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Steric Aspects of Adrenergic Drugs

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... Concepts are extremely useful to the human mind, but concepts may change and change very fast. What remains are the facts, the experimental facts, the discoveries. If a new concept leads to a discovery, you may award a prize without hesitation. But a concept without a discovery would have little chance. Concepts are instruments in scientific research. They help you to make new discoveries. If they don't, they have no justification. And the only way you can prove the justification of a concept—the correctness of a concept—is to see if it not only explains already known facts, but also leads to a new and unexpected discovery. That proves that there is something new in the concept....

Arne Tiselius
(Nobel Laureate)

Adrenergic drugs can almost serve as a prototype for the classical development of a group of medicinally active compounds. The initial lead arises from the observation of physiological activity associated with a natural product. Eventually, the chemical structure of the active substance is revealed, synthetic analogs are prepared, and their pharmacological activities are determined. An empirical body of structure–action relationships develops as a result of these studies, which leads to the synthesis of more compounds and, as knowledge of structural chemistry and pharmacological mechanisms becomes more refined, ideas concerning the relationship of chemical structure and pharmacological action become less empirical.

All these steps have been followed in developing the area of adrenergic drugs to its present state. It is particularly notable that the lead compound, epinephrine, is optically active. The early syntheses leading to racemic material and the pharmacological evaluation of the racemate made it apparent that some stereoselectivity was involved since the racemate was less active than the optically active natural product. Thus, at a very early stage in the development of adrenergic drugs, steric effects were observed. It is surprising that more resolutions were not performed and absolute configurations were not determined for many of the major synthetic analogs until recently. However, some attempts have

been made to review natural asymmetry and pharmacologic action (1, 2). The current revival of interest in this area makes it desirable to review what is known about steric effects and adrenergic agents.

A detailed discussion of adrenergic mechanism would be out of place here, but a brief review of some basic concepts is essential for any meaningful discussion of steric aspects.

Ahlquist (3) suggested the terms α and β for two different types of adrenergic receptors. This classification was based on the comparative effectiveness of the compounds epinephrine, norepinephrine, and isoproterenol on various tissues. In those tissues where the order of activity was epinephrine $\geq$ norepinephrine $\gg$ isoproterenol, the receptors were called α and associated with excitation or contraction. In tissues where the order was isoproterenol $\gg$ epinephrine $\geq$ norepinephrine, the receptors were designated β and associated with inhibition of function or relaxation. The validity of this concept, at least with respect to certain tissues, has been supported by experiments with selective antagonists, some of which block only α-effects and some of which block only β-effects. Other types of adrenergic receptors have been suggested, but most of the experimental work to date has been done with so-called α-agonists and β-agonists and their respective antagonists. A more complete discussion of the concepts of α- and β-receptors may be found in a recent publication (4).

The concept of direct and indirect action has received ample experimental verification. In its simplest sense, it suggests that sympathomimetic agents may act: predominantly directly, that is, at the effector site; predominantly indirectly, by releasing endogenous norepinephrine; or by a combination of these processes. Actually, there is evidence that the mechanism of action of indirect-acting sympathomimetic amines is more complex. Many indirect acting agents appear to block the uptake of norepinephrine as well as cause its release (5).

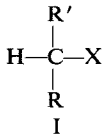
Regardless of the intricacies of the mechanism of action of indirect-acting sympathomimetic agents, it is important to keep in mind that drugs which are closely related structurally, such as the phenethylamines, may produce pharmacologically identical effects by different mechanisms. Analyses of structural or stereochemical requirements for activity are considerably complicated by this fact, and comparative studies of sympathomimetic amines done prior to the establishment of this concept must be reevaluated in modern terms.

Uptake of catecholamines by adrenergic nerves has been shown to proceed against a concentration gradient, and it seems that a saturable active transport system is involved. Iversen (6) has defined two uptake processes: uptake_1 and uptake_2. Uptake_1 is operative at low perfusion concentrations, accumulated norepinephrine is not readily washed out by perfusion with norepinephrine-free medium, and the process appears to be stereoselective for the enantiomers of both norpinephrine and epinephrine. Uptake_2 is operative at higher concentrations. The uptake is characterized by rapid washout of accumulated norepinephrine and complete lack of stereoselectivity. After exogenous catecholamines pass through the neuronal membrane, they may be retained by storage granules inside the neurone. There is considerable evidence for the existence of more than one pool of stored catecholamines within the neurone. Perhaps the most complete information available deals with norepinephrine storage granules isolated from bovine splenic nerves and the adrenal medulla (7). The ability of these isolated storage granules to accumulate the isomers of norepinephrine, epinephrine, and some related compounds has been studied and will be discussed later.

Before leaving the subject of uptake and storage, the effects of cocaine and reserpine must be mentioned. These two drugs have been widely used as pharmacological tools in the study of adrenergic mechanisms. Cocaine apparently acts by inhibiting uptake of catecholamines and related compounds at the adrenergic neurone membrane. On the other hand, reserpine appears to exert its effect on the intraneuronal storage mechanisms and acts to prevent accumulation of norepinephrine or related compounds. Reserpine does not affect neuronal membrane uptake. The recently published monograph by Iversen (6) provides a detailed and extensively documented discussion of various aspects of the uptake and storage processes.

STEREOCHEMICAL NOTATION

Any discussion of stereochemical aspects of drug action must involve the proper use of notation to identify and specify the actual three-dimensional characteristics of the molecules considered. The small capital letters D and L are generally used to denote the configuration of an asymmetric carbon atom (Structure I) in a molecule of the type:

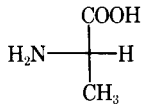


I

The molecule is oriented so that the number 1 carbon of the principal chain is at the top in the usual Fischer projection (8). This is sometimes stated differently, that is, that the carbon in the highest oxidation state is at the top, but since this would be the number 1 carbon, there is no conflict between these statements. If, when the molecule is so oriented, X is on the right, the configuration is said to be D; if X is on the left, the term L is used. These letters should *never* be used to denote sign of rotation. In many cases the lower case letters *d* and *l* (*dextro* and *levo*) have been used to specify sign of rotation, but this can lead to ambiguities. Some authors, particularly in the biological literature, use the upper case and lower case symbols interchangeably. For example, in some studies of sympathomimetic amines, the more active enantiometer of norepinephrine has been referred to as the L-isomer. In these cases, this means levorotatory; but most chemists would interpret this as meaning the "L" configuration, and the L isomer of norepinephrine is dextrorotatory. This example should suffice to show that when either upper case or lower case symbols of this type are used, the reader cannot be sure which enantiomer is being discussed.

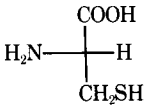
The most reliable notation for specifying absolute configurations is usually referred to as the "sequence

rule." This method has been described in the literature (9) and has been widely accepted. An important distinction between the sequence rule and DL nomenclature is that while the symbols D and L relate the configuration of a molecule to some arbitrary standard, the sequence rule notation is self-consistent for the molecule in question but cannot be used to relate a series of compounds. This can be most clearly seen by considering the following compounds (Structures II and III):



L-alanine

II



L-cysteine

III

These two compounds are obviously related configurationally and have the same absolute configuration. However, due to the difference in atomic number of the substituents, L-alanine has the S-configuration while L-cysteine has the R-configuration.

Medicinal chemists and pharmacologists are often interested in describing structure–action relationships in a series of structurally related drugs. When this is done, it makes little difference which method is used to describe the compounds evaluated as long as the method is unambiguous. For this reason, the symbols D, L, *d*, and *l* should not be used in these cases. Rotations, if desired, should be indicated by either (+) or (−), and absolute configurations by either R or S. Throughout this review, isomers are identified by the signs (+) or (−).

FACTORS COMPLICATING INTERPRETATION OF RESULTS

In every review or symposium, it has become increasingly important to discuss factors that can influence the interpretation of results. This review on optical isomers cannot be an exception. First and most important, when the activity of (−)- and (+)-isomers is compared, it is assumed that both forms are pure. However, this may not be the case. The activity ratios between (−)- and (+)-isomers of isoproterenol were reported as 11.8 for cat blood pressure (10) and 87.45 for dog blood pressure (11). Subsequently, Lands *et al.* (12) obtained higher activity ratios, 1000 for cat blood pressure and 450 for dog blood pressure. They pointed out that constant specific rotation or melting point is not the best criterion for optical purity, but that constant biological activity is.

Occasionally, pharmacologic activity of (−)- and (+)-isomers is expressed in mcg./kg. or mcg./ml. If both isomers have identical salts, it should not influence the calculation of the activity ratio. However, if one isomer is bitartrate salt and the other is hydrochloride salt, it will obviously affect the expression of the activity ratio. Hence, the dose or concentration should be expressed in molar terms, which necessitates knowing the molecular weight of a given drug. Further, it is interesting to know that different physical forms can have different biologic activity. Lands *et al.* (12) found that (+)-isoproterenol as a bitartrate salt is more toxic than when given as a base dissolved with dilute hydrochloric acid. Unfortunately, where the isomers are obtained from outside sources, frequently the physical form of the isomers is not explicitly stated.

Pharmacologic activity of the sympathomimetic agents at adrenergic synapses is complicated by functional integrity of the uptake site (or transfer site) or storage site or by the presence of both α- and β-adrenergic receptors. The enzymes, dopamine-β-hydroxylase, monoamine oxidase (MAO), and catechol-*o*-methyl transferase (COMT), further complicate the situation. Optical isomers of adrenergic drugs are known to interact differently with many of these factors that influence pharmacologic activity. Depending upon the objective of the investigator in a given problem dealing with optical isomers, the following modifications can be made:

1. Reserpine pretreatment can be given to an animal to eliminate stored norepinephrine. Sufficient time must be allowed between reserpine pretreatment and depletion of endogenous norepinephrine. Korol *et al.* (13) studied optical isomers of octopamine on the cardiovascular system of the dog and concluded that both isomers of octopamine are acting directly. However, only 1 hr. was allowed between reserpine treatment and testing of the isomer. It is well known that during the early phase of action of reserpine, the effects of tyramine or phenethylamine are potentiated.
2. Cocaine can be used to inhibit uptake at the adrenergic neuronal membrane.
3. If the pharmacologic effects of closely related agents such as (+)-isomers and corresponding deoxy derivatives[1] are to be studied, it is important that the enzyme, dopamine-β-hydroxylase, be inactivated by a suitable inhibitor. Many deoxy derivatives are good substrates for this enzyme. Shore (14) and Patil *et al.* (15) used a similar approach when activity of deoxy derivatives was compared with corresponding (−)- or (+)-isomers.
4. Comparative pharmacologic effects of α-methylated and non-α-methylated sympathomimetic amines should be studied under the influence of MAO inhibitor because the latter amines are quickly inactivated by enzyme monoamine oxidase. Selection of monoamine oxidase can add one more variable in experimental design. The enzymatic action can be avoided by studying α-methylated amines only.
5. Although in some tissues the role of COMT in termination of pharmacologic effect of (−)-norepinephrine may be negligible, its role for that of (+)-isomer is yet undefined. Recently, fairly stable inhibitors of this enzyme were available. Comparison of activity of both isomers of norepinephrine in the presence of cocaine and under the influence of COMT inhibitor should give a better idea regarding the activity ratio of these antimers. It is difficult to talk about the receptor level when drug effects on a complex parameter such as blood pressure are studied. Studies on isolated tissues are better for controlling the variables.
6. While studying α-adrenergic receptors, β-receptors should be blocked by a suitable β-blocker which has a minimal α-adrenergic blocking property. Rat vas

[1] Amine without β-hydroxyl group. The term deoxy derivative will be used interchangeably with the other name of the amine, for example, deoxymetaraminol or α-methyl-*m*-tyramine.

deferens, seminal vesicles, rabbit aorta, cat spleen, mouse spleen, and cat nictitating membrane are generally used for testing α-adrenergic drug activity. Guinea pig atria, trachea, and rat uterus are generally used for testing β-adrenergic activity. If desired, different tissues from the same species could be used. Every test tissue has its own characteristic. Some sympathomimetic amines produce tachyphylaxis (acute tolerance), and the rate of tachyphylaxis varies with different isomers (16, 17). Two tachyphylactic amines, therefore, cannot be assayed on the same tissue. This introduces some variability in testing procedure and, hence, in the interpretation of results. By taking care of variables in experimental design, facts are clearly exposed. Furchgott (18) has critically evaluated various problems in a study of adrenergic drugs.

Frequently, biological activity of a single isomer is derived from that of the racemic form. However, some wrong assumptions may be made. Various possibilities can occur:

1. Only one isomer is active while the other isomer is practically inactive; for example, (−)-isopropylmethoxamine is a potent β-adrenergic blocker on trachea while (+)-isopropylmethoxamine is practically inactive (15).

2. One isomer is relatively more active than the other isomer. Both the isomers of sotalol are active in blocking β-adrenergic receptors. The pA_2 value for the (−)-isomer is 6.8 and that of the (+)-isomer is 5.15. The effect is stereoselective[2] (19).

3. Both the optical isomers can be equiactive. On the isolated rat vas deferens, isomers of hydroxyamphetamine produce equal effects which do not differ from racemic hydroxyamphetamine (20).

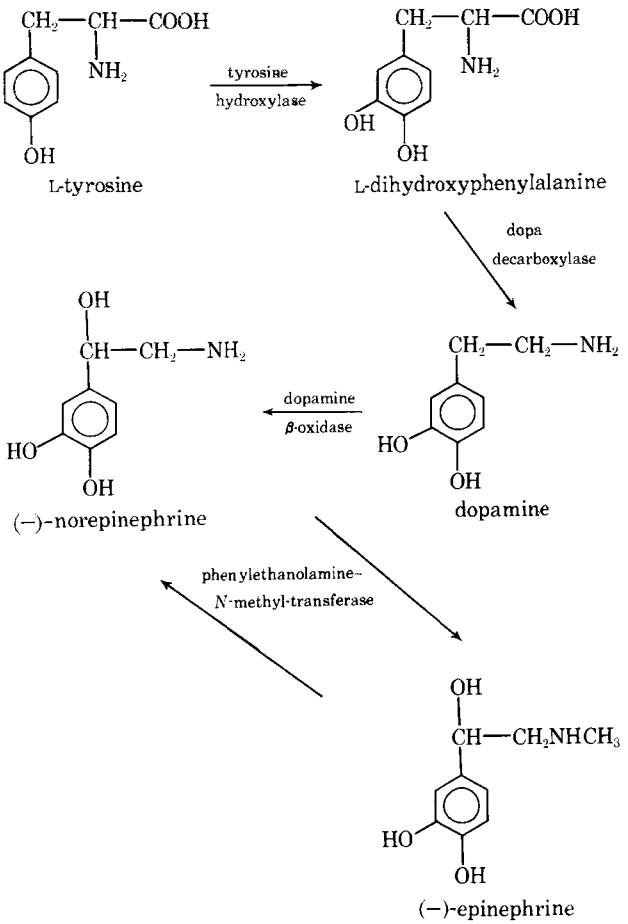
4. A given isomer may not produce any apparent effect, but it may be antagonistic to the pharmacological effects of other isomers. Luduena (21) reported that (+)-isoproterenol antagonizes the effects of (−)-isoproterenol.

5. Similarly, Fielden and Green (22–24) found that (+)-*N*-(1-phenethyl)guanidine has little effect of its own but antagonizes the potent adrenergic neurone-blocking effect of the (−)-form.

6. A very unusual finding reported by Porter *et al.* (25) is that racemic α-methyl-*p*-tyrosine causes greater depletion of norepinephrine from tissues than either isomer alone. In other words, the racemic form can behave as the third molecular species.

Occasionally, the relationship between the activity of (+)- and (−)-isomers may be different when different parameters 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 the adrenergic neurone-blocking agent. Such findings might be very useful in analyzing the mechanism of drug action. These observations clearly state that the norepinephrine depletion caused by the drug and the antagonism of the adrenergic neurone blockade are two different mechanisms (26).

Over the years, radiolabeled agents have facilitated research projects on adrenergic drugs, but they also have added another complication. Many labeled sympathomimetic drugs are only available in racemic form. When single desired isomers of high specific activity become available, it will be necessary to reevaluate some previous studies with the racemic form. The role of single molecular species then will be clarified.



Scheme I

BIOSYNTHESIS

The biosynthetic pathway by which norepinephrine and epinephrine are formed has been intensively studied, and a recent review summarizing this work has appeared (27).

All of the work to date has confirmed the original postulation of Blaschko (28) with respect to the major pathway in animals (Scheme I).

The substrates for the first two steps are optically active compounds. Dopamine, while not optically active, yields an optically active product, (−)-norepinephrine, which can then be converted to another optically active compound, (−)-epinephrine. Each of these reactions may, therefore, exhibit some stereochemical features which will be considered at this point.

Tyrosine → Dihydroxyphenylalanine

The action of tyrosine hydroxylase has been studied by several groups, and the properties of the enzyme have been defined (29). D-Tyrosine, tyramine, DL-*m*-tyrosine, and L-tryptophan were found to be inactive as sub-

[2] This term is preferred over stereospecificity.

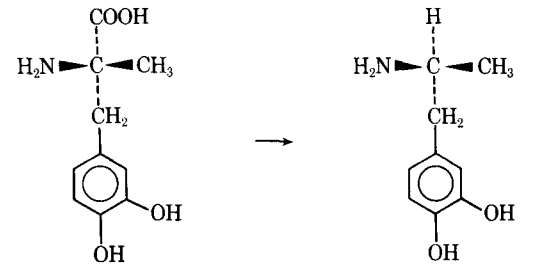
strates, indicating a high degree of structural and stereochemical specificity (30).

Dopa → Dopamine

The enzyme responsible for this conversion is best known as dopadecarboxylase, although the term aromatic L-amino acid decarboxylase is probably more correct (31). As this name suggests, the enzyme is stereospecific because L-aromatic amino acid does not decarboxylate D-dopa (28, 32, 33). It has been shown, however, that D-dopa is converted to dopamine *in vivo*, presumably by conversion to the keto acid followed by transamination to give L-dopa (34).

In addition, it has been demonstrated (35) that the enzymatic decarboxylation of amino acids proceeds with retention of configuration. Obviously, this point is not a major one in the reaction dopa → dopamine, since dopamine contains no asymmetric carbons. It is of major importance, however, in the reaction of α-methylated amino acids, where both the substrate and the product contain asymmetric carbon atoms. The α-methylated analogs of many sympathomimetic amines have been the subject of widespread interest because of their ability to act as false adrenergic transmitters (36).

Biochemically active α-methyldopa has been shown to have the S-configuration (37). This corresponds to the L-configuration of dopa. Decarboxylation of this compound with retention of configuration should yield α-methyldopamine, which also has the S-configuration (Scheme II).



S(−)-α-methyldopa S(+)-α-methyldopamine

Scheme II

The observation that decarboxylation of L-α-amino acids produces dextrorotatory α-methylamines (38), coupled with the proof that the absolute configuration of levorotatory α-methyldopamine (not the natural metabolite) is R (39), establishes the absolute configuration of the natural metabolite as S and serves as proof for the retention of configuration in the α-methyl series.

Dopamine → Norepinephrine

This transformation is catalyzed by the enzyme dopamine-β-oxidase, whose properties have been recently reviewed (40).

The substrate specificity of dopamine-β-oxidase is rather low. In the case of the conversion of dopamine to norepinephrine, substrate stereospecificity is not a factor since the substrate contains no asymmetric carbons. The product, (−)-norepinephrine, has the R-configuration.

In the case of substrate with an α-methyl group, substrate stereospecificity is involved, and several studies have demonstrated that only one enantiomer is β-hydroxylated (41–44).

In the series under discussion, where the sequence rule priorities of functional groups remain essentially the same, those enantiomers with the S-configuration at the β-carbon are active substrates while those with the R-configuration are not. The introduction of the hydroxyl group at the β-carbon is apparently stereospecific, yielding compounds whose absolute configurations at the β-carbon are the same as R(−)-norepinephrine. Thus, S(+)-α-methyldopamine is converted to αS, βR-(−)-α-methylnorepinephrine.

Norepinephrine ⇌ Epinephrine

This interconversion is catalyzed by phenethanolamine-*N*-methyltransferase, which can utilize both enantiomers as substrates, those with the R-configuration showing greater activity (45).

SELECTIVITY OF UPTAKE AND STORAGE MECHANISM FOR OPTICAL ISOMERS

After reviewing different papers on this subject, a need for consistent terminology became evident. For the sake of uniformity, the term accumulation is preferred over deposition, uptake site over transfer site, and release over efflux or chemorelease. In many instances the drug was injected by various routes, and tissue accumulation was observed at a fixed time. This type of tissue accumulation will not distinguish between accumulation by adrenergic nerve endings and extraneuronal uptake. Further, uptake by nerve endings is a two-stage process: (*a*) uptake by neuronal membrane in the cytoplasm, and (*b*) uptake by granular membrane into granules.

In 1963, Kopin and Bridgers (46) investigated the biological differences in (−)- and (+)-isomers of norepinephrine. Rats were subcutaneously injected with equal amounts of either the (±)-norepinephrine-^{14}C and (±)-norepinephrine-^{3}H or the (−)-norepinephrine-^{14}C and (±)-norepinephrine-^{3}H solutions. Animals were killed 1 or 24 hr. later; the ^{3}H–^{14}C ratio in hearts and spleens was determined. From these experiments, the relative role of (−)- and (+)-norepinephrine in tissue uptake and retention was defined. They concluded that both isomers are bound to the tissue to the same extent, and that the disappearance of (+)-norepinephrine-^{3}H is more rapid than that of (−)-isomer. Subsequently, Maickel *et al.* (47) reported that 5 min. after the intravenous injection of (±)-norepinephrine-^{3}H, the ratio of (−)-norepinephrine-^{3}H–(+)-norepinephrine-^{3}H in the rat heart was 11:1. This ratio indicated a higher affinity of uptake mechanism for (−)-norepinephrine. The lack of detection of selectivity of the uptake mechanism by Kopin and Bridgers (46) was then due to methodology. Maickel *et al.* (47) separated the isomers of norepinephrine-^{3}H by an isotope dilution technique. Kopin and Bridgers tested the differences after 1 hr. as compared to 5 min. by former investigators.

More details on the kinetic analysis of uptake of norepinephrine isomers were presented by Iversen (48). Isolated rat heart was perfused for various times with

different concentrations of norepinephrine isomers. The initial rates of uptake by various concentrations obeyed Michaelis-Menton kinetics. On the basis of the Michaelis constant, K_m, (+)-norepinephrine has only one-sixth the affinity for uptake as has (−)-norepinephrine. It is interesting, however, that the $v_{max.}$ for (+)-norepinephrine is higher than that for the (−)-isomer. Under similar experimental conditions, when hearts were perfused for 5 min. at 100 ng./ml. of epinephrine isomers, the tissue accumulation of (+)-epinephrine was only one-third as compared to that of (−)-epinephrine (49). This indicated differences in uptake for isomers of epinephrine. Although accumulation of (−)-norepinephrine in the adrenergic neurone is considered to be an active process (*i.e.*, against a concentration gradient), Born (50) has argued against this concept. The uptake of (−)-norepinephrine in the human erythrocytes occurs by simple diffusion. This process is also stereoselective (51). However, in contrast to the accumulation of (−)-norepinephrine in the adrenergic neurone, the accumulation in the erythrocytes is not inhibited by cocaine. Thus there appears to be some differences between the process of accumulation in these two systems. Selectivity of accumulation of norepinephrine isomers was also studied in the guinea pig heart (52). After intravenous injection of 1 μmole/kg. of the appropriate isomer in the anesthetized guinea pig, the norepinephrine content in the heart and aortic blood was examined. One minute after injection the net uptake of (−)-norepinephrine by the heart was higher than that of (+)-norepinephrine. However, 60 min. afterwards, the net uptake for both isomers appeared to be the same. Possibly because of the small number of observations and the large variability in results, selectivity in uptake might not have been detected.

Westfall (53) used a systematic approach to study the stereoselectivity of epinephrine accumulation in the rat heart. After equal intramuscular doses, 1 mg./kg., both (−)- and (+)-epinephrine caused a greater accumulation of (−)-isomer in the heart, with concomitant greater loss of endogenous norepinephrine. The granular fraction exhibited greater selectivity than other tissue fractions from the heart. Selectivity in accumulation of the physiologically more active isomer of epinephrine was also demonstrated in the mouse heart (54). Approximately 1 hr. after 2-mg./kg. doses, i.p., of epinephrine isomers, the accumulation of (+)-isomer was only one-third of that of (−)-isomer. In mouse femoral muscle, the accumulation difference between (−)- and (+)-isomers was very small.

Work demonstrating the stereoselectivity of norepinephrine isomer uptake and storage has continued to be reported. At relatively high concentrations of norepinephrine isomers, Mueller and Schideman (55) were unable to demonstrate the stereoselectivity in the particular fractions of cat atria. Reinvestigation of the problem with a modified technique eventually did show the differences at low concentrations of these isomers (56). The accumulation of total tritium from (±)-norepinephrine was relatively more inhibited by unlabeled (−)-isomer. Green and Miller (57) reported that *in vitro* labeled epinephrine accumulates in the rat uterus and that (−)-norepinephrine causes greater release of labeled epinephrine while (+)-norepinephrine is significantly less effective. These results indicate a possible stereoselectivity for uptake and/or release in norepinephrine isomers. Tissue perfusion provides a better test system to demonstrate selectivity for uptake than studies in intact animals with various routes of administration. Kirpekar and Wakade (58) used perfused cat spleen to investigate several factors influencing norepinephrine uptake. When (+)-norepinephrine was infused at 0.51 mcg./min. into the arterial supply of the spleen, 55% of the infused amount was recovered in the venous effluent. The recovery for that of (−)-norepinephrine was only 34%. Results indicate a 66% accumulation of (−)-norepinephrine by spleen and only 45% for (+)-norepinephrine.

Greater accumulation of exogenous norepinephrine can be demonstrated in the tissues if endogenous norepinephrine is depleted by a suitable agent which does not drastically block or impair the storage mechanism. Mackenna (59) used prenylamine-treated rabbits to study the uptake of norepinephrine and epinephrine isomers. After injection of 0.41 mg./kg. i.m. of either isomer, as compared to (+)-isomers, the accumulation and retention of (−)-isomers in the heart were higher, with longer duration. Similarly, von Euler and Lishajko (60) investigated the uptake of catecholamines in rabbit hearts depleted by decaborane. When the (−)- and (+)-epinephrines were given with (−)-norepinephrine, simultaneously and in equal amounts, relative to the uptake of (−)-norepinephrine the uptake of (−)-epinephrine was 2–3 times greater than that of (+)-epinephrine. It is suggested that when isomers are taken up in specific stores, the (−)- and (+)-isomers are released at the same rate.

The amine uptake in adrenergic nerve granules from bovine splenic nerves deserves special comment, since the study of specificity of uptake on such a simple system is more meaningful. The spontaneous depletion of endogenous norepinephrine from bovine splenic nerve granules is prevented more by (−)-norepinephrine than by the (+)-isomer. The amine uptake is enhanced by adenosine 5′-triphosphate (ATP). It is concluded that uptake is stereoselective at low concentrations (1 mcg./ml.) for naturally occurring isomers of both norepinephrine and epinephrine (61). The "affinity ratio," (−)-norepinephrine–(+)-norepinephrine, is reported as 5.9:1 (62).

However, reuptake of spontaneously released norepinephrine from the surrounding medium previously distorted the calculations of the affinity ratio of norepinephrine isomers. Von Euler and Lishajko (63) overcame this difficulty by studying the affinity ratio for uptake in the presence of potassium ferricyanide, an agent which continuously removes norepinephrine from the incubation medium of adrenergic nerve granules. The mean value for the affinity ratio found by the new method was 9.4. In the partially purified preparation of norepinephrine storage granules from rat heart, Potter and Axelrod (64) tried to demonstrate the affinity differences between isomers of norepinephrine. However, the differences did not appear to be great or significant.

Under proper conditions, condensation of formaldehyde with tissue catecholamines produces green fluores-

cent adrenergic nerves. Reserpine pretreatment depletes norepinephrine and, hence, fluorescence disappears. It can be reinstituted in the nerves by a suitable catecholamine. In catecholamine-depleted rat iris and salivary glands, after equal doses of either isomers of norepinephrine, an identical fluorescent intensity was observed in the nerves (65–67). Since the method is semiquantitative, small differences in the uptake might not have been detected. The optical isomers may differ in the rate of uptake and show identical accumulation at the end of a fixed time interval. On the other hand, it may be that the uptake mechanism in the rat iris and salivary gland and that in the heart are dissimilar. In his recent study, Iversen (68) was unable to observe differences between (−)- and (+)-isomers of amphetamine in regard to the affinity in the rat iris, but the same isomers showed a 20-fold difference in heart tissue. On this basis, he concluded that the uptake mechanism in two tissues from the same species may be different.

Although (−)- and (+)-isomers of (±)-*erythro*-levonordefrin[3] are resolved, (±)-pseudolevonordefrin or (±)-*threo*-levonordefrin are as yet unresolved. Hence, the question regarding stereospecificity of the levonordefrin molecule for uptake and storage is only partly answered. Muscholl and Lindmar (69) compared the uptake and binding of (−)-levonordefrin and (±)-*threo*-levonordefrin in the perfused rabbit heart. Like (−)-levonordefrin, (±)-*threo*-levonordefrin was readily taken up and retained by the heart with concomitant equivalent loss of norepinephrine. While initial uptake for (−)-levonordefrin, (±)-levonordefrin, and (±)-*threo*-levonordefrin appeared to be the same, their release rates were different. Similarly, in the mouse heart at various times after injection of 20 mcg./kg. i.v. of (±)-*threo*-^{3}H-levonordefrin or either (±)-*erythro*-^{3}H-levonordefrin, there was essentially equal uptake of both *erythro*- and *threo*-levonordefrin (70). But (±)-*threo*-levonordefrin (half-life, 20 hr.) appeared to leave faster than the (±)-*erythro*-form (half-life, 72 hr.). In the catecholamine-depleted rat iris, Patil and Jacobowitz (71) studied reinstitution of fluorescence caused by isomers of levonordefrin. It was observed that (−)- and (+)-levonordefrin produced equal fluorescence intensity, indicating possible equal affinity for uptake and/or storage sites. However, even higher doses of (±)-*threo*-levonordefrin failed to produce fluorescence in the nerves. Thus, histochemical evidence in rat iris is against that found in the heart. As previously stated, it is very likely that uptake characteristics are very different in the two tissues.

Under the influence of the MAO inhibitor, nialamide, the release of labeled (±)-*erythro*-levonordefrin from the mouse heart is not influenced, while (±)-*threo*-levonordefrin is virtually lost after 18 hr. (72). During the first 6 hr. after infusion, (±)-*erythro*-*N*-methyl-levonordefrin[4] retained by the heart was lost with a half-time of 5 to 6 hr. Likewise, (±)-*threo*-*N*-methyl-levonordefrin was taken up by heart and spleen. However, the concentrations did not decrease exponentially with time and the initial rates of loss were greater than those observed with (±)-*erythro*-*N*-methyl-levonordefrin (73, 74). Histochemical work of Patil and Jacobowitz (71) indicates that (±)-*erythro*-*N*-methyl-levonordefrin restores the fluorescence in catecholamine-depleted rat iris, while (±)-*threo*-*N*-methyl-levonordefrin does not. These findings are similar to those for (±)-*erythro*- and (±)-*threo*-levonordefrin.

Shore and Alpers (75) developed a sensitive and specific fluorometric method for the estimation of metaraminol in tissues. Since chemical methods do not distinguish between optical isomers of metaraminol, it has provided a valuable tool for studying stereoselectivity of these molecules. It was observed (76) that after injection of 50 mcg./kg. i.v. of either (−)-metaraminol, (+)-metaraminol, or (±)-deoxymetaraminol, only (−)-metaraminol was retained in the rat heart for up to 24 hr. The tissue concentration of (+)-metaraminol and (±)-deoxymetaraminol fall sharply 10–15 min. after administration. Lack of accumulation of (−)-metaraminol in immunosympathectomized animals indicated that accumulation of (−)-metaraminol in normal animals was in sympathetic nerves only. Intracellular distribution of these agents in different fractions from the heart revealed that (−)-metaraminol displaces heart norepinephrine and is significantly associated with particulate cell fraction, whereas (±)-deoxymetaraminol neither depletes norepinephrine nor is associated significantly with cell particles. Although (+)-metaraminol does not deplete norepinephrine, it shows some association with cell particles (77). *In vitro*, rabbit heart slices also exhibit selective accumulation of (−)-metaraminol over (+)-metaraminol or (±)-deoxymetaraminol. Pretreatment of these slices with imipramine or ouabain markedly inhibited accumulation of both isomers of metaraminol (78). Reserpine pretreatment does not prevent accumulation of (−)- and (+)-metaraminol. But, combined treatment of reserpine and ouabain markedly prevents the accumulation of (−)-metaraminol more than ouabain alone. A similar combination is not synergistic for prevention of the accumulation of (+)-metaraminol. On this basis, it is suggested that reserpine, perhaps by creating local ionic imbalance in the cell, allows ouabain to exert a greater effect on the local Na^+-K^+-ATPase-amine pump-linked mechanism which normally allows the (−)-form of catecholamine to be accumulated more rapidly than the (+)-form (79). Both in the normal and the reserpine-pretreated heart slices, the washout rate for amines is (±)-deoxymetaraminol > (+)-metaraminol > (−)-metaraminol. However, amines are washed out more easily in the reserpine-pretreated animals (80). Using (±)-metaraminol-^{3}H, Lundborg (81) and Lundborg and Stitzel (82) elaborated on the stereoselectivity of (−)- and (+)-metaraminol in the different tissue fractions from mouse heart. The two isomers of metaraminol were able to displace (±)-metaraminol-^{3}H from subcellular fractions of the mouse heart when given 15 min. after administration of the labeled amine. The (−)-form was more effective. If unlabeled isomers were given 24 hr. after (±)-metaraminol-^{3}H, only the (−)-isomer was an effective agent. In the adrenal medullary granules, (−)-metaraminol was more effective in preventing the uptake of (±)-metaraminol-^{3}H than the (+)-form (83).

[3] Cobefrin.
[4] α-Methylepinephrine or dihydroxyephedrine.

Table I—Release of ^{3}H-Norepinephrine (^{3}H-NE) from Mouse Hearts by Optical Isomers and Deoxy Derivatives of Sympathomimetic Agents

Agent	Steric Structure	Dose,[a] mg./kg. s.c.	^{3}H-NE in Heart[b] — % Control	% Depletion
(−)-Norepinephrine bitartrate	1R	2.5	33	67
(+)-Norepinephrine bitartrate	1S	2.5	57	43
Deoxynorepinephrine	—	5	50	50
(−)-Epinephrine bitartrate	1R	2.5	36	64
(+)-Epinephrine bitartrate	1S	2.5	62	38
Deoxyepinephrine	—	5	55	45
(−)-Levonordefrin	1R	2.5	20	80
(±)-Deoxylevonordefrin (methyldopamine)	—	5	39	61
(−)-Metaraminol bitartrate	1R	5	22	78
(±)-Deoxymetaraminol (α-methyl-*m*-tyramine)	—	5	38	62
		10	34	66
(−)-*m*-Octopamine tartrate (*m*-tyramine)	1R	5	38	62
Norephedrine	?	10	68	32
(−)-Deoxynorephedrine	2R	10	86	14
(+)-Deoxynorephedrine	2S	10	58	42
Phentermine	—	10	95	5
Ephedrine HCl	?	10	91	9
(+)-Pseudoephedrine	1S,2R	10	84	16
(+)-Deoxyephedrine	2R	10	62	38
Mephentermine	—	10	100	0

[a] Drugs were administered 60 min. after intravenous ^{3}H-NE. [b] Two hours after drug administration. Data after Daly *et al.* (89).

Histochemically, the accumulation of isomers of dopa has been studied in several tissues. The number of green fluorescent enterochromafinlike cells that could be induced by administration of the (+)-isomer of dopa was usually smaller than that after the (−)-isomer (84). The transport of amino acid into islet cells of the rat pancreas was selective, since accumulation of (+)-dopa could not be demonstrated histochemically even after pretreatment with nialamide. In contrast to this, fluorescence developed in the exocrine cells, both after administration of (−)- and (+)-dopa (85, 86). Similarly, the parafollicular cells of mouse thyroid (87) and the endothelial cells in the capillaries of mouse brain (88) selectively accumulate the (−)-isomer of dopa. When the rat iris is incubated with (−)- or (+)-dopa, a marked diffused fluorescence is seen after (−)-dopa, while (+)-dopa is selectively accumulated in the iris capillaries.[5]

STERIC STRUCTURE–ACTIVITY RELATIONSHIPS FOR RELEASE OR DEPLETION OF ENDOGENOUS AMINES BY SYMPATHOMIMETIC AGENTS

Tissue norepinephrine-depleting effects of certain isomers are described in the previous section. Many studies, which are summarized in this section, were designed for obtaining information regarding a structural requirement for releasing biogenic amines. Daly *et al.* (89) investigated the norepinephrine-releasing potencies of a wide variety of sympathomimetic amines and related compounds. Endogenous cardiac norepinephrine was prelabeled with 5 μc. of (±)-norepinephrine-7-^{3}H with a compound that caused loss of tritiated norepinephrine. Relative activities of some selected agents are presented in Table I. It is clear that monophenolic or diphenolic amines with 1R stereochemistry are better at depleting cardiac norepinephrine than are corresponding isomers with 1S stereochemistry. Nonphenolic amines, in general, were very weak in depleting norepinephrine-7-^{3}H. (+)-Deoxynorephedrine appeared to be a better depleting agent than (−)-deoxynorephedrine. Because enzymes in biotransformation of these agents were not inhibited, it is open to question whether observed differences between (+)- and (−)-deoxynorephedrine are valid. Loss of asymmetry by substitution of one more α-methyl group in deoxynorephedrine and deoxyephedrine made molecules very weak in depleting cardiac ^{3}H-norepinephrine.

Shore (14) compared the norepinephrine-depleting activity of various sympathomimetic amines in which a limited number of optical isomers was included. Drugs were given intraperitoneally to rats; 3 hr. later the change in heart norepinephrine was measured. Drugs used were optical isomers of *p*-hydroxyamphetamine, levonordefrin, and metaraminol. The structure–activity relationships showed again that (−)-metaraminol (1R) in relatively low doses, 1 mg./kg., is the most effective in depleting cardiac catecholamines. Higher doses of deoxymetaraminol (10 mg./kg.) were also effective in lowering the tissue norepinephrine, but this activity was inhibited by benzyloxyamine, an inhibitor of dopamine-β-hydroxylase.

In the isolated perfused rat heart, after labeling endogenous norepinephrine stores, release of tritiated norepinephrine by optical isomers of catecholamines was investigated by Nash *et al.* (90). The release rate for (−)-isomers of norepinephrine, epinephrine, and isoproterenol was higher than for corresponding (+)-isomers. *In vivo*, tyramine is rapidly converted to its β-hydroxylated product, octopamine. Carlsson and Waldeck (91) used this approach to study structure–activity relationships for release of ^{14}C-octopamine that was formed from ^{14}C-tyramine. Mice were injected with 0.2 mg./kg. i.v. of ^{14}C-tyramine. Fifteen minutes was allowed for conversion of ^{14}C-tyramine to ^{14}C-octopamine. Various amines were injected, and after 15 min. the loss of ^{14}C-octopamine from the heart was determined. The steric structure–activity relationship for releasing ^{14}C-octopamine is very similar to that for releasing norepinephrine.

Availability of all four isomers of ephedrine enabled Abdallah *et al.* (17, 92) to investigate the norepinephrine release by these agents. In the perfused rabbit heart, (−)-ephedrine is more effective in both the rate-accelerating effect and releasing the cardiac norepinephrine in the perfusate. However, at equivalent doses, (+)-ephedrine, (−)-pseudoephedrine, and (+)-pseudoephedrine released approximately the same amount of norepinephrine in perfusate, but their heart rate-accelerating effects were not the same.

Recently, synthesis, resolution, and pharmacology of (±)-*threo*-metaraminol and related agents have been carried out by Saari *et al.* (93), Torchiana *et al.* (44), Waldeck (94), and Carlsson *et al.* (95). (−)-α-Methyldopamine (deoxylevonordefrin) is approximately 3 times as potent as its (+)-form in causing norepinephrine depletion from mouse heart, while for similar effects (+)-α-methyl-*m*-tyramine is more active than its (−)-form. A reasonable and logical explanation for the

[5] B. Hamberger and T. Malmfors, personal communication, 1969.

higher norepinephrine-depleting effects of (+)-α-methyl-*m*-tyramine (deoxymetaraminol) is that this agent is converted to (−)-metaraminol, which is a very potent agent in depleting endogenous norepinephrine. The (−)-form may not undergo similar biotransformation; in addition, the (−)-form might be inactive as such. The norepinephrine-depleting effects of the (−)- and (+)-forms of α-methyldopamine are quite opposite to those of isomers of α-methyl-*m*-tyramine. Under *in vitro* conditions when (+)-forms are incubated with semipurified dopamine-β-hydroxylase from bovine adrenal medulla, the corresponding (−)-forms of amine metabolites [namely (−)-levonordefrin from (+)-α-methyldopamine and (−)-metaraminol from (+)-α-methyl-*m*-tyramine] can be obtained. The (−)-forms of both α-methyldopamine and α-methyl-*m*-tyramine failed to yield corresponding β-hydroxylated products. This indicates that marked norepinephrine-depleting effect of (−)-α-methyldopamine might be caused by the parent molecule itself. Furthermore, it is probable that dopamine-β-hydroxylase is also inhibited by this agent. However, exogenous administration of (±)-*threo*-levonordefrin can cause dose-dependent reduction of cardiac norepinephrine, with a concomitant fall of blood pressure in rats (96). As compared to (±)-*threo*-levonordefrin, (±)-*threo*-metaraminol appears to be much less active in depleting cardiac norepinephrine from the heart.

So far, only a limited number of optical isomers have been studied. Validity of any structure–activity relationship can only be studied by investigating structurally similar isomers on one system under well-controlled experiments. Norepinephrine depletion should not only be studied at equimolar doses but at a dose which will cause equivalent depletion or release. The former criterion is easier than the latter one.

INHIBITION OF UPTAKE

Several drugs are known to inhibit the uptake of exogenous norepinephrine into the sympathetic nerve endings. In the isolated perfused rat heart, Burgen and Iversen (97) made a systematic study of chemical structure of sympathomimetic amines and its relationship to inhibition of (±)-^{14}C-norepinephrine uptake. Similarly, in the perfused rabbit heart, Muscholl and Weber (98) studied the inhibition of uptake of levonordefrin by some sympathomimetic amines. The following generalizations can be made regarding steric structure–action relationships: (*a*) In phenolic amines, deoxy derivatives are more potent than their corresponding (−)-isomers which are in turn more potent than (+)-isomers. (*b*) In nonphenolic amines such as ephedrine and norephedrine, deoxy derivatives are also more potent than (−)-isomers. But in the latter case, the situation is complicated by two asymmetric carbons. (*c*) (+)-Deoxynorephedrine is about 20 times more potent in inhibiting norepinephrine uptake than that of (−)-deoxynorephedrine. (*d*) (+)-Norpseudoephedrine is more potent than either (−)-norephedrine or (+)-deoxynorephedrine (Table II). Ross and Renyi (99) investigated effects of a series of amines on accumulation of (±)-norepinephrine-^{3}H in cortex slices from mouse brain. As in the heart, uptake of tritiated norepinephrine resembles that of the enzymatic reaction according to Michaelis-Menton. In a concentration-dependent manner, various sympathomimetic amines prevented accumulation of tritiated norepinephrine in brain slices. However, the slopes of these dose–response curves varied considerably. Isomers, (−) and (+), of metaraminol exhibited distinct differences; the (−)-form was more potent. At the lowest concentration, 0.1 mcg./ml. of (−)-norepinephrine and (±)-norepinephrine, the inhibition of uptake was identical. This possibly indicates that (−)- and (+)-isomers of norepinephrine may not differ in preventing accumulation of tritiated norepinephrine. It is difficult to understand why isomers of metaraminol continue to exhibit stereospecificity at various concentrations while isomers of norepinephrine, amphetamine, and norphenylephrine did not differ in this respect.

Table II—Inhibition of Norepinephrine Uptake by Sympathomimetic Amines in the Rat Isolated Heart

Agent	Steric Configuration	ID_{50},[a] *M*	Relative Inhibition of Uptake[b]
Phenethylamine	—	1.1×10^{-6}	1.00
(−)-Norepinephrine	1R	2.7×10^{-7}	4.07
(+)-Norepinephrine	1S	1.4×10^{-6}	0.78
Deoxynorepinephrine	—	1.7×10^{-7}	6.50
(−)-Epinephrine	1R	1.0×10^{-6}	1.10
(±)-Epinephrine	—	1.4×10^{-6}	0.78
Deoxyepinephrine	—	7.6×10^{-7}	1.45
(−)-Levonordefrin	1R,2S	2.0×10^{-7}	5.50
(±)-Levonordefrin	—	4.2×10^{-7}	2.60
(±)-Deoxylevonordefrin (methyldopamine)	—	1.8×10^{-7}	6.10
(−)-Metaraminol	1R,2S	7.6×10^{-8}	14.40
(−)-Ephedrine	1R,2S	2.2×10^{-6}	0.50
(+)-Deoxyephedrine	2S	6.7×10^{-7}	1.65
Mephentermine	—	1.0×10^{-6}	1.10
(+)-Deoxynorephedrine	2S	1.8×10^{-7}	6.10
(−)-Deoxynorephedrine	2R	3.7×10^{-6}	0.30

[a] Drug concentration producing 50% inhibition of norepinephrine uptake. [b] Relative inhibition = (ID_{50} of phenethylamine)/(ID_{50} of drug). Data in part taken from Burgen and Iversen (97).

Independently and simultaneously, Thoenen *et al.* (100) and Ross *et al.* (101) have observed that tissue accumulation of labeled nonphenolic agents such as amphetamine, norephedrine, and phenylethanolamine are not influenced by cocaine. They have suggested that tissue accumulation of nonphenolic amines is a function of their physical–chemical properties, while that of monophenolic or diphenolic amines is a function of the hydroxyl group. These studies suggest that some precautions are needed in comparing uptake inhibition by phenolic and nonphenolic amines. It is reasonable to compare activity of (−)- and (+)-isomers on any single parameter because their physical–chemical properties are identical. However, to make any generalizations regarding steric structure–activity, a study of a large number of antimeric pairs of both phenolic and nonphenolic amines is required.

IMPLICATIONS OF UPTAKE, STORAGE, INHIBITION OF UPTAKE, AND RELEASE OF ADRENERGIC DRUGS

Endogenously released or exogenously administered (−)-norepinephrine is mainly inactivated by rapid uptake into the adrenergic nerve endings. (+)-Norepineph-

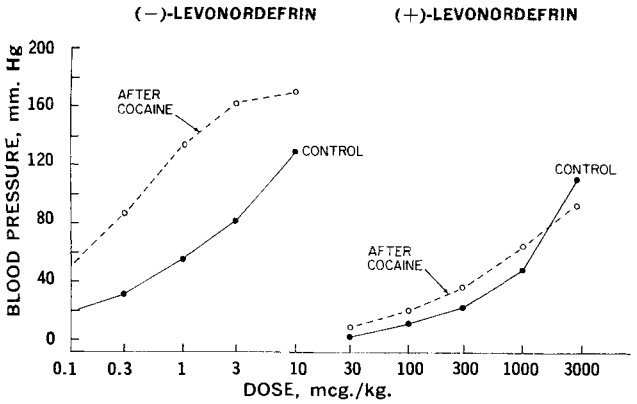


Figure 1—*Stereoselective potentiation of isomers of levonordefrin by cocaine (5 mg./kg. i.v.) in the spinal cat. Data in part obtained from Tye* et al. *(109).*

rine has very little affinity for both uptake and storage site (see *Inhibition of Uptake*). The physiologic implications of these findings are reflected in the transmitter economy. The naturally occurring (−)-norepinephrine and (−)-epinephrine are most efficiently utilized. One important concept that has emerged from the study of uptake and storage mechanisms is the concept of "false neurochemical transmitters." It is possible to substitute a molecule which is weaker than the natural neurohormone in activating pharmacologic receptors. These agents have proved to be very valuable substances in the treatment of hypertension. The classical example is that of α-methyldopa. The biotransformation and the formation of the false neurochemical transmitter are discussed in the previous section.

Because of modern instrumentation and laboratory facilities, many research projects are being carried out at a much faster rate than they were 20–30 years ago. There is also no doubt that many recent studies are better designed than before; however, it is of historical importance to comment on early reports concerning optical isomers of various adrenergic drugs. Tainter (102) and Luduena *et al.* (103) reported that pressor effects of isomers of norepinephrine and epinephrine were potentiated by cocaine. On the other hand, pressor effects of (+)-levonordefrin and (+)-phenylephrine were unaffected or desensitized by cocaine (104, 105). The intestinal smooth muscle of an unanesthetized dog is relaxed by (−)-norepinephrine and (−)-epinephrine. This effect was potentiated by cocaine, while that of (+)-isomers was unaffected or slightly antagonized by cocaine (106).

Trendelenburg (107) and Trendelenburg *et al.* (108) studied sensitization by cocaine for (−)- and (+)-isomers of norepinephrine and epinephrine. The cat nictitating membrane effect caused by (−)-isomers was sensitized more than the corresponding (+)-isomers. Independently, Tye *et al.* (109) also investigated the influence of cocaine on the isomers of norepinephrine, epinephrine, levonordefrin, and phenylephrine. In both normal and catecholamine-depleted cats, the nictitating membrane and blood pressure effects of (−)-isomers were sensitized by cocaine. When studied similarly, the effects of (+)-isomers were less sensitized or desensitized. Unequal potentiation of optical isomers was explained on the basis of previously reported unequal rates of uptake of these agents into the adrenergic neurone. In terms of the structure–activity relationship, it is clear that 1R stereochemistry is necessary for stereoselective or stereospecific sensitization by cocaine. However, the role of the β-hydroxyl group becomes secondary, if the molecule is heavily substituted on nitrogen. (−)-Isoproterenol has 1R stereochemistry at C_1 but is not potentiated by cocaine. Draskóczy and Trendelenburg (110) found that over the wide range of concentrations, (−)- or (+)-norepinephrine is removed from perfusate by the heart at approximately equal rates. In the same tissue, however, cocaine did produce marked stereospecific supersensitivity in favor of the (−)-isomer. Since perfused rabbit heart did not exhibit stereospecific uptake and since cocaine did not potentiate (+)-norepinephrine, they proposed that the lack of potentiation of (+)-norepinephrine by cocaine is caused by low potency of this isomer, which exerts pharmacological effects only in concentrations that saturate the uptake mechanism. The effect of cocaine becomes negligible when the uptake site is saturated (111). It was also demonstrated that in the lower concentration, (+)-norepinephrine, which caused little effect, markedly potentiated the positive inotropic effects of the (−)-isomer. According to these observations, the stereospecificity of the sensitizing effect of cocaine can no longer be ascribed to differences in the rate of uptake of the isomers. Seidehamel *et al.* (112) selected dopamine in an attempt to determine the importance of the positional role of the β-hydroxyl group. This agent is structurally similar to norepinephrine, except it lacks the β-hydroxyl group. The sensitization of the nictitating membrane to these agents produced by cocaine has the following sequence: (−)-norepinephrine > dopamine = (+)-norepinephrine. West *et al.* (113) also attempted to explain the unequal potentiation by cocaine of (−)- and (+)-isomers of norepinephrine, metaraminol, and octopamine in relation to degree of uptake. Cocaine-induced supersensitivity in atria and the rate of uptake occurred in a diminishing fashion: (−)-norepinephrine > (+)-norepinephrine > (−)-metaraminol > (−)-octopamine > (+)-metaraminol > (+)-octopamine. Here, again, saturation of uptake rather than rates of uptake might be an important factor in explaining the rank order. Desipramine, a cocainelike agent, is reported to produce stereoselective sensitization of norepinephrine isomers on rat vas deferens (114). Two other agents, namely prenylamine (115) and α,α′-bis(dimethylammoniumacetaldehyde diethylacetal)-*p,p′*-diacetylbiphenylbromide (DMAE), also behave like cocaine (116). These agents markedly potentiate pressor effects of (−)-norepinephrine and produce little or no potentiation of (+)-norepinephrine. In anesthetized cat nictitating membrane, after the administration of DMAE or cocaine, Wong and Long (116) did not observe a stereospecific sensitization for (−)- and (+)-epinephrine. Thus, it appears that stereospecific or stereoselective sensitization after cocaine or cocainelike agents would be best demonstrated in an antimer which exhibits high isomeric ratio in activation of pharmacologic receptors, as well as high affinity difference for uptake sites [*e.g.*, (−)- a nd (+)-isomers of levonordefrin] (Fig. 1). As compared to other antimers, the isomeric

ratio and difference in uptake for (−)- and (+)-epinephrine are small. Hence, it may be difficult to obtain a clearcut separation by sensitizing effects of one isomer over another.

Before leaving this section, it should be emphasized that stereoselective potentiation of norepinephrine isomers by a given agent should not be taken as a criterion for cocainelike effects. A ganglionic blocker, mecylamine, which does not resemble cocaine in any respect, also causes stereoselective potentiation of norepinephrine isomers (117) (Fig. 2).

Various drug treatments and surgical procedures are known to produce supersensitivity to certain sympathomimetic agents. For further information, readers are referred to an excellent review on this subject (118). Surgical removal of sympathetic ganglia causes degeneration of postganglionic fibers. A few days after the procedure, there is a marked loss of transmitter. The end organ continues to develop a supersensitivity, at least until the 28th postoperative day (119). Denervation supersensitivity has two components: (*a*) cocainelike and (*b*) nonspecific, such as described for decentralization. With any agent that interferes with the release of the neurotransmitter, the end organ develops a supersensitivity which is more like that produced by surgical decentralization. Chronic treatment with reserpine produces a decentralization type of supersensitivity.

The idea of utilizing optical isomers of sympathomimetic amines to study supersensitivity caused by denervation can be traced to as early as 1939 (120). However, it was only after the role of these agents for uptake, binding, and release had been clarified that they became useful tools for analysis of various types of supersensitivities. Trendelenburg (107) reported that 7 days after excision of the superior cervical ganglia of a cat, the denervated nictitating membrane was nearly 100 times more sensitive to (−)-norepinephrine than the corresponding normal side of the same animal. The sensitivity to (+)-norepinephrine, on the other hand, increased only by a factor of 3.5. Soon after this, it was realized that the variable tone of the denervated nictitating membrane of the spinal cat, determined under ether, complicates the accurate estimation of supersensitivities (121). Seidehamel *et al.* (122), therefore, studied the various types of supersensitivities in both normal spinal cat as well as preparations in which tone of the nictitating membrane was reduced to a relatively stable level by acute treatment with reserpine. It was concluded that supersensitivities caused by decentralization and chronic treatment with reserpine cause stereoselective potentiation in favor of (−)-norepinephrine. However, denervation supersensitivity appeared to be nonstereoselective, because both isomers of norepinephrine were equally sensitized by this procedure. The role of COMT becomes important when uptake mechanism is lost. Thus, during the investigation of stereoselectivity and nonstereoselectivity of denervation supersensitivity, the enzyme COMT should be inhibited by a stable enzyme inhibitor like tropolone. Langer *et al.* (119) reported equal sensitizing effects of dopamine and (+)-norepinephrine. Green and Fleming (123) observed that denervated cat spleen *in vitro* produced three- to fourfold potentiation of (−)-norepinephrine, while that

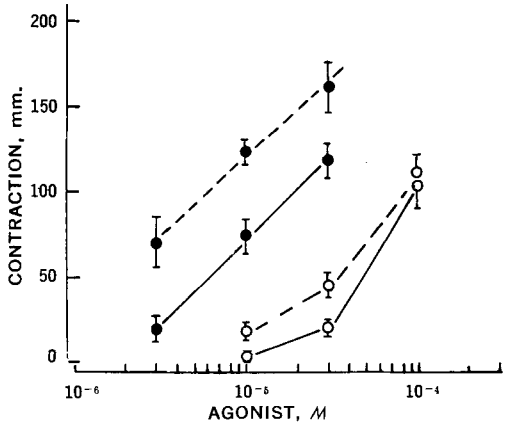


Figure 2—*Stereoselective potentiation of isomers of norepinephrine (NE) by mecamylamine (3-min. incubation). Isolated rat vas deferens was the test organ. Vertical lines are* SEM. *Data from Patil* et al. *(117). Key: ——, control; - - -, with mecamylamine, 3 × 10⁻⁴ M; ●, (−)-NE; and* ○, *(+)-NE.* n = 8.

of (+)-isomer was slightly desensitized. The supersensitivity resulting from chronic reserpine treatment could not be demonstrated in this tissue. Failure to demonstrate such a sensitivity *in vitro* was attributed to the isolational procedural stress. From these reports it appears that the whole problem of change in the activity of optical isomers of norepinephrine in relation to denervation supersensitivity deserves reinvestigation under well-controlled experimental conditions. After denervation, if there is a qualitative change in α-adrenergic receptors (*i.e.*, change in the configuration), it should be reflected in isomeric ratios of norepinephrine. Hence, the isomeric ratio after denervation should be different from that in the normal tissue after administration of cocaine (124).

After a series of well-controlled experiments in reserpine-pretreated spinal cat, Trendelenburg (125) reported an interesting finding with (−)- and (+)-isomers of norepinephrine. During nerve stimulation, after equieffective doses of isomers of norepinephrine, the nictitating membrane effects of (−)-norepinephrine were potentiated more than those of the (+)-isomer. These observations were explained on the basis that during nerve stimulation, injected (−)-norepinephrine is immediately available for release. The (+)-isomer has less affinity for the uptake site and, therefore, will not be stored. Hence, no potentiation will result.

Many sympathomimetic amines and related agents inhibit the uptake of norepinephrine into the sympathetic nerve endings. This implies that relatively more concentration of norepinephrine will then be available for activation of pharmacologic receptors. Since various sympathomimetic amines displayed varying inhibition of uptake of norepinephrine (97), it was anticipated that different sympathomimetic amines should produce varying potentiation of norepinephrine effects. In the isolated rat vas deferens, Swamy *et al.* (126) studied potentiation of (−)-norepinephrine by (+)- and (−)-amphetamine. At all concentrations, 10^{-7}–10^{-5} *M*, of amphetamine, the potentiation of exogenous norepinephrine was identical. In the presence of amphetamine, responses to the effects of endogenously released norepinephrine can be potentiated. Day (127) carried out ex-

periments on the isolated guinea pig vas deferens. The effects of (+)-, (−)-, and (±)-amphetamine were compared on the maximal response to sympathetic nerve stimulation over the frequency range 2–50 pulses/sec., using both pre- and postganglionic stimulation. It was found that both optical isomers and racemic mixture produced identical results. Thus, it appears that on vas deferens, isomers of amphetamine produce identical potentiation of exogenous or endogenous norepinephrine.

Swamy *et al.* (128) used a systematic pharmacologic approach to define some stereochemical characteristics of uptake site. An assumption was made that potentiation of exogenous norepinephrine is a faithful reflection of the ability of molecules to inhibit the uptake of norepinephrine. Only metabolically stable α-methylated amines were selected. Two isolated tissues from rat, vas deferens and right atria, were used to study the potentiating abilities of these agents. On both tissues at relatively low concentrations, 10^{-6} *M*, these drugs produced a potentiation of exogenous norepinephrine. The isomers were ranked according to their abilities to potentiate norepinephrine. In the isolated rat vas deferens, there was not a clearcut separation of steric structure and ability to potentiate norepinephrine. (−)-Metaraminol, which has the highest affinity for uptake site in the heart, was ranked 20th on the vas deferens. (−)-Amphetamine, which has a poor affinity for uptake, was ranked first. Rat vas deferens contains mainly α-adrenergic receptors. A given sympathomimetic amine can compete for both uptake and direct α-adrenergic receptors. If a given agent has higher affinity for both uptake and direct sites, then observed potentiation may not be a true reflection of the amine to inhibit uptake. In other words, norepinephrine, which is spared, will not produce its full effect on α-adrenergic receptors which might be occupied by the amine. In the isolated rat atria, which mainly contains β-adrenergic receptors, the isomers of various amines exhibited distinct stereochemical differences: (*a*) (−)-metaraminol ranked first in 20 agents tested; (*b*) (−)-isomers of norephedrine, ephedrine, and metaraminol ranked higher than their corresponding (+)-isomers; and (*c*) deoxy derivatives, such as α-methyl-*m*-tyramine, α-methyldopamine, amphetamine, and methamphetamine, ranked higher than their corresponding β-hydroxylated (+)-isomers. Thus, if potentiation is the faithful reflection of ability of an amine to inhibit uptake of (−)-norepinephrine, the obvious conclusion is that uptake characteristics differ in rat vas deferens as compared to atria. This view would be consistent with a recent report by Iversen (68).

As early as 1923, Gottlieb (129) studied the optical isomers of cocaine for local anesthetic activity. But a complete steric structure-activity of the cocaine molecule for inhibition of norepinephrine uptake is yet to be defined. Synthesis and/or resolution of these agents appear to be the main task. Schmidt *et al.* (130) investigated central and peripheral effects of (−)-cocaine and (+)-pseudococaine. Only (−)-cocaine was effective in both, producing central sympathetic stimulation and potentiating epinephrine. It can be concluded from these experiments that (+)-pseudococaine might not be an effective inhibitor of epinephrine uptake. However, (−)-cocaine and (+)-pseudococaine have identical local anesthetic properties. This indicates that inhibition of uptake and local anesthetic effects may not be causally related. (−)-Norepinephrine-potentiating activity of close structural analogs of cocaine, tropacocaine, and pseudotropacocaine was investigated on the isolated rat vas deferens (131). Pseudotropacocaine appeared to be slightly more active than tropacocaine. Both of these agents were only 1/30th–1/100th as active as (−)-cocaine. The effects of tyramine were affected differently. A large body of evidence indicates that cocaine, norepinephrine, and tyramine compete for the same site at the adrenergic nerve endings. As expected, effects of norepinephrine were equally shifted by cocaine to the left and right, respectively. However, tropacocaine and pseudotropacocaine did not affect norepinephrine and tyramine response to an equal degree. Tropacocaine caused a slight shift of norepinephrine responses to the left. On the other hand, the same concentration of tropacocaine markedly shifted the effects of tyramine to the right with reduction in maxima. Pseudotropacocaine did not affect tyramine at all, but the effect of norepinephrine was slightly potentiated. These results indicate that a noncompetitive interaction of tropacocaine and pseudotropacocaine with receptors in the adrenergic nerves cannot be excluded.

Because of two asymmetric centers, the antidepressant drug methylphenidate can be exhibited in four stereoisomeric forms. Clinically, only the (±)-*threo*-form is used. The (±)-*erythro*-form is devoid of central effects. Buckner *et al.* (132) evaluated comparative peripheral effects of (±)-*erythro*- and (±)-*threo*-forms of methylphenidate. On the rat vas deferens in potentiating (−)-norepinephrine at equiactive concentrations, (±)-*erythro*-methylphenidate has 1/300th the activity as that of the (±)-*threo*-form. A histochemical technique demonstrated that in rat iris the (±)-*threo*-form markedly inhibited uptake of levonordefrin while the (±)-*erythro*-form was without effect. Implications of these findings are that if CNS stimulant effects are causally related to inhibition of uptake of norepinephrine, a greater potency of (±)-*threo*-methylphenidate over that of the (±)-*erythro*-form explains its higher CNS stimulant activity. The absolute configurations, methylphenidates, pipradrols, and pheniramines, have been determined recently (133).

MECHANISM OF ACTION OF "INDIRECTLY" ACTING β-HYDROXYLATED (+)-ISOMERS AND THEIR CORRESPONDING DEOXY DERIVATIVES

Muscholl (5) has pointed out that "indirectly" acting amines not only cause release of stored norepinephrine but also cause inhibition of reuptake of released norepinephrine. Previously, Patil *et al.* (20) observed that in the normal vas deferens, indirectly acting deoxy derivatives always produced a greater magnitude of pharmacologic effects than their corresponding β-hydroxylated (+)-isomers. For example, deoxylevonordefrin (α-methyldopamine) produced greater effects than (+)-levonordefrin. These results were explained on the basis that deoxy derivatives enter the intraneuronal stores at a faster rate and displace the stored norepinephrine at a faster rate than the corresponding β-hydrox-

ylated (+)-isomer. An incorrectly oriented alcoholic hydroxyl group in (+)-isomer was believed to cause hindrance in uptake of these agents. However, it can be argued that both deoxy derivative and (+)-isomer are taken up at an equal degree and displace equal amounts of norepinephrine. The higher activity of deoxy derivatives then can be explained by greater inhibition of reuptake by the agent. The lower activity of the (+)-isomer is caused by its ability to inhibit the reuptake 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 deoxy derivative and (+)-isomer were the same as those in which they exhibit unequal pharmacological effects on the normal tissue. The deoxy derivative and corresponding (+)-isomer were tested on the contralateral vas deferens of the same reserpine-pretreated rat. It was observed that the deoxy derivative and corresponding (+)-isomer caused equal potentiation of exogenous norepinephrine (134). These observations strengthen the original suggestion that in the rat vas deferens greater indirect effects of the deoxy derivative over the corresponding (+)-isomer is caused by either faster uptake of the agent and/or faster displacement of norepinephrine. The (+)-isomer possibly lacks both effects. This hypothesis is illustrated in Fig. 3.

Since tissue accumulation of nonphenolic amines is not affected by cocaine (100, 101), a very basic question was raised. "Do all indirectly acting agents release the stored norepinephrine by the same mechanism?" Experiments were designed on the rat vas deferens to seek the answer. A group of phenolic and nonphenolic amines was selected. From previous studies (20), it was also known that certain indirectly acting amines produce different maximal effects. To get some clue regarding similarity and dissimilarity of closely related indirectly acting agents, their maximal effects were superimposed. Phenolic amines and nonphenolic amines did show dissimilar behavior. Thus, the results support the hypothesis that all indirectly acting agents do not act by the same mechanism.

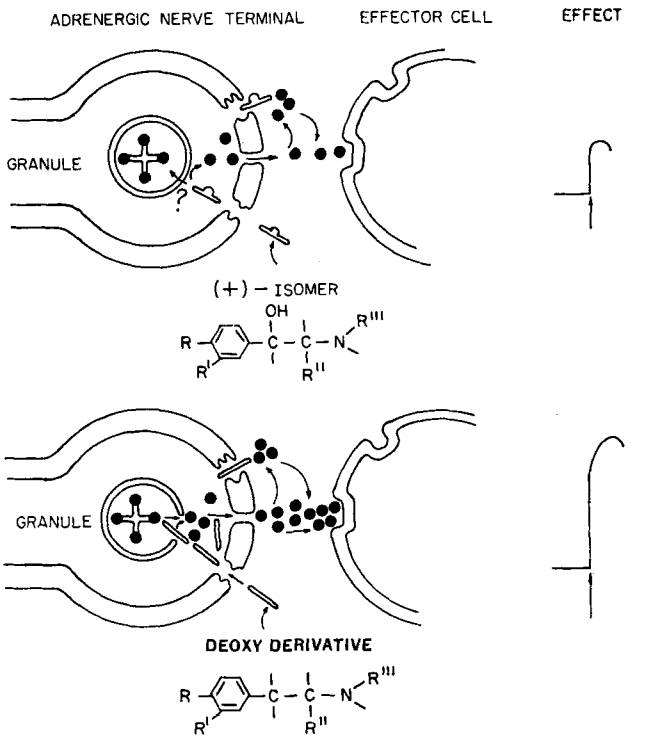


Figure 3—*Illustration of a possible cause of low activity of (+)-isomers when compared with the corresponding deoxy derivatives. Since exogenous norepinephrine (●) is equally potentiated by "indirectly" acting (+)-isomers and their corresponding deoxy derivatives, the inhibition of reuptake of endogenously liberated neurohormone may be the same for both agents. Thus, higher rate of neuronal uptake and/or increase in the basic release mechanism may be the cause for higher amplitude of contraction of deoxy derivative over that of (+)-isomer. In (+)-isomers the OH-group may be incorrectly oriented with respect to transport mechanisms, and the rate of transport, therefore, is slower than that of the corresponding deoxy derivative where such a group is absent. R = H, R′ = OH, R″ = CH_3, R‴ = CH_3. Reproduced, with permission, from Patil* et al., Arch. Int. Pharmacodyn. Ther., *189, 32(1969), and St. Catherine Press, Brugge, Belgium.*

PHARMACOLOGICAL EFFECTS OF OPTICAL ISOMERS OF ADRENERGIC DRUGS— α- AND β-ADRENERGIC AGONISTS

Great credit should go to Cushny (135–137) for his pharmacologic studies on the optical isomers of various substances. He has carefully documented earlier reports concerning optical isomers of many naturally occurring substances (135). Soon after the presence of the epinephrine was demonstrated in adrenal medulla, Cushny (136) examined the pressor effects of naturally occurring (−)-epinephrine and synthetic (±)-epinephrine in the anesthetized dog. The (±)-epinephrine was one-half as active as (−)-epinephrine. From these experiments, it became evident that optical isomers of epinephrine showed selectivity in pharmacologic effects. In the following year, 1909, Cushny (137) compared individual (−)- and (+)-isomers of epinephrine. He found that the (+)-isomer was 1/12th–1/15th as active as (−)-isomer in raising blood pressure.

The single most important contribution in explaining the behavior of optical isomers toward the specific pharmacologic receptors came from the reports of Easson and Stedman (138). The theory was proposed that in an asymmetric molecule like (−)-epinephrine, three of the four groups linked to the asymmetric carbon are concerned in the attachment with the receptor. These groups are: (*a*) the basic nitrogen; (*b*) the aromatic group (with *m*- and *p*-hydroxyl groups which determine the intensity of attachment); and (*c*) the alcoholic hydroxyl group. In the (+)-isomer, since the alcoholic hydroxyl group is oriented in the wrong position, only two-point interaction is expected. This view was strengthened by the fact that deoxyepinephrine, which lacks the alcoholic hydroxyl group, is equiactive with (+)-epinephrine. This theory was elaborated by Blaschko (139) and Beckett (140). It can be illustrated as shown in Fig. 4. On guinea pig ileum, Wilson (141) tested (−)-epinephrine and (+)-epinephrine. According to this theory, the (−)-isomer was more active than the (+)-isomer and epinine was equiactive to (+)-epinephrine. However, Badger (142) collected the results from a number of reports and pointed out that in many cases the activity of deoxy derivatives and (+)-isomers was

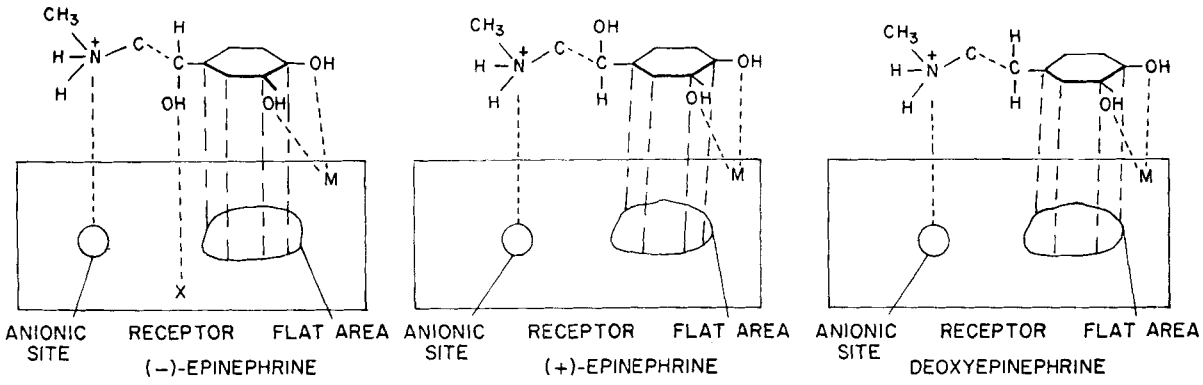


Figure 4—*Suggested interactions of optical isomers of epinephrine and deoxyepinephrine with adrenergic receptors [after Easson and Stedman (138), Belleau (199), and Beckett (140)]. According to Easson and Stedman, the correct orientation of the β-hydroxyl group of (−)-isomer results in higher activity while incorrect orientation of this group in (+)-isomer or the lack of the group in deoxy derivative would result in lesser but equal intensity of effect. In other words, (+)-isomer acts as if the β-hydroxyl group is missing (140).*

not in harmony with the Easson-Stedman hypothesis. For many years, Badger's argument against the theory remained unanswered.

In the meantime, the concept of directly and indirectly acting amines has been widely accepted. Patil *et al.* (20, 143) selected a series of (−)- and (+)-isomers and their deoxy derivatives of sympathomimetic amines in order to test the Easson-Stedman hypothesis. Vas deferens from normal as well as reserpine-pretreated rats were used for the experiments. On the normal vas deferens, the (−)-isomer was always more active than the (+)-isomer, but many corresponding deoxy derivatives were more active than (+)-isomers. When the endogenous norepinephrine in the vas deferens was depleted by reserpine treatment, a very interesting result was obtained. The (−)-isomers retained their higher activity, as in normal tissue, but many (+)-isomers and corresponding deoxy derivatives appeared to have large, unequal, indirect components of action. Both the (+)-isomer and the deoxy derivative were equiactive in the reserpine-pretreated tissues. Results are illustrated in Fig. 5. In other words, in catecholamine-depleted tissues, results were in harmony with the Easson-Stedman hypothesis. These observations suggest that the hypothesis holds true for sites of direct action only (α- or β-adrenergic).

From this study, another question emerged. When catecholamine stores are intact, why are deoxy derivatives more active than the (+)-isomers? A possible explanation is that in (+)-isomers, orientation of the β-hydroxyl group is such that it may cause hindrance of transfer from extraneuronal sites to intraneuronal sites, thereby liberating lesser amounts of neurohormone at slower rates. With the deoxy amines, which lack the β-hydroxyl group, there is no such hindrance to transfer and these amines will be taken up by the storage site more easily and produce greater pharmacologic effects because of a faster rate of release of neurohormone. (This view is discussed and illustrated in a previous section.) Even *in vivo* systems, after depletion of catecholamines, (+)-isomers, and their deoxy derivatives, appear to produce equal pharmacologic effects (16, 144, 145). Dopamine causes biphasic or vasodepressor response in the anesthetized rabbit. Under similar conditions, (+)-norepinephrine produces a pressor effect. It was anticipated that a similar activity difference could be detected on the rabbit aorta. Although the shapes of the dose–response curves of (+)-norepinephrine and dopamine were slightly different, the ED_{50} values were similar (146). (−)-Norepinephrine causes relaxation of the rat fundus strip. Both (+)-norepinephrine and dopamine have some 1/250th the activity of (−)-norepinephrine.[6] The activities of (+)-norepinephrine and deoxynorepinephrine are identical, indicating a possible similar interaction of these drugs as suggested by Easson and Stedman. Furthermore, this theory holds true for the drug effects on skeletal muscle (147).

By use of isomers of norepinephrine, an attempt has been made to characterize the nature of the receptor material. Because cell walls are composed of phospholipids, it is implied that these lipids might be involved in the transport of drug in the adrenergic neurone or in the interaction of drugs at the pharmacologic receptors. For the *in vitro* system, isomers of norepinephrine failed to show differences in their interaction with lecithin (148). This is in contrast to the well-known stereoselectivity of the pharmacologic receptors. Failure of an *in vitro* system to demonstrate a difference should not be taken as evidence against the implication of phospholipids as the receptor material.

On the basis of similar patterns of potency of agonists and dissociation constants of antagonists, Furchgott (18) suggested that α-adrenergic receptors in rabbit aorta, muscle from the corpus of the stomach, and duodenum appear to be of a single type. This suggestion can be reexamined on the basis of activity ratio of optical isomers and the activity ratio of (+)-isomers and the deoxy derivative. If α-adrenergic receptors are identical in different tissues under proper conditions, one should obtain a similar isomeric potency ratio in all these tissues (124). Furthermore, if the Easson-Stedman hypothesis is true for interaction of agonists with α-adrenergic receptors, the activity of (+)-isomers and deoxy derivatives should be identical. Data in Table III are obtained from different reports, because the criteria for obtaining an isomeric ratio for antimers of norepinephrine are relatively constant.

[6] J. R. Vane, personal communication, 1969.

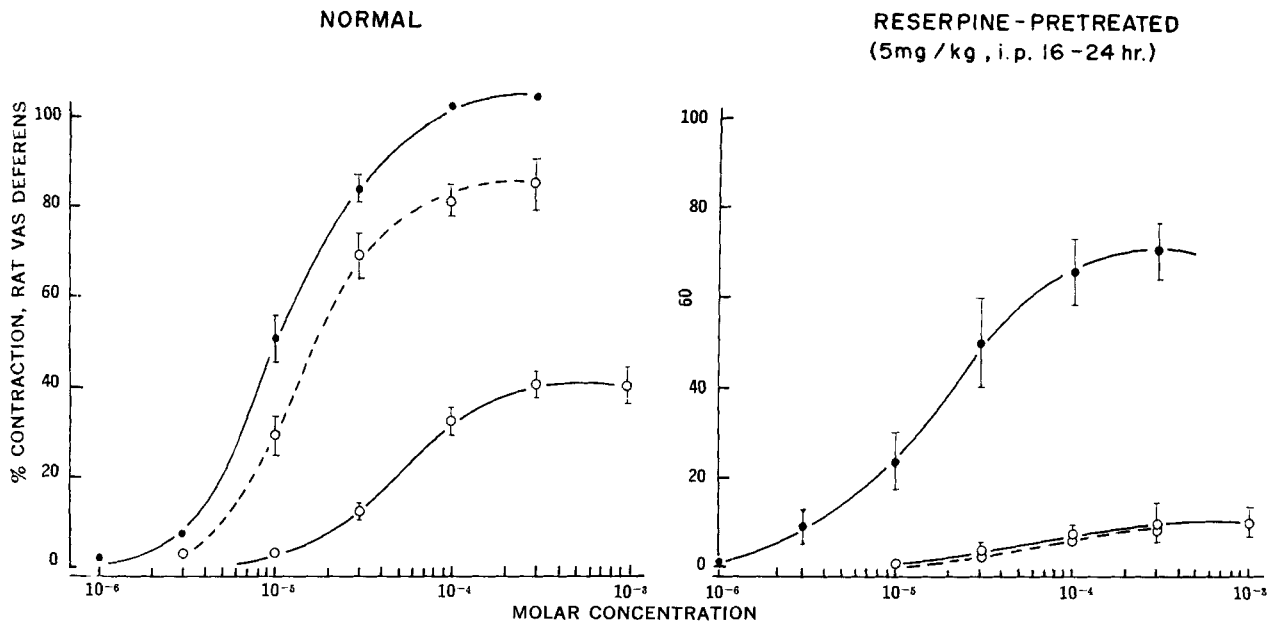


Figure 5—*Dose-response curves of (−)-levonordefrin (—●—), (+)-levonordefrin (—○—), and (+)-deoxylevonordefrin (- -○- -) in normal and reserpine-pretreated rat vas deferens. Note that the Easson and Stedman hypothesis only holds true in catecholamine-depleted tissue. Data after Patil and Jacobowitz (71).*

Fortunately, the isomeric ratios for isomers of norepinephrine from seven different test preparations containing mainly α-adrenergic receptors were readily available. In tissues which contain a high density of adrenergic nerves, the isomeric ratio for norepinephrine should be compared in the presence of cocaine. It can be seen from Table III that within the limits of experimental error, the isomeric ratios for norepinephrine isomers are approximately equal. Furthermore, the activities of (+)-norepinephrine and deoxynorepinephrine were approximately equal. On the basis of this data, new experiments were designed to test the validity of this concept. It was observed that after block of uptake by cocaine, isomeric ratios of norepinephrine on rat vas deferens, seminal vesicles, rabbit aorta, ileum, and spleen are equal (151). Since stereoselectivity is one of the best established properties of receptors, determination of isomeric ratios should provide an excellent criterion to differentiate the receptors. It seems probable, therefore, that the α-adrenergic receptors in all of the mentioned tissues are of a single type. The suggestion by van Rossum (149) regarding different types of α-adrenergic receptors in different tissues appears less tenable.

Conversely, if β-adrenergic receptors are of dissimilar types, the isomeric potency ratio of a given pair of isomers in different tissues containing β-adrenergic receptors should be different (124). The isomeric ratios between (−)- and (+)-norepinephrine for rate-accelerating effects in atria from cat, dog, rabbit, and guinea pig are 60, 10, 10, and 30, respectively.[7] Different isomeric ratios from different species, at least in part, might be a reflection of unequal density of adrenergic enervation. Unfortunately, the effects of isomeric potency ratios are only available from two tissues, heart and bronchioles (Table IV).

Blinks (155) investigated (−)- and (+)-isomers of norepinephrine, epinephrine, and isoproterenol and their deoxy analogs, dopamine, epinine, and deoxyisoproterenol, respectively. Even in the tissues containing β-adrenergic receptors, guinea pig atria, the results were in accordance with the Easson-Stedman hypothesis. Deoxyisoproterenol and (+)-isoproterenol are equipotent weak agonists of β-adrenergic receptors. Both of these agents also have an identical α-adrenergic receptor blocking activity (156, 157). Similarity of interactions of (−)-, (+)-, and their deoxy derivative in tissue containing both α- and β-adrenergic receptors suggests that many molecular features of these sites must be similar. The dissimilarity between α- and β-adrenergic receptors becomes apparent when effects of (−)- and (+)-levonordefrin and (+)-deoxylevonordefrin are examined on both of the adrenergic receptors. (+)-Deoxylevonordefrin and (+)-levonordefrin are equiactive on the reserpine-pretreated rat vas deferens; while on the guinea pig trachea, (+)-deoxylevonordefrin is far more active than (+)-levonordefrin (71).

Apart from these observations, some qualitative differences between deoxy derivatives and (+)-isomers have been reported. Evidence suggests that there may be receptors for initiation of renal vasodilation which are affected by dopamine (deoxynorepinephrine). The renal vasodilatory effects of dopamine are not blocked by any classical antagonist, while those of (+)-norepinephrine are blocked (158). Similarly, it appears from the report of van Rossum (149) that deoxysynephrine causes stimulation of the rabbit jejunum, while (+)-synephrine causes inhibition. On any given tissue, the deoxy derivative and (+)-norepinephrine may interact identically with pharmacologic receptors, as suggested by Easson and Stedman; but if only the deoxy derivative liberates other substances such as histamine or serotonin, then the observed activity difference of (+)-isomer and the deoxy derivative may not appear equal.

[7] J. R. Blinks, personal communication, 1969.

Table III—Relative Activities of Optical Isomers of Norepinephrine (NE) and Deoxynorepinephrine (Dopamine) on Various Tissues which Mainly Contain α-Adrenergic Receptors

Test Parameter	Procedure	Approximate Ratio[a] (+)-NE / (−)-NE	Approximate Ratio[a] (+)-NE / Deoxy NE	Reference No.
Cat blood pressure	Normal	40	1	109, 122,
	Reserpine[b]	47	1	150
	Cocaine	60		
Cat nictitating membrane	Normal	8	1	109, 112,
	Reserpine[b]	8	1	122
	Denervation (R)[b]	128	3	
	Cocaine (R)[b]	80	1	
	Chronic reserpine[c]	58	1	
Cat spleen	Normal	2	—	123
	Cocaine (R)[b]	65	—	
Rabbit aorta	Normal	42	1	146
Rabbit jejunum	Normal	64	10	149
Rat vas deferens	Normal	5	1	20
	Reserpine[b]	5	1	143
	Desipramine	50	—	114

[a] A dose that will cause equivalent effect was selected as a criterion for calculation of dose ratio. [b] Reserpine, 3–5 mg./kg. i.p., was used to deplete catecholamine. [c] Reserpine, 0.1 mg./kg./day for 14 days.

Reuter and Wollert (159) studied the effects of 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 contractility and influx of ^{45}Ca. It was concluded that increase of Ca-influx during excitation is responsible for the positive inotropic effects of these agents. If there is a transport system at the site of pharmacologic receptors, it seems likely that calcium is required in transport of these amines at the receptors for the effect; and 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 role of divalent metals and their chelates in relation to pharmacologic effects of sympathomimetic amines and their optical isomers should provide an interesting chapter on future developments in understanding the basic mechanisms of drug action. The possible role of metals in the transport of norepinephrine has been discussed by Colburn and Maas (160, 161).

Table IV—Isomeric Ratios of Optical Isomers of Some Sympathomimetic Amines on Tissues Containing Mainly β-Adrenergic Receptors[a]

Isomer	Approx. (+)/(−) Ratio: Rabbit Heart (Rate)	Approx. (+)/(−) Ratio: Perfused Guinea Pig Lung (Bronchodilatation)
(−)-Isoproterenol (−)-Isoproterenol	1500	>800
(−)-Epinephrine (+)-Epinephrine	110	45
(−)-Norepinephrine (+)-Norepinephrine	60	70
(−)-Levonordefrin (+)-Levonordefrin	2600	>3000
(−)-Phenylephrine (+)-Phenylephrine	>10	—

[a] Data condensed from Brown and Lands (152), Luduena and Snyder (153), and Luduena *et al.* (1954).

Table V—Relative Pressor Activity of (−)- and (+)-Isomers of Nonphenolic Sympathomimetic Amines

Isomer	Pressor Effects: Ratio of Activity	Pressor Effects: Isomeric Ratio	Reference No.
(−)-Ephedrine (+)-Ephedrine	1 (pithed cat) 0.3	3	163
(+)-Pseudoephedrine (−)-Pseudoephedrine	1 (pithed cat) <0.1	>10	163
(−)-Norephedrine (+)-Norephedrine	1.0 (pithed dog) 0.68	<2	164
(−)-Norpseudoephedrine (+)-Norpseudoephedrine	1.0 (pithed dog) 0.87	1	164
(−)-Deoxyephedrine (+)-Deoxyephedrine	1.0 (pithed dog) 0.71	1	164
(−)-Deoxynorephedrine (+)-Deoxynorephedrine	1.0 (dog) 0.71	1	164
(−)-Phenylethanolamine (+)-Phenylethanolamine	1.0 (dog) 0.3	3	165
(−)-*N*-Methylphenethanolamine (+)-*N*-Methylphenethanolamine	1 0.25	4	166
(−)-Cyclohexylisopropylamine (+)-Cyclohexylisopropylamine	1.0 (dog) 0.5	2	167
(−)-Cinnamylephedrine (+)-Cinnamylephedrine	1.0[a] (cat) 0.5[a]	2	168

[a] Fall in the blood pressure.

OPTICAL ISOMERS OF NONPHENOLIC AMINES

Since the discovery of ephedrine, many closely related amines have been synthesized and tested for sympathomimetic effects. In early days, the main interest in nonphenolic amines was caused by their central stimulant and prolonged pressor effects. The classical work of Badger and Dale (162) describes pressor activity of many structurally similar amines. Along with these developments, the optical isomers of some nonphenolic amines were also investigated. Many earlier studies were semiquantitative. Hence, an attempt to obtain a coherent summary except for blood pressure effects was a failure. A short report regarding the pressor effects of the isomers is presented in Table V. The pressor effects of nonphenolic amines are now recognized as being mediated through the release of catecholamines from the sympathetic nerve endings. Readers interested in earlier studies regarding optical isomers of nonphenolic amines are urged to read other reports (169–174).

Among nonphenolic amines, the ephedrine isomers present a unique opportunity to study steric structure–activity relationships. Although heart rate and pressor effects of naturally occurring (−)-ephedrine were considered as mixed actions, the investigation of all four isomers of ephedrine revealed that this property is not shared by (+)-ephedrine, and for (+)-pseudoephedrine was mainly "indirect" through the release of endogenous norepinephrine (16). De Meyts and Cession-Fossion (175) extended these observations in rats, that pressor effects of (−)-ephedrine were direct as well as indirect while those

of (+)-pseudoephedrine were mainly indirect. Fifty milligrams per kilogram, i.p., of (−)-ephedrine caused a significant decrease in myocardial catecholamines in rats, while under similar conditions (+)-pseudoephedrine was inactive. Both (−)-ephedrine and (+)-pseudoephedrine did not influence adrenal catecholamines. Light *et al.* (176) examined the vascular effects of ephedrine isomers in dogs. The intraarterial injection of (−)-ephedrine reduced the blood supply to all vascular beds studied, in contrast to the dilation produced by (−)-pseudoephedrine. Renal and vertebral arterial flows were increased and the carotid flow decreased by the (+)-isomers of both ephedrine and pseudoephedrine. Limb flow increased by (+)-ephedrine but decreased by the (+)-pseudoephedrine. These vascular effects probably indicate the indirect or direct activation of either α- or β-adrenergic receptors.

The pattern of the indirect pharmacologic activity of all four ephedrine isomers in the rat vas deferens appears as (−)-ephedrine > (+)-ephedrine ≥ (+)-pseudoephedrine ≫ (−)-pseudoephedrine. The pattern of the potentiation of exogenous norepinephrine by these agents in the reserpine-pretreated tissues also appears to be the same (Table VI). On the isolated rabbit aorta, (−)-ephedrine produces a marked contractile effect while other isomers produce little or no effect (177).

(−)-Ephedrine was introduced in therapeutics as a bronchodilator drug (178) and has been widely used as such. Tye *et al.* (179) investigated the effects of ephedrine isomers on tracheal smooth muscle of guinea pig. All isomers appear to be partial agonists and (−)-ephedrine and (−)-pseudoephedrine were mainly direct acting, the smooth muscle relaxant effects of (+)-ephedrine and (+)-pseudoephedrine were considerably reduced by reserpine-pretreatment. Propranolol, a β-adrenergic blocker, reduced the effects of all isomers. Ephedrines were studied in the presence of tone induced by methacholine. Muscle relaxant effects, therefore, may partly be attributed to competition of ephedrine molecules with that of methacholine.[8]

Pendular movements of rabbit ileum are inhibited only by (−)-ephedrine; the other three isomers produce little or no effect and are antagonistic to the α-adrenergic inhibitory effects of (−)-norepinephrine. The stereospecificity in such antagonism is not very marked. For example, (+)-ephedrine and (−)-pseudoephedrine vary considerably in steric structure, but produce similar antagonistic effects to (−)-norepinephrine (181). (−)-Phenylethanolamine produces inhibition of the rabbit ileum. (+)-Phenylethanolamine and the deoxy derivative, phenethylamine, do not have intrinsic activity, but both agents produce an equal antagonism to norepinephrine effects (182). These observations are consistent with the Easson-Stedman hypothesis (138).

LaPidus *et al.* (183) pointed out the stereochemical similarities between (−)-ephedrine and (−)-pseudoephedrine. In both of these molecules, the functional groups, the phenyl ring, β-hydroxyl group, and the amino group could fit the same three points on a hypothetical receptor. In the anesthetized cat, the pressor and nictitating membrane effects of (−)-ephedrine were promptly terminated by (−)-pseudoephedrine. The pretreatment of an animal with (−)-pseudoephedrine can also prevent the effects of (−)-ephedrine (177). However, the antagonism by (−)-pseudoephedrine can be extended to other indirectly acting amines such as amphetamine and tyramine. The antagonism between (−)-ephedrine and (−)-pseudoephedrine on the cat nictitating membrane or blood pressure may even be physiological.

Table VI—Effects of "Indirectly" Acting Ephedrine Isomers on the Normal Vas Deferens and the Potentiation of Norepinephrine Responses by the Same Agents in the Reserpine-Pretreated Tissues

Isomer	Normal Vas Deferens,[a] % Contraction[b] 10^{-5} *M*	*n*[d]	Reserpine-Pretreated Vas Deferens Response to 3×10^{-7} *M* Norepinephrine in the Presence of 10^{-5} *M* of the Drug[c] % Contraction[e]	*n*[d]
(−)-Ephedrine	25 (±4)	8	49 (± 5)	10
(+)-Ephedrine	11 (±2)	8	20 (±3)	10
(+)-Pseudoephedrine	8 (±2)	8	26 (±4)	10
(−)-Pseudoephedrine	0	8	5 (±1)	10

[a] Data taken from Patil *et al.* (20). [b] With reference to maximal response to norepinephrine = 100. [c] Incubation time 3 min. [d] Number of observations. [e] With reference to maximal response to 3×10^{-4} *M* norepinephrine after the procedure = 100. Data taken from Patil and Patel (180).

Because the pattern of effects of ephedrine isomers varies so greatly from one tissue to another, Kier (184) postulated a pattern of complementary features which represent the α-adrenergic receptor that is activated by ephedrine isomers. Molecular orbital calculations were used to map α-adrenergic receptors. These calculations present an interesting theoretical approach which would gain more recognition if the hypothesis were tested over a wide variety of related molecules and by some experimental means.

Several attempts were made to synthesize the ephedrinelike molecules with a rigid structure. Yelnosky and Katz (185) reported a sympathomimetic action of *cis*-2-amino-4-methyl-5-phenyl-2-oxazoline. Comparison of the structure of this agent with that of ephedrine illustrates a marked similarity in the functional groups. The pharmacological effects are also similar to ephedrine's. Meyer *et al.* (186) synthesized norephedrine homologs, 2-aminotetralol, with a rigid molecular structure. Like norephedrine, this agent could exist in four possible isomers: (+), (−)-*cis*-form and (+), (−)-*trans*-form. Only the *cis*-form was effective as a pressor agent. (+)-*cis*-2-Aminotetralol was about 5 times as active as (−)-*cis*-2-aminotetralol. The arrangement of functional groups in (+)-*cis*-2-aminotetralol was claimed to be like that in the most active form of norephedrine. It should be emphasized that higher pressor potency of these two agents reflects their possible catecholamine-releasing effects. Smissman and Chappell (187) reported on conformationally rigid derivatives of ephedrine. Pharmacological testing on the isolated rat vas deferens revealed that there were no intrinsic effects from all of the agents. However, in 10^{-4} *M* concentrations, effects of exogenous norepinephrine were

[8] R. F. Furchgott, personal communication, 1969.

potentiated. This potentiation indicates that these agents possibly interact with uptake sites. Bulky substituents possibly retard the catecholamine-releasing activity which is seen in the parent ephedrine molecules. It would be interesting to synthesize and test the conformationally rigid analogs of catecholamines such as norepinephrine and epinephrine.

TACHYPHYLAXIS (ACUTE TOLERANCE)

It is outside the scope of this report to review various theories or explanations for tachyphylaxis. Only work pertinent to the optical isomers will be referred to. In the early years, tachyphylaxis to the sympathomimetic amines, such as ephedrine or amphetamine was said to be caused by receptor saturation (188). But when the various sites such as uptake, binding, and direct action were known, it was important to explain the phenomenon of tachyphylaxis in relation to these sites. Abdallah *et al.* (17) studied tachyphylaxis to the ephedrine isomers in the perfused rabbit heart and found that under identical experimental conditions, the rate of development of tachyphylaxis varied with the isomers. (−)-Ephedrine was the most potent tachyphylactic isomer studied. The correlation between norepinephrine recovered in the perfusate and loss of chronotropic effect was not parallel. None of the isomers significantly influenced the total cardiac norepinephrine. *In vivo*, at "equipressor" doses, the tachyphylactic tendencies for ephedrine isomers were (+)-pseudoephedrine > (+)-ephedrine > (−)-ephedrine (16), which is the reverse of the ranking observed *in vitro*. This ranking might be caused by differing disposal rates for the ephedrine isomers *in vivo*; but since cardiovascular reflexes may so greatly influence blood pressure, comparisons between *in vitro* and *in vivo* data are difficult to make. There are some doubts regarding participation of adrenal medulla during ephedrine tachyphylaxis (189). However, in an anesthetized cat, pretreatment with a total of 6 mg./kg. of *N,N*-diisopropyl urea, *N'*-isoamyl urea, and *N'*-diethylaminoethyl urea (P. 286), a specific adrenal medullary blocker, did not influence the pressor effects or the tachyphylactic effects caused by ephedrine isomers (190). If a given indirectly acting amine has a greater affinity for the uptake sites and is not washed off the tissue during perfusion, it may inhibit its own uptake during subsequent injections. This inhibition will result in a reduced displacement of catecholamines from storage sites and a consequent diminishing of tissue response. Similar suggestions have been made earlier by Blaschko (191) and Fawaz and Simaan (192). In other words, different rates of development of tachyphylaxis may be indicative of an amine's ability to prevent its own uptake into the sympathetic nerve endings.

Hanna (193) and Harvey *et al.* (194) found that the tachyphylactic tendencies of amphetamine isomers on dog blood pressure and on rabbit aortic strips were the same for both isomers. The major structural difference between amphetamine and ephedrine is that the latter possesses an alcoholic hydroxyl group. The different rates of development of tachyphylaxis observed with ephedrine isomers in this study may well depend on the orientation of this group. (+)-Pseudoephedrine, a *threo*-isomer, appeared to be less tachyphylactic than (−)-ephedrine and (+)-ephedrine, which are both *erythro*-isomers. Lindmar *et al.* (74) have reported that (±)-pseudo dihydroxyephedrine, a *threo*-form, is washed off the heart more easily than (±)-dihydroxyephedrine, an *erythro*-form. (+)-Pseudoephedrine's lack of tachyphylactic power then may well be caused by its *threo*-conformation which allows it to be more easily washed off. These observations lead to the conclusion that the relative orientation of the β-hydroxyl group and the α-methyl group must be of critical importance in the development of tachyphylaxis in the ephedrine isomers.

Hornykiewicz and Obenaus (195) elaborated on some of the previous observations regarding tachyphylaxis to direct acting amines. In the anesthetized rats, infusion of large amounts of (+)-epinephrine, (+)-norepinephrine, epinine, and (−)- or (+)-phenylephrine induces tachyphylaxis to vasopressor effects of (−)-epinephrine, (−)-norepinephrine, and tetraethylammonium. The tachyphylactic potency of the amines was (−)-phenylephrine > epinine > (+)-norepinephrine = (+)-epinephrine > (+)-phenylephrine. Other peripheral effects of (−)-isoproterenol and (−)-epinephrine were also reduced by infusion of (+)-epinephrine. From these results, it was concluded that tachyphylaxis could result from saturation of α- or β-adrenergic receptors by less active isomers of catecholamines and related agents. In the isolated heart of *venus mercenaria*, (−)-norepinephrine produces negative inotropic effects. The repetition of the same dose produces tachyphylaxis. Optical isomers of amphetamine and ephedrine can prevent the tachyphylactic effects caused by (−)-norepinephrine. (−)-Ephedrine was found to be more potent than (+)-ephedrine, and (+)-amphetamine was found to be more potent than (−)-amphetamine (196, 197).

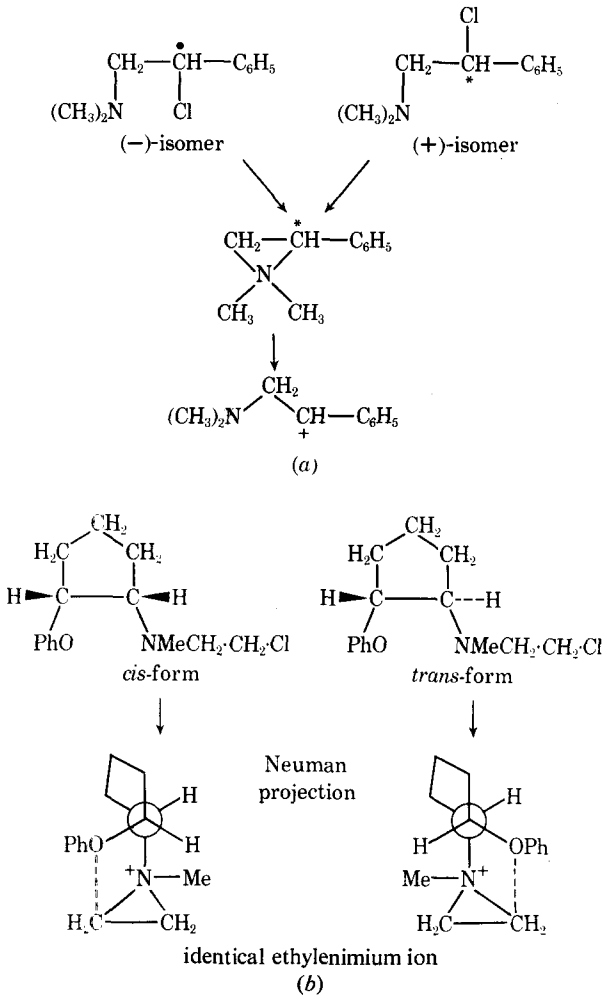
Thus, it appears that sympathomimetic amines can produce tachyphylaxis by the following mechanism or combination of the following mechanisms: (*a*) depletion of small available stores of norepinephrine; (*b*) inhibition of their own uptake by neuronal membrane; (*c*) saturation of α- and/or β-adrenergic receptors; and (*d*) slow excretion or metabolic disposition. The optical isomers of sympathomimetic amines provide a good tool for exploring various mechanisms of sympathomimetic amines.

α-ADRENERGIC BLOCKERS

At present, very little information is available regarding steric aspects of the reversible competitive antagonist of α-adrenergic receptors. Therefore, our knowledge regarding α-adrenergic receptor antagonists is derived solely from irreversible blockers. Nickerson (198) suggested that in the case of β-haloalkylamines, the development of a stable drug receptor bond can be related directly to the chemical reactivity of ethylenimium intermediate formed at physiological pH. Belleau (199) later introduced a concept of isosterism. He suggested a similarity of interaction of (−)-norepinephrine and ethylenimium ion from *N*-(2-chloroethyl)dibenzylamine at the α-adrenergic receptor. It is of interest to note that the suggested interaction of agonist, (−)-noradrenaline, with the α-adrenergic receptor is essentially the same as that postulated by Easson and Stedman (138).

Belleau and Triggle (200) synthesized and resolved two optical isomers of *N,N*-dimethyl-β-chlorophenethylamine.

Equipotent antiadrenaline effects of the two isomeric forms of *N,N*-dimethyl-β-chlorophenethylamine indicate a possible common reactive symmetric molecular species at adrenergic receptors (Scheme III*a*). Similarly, *cis*- and *trans*-isomers of *N*-methyl-*N*-(2′-phenoxycyclopentyl)-2-chloroethylamine yield similar reactive molecular species, and hence they are approximately equipotent with respect to the reactive α-adrenergic site (Scheme III*b*)



Scheme IIIa, b—Common reactive ethylenimium ion from the optical isomers of N,N-*dimethyl-β-chlorophenethylamine and* N-*methyl*-N-(*2′-phenoxycyclopentyl*)-*2-chloroethylamine, respectively.*

(201). There are several thought-provoking reviews available on this subject (202–205).

β-ADRENERGIC BLOCKERS

Since the introduction of dichloroisoproterenol as an antagonist to the inhibitory effects of catecholamines, a number of structurally related chemicals have been synthesized and tested for β-adrenergic blocking properties. All of those agents which produced significant β-adrenergic blockade have asymmetric carbon or carbons. The optical isomers of dichloroisoproterenol (206), pronethalol (206, 207), propranolol (208–211), methoxamine and its derivatives (15, 19, 212), INPEA (213–215), α-methyl-INPEA (216), sotalol (19, 217), butedrine (218–221), and H56/28 (222) have been synthesized, resolved, and tested for pharmacologic activity. The chemical structure and β-adrenergic blocking properties are summarized in Table VII. Examination of data in Table VII reveals that all potent β-blockers markedly resemble the agonists (−)-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 β-adrenergic blockers are relatively more specific in their attachment to the β-receptor than α-adrenergic antagonists to the α-receptors. However, there is a marked similarity between interaction of α- and β-adrenergic agonists with their respective receptors. Substitution of the methyl group adjacent to the carbon-carrying alcoholic hydroxyl group can hinder the effective interaction of agonist or antagonists with the receptor. For example, (−)-pseudobutoxamine fulfills all the structural requirements for a β-adrenergic blocker, but it does not appear to block β-adrenergic receptors. A very interesting study on the conformational aspects of the ephedrine isomers has been carried out by Portoghese (223). 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 (−)-pseudoephedrine 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 β-adrenergic receptor and (−)-pseudoisomer 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 equiactive.

(−)-Propranolol is more potent in antagonizing isoproterenol-induced tachycardia while its (+)-isomer is much less active. The deoxy analog of propranolol is approximately as active as (+)-propranolol (208). Thus, β-adrenergic blockers are in line with Easson and Stedman's suggestions 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 location of adrenergic receptors in tissue is not known. If β-

Table VII—Isomeric Activity Ratio of β-Adrenergic Blockers

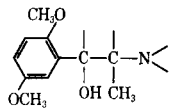
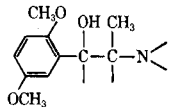
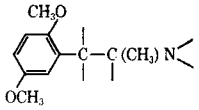
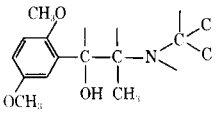
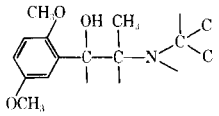
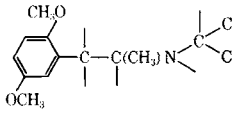
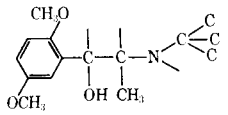
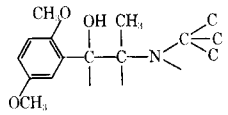
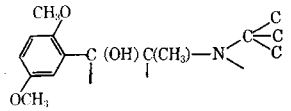
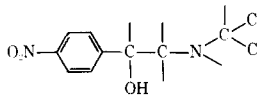
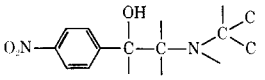
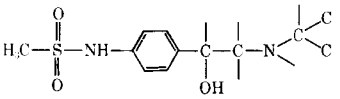
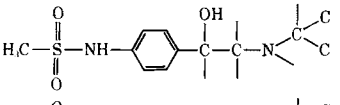
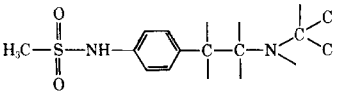
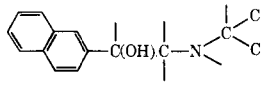
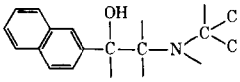
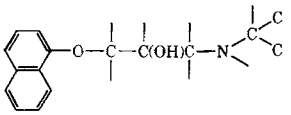
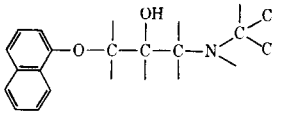
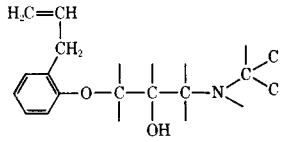
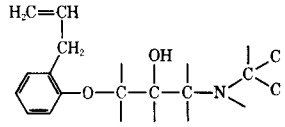
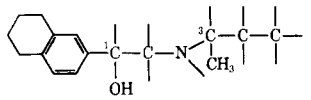
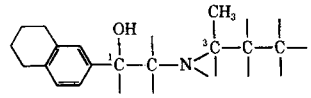
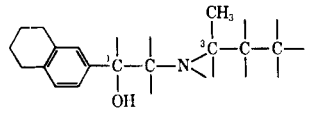
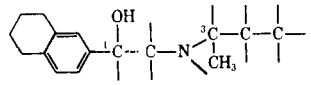
Chemical Structure	Name	pA_2	Isomeric Ratio
	(−)-Methoxamine	6.30	
			77[a]
	(+)-Methoxamine	4.37	
	(±)-Deoxymethoxamine	5.09	
	(−)-Isopropylmethoxamine	6.53	
			>1100[a]
	(+)-Isopropylmethoxamine	<3.50	
	(±)-Deoxyisopropylmethoxamine	4.85	
	(−)-Butoxamine	7.20	
			>1700[a]
	(+)-Butoxamine	<4.0	
	(±)-Pseudobutoxamine	<4.0	
	(−)-INPEA	6.50	
			190[a]
	(+)-INPEA	4.22	
	(−)-Sotalol	6.80	
			44[a]
	(+)-Sotalol	5.15	
	Deoxysotalol	3.88	
	(±)-Pronethalol	7.30	
			>120[a]
	(+)-Pronethalol	5.20	
	(±)-Propranolol	8.50	
			>100[b]

Table VII—*Continued*

Chemical Structure	Name	pA_2	Isomeric Ratio
	(+)-Propranolol	6.50	
	(−)-H56/28	—	
	(+)-H56/28	—	100[c]
	(−)-Butedrine 1R,3R	7.85	
	(+)-Butedrine 1S,3R	<6.00	>70[b]
	(+)-Butedrine 1R,3S	6.70	
	(+)-Butedrine 1S,3S	<5.50[d]	>15[b]

[a] On guinea pig trachea (15, 19). [b] On guinea pig atria for heart rate (222). [c] In anesthetized cat for heart rate (221). [d] Causes marked cardiac depression (220).

receptors 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.

Different types of β-receptors complicate interpretation of structure–activity study from one tissue to another (224, 225). Substitution of $-CH_3$ group on α-carbon is known to decrease ability of molecules to block β-receptors in the heart. The β-adrenergic receptors in the skeletal muscle are classified as Type β_2. Tremors of skeletal muscles produced by infusion of catecholamines in man are blocked by (±)-propranolol but are unchanged by (+)-propranolol (226). Whether all these β-receptors are different or whether the effects are due merely to physical-chemical properties of β-blockers is not known.

In addition to blocking β-adrenergic receptors, many of these blockers produce direct myocardial depression. Levy and his colleagues (227–230) attempted to correlate the physical-chemical properties with the cardiac effects of various β-blockers and their isomers. However, there appears to be no simple relationship between *in vitro* β-adrenergic blocking action and myocardial depressant effects.

On the other hand, the inhibition of Ca^{++} uptake in cardiac sarcoplasmic reticulum fractions by (−)- and (+)-propranolol correlates with their ability to decrease cardiac contractility (231).

Recently, Serrano and Hardman (232) suggested that a nonionized form of the drug might be essential for the production of β-adrenergic blockade. While this suggestion will require further proof, an interesting experiment could be made by testing (−)- and (+)-isomers of β-adrenergic blockers. Since pKa values of (−)- and (+)-isomers are identical, regardless of change in pH, the relative number of nonionized forms of both drugs should be the same. This would indicate that the difference in pharmacological activity between (−)- and (+)-isomers should remain the same at different pH values.

An attempt has been made to characterize the nature of the β-adrenergic receptor in the guinea pig atria (233). A potent and long-acting β-blocker, propranolol, was used as a tool to tag the receptors. However, uptake of the less active (+)-propranolol was the same as that of the more potent racemate or (−)-isomer. This nonspecific uptake indicates that the antagonist was largely bound to nonspecific sites and that the amount of antagonist bound to the specific sites must be very small. Thus, attempts to identify the pharmacologic receptors have achieved limited success.

Because of their antiarrhythmic effect, there has been considerable clinical interest in β-adrenergic blockers. However, the mechanism of antiarrhythmic action of

these drugs is complicated by their local anesthetic, "quinidinelike," and β-adrenergic blocking effects. Results clearly indicate that adrenergically induced cardiac arrhythmias can be promptly terminated by low doses of (−)-isomers of β-adrenergic blocker. On the other hand, much higher and nearly equivalent doses of both (−)- and (+)-isomers of β-adrenergic blockers are required to prevent or prolong the cardiac arrhythmia induced by ouabain (234–241). Posttetanic potentiation of cat soleus is equally depressed by optical isomers of the β-blocker. On this basis, Standaert *et al.* (242) suggested that termination of digitalis-induced arrhythmia might be a neural phenomenon. In the guinea pig, when arrhythmias were produced by infusion of ouabain, Dohadwalla *et al.* (243) showed that (±)-propranolol is slightly more effective than (+)-propranolol. They attributed this unequal antiarrhythmic effect to the unequal β-adrenergic blocking property and not to the local anesthetic effect. Stickney and Lucchesi (244) elaborated on the ventricular arrhythmias elicited in dogs by central administration of acetylstrophanthidin. Racemic propranolol, but not (+)-propranolol, attenuated the arrhythmias caused by the glycoside.

Some β-adrenergic blockers are known to have a hypotensive effect in man. Kelliher and Buckley (245) studied the possible mechanism of this hypotensive effect. When administered directly into the left lateral ventricle of cat, both (+)- and (±)-propranolol produced nearly equal hypotensive effects. It was concluded that the central hypotensive effect is independent of β-adrenergic blockade. This study should be extended to the other optical isomers which are not local anesthetics. Furthermore, prevention of adrenergically induced arrhythmias may not necessarily be related to local anesthetic effects of β-blockers. For example, (−)-sotalol lacks local anesthetic effect but is a very effective agent in preventing the adrenergically induced arrhythmia. The mechanism of termination of nonadrenergically induced arrhythmia is yet to be clarified. Both the isomers of propranolol cause equal myocardial depression and the local anesthetic effect is also identical. In order to separate local anesthetic effect from quinidine-like effect, the use of deoxypropranolol has been suggested by Ariens (246). Parmley and Brunwald (247) compared myocardial depressant and antiarrhythmic properties of (±)-propranolol, (+)-propranolol, and quinidine. Their study suggests that (+)-propranolol might be a very useful drug in the treatment of certain arrhythmias where β-adrenergic blockade is not desired. Quinidine lowers arterial blood pressure, while (+)-propranolol is without such a clinically undesired effect.

Peripheral vascular effects of propranolol isomers have been studied in the anesthetized dog. Direct intracoronary injections of small doses, in the anesthetized dog, of (+)-propranolol produces transitory reduction in coronary vascular resistance; under similar conditions, (−)-propranolol only increases coronary vascular resistance. This increased resistance may be a reflection of β-blocking effect of (−)-isomer (248). Both isomers of propranolol, when injected directly in the external ilial artery, increased blood flow through the artery to the same extent. This effect is, however, attributed to the local anesthetic effects of the agents (249).

Studies on effects of isomers of adrenergic drugs on metabolic processes are relatively few. Isoproterenol is very potent in releasing free fatty acids *in vitro* from adipose tissue. The (+)/(−) isomeric ratio is approximately 4000 (250). β-Adrenergic blockers competitively inhibit the free fatty acid mobilization stimulated by the adrenergic agents. (−)-Isomers of INPEA, isopropylmethoxamine, and methoxamine are some 100 times more potent than their (+)-isomers (251–255). High concentrations of both (−)- and (+)-INPEA equally reduced the incorporation of labeled glucose in the fat cells. Thus, the phenomenon appears to be nonspecific and unrelated to the β-adrenergic blockade (256).

Any adrenergic drug with a basic chemical structure such as phenethylamine exhibits a variety of characteristic effects at adrenergic synapses. β-Adrenergic blockers are basically derivatives of phenethylamines. It is possible, therefore, that β-receptor antagonists can interact at the same two points (Sites A and B), as suggested for *N*-(2-chloroethyl)dibenzylamine, and can protect α-receptors from the latter (Fig. 6). This two-point interaction would occur equally well with (−)- or (+)-isomers of β-antagonists, and thus the degree of stereoselectivity in protection of α-receptors by β-receptor antagonists would not be marked (257). If β-adrenergic blockers compete with norepinephrine for α-adrenergic receptors, these agents should shift the dose–response curve for norepinephrine to the right in a parallel fashion. Most agents, except isomers of H56/28, produced a potentiation of the effects of exogenous norepinephrine and shifted the curve to the left. This paradoxical observation can be explained by differential interplay between two main factors operative at sympathetic neurones: (*a*) inhibition of uptake, and (*b*) competition at α-adrenergic receptors. Whenever effects of norepinephrine are potentiated by a given blocker, the optical isomers do not differ in this respect. It indicates that inhibition of uptake of norepinephrine by (−)- and (+)-isomers of β-blocker must be similar. Results from biochemical studies tend to support this notion. Either in the adrenergic nerve granule or in the perfused heart, inhibition of uptake of exogenous norepinephrine or epinephrine by (−)- and (+)-isomers of β-blocker is similar (258–261). Since (−)- and (+)-isomers of β-blocker do not differ in either competition at α-receptor or inhibition of uptake and since (−)-isomers are the most potent blockers, the observed effects are not causally related to β-adrenergic blockade. Furthermore, on rabbit aorta, where uptake of agonist is not a critical factor in the determination of competitive antagonism, the pA_2 values of (−)- and (+)-INPEA are equal (262).

All four optical isomers of butedrine were also investigated (263) on the rat vas deferens. Phenylephrine was used as agonist. In the presence of butedrines, the dose–response curve of phenylephrine was depressed. The pD_2' values for noncompetitive antagonism varied from 3.7 to 4.20. This indicates a very small difference in butedrine isomers for the noncompetitive interaction with α-agonists, hence, unrelated to their β-blocking properties.

β-Adrenergic blockers are known to enhance the respiratory difficulty in asthmatics. With the aid of the optical isomers of INPEA, Murmann (264) attempted to analyze the underlying mechanism. He found that except in one species of mice, LD_{50} of histamine was not significantly altered by pretreating mice with either isomer of INPEA. He tentatively, then, suggested that β-adrenergic blockade may not be the cause for the enhancement of respiratory difficulty in asthmatics. However, it appears that in the bronchioles, when the smooth muscle relaxation is blocked by β-adrenergic blockers, there could be bronchoconstriction caused by overpowering of parasympathetics, as well as that of α-adrenergic receptors. Hence, in order to define the role of β-adrenergic blockade in asthmatics, two types of experiments remain to be done: (*a*) to see if (+)-isomers of INPEA will enhance the bronchoconstriction in asthmatics; and (*b*) to compare endotracheal pressure changes after pretreatment of either (−)- or (+)-isomers of β-blockers in experimental animals. The two phases of nicotinic blood pressure effects of acetylcholine in atropine-pretreated animals are well known. The first phase, caused by stimulation of sympathetic ganglia, is selectively antagonized by (−)-INPEA, while (+)-INPEA is ineffective. It is concluded that selective β-adrenergic blockade might be involved at the level of sympathetic ganglia (215).

METABOLIC ASPECTS

Only a limited number of reports concerning this facet are available at present. Epinephrine increases blood sugar; this effect is stereoselective in favor of (−)-isomer. In rabbits, 0.05 mg./kg. of (−)-epinephrine and 1 mg./kg. of (+)-epinephrine produce approximately an equivalent rise in the blood sugar. The effects of the (+)-isomer are transitory, while those of the (−)-isomer last many hours (265). Bowman and Raper (266) studied drugs affecting carbohydrate metabolism on contractions of the rat diaphragm. Potassium-induced depression of the skeletal muscle contraction is readily reversed by sympathomimetic agents. In this respect, (−)-norepinephrine and (−)-epinephrine are 500–1000 times more potent than their respective (+)-isomers. These effects, as they claim, are probably caused by activation of the β-receptor. Anderson and Chen (267) screened the hyperglycemic action of 40 amines. After equimolar doses (0.1 ml. of 0.1 *M* i.v.) in rabbits, the rise in blood sugar was determined. According to this test, the activity of ephedrines was: (−)-pseudoephedrine > (+)-ephedrine = (+)-pseudoephedrine > (−)-ephedrine. The differences between (−)-pseudoephedrine and (−)-ephedrine were striking. The onset of action of (−)-pseudoephedrine was very slow and as much as 40 min. was required for the peak effects. On the other hand, only 10 min. was required for the peak effects of the less active (−)-ephedrine.

A highly attractive hypothesis regarding the role of cyclic 3′,5′-AMP in response to catecholamines has been put forward by Sutherland and his colleagues (268–270). It has been suggested that cyclic 3′,5′-AMP plays an essential role in the hyperglycemic response

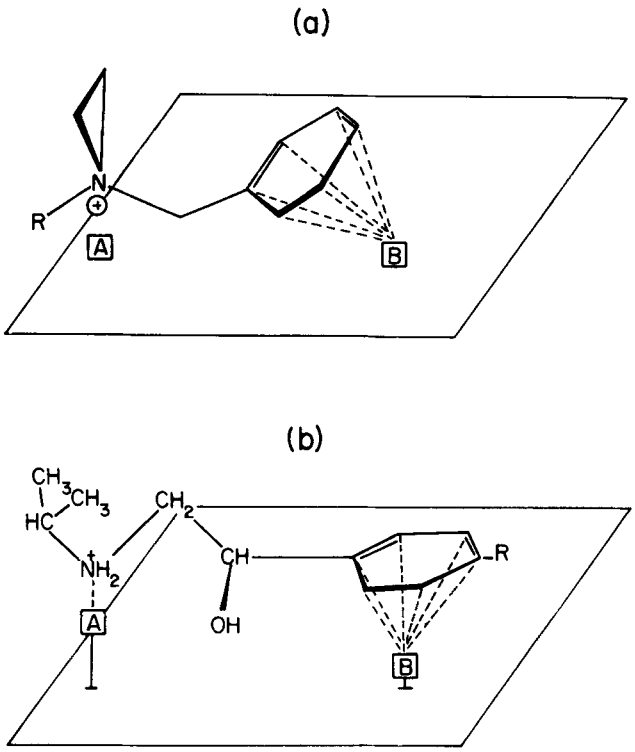


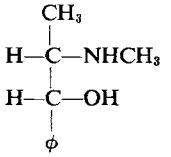
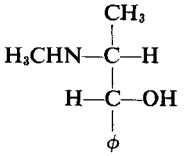
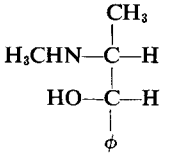
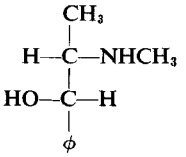
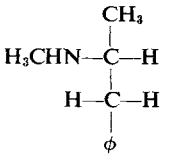
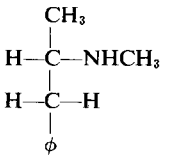
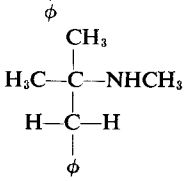
Figure 6—(*a*) *The initial interaction of the ethylenimium ion from* N-(*2-chloroethyl*)*dibenzylamine* ($R = C_6H_5CH_2$) *and the α-adrenergic receptor as suggested by Belleau (199) and Triggle (202).* (*b*) *The possible interaction of β-adrenergic blockers like INPEA* (*where* $R = NO_2$) *and explains the protective action of β-adrenergic blockers against block by* N-(*2-chloroethyl*)*dibenzylamine. Note that the β-hydroxyl group is not involved and thus explains a similar protective effect of* (−)- *and* (+)-*INPEA. Reproduced with permission from Patil* et al., J. Pharmacol. Exp. Ther., *163, 309(1968), and the Williams & Wilkins Co., Baltimore, MD 21202*

to epinephrine, principally through its effect on phosphorylase. Furthermore, it has been been suggested that cyclic 3′,5′-AMP might also be involved in the positive inotropic effects of catecholamines in the heart. The ability of (−)-isoproterenol, (−)-epinephrine, and (−)-norepinephrine to stimulate formation of cyclic 3′,5′-AMP and to produce positive inotropic responses is of a similar nature. (−)-Epinephrine, as compared to its (+)-isomer, is much stronger both in formation of cyclic 3′,5′-AMP and in positive inotropic effects. Recently, Weiss and Costa observed a stereospecific activation of adenylcyclase (271).

McNeill and Brody (272) determined the stereoselectivity of norepinephrine isomers for rat cardiac phosphorylase. Although both forms activated the enzyme to the same extent, (+)-norepinephrine was found to be 1/30th as active as its (−)-form. Thus, adenylcyclase and cyclic 3′,5′-AMP and phosphorylase exhibit stereoselectivity.

A study of the metabolic fate of optical isomers provides an interesting approach to elucidating the nature of various mechanisms that are involved in biotransformation and excretion of the drugs. Considering the specificity of various enzymes, it is unlikely that optical isomers are handled identically by the body. Development of a sensitive and specific analytical method, however, poses a problem. Many methods that are available today do not distinguish between (−)- and

Table VIII—Kinetic Constants for the *In Vitro* *N*-Demethylation of a Series of Sympathomimetic Amines by the 9000×*g* Supernatant Fraction from Rabbit Liver

Drug	Structure	Average K_m (× 10^4 *M*)	Average $\nu_{max.}$[a] (× 10^3)
(−)-Ephedrine (1R,2S)		1.2[b]	1.3
(−)-Pseudoephedrine (1R, 2R)		1.7	1.1
(+)-Ephedrine (1S,2R)		2.7	1.3
(+)-Pseudoephedrine (1S,2S)		2.2	1.8
(−)-Methamphetamine (2R)		3.0	2.6
(+)-Methamphetamine (2S)		4.5	2.6
Mephentermine		5.7	1.7

[a] μmole of HCHO/min./mg. protein. [b] The *Km* and $\nu_{max.}$ values shown are the mean values determined from two or three experimentally independent $1/v$ *versus* $1/s$ plots. The experimental points on each of these plots represented the mean of duplicate rate measurements for each substrate level. The standard error of the mean for the values in the table was about 10%. Data from Dann (282).

(+)-isomers of the drug. Hence, the metabolic fate of (−)- and (+)-isomers from the injected racemate cannot be precisely studied.

The fate and urinary excretion of amphetamine isomers 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 are not great (273–275). Gunne (276) used the gas chromatographic resolution method (277) for amphetamines and found that after administration of (±)-amphetamine, all subjects excreted approximately equal amounts of both isomers during the first 12 hr. Urine collected after 12 hr. 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 (42). In rats, 2 days after dosing with equal amounts of (+)- and (−)-amphetamine, the quantity of *p*-hydroxyamphetamine is 48 and 63% of the initial dose, respectively. Relatively more (+)-*p*-hydroxyamphetamine is converted to its β-hydroxylated product, (−)-*p*-hydroxynorephedrine (43) by enzyme dopamine-β-hydroxylase; hence less (+)-*p*-amphetamine 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 while (+)-ephedrine is not (278). There are marked species differences in the metabolism of amphetamine isomers. Axelrod (279) 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 substrate. However, neither (−)- nor (+)-amphetamine is metabolized by a microsomal preparation of rat liver (280). The urinary excretion kinetics of a close structural analog of amphetamine, methamphetamine, was also studied in man (276, 281). The excretion patterns of (+)- and (−)-methamphetamine are similar. Most of the drug is excreted unchanged in 24 hr.; however, a very small amount of the drug is *N*-demethylated. Because more (+)-amphetamine occurs in the urine after (±)-methamphetamine, it is suggested that enzymatic *N*-demethylation may be stereospecific. Similarly, (−)-ephedrine is *N*-demethylated while (+)-ephedrine is not (278). Recently, Dann (282) has investigated demethylation rates of ephedrine isomers. Isolated rabbit liver microsomes were used to determine the enzymatic kinetics. It was found that the $\nu_{max.}$ is the same for all ephedrine isomers, but K_m values for (−)-ephedrine and (−)-pseudoephedrine were approximately twice those of (+)-ephedrine and (+)-pseudoephedrine (Table VIII).

MAO and COMT are the two major enzymes intimately involved in adrenergic drug effects. Although stereochemical substrate specificity was not detected in the early semipurified enzymatic preparations (283–286), it is now demonstrated that indeed these enzymes do show selectivity for the (−)-isomers of norepinephrine, epinephrine, and *m*-octopamine which are better substrates than their corresponding (+)-isomers (287). Kynuramine oxidation by rat liver monoamine oxidase was inhibited more by (+)-isomers (2S) of amphetamine, 2,4-dichloroamphetamine, 4-chloro-*N*-methylamphetamine, than their corresponding (−)-isomers (288). Although available reports indicate that both (−)- and (+)-epinephrine are equally good substrates for COMT (289, 290), a more definite study is needed to establish the stereoselectivity in the highly purified enzyme.

CNS EFFECTS

In 1939, Alles reported that (+)-amphetamine was much superior to its (−)-isomer in antagonizing chloral hydrate hypnosis in rabbits (291). Later, Prinzmetal and Alles (292) confirmed these observations in humans. They concluded that (+)-amphetamine was 2–4 times as

active as (−)-amphetamine in producing CNS stimulation. Tainter *et al.* (293) studied analeptic potency of a series of sympathomimetic amines in rats against hypnotic action of tribromoethanol, chloral hydrate, and pentobarbital. The time required for recovery of the corneal and righting reflexes after a fixed dose of the hypnotic was compared with that observed under the same conditions when various of the supposed analeptics were administered also. Both (−)- and (+)-ephedrine (60 mg./kg.) did not produce any analeptic effects. However, (−)-pseudoephedrine produced a shortening of some of the reflexes under tribromoethanol and chloral hydrate which indicated that with this isomer there was a definite analeptic power. Fairchild and Alles (294) systematically investigated the locomotor activities of all optical isomers of ephedrine, norephedrine, and amphetamine. In mice, the central locomotor activity of the most potent (+)-amphetamine was assigned as 1. The activity ratios of (−)-amphetamine, (+)-norpseudoephedrine, (−)-ephedrine, and (−)-norpseudoephedrine were 4.2, 10, 24.4, and 42.8, respectively. Other isomers, (−)-norephedrine, (+)-norephedrine, (−)-pseudoephedrine, (+)-pseudoephedrine, and (+)-ephedrine, were considered as less effective as central locomotor stimulants and produced measurable activity only at doses approaching lethal amounts. Schulte *et al.* (295) used the jiggle cage to record the central activity of various sympathomimetic amines in rats. When compared at threshold, stimulant doses of (+)-amphetamine were 8 times as potent as its (−)-form. Both forms of pseudoephedrine were much less active than (−)-ephedrine.

The ratio of central stimulant activity using (+)- and (−)-methamphetamine varies from 4 to 8, with the (+)-form more active (296). This activity difference is the same as that exhibited between isomers of amphetamine.

Considering the complexity of the CNS, more than one criterion is needed to obtain a full profile of central stimulant activity. Lanciault and Wolf (297) carefully examined the neuropharmacological properties of the ephedrine isomers. Several standard techniques, including low-frequency electroshock and chemoshock threshold determinations, hexobarbital sleep-time alteration, and a behavioral rating scale, were employed. It was concluded that (−)- and (+)-ephedrines were considerably more potent than (−)- and (+)-pseudoephedrine. None of these agents affected the hexobarbital sleep-time. Interestingly enough, in one test, a given stimulant raises a threshold while the same agent in other tests lowers the seizure threshold. For example, (±)-amphetamine raises the seizure threshold of the low-frequency electroshock test while pentylenetetrazol seizure threshold is lowered by (±)-amphetamine. According to both tests, low-frequency electroshock and chemoshock threshold determinations, (−)- and (+)-ephedrines were more potent than (−)- and (+)-pseudoephedrines. In rabbits, however, (+)-amphetamine was more effective in raising the threshold to electrical convulsions and (−)-amphetamine and (−)- and (+)-ephedrines did not produce any change (298).

Central effects of the optical isomers of phenolic amines such as norepinephrine and epinephrine have been investigated (299). Since these agents produce marked pressor effects, there is the problem of separating peripheral effects from central effects. Moreover, these amines penetrate very poorly into the CNS. To overcome this difficulty, drugs were applied iontophoretically in order to study responses to brainstem neurones in decerebrate cats. The effects of (−)-norepinephrine on the firing rate of spontaneously active neurones have been found to conform to certain well-defined patterns. There are two types of patterns observed with (−)-norepinephrine, excitatory and/or inhibitory. (+)-Norepinephrine inhibited certain neurones which were also inhibited by (−)-norepinephrine; but on neurones excited by (−)-norepinephrine, its effect was weaker or absent. Thus, the excitatory effect shows stereoselectivity whereas inhibitory effect does not. The classical adrenergic blockers did not modify the responses. It is concluded that receptors for norepinephrine on brainstem neurones are of more than one kind and that they do not fit into the α- and β-classification applied to peripheral receptors (300).

An interesting approach to the study of central effects of catecholamines which do not pass the blood–brain barrier is to study them in young chickens where the blood–brain barrier is imperfect or nonexistent. Dewhurst and Marley (301, 302) used this approach to examine central effects of certain phenolic amines. The behavioral, electrocortical, and electromyographic activities were recorded. The phenolic amines produced depressant effects. Levonordefrin was at least 4 times as potent as the (+)-form. (−)-Norepinephrine was twice as potent as (+)-norepinephrine; however, dopamine was more potent than (−)-norepinephrine or (+)-norepinephrine. This pattern differs from that of peripheral sites 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.

β-Adrenergic blockers produce variable effects on the CNS. With the aid of optical isomers of INPEA, Murmann *et al.* (303) concluded that, since both isomers were equipotent in producing CNS stimulation and since only one isomer is a potent β-adrenergic blocker, the central effects of β-blocker are unrelated to the blockade of β-adrenergic receptors.

The role of endogenous catecholamines in the peripheral effects of many nonphenolic amines is well defined. However, controversy still exists regarding the role of endogenous catecholamines in central effects of amphetamine and related agents. Wolf *et al.* (304) investigated optical isomers of several nonphenolic amines in the normal, reserpine-pretreated, and α-methyl-*m*-tyrosine-pretreated mice. Ability of the drug to lower chemoconvulsive threshold was used as an index of the central activity. All agents except (−)-pipradrol and (−)-pseudoephedrine demonstrated significant central effects. Reserpine pretreatment lowered the chemoconvulsive threshold to (+)-amphetamine, (−)-amphetamine, (+)-pipradrol, (−)-ephedrine, and (+)-ephedrine, while that after (+)-pseudoephedrine, (−)-norephedrine, and (+)-norpseudoephedrine was not affected. On the other hand, in the α-methyl-*m*-tyrosine-pretreated animals, the chemoconvulsive thresholds

after (+)-amphetamine, (−)-amphetamine, (−)-ephedrine, and (+)-pseudoephedrine were increased. Results from α-methyl-*m*-tyrosine-pretreated animals were assumed to be a better reflection of central catecholamine depletion. It was concluded that central effects of (+)- and (−)-amphetamine, (−)-ephedrine, and (+)-ephedrine possess large indirect components in their activity while those of (+)-pipradrol, (−)-norephedrine, and (+)-norpseudoephedrine are mainly direct. The effects of the optical isomers of amphetamine were also investigated on brain dopamine levels (305, 306), tissue respiration (307), and liver monoamine oxidase inhibition (284). However, both forms of amphetamine isomers produced similar results, indicating that these effects are not causally related to the central effects.

Infusion of (−)-α-methyldopa into the vertebral artery of the cat produces lowering of the blood pressure while (+)-α-methyldopa produces no such effect (308).

No clearcut structure–activity relationship as yet can be formulated for the central effects of sympathomimetic amines. In spite of this, as in the periphery, there are many agents which exhibit stereoselectivity in their central effects.

Amphetamine is well known to produce aggregation toxicity in mice. This effect is more pronounced with (+)-amphetamine. The potency ratio of the isolated/aggregated LD_{50} 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 (+)-isomer in 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 (309). Selectivity of aggregation toxicity was also studied for ephedrine isomers. Except for (+)-pseudoephedrine, all isomers of ephedrine exhibited this phenomenon, but in a magnitude which is much lower than that of (+)-amphetamine (310). The LD_{50} potency ratio (isolated/aggregated) for the most potent ephedrine isomer was only 1.5.

There has been considerable clinical interest in the anorexigenic response of the drugs affecting the CNS. A reliable eating response can be obtained on injection of small quantities of (−)-norepinephrine into the rostral hypothalamus of the rat. (+)-Norepinephrine induces a negligible response, indicating a stereoselective effect. Booth (311) postulated that α-adrenergic modulation of postsynaptic activity by norepinephrine from the nerve ending is involved in the hypothalamic control of feeding in the rat. The stereoselectivity in the feeding behavior for isomers of norepinephrine was also observed by Margules (312). Roszkowski and Kelley (313) 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. Abdallah (314) found (−)-ephedrine to be the most potent of the ephedrine isomers in causing a reduction of food intake in mice. Several other reports indicate that the (+)-form of amphetamine is more of an appetite depressant than the (−)-isomer (315–317). Between the isomers there is crosstolerance to the anorexic effect (318). Thus, it has been difficult to separate the central stimulant effects from the anorexigenic effects of these agents.

Greater loss of sodium from tissue could cause a great 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 (319).

The potent CNS-stimulating effect of (+)-amphetamine can be demonstrated by increased oxygen consumption in morphine-pretreated dogs or in humans. In this respect, (−)-amphetamine is much less active than the (+)-form (320, 321). *p*-Chloro-substituted amphetamines show some promise as anorexic agents. (+)-*p*-Chloroamphetamine is less of a central stimulant but is a longer-acting anorexigenic agent than (+)-amphetamine (322). (−)-*p*-Chloroamphetamine is more active than (−)-amphetamine; both agents are less active than their respective (+)-forms. The anorexigenic effect of (+)-*p*-chloroamphetamine is unrelated to depletion of brain serotonin because both optical isomers are equally effective in causing depletion of brain serotonin (323). Phenmetrazine and phendimetrazine are known to be effective anorexigenic agents. There is an interesting stereochemical relationship between ephedrine and the phenmetrazine molecule (324, 325). Most observations to date tend to support the view that the anorexigenic effect of (+)-amphetamine may be of central origin.

CONCLUDING REMARKS

An attempt is made to survey a highly scattered and occasionally fragmentary literature on the optical isomers of adrenergic drugs. The facts from the old literature as well as those from the recently published manuscripts are blended in a proper perspective. At adrenergic synapse, stereoselectivity has been observed for: (*a*) all biosynthetic pathways; (*b*) transport in adrenergic neurone; (*c*) prevention of uptake at the neurone; (*d*) binding and retention in the granule; (*e*) enzyme monoamine oxidase; and (*f*) pharmacologic α- and β-adrenergic receptors. Previously, adrenergic blockers were used to classify the pharmacologic receptors. However, blockers don't have to react with the exact configuration of the receptor. In addition, physical–chemical properties of one blocker could differ from those of the other. So far as (−)- and (+)-isomers are concerned, their physical–chemical properties are identical. The evidence obtained from blockers could be regarded as an indirect one. Agonists have to interact with the exact configuration of the receptor to produce the pharmacologic effect. The isomeric activity ratio should serve as a better criterion to differentiate receptors. Even if the adrenergic receptor is isolated, its similarity to that in the tissue will not be proven until isomeric affinity ratios are obtained and compared with the isomeric activity ratio. Thus, optical isomers provide a valuable tool to analyze drug effects at basic levels.

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RESEARCH ARTICLES

Interaction of Isoniazid with Magnesium Oxide and Lactose

WEN-HUNG WU, TING-FONG CHIN, and JOHN L. LACH

Abstract □ Interactions of isoniazid with magnesium oxide and with lactose were investigated in the solid state. In the isoniazid–magnesium oxide system, chemisorption as well as physical adsorption of isoniazid molecules onto the magnesium oxide surface was confirmed by diffuse reflectance spectroscopic data. An absorption maximum due to chemisorbed isoniazid molecules was found to occur at 325 mμ, whereas physical adsorption was detected at 268 mμ. The mechanism of surface chemisorption is different from that of the formation of the isoniazid–metal-ion complex in solution. The browning reaction of the isoniazid–lactose system in solid state was also studied using diffuse reflectance spectroscopy. The rates of browning at 95, 100, 105, and 110° were followed by measuring the reflectance at 450 mμ. From the data obtained, the approximate time needed for browning to be perceptible has been predicted. TLC separations of the reaction products confirmed the presence of isonicotinoyl hydrazones of lactose and hydroxymethylfurfural.

Keyphrases □ Isoniazid interaction—magnesium oxide, lactose □ Lactose–isoniazid systems—browning rates □ Magnesium oxide, chemisorption—isoniazid □ Diffuse reflectance spectroscopy—analysis □ IR spectrophotometry—identity

Difficulties in formulating a new pharmaceutical dosage form have often been experienced because of the interactions between the supposed inert adjuvants and the active ingredient itself. Although the nature and intensity of these interactions vary, such interactions may alter the stability, dissolution rate, and, consequently, the absorption of the drug. A literature survey indicates that such interactions involving the formation of complexes have been studied extensively in aqueous solution; relatively few studies have been carried out in the solid state.

Stearic acid and calcium stearate have been shown by Kornblum and Zoglio (1) to catalyze the degradation of aspirin. Ribeiro *et al.* (2) studied the influence of lubri-