state studies, the preferred conformation of a large number of the phenethylamine derivatives has been shown to favor the *trans* conformation, *e.g.*, phenethylamine (397), ephedrine (306), (−)-norepinephrine (75), and dopamine (47). These structures, along with other important amines including amino acids found in the adrenergic nervous system, have been reviewed by Bergin (46). The conformation of isoproterenol in the solid state has been reported to be similar to that of norepinephrine (252).

The inferences one can make as to the preferred conformation in the gas, liquid, or solid states to the conformation required to interact with the active site of a receptor in a physiological state are dubious. Until more is known about the drug-receptor interaction and its relationship to a specific biological action, it would

appear to be inappropriate to assign any great importance to a specific conformation, in a conformationally mobile molecule, and its ability to produce a biological action (69, 158, 214, 257).

An interesting approach to attempt to delineate the conformational requirements of adrenergic drugs has been to synthesize rigid and semirigid molecules in which the relative positions of the various functional groups are fixed. Some of the initial work in preparing conformationally rigid analogs of phenethanolamines has been carried out by Smissman and co-workers (360–363) and Nelson and Miller (277). Among the conformationally rigid diastereoisomers to be investigated thus far include the four isomeric 3-amino-2-phenyl-*trans*-2-decalols (363), the two isomeric 3-phenyl-3-hydroxy-*trans*-decahydroquinolines (362), the four isomeric 3-amino-2-(3,4-dihydroxyphenyl)-*trans*-2-decalols (360, 361), and the four isomeric 9-hydroxy-10-amino-1,2,3,4,4a,9,10,10a-(*trans*-4a,10a)-octahydrophenanthrenes (277). The major criticism of this work is directed against the bulk added to the molecules in order to form conformationally rigid systems (142, 206). Because of the low biological activity of these molecules, the added hydrocarbon skeleton must in some manner either be preventing the drug from getting to the adrenergic effector sites or not allowing for a favorable interaction at the adrenergic effector site. Thus, it may be possible for some of these rigid phenethanolamines to have a correct orientation of the important functional groups but the added hydrocarbon prevents them from interacting with the adrenergic effector sites to produce the pharmacological response.

More recently, conformationally restricted molecules with a minimum of structural change in going from the parent to the restricted analog have been studied. This approach of studying small conformationally restricted molecules on the cholinergic muscarinic receptor has been applied successfully by Armstrong and Cannon (16). Small conformationally rigid analogs of drugs affecting the adrenergic nervous system have met with some success. For example, the case of *cis*- and *trans*-2-phenylcyclopropylamine being viewed as a derivative of amphetamine has aided studies in conformational requirements of uptake (181, 262). Other conformationally restricted phenylethanolamine analogs which have been prepared are *cis*- and *trans*-3-phenyl-2-methyl-3-azetidinols, *cis*- and *trans*-2-aminoindanes (321), 2,3-dihydroxy-*cis*- and *trans*-6-amino - 6,7,8,9 - tetrahydro - 5H - benzyocyclohepten-5-ols (220), 1,2,3,4,-tetrahydroisoquinine-6,7-diol and 2-aminoindane-5,6-diol (158). Possibly, this approach of using conformationally restricted analogs, besides giving a better understanding of what the conformational requirements are of adrenoreceptors, will also provide a selective agonist which could be of value in classifying receptors.

XIV. Isomers of Phenolic and Nonphenolic Amines

A. Some Peripheral and Central Effects

Many nonphenolic amines are mainly indirectly acting drugs, hence the pharmacological effects are mainly dependent on the release and the availability of the neurotransmitter norepinephrine. The peripheral pharmacological effects of ephedrine, norephedrine, amphetamine, methamphetamine, and other nonphenolic amines have been studied (79, 224, 291, 296, 324, 379, 401). Although many amines produce pressor effects, the differences between the isomers are small. When the doses of amines are repeated at short intervals, tachyphylaxis develops to many pharmacological effects. The rate of development of tachyphylaxis varies with the isomer used (2, 296). For example, Gerald and Hsu (143) made an interesting observation related to the tachyphylactic effects of

amphetamine isomers on skeletal muscle. Within a narrow concentration range of 1×10^{-4}M to 8×10^{-4}M, both isomers exhibit an equal degree of facilitatory effect and, with increasing doses, of inhibitory effect. Tachyphylaxis was observed to the effects of (+)-amphetamine and not to those of the (−)-isomer. Further analysis indicated that the neuromuscular effects of amphetamine isomers were highly complex.

Amphetamine is well known to produce "aggregation toxicity" in mice. This effect is more pronounced with (+)-amphetamine. The potency ratio of the isolated/aggregated LD50 is 4.9 for (+)-amphetamine and 1.2 for (−)-amphetamine. There was a marked dose-dependent reduction of the brain and heart norepinephrine content after (+)- or (−)-isomers of amphetamine. It was, however, only after the administration of the (+)-isomer to aggregated mice that the norepinephrine-depleting effect was enhanced. Hence, it was concluded that the release of endogenous stores of norepinephrine plays a role in the enhanced toxicity of (+)-amphetamine in mice (264). Selectivity of aggregation toxicity was also studied for ephedrine isomers. Except for (+)-*pseudo*ephedrine, all isomers of ephedrine exhibited this phenomenon, but at a level which is much lower than that of (+)-amphetamine (418). The LD50 potency ratio (isolated/aggregated) for the most potent ephedrine isomer was only 1.5. There were some conflicting reports regarding the involvement of body temperature in amphetamine-aggregation toxicity. Wolf and Bunce (416) observed that both isomers of amphetamine can produce hyperthermia of the same magnitude in isolated or aggregated mice. (−)-Amphetamine produced hypothermia in both isolated and aggregated mice before elevating body temperature. Thus, the data do not support the concept that amphetamine-induced lethality is dependent upon body temperature exceeding a critical threshold.

Gerald and Riffee (144) observed the effects of acute and chronic treatment with amphetamine isomers on pentylenetetrazol-induced seizures. The seizure threshold was expressed as the ratio of the mean dose of pentylenetetrazol required to produce seizures in amphetamine-treated mice divided by the mean dose needed in animals without amphetamine. The threshold to the minimal clonic seizures was lowered by both isomers. However, (−)-amphetamine lowered the threshold to the maximal tonic seizures and the (+)-form raised it. These differential effects of the isomers might be very useful in elucidating the physiological mechanism of seizure. On chronic administration of both isomers, a tolerance developed to the effect.

An interesting approach to the study of the central effects of catecholamines which do not pass the blood-brain barrier is to study them in young chickens whose blood-brain barrier is imperfect or nonexistent. Dewhurst and Marley (103, 104) used this approach to examine the central effects of certain phenolic amines. The behavioral, electrocortical, and electromyographic activities were recorded. The phenolic amines produced depressant effects. Racemic α-methylnorepinephrine was at least four times as potent as the (+)-form. (−)-Norephinephrine was twice as potent as (+)-norepinephrine; however, dopamine was more potent than (−)-norepinephrine or (+)-norepinephrine. This pattern differs from that at peripheral α-adrenoreceptors where (+)-norepinephrine and dopamine are almost equiactive. The nonphenolic amines, such as amphetamine, produced excitatory effects. The (+)-form was more active than the (−)-form of amphetamine.

The effects of (−)-norepinephrine on the firing rate of spontaneously active neurons have been found to conform to certain well defined patterns. There are two types of patterns observed with (−)-norepinephrine, excitatory and/or inhibi-

tory. (+)-Norepinephrine inhibited certain neurons which were also inhibited by (−)-norepinephrine, but on neurons excited by (−)-norepinephrine its effect was weaker or absent. Thus, the excitatory effect shows stereoselectivity whereas, the inhibitory effect does not. The classical adrenoreceptor blockers did not modify the responses. It is concluded that receptors for norepinephrine on brainstem neurons are of more than one kind and that they do not fit into the α- and β-classification applied to peripheral receptors (58).

There has been considerable clinical interest in the anorectic response to drugs affecting the central nervous system. A reliable eating response can be obtained on injection of small quantities of (−)-norepinephrine into the rostral hypothalamus of the rat. (+)-Norepinephrine induced a negligible response, indicating a stereoselective effect. Booth (61) postulated that α-adrenoreceptor modulation of postsynaptic activity by norepinephrine released from the nerve ending is involved in the hypothalamic control of feeding in the rat. Stereoselectivity in the feeding behavior for isomers of norepinephrine was also observed (250). Roszkowski and Kelley (325) developed a screening method for assessing drug inhibition of feeding behavior. (+)-Amphetamine is very effective in producing an inhibition of broth consumption in rats. (−)-Amphetamine was ineffective. Tolerance and cross-tolerance to the anorexic effect is exhibited by these isomers (380). Abdallah (1) found (−)-ephedrine to be the most potent of the ephedrine isomers in causing a reduction of food intake in mice. Thus, it has been difficult to separate the central stimulant effects from the anorexigenic effects of these agents.

Loss of sodium from tissue could cause loss of body water which in turn reduces the weight of an animal. Because isomers of amphetamine do not significantly differ from one another in causing sodium loss, it cannot be an important factor in the weight-reducing effect of (+)-amphetamine (335).

Substituted amphetamines show some promise as anorectic agents. (+)-Chloramphetamine is less of a central stimulant, but is a longer-acting anorectic agent than (+)-amphetamine (279). (−)-Chloroamphetamine is more active than (−)-amphetamine; both agents are less active than their respective (+)-forms. Whether the observed effect of (+)-*p*-chloroamphetamine is related to depletion of brain serotonin or norepinephrine is yet to be decided (113, 135, 266). Phenmetrazine and phendimetrazine are known to be effective anorectic agents. There is an interesting stereochemical relationship between ephedrine and the phenmetrazine molecule (118, 176). Costa *et al.* (86) observed that in rats a small dose of 0.2 mg/kg i.v. of the (+)-amphetamine and 1 mg/kg i.v. of (−)-amphetamine produced anorectic effects without any increase in motor activity. Although motor and anorectic effects of the isomers could be separated, the turnover rates of central dopamine and norepinephrine were unaltered. An attempt has been made to correlate the absorption of triglycerides after the administration of amphetamine isomers. The isomers in doses having only a mild effect on plasma triglycerides in normal rats strongly inhibited the rise in plasma triglycerides elicited by olive oil ingestion. The (−)-isomer proved to be more effective (162). The significance of these findings in relation to differential anorectic effects remains to be clarified.

Toxic and anorectic effects of isomers of the newer drug fenfluramine were compared in rats. The isomeric (−)/(+) ratio for the toxicity is 1.5 while that for anorexia is 3. Thus, the (+)-form of the drug appears to be a potentially useful agent (45). The lesser potency of the (−)-isomer may be due to rapid metabolism (86).

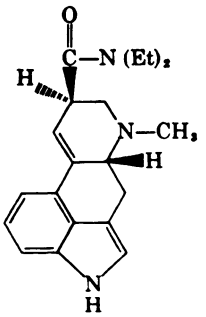
Attempts have been made at correlating

the absolute configuration of hallucinogenic amphetamines with that of (+)-lysergic acid diethylamide (LSD) (79a, 355, 356). The absolute configuration of (+)-LSD is known to be 5R, 8R (226). Shulgin (355) has demonstrated a stereospecificity for the hallucinogenic effects in man by using the isomers of 2,5-dimethoxy-4-methyl amphetamine (DOM or STP). It appears that only the (R)-(−)-DOM has hallucinogenic activity and that it is twice as potent as the racemate. It has been postulated that since the R-isomers of the substituted amphetamines correlate with the configuration at C-5 of (+)-LSD that there is an asymmetric site associated with the action of hallucinogens. In contrast to the hallucinogenic amphetamines, it is the (S)-(+)-isomer of amphetamine which is the most active isomer in producing central nervous system stimulation. An interesting observation by Barfknecht and Nichols (24) was that the racemate of 3,4-dimethoxyamphetamine produced a mescaline-like action in rats which neither of the pure optical isomers could produce alone. Bennington *et al.* (44) have also reported, by using a Sidman avoidance schedule in rats, that the psychotomimetic potency is possessed primarily by the (R)-(−)-isomer of DOM and 2,5-dimethoxy-4-bromoamphetamine.

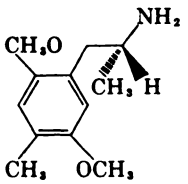
B. Metabolic Aspects

Significant contributions to the understanding of the metabolism of nonphenolic amines was made by Beckett and his colleagues (29–35). Recently, the influence of stereochemical factors on drug disposition was reviewed (193a).

The absorption rate and urinary excretion of amphetamine isomers and related drugs have been studied in various species and in man. Although total urinary excretion of these drugs is pH dependent, the differences in the excretion pattern between a pair of isomers are not great (29, 31, 32, 35). Gunne (161) used the gas chromatographic resolution method (153) for amphetamines and found that after administration of (±)-amphetamine, all subjects excreted approximately equal amounts of both isomers during the first 12 hours. Urine collected after 12 hours contained a continually decreasing proportion of the (+)-isomer. This slow excretion might be a reflection of higher tissue deposition of (+)-amphetamine (or its metabolites) over that of (−)-amphetamine. In man, after oral administration of diethylpropion, the drug was rapidly and extensively metabolized by N-de-ethylation and stereoselective carbonyl reduction. The major metabolites in the urine, representing 70% of the dose administered, were N-ethylaminopropiophenone, (+)-N-diethylnor*pseudo*ephedrine, (+)-N-ethylnor*pseudo*ephedrine, (−)-norephedrine, and (−)-nor*pseudo*ephedrine. The results are discussed in relation to the central anorectic effects of the drug (385). The metabolic effect of increasing N-alkyl chain length on amphetamine isomers were studied. The total metabolism of the (+)-iso-



(5R,8R)-(+)-LSD



(R)-(−)-DOM

mers of methyl, ethyl, and n-propylamphetamine was greater than that of the corresponding (−)-isomers, but there was no difference in the total metabolism of (+)- and (−)-n-butylamphetamine. The (+)-isomers of methyl- and ethylamphetamine were N-dealkylated more than their (−)-enantiomorphs, but (−)-n-propylamphetamine was N-dealkylated more than the (+)-isomer. This finding emphasizes the importance of alkyl chain length and stereochemistry in the metabolism of amphetamines (33). In rats, two days after treatment with equal amounts of (+)- and (−)-amphetamine, the quantity of *p*-hydroxyamphetamine was 48 and 63% of the initial dose, respectively (111, 112). Relatively more (+)-*p*-hydroxyamphetamine is converted to its β-hydroxylated product, (−)-*p*-hydroxynorephedrine, by the enzyme dopamine β-hydroxylase, hence less (+)-*p*-hydroxyamphetamine will appear in the urine. It is interesting, however, that the ring-hydroxylating enzyme does not appear to show selectivity for amphetamines. This lack of selectivity is in contrast to the fact that (−)-ephedrine is ring hydroxylated in rats whereas (+)-ephedrine is not (275).

Baba *et al.* (22, 23) studied the distribution and identification of metabolites formed from (−)- and (+)-ephedrine ($\alpha^{14}C$) in rats. The initial blood concentration of the (+)-isomer was higher than that of the (−) form, while tissue retention of the (−)-form was higher. As the result the (+)-form was excreted at the faster rate. In rabbit liver slices, when incubated with either (−)- or (+)-ephedrine for five hours, the recovery of the unchanged (−)-ephedrine was only 8%, while relatively large amounts (42%) of (+)-ephedrine were recovered from that incubated with the (+)-form. Although N-demethylation was approximately the same for both isomers, more hippuric acid was formed from (−)-ephedrine. Feller and Malspeis (130) studied the metabolism of ^{14}C-labeled (−)- and (+)-ephedrine. In rabbits, after 3 mg/kg i.p. of either isomer, about 50% of the drug is excreted in the urine over 24 hours as benzoic and hippuric acid. In liver microsomes from the same species, the conversion to benzoic acid from (−)-ephedrine appeared to be two to three times faster than that after (+)-ephedrine. The total metabolism of (−)-ephedrine is faster than that of the (+)-form.

There are marked species differences in the metabolism of amphetamine isomers (112). Axelrod (19) reported that an enzyme system in rabbit liver microsomes catalyzes the deamination of amphetamine to yield phenylacetone and ammonia. This enzyme system prefers (−)-amphetamine as a substrate (175); however, neither (−)- nor (+)-amphetamine is metabolized by a microsomal preparation of rat liver. The urinary excretion kinetics of a close structural analog of amphetamine, methamphetamine, was also studied in man (32, 161). The excretion patterns of (+)- and (−)-methylamphetamine are similar. Most of the drug is excreted unchanged in 24 hours; however, a very small amount of the drug is N-demethylated. Because more (−)- than (+)-amphetamine occurs in the urine after (±)-methamphetamine, it is suggested that enzymatic N-demethylation is stereoselective (161). In hepatic microsomes from phenobarbital-pretreated rabbits, (+)-benzphetamine is N-demethylated 30% faster than the (−)-isomer (175). Similarly, (−)-ephedrine is N-demethylated whereas (+)-ephedrine is not (259, 275). Dann (97) has investigated demethylation rates of ephedrine isomers. Isolated rabbit liver microsomes were used to determine the enzymatic kinetics. It was found that the V_{max} is the same for all ephedrine isomers, but K_m values for (−)-ephedrine and (−)-*pseudo*ephedrine were approximately twice those of (+)-ephedrine and (+)-*pseudo*ephedrine. A discriminatory effect of (+)- and (−)-amphetamine was noted for another liver enzyme, alcohol dehydro-

genase. The inhibition of the enzyme by the (−)-isomer was significantly more pronounced than that by the (+)-form (358).

XV. Summary and Concluding Remarks

The literature on the relationship between stereochemical structure and its influence on the biochemical and pharmacologic activity of adrenergic drugs has been reviewed. For the proper appreciation of the steric-structure activity relationship, stereochemical definitions and notations are provided. Throughout the review, the value of stereoisomers as a tool to study and dissociate various drug actions has been emphasized.

At the adrenergic neuroeffector junction the enzymes which are involved in the biosynthesis and metabolic degradation of norepinephrine show marked stereoselectivity. As a matter of fact, stereoisomers of tyrosine were used in early studies to dissociate activity of tyrosinase from that of tyrosine hydroxylase. Only the latter enzyme exhibits a high degree of stereoselectivity (276). The enzymatic decarboxylation proceeds with the retention of configuration at the α-carbon atom of (−)-α-methyldopa (38) and the β-hydroxylation is stereospecific (152, 208, 389). These processes are very important in understanding the formation of (−)-α-methylnorepinephrine from (−)-α-methyldopa or formation of corresponding false neurotransmitters from (−)-α-methyl-*m*-tyramine and (+)-amphetamine. Recently, the isolation of isoquinoline alkaloids has been demonstrated from urine of a patient treated with (−)-dopa (334). A study of the uptake and retention of stereoisomeric isoquinoline alkaloids by the storage vesicles should be rewarding.

The enzymatic degradation of the stereoisomers of catecholamines or monophenolic amines by monoamine oxidase is stereoselective favoring the R(−)-isomers. It should be informative to study the substrate stereoselectivity of various subtypes of monoamine oxidases. If these isoenzymes differ in the geometry of the active sites, the stereoselectivity of the various isoenzymes towards a given pair of isomers of catecholamine should differ.

Although the stereoisomers of cocaine and methylphenidate exhibit distinct differences for the inhibition of neuronal uptake of norepinephrine (66, 25, 5, 218), insofar as the stereoselectivity of the neuronal membrane for the isomers of norepinephrine is concerned, the best available evidence indicates that some organs exhibit a small degree of stereoselectivity of uptake while others do not. For some organs the evidence is contradictory. Much of the evidence does not meet the strict requirement of being based on determinations of initial rates. Moreover, for some cardiovascular tissues of the rabbit, a reversed stereoselectivity of uptake has been described; namely a preference for the (+)-isomer of norepinephrine (169). Thus, the study of drugs on properties of the adrenergic neurone still presents a challenging problem.

Theoretical calculations in solution and in solid state have been performed for the interaction of drugs at various sites of adrenergic neuroeffector junction. In most cases the *trans* conformational relationship between the phenyl ring and amino functional groups has been observed. Although these findings have led to various hypotheses as to what the receptor makeup should be, it has been pointed out that such speculations may be inappropriate. Studies have been performed using conformationally restricted analogs of phenethylamine derivatives to gain better insight into the conformational requirements. These studies have met with some success. It would appear that further studies need to be carried out to gain a better knowledge of the conformational requirements for uptake, release, metabolic and effector sites in the sympathetic nervous system.

Since the initial adrenoreceptor interacting components for catecholamines remain unidentified, the old theory of Easson and

Stedman still appears to be attractive to explain the differences in the pharmacologic activity of (−)- and (+)-isomers and the β desoxy derivative. On many peripheral tissues examined for either the α- or β-adrenoreceptors, the pharmacologic activity of the (−)-isomer >> (+)-isomer = the β desoxy form (290, 288). If the desoxy form of a catecholamine such as dopamine differs from the activity of the (+)-norepinephrine, a criterion for the presence of the dopamine receptor is suggested. The use of stereoisomers of catecholamines in the pharmacologic characterization of the adrenoceptor is discussed and it is emphasized that similarity of the receptors should generate similar isomeric activity ratios.

Although only limited studies have been carried out on the isomers of α-adrenergic blockers, the pharmacology of the stereoisomers of reversible competitive antagonists of β-adrenoreceptors has been thoroughly studied. Only R(−)-isomers are potent antagonists, which differ in the magnitude of pharmacological activity from the corresponding S(+)-isomer by approximately 100-fold. In addition to their antiarrhythmic effects, β-adrenoreceptor antagonists produce a variety of effects such as hypotension, local anesthesia, weak α-adrenoreceptor block, blockade of adrenergic neurons, lowering of intraocular pressure and central nervous system depression; with the aid of isomers of antagonists it was possible to dissociate the β-adrenoreceptor mechanism from the nonspecific mechanism.

The stereoisomers of catecholamines or of adrenergic blockers should prove to be valuable substances for the characterization of the isolated receptor macromolecule. A receptor macromolecule which binds adrenergic drugs with high affinity and high capacity may be a misleading criterion for isolating the receptors, but one which exhibits stereoselectivity in binding might be a better one. Since the pharmacological effects of stereoisomers exhibit a high degree of stereoselectivity, *the same degree* of stereoselectivity is expected in binding of stereoisomers by the isolated adrenoreceptor macromolecule (294). Although it has been impossible to show stereoselectivity in binding of stereoisomers of catecholamines to fat cell membrane and liver cell membrane, the immediate intracellular biochemical event after the receptor interaction exhibits a high degree of stereoselectivity (50, 102). In the future, stereoisomers of drugs should prove to be valuable tools in isolating, characterizing and mapping the active site of the adrenoreceptors.

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