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REVIEW

Substrates and Inhibitors of Aromatic Amino Acid Decarboxylase

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Aromatic L-amino acid decarboxylase (AADC; EC 4.1.1.38) is the enzyme responsible for the decarboxylation of dihydroxyphenylalanine (dopa)[1] to dihydroxyphenyl ethylamine (dopamine). The enzyme is widely distributed in mammalian tissues and requires pyridoxal 5′-phosphate (PLP) as cofactor. In addition to dopa, it decarboxylates a number of other aromatic amino acids, both natural and synthetic; 5-hydroxytryptophan (5-HTp), the precursor of serotonin, is among the most important of these other substrates (*1*). Although solid experimental evidence indicates that AADC is a single homogeneous enzyme (*2, 3*), several puzzling facts such as pH dependence of the activity on different substrates (*4*), substrate sensitivity on treatment with denaturants (*5*), and kinetic behavior toward reversible and irreversible inhibitors (see below) could indicate the existence of multiple forms. The enzyme purified from hog kidney has a molecular weight of 110,000 and consists of two units which are most probably nonidentical (*2, 6, 7*). The enzyme has a high affinity for PLP; in the absence of exogenous PLP, there is only one cofactor molecule per active enzyme (*3, 7*). Addition of exogenous PLP, however, stimulates enzyme activity two- to fivefold. The nature of this activation is not yet elucidated. It has been proposed that this PLP may bind to an allosteric site (*2*), or to a second active site (*8*). Alternatively, this PLP may compensate for the formation of pyridoxamine 5′-phosphate during the abortive decarboxylation-dependent transamination, as is discussed later.

The long-standing interest in finding inhibitors for this enzyme is justified by its role in the synthesis of the known neuotransmitters dopamine, noradrenaline, and serotonin both in central and peripheral neurons (*9*). In addition, as recognized more recently, the enzyme is also present in neurons which do not contain any of the classical biogenic amines (*10*). AADC exists also in insects where dopamine in its *N*-acetylated form is the cuticular sclerotizing agent and inhibitors of AADC are toxic to larvae (*Lucilia cuprina*) at the time of molting (*11*). In the present

[1] Abbreviations used: dopa, dihydroxyphenyl alanine; dopamine, dihydroxyphenyl ethylamine; PLP, pyridoxal 5′-phosphate; 5-HTp, 5-hydroxytryptophan.

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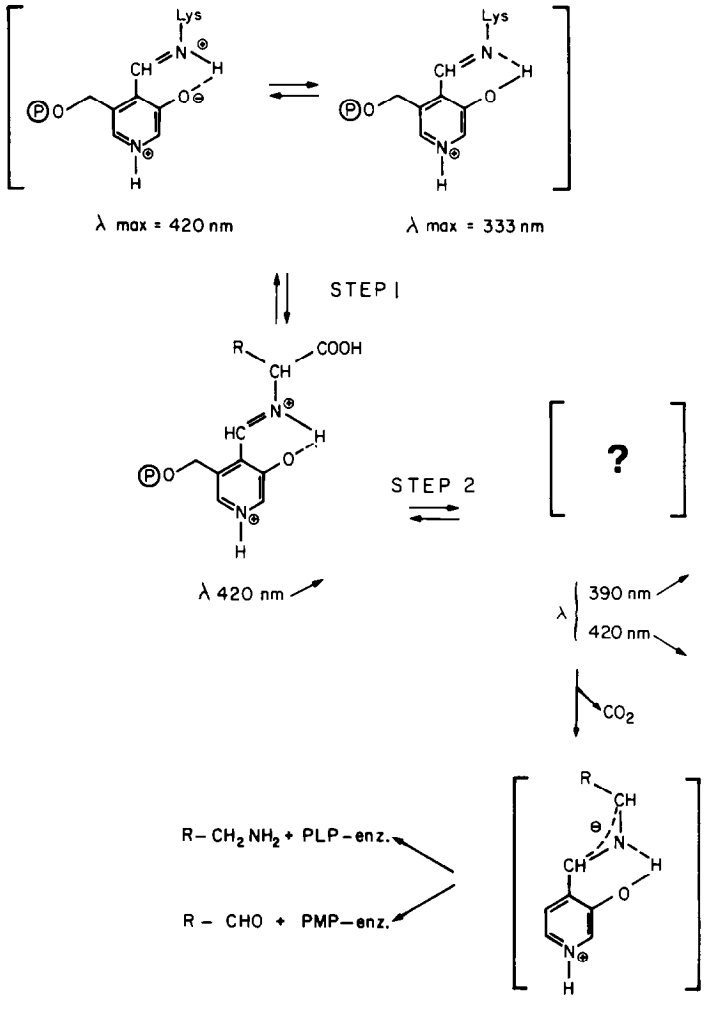


FIG. 1. Fast-kinetics studies on the interaction of AADC with its substrate.

study, however, we concentrate on molecular and mechanistic aspects of the mammalian enzyme.

(*A*) *Reaction with* L-*and* DL-*Amino Acids*

The native enzyme shows two uv absorption bands at 333 and 420 nm (*12*). These bands indicate that the coenzyme is present in at least two forms. The ratio between the two bands is pH-dependent, and the 333-nm absorbing species becomes more important as the pH is raised. A likely explanation of this observation is the presence of an equilibrium between a protonated aldimine (420 nm) and the unprotonated form (333 nm) (*13*). Upon addition of L-dopa, three events can be followed by stop-flow techniques (*13*) (Fig. **1**). There is first a very rapid ($t1/2 < 5$

ms) increase of absorption at 420 nm and decrease at 333 nm which has been attributed to formation of the aldimine with the substrate in its protonated form. During the second phase ($t1/2 \sim 20$ ms), the absorption at 420 nm decreases while a species that absorbs at 390 nm appears. This step, which is not well characterized, precedes decarboxylation and may represent a tautomery facilitating decarboxylation. The final and slowest step ($t1/2 \sim 0.2$ s) is proposed to be the decarboxylation and is characterized by a further decrease at 420 nm. No quinonoid species (absorption expected around 500 nm) could be detected, and this may be because the protonation is too rapid to be detectable. If protonation takes place on the α-carbon, then product amine is released and the native enzyme is regenerated. It was noted, however, that when AADC is incubated with dopa in the absence of exogenous PLP, the reaction stops after a few minutes before exhaustion of substrate (*1*) and dihydroxyphenyl acetaldehyde is released (*14*). Detailed investigation of this mechanism (*15*) led to the conclusion that AADC catalyzes, in addition to the normal decarboxylation, a decarboxylation-dependent transamination resulting from protonation on the methylene group of the cofactor instead of the α-carbon of the substrate. This abnormal event takes place every 5000 turnovers with dopa or *m*-tyrosine as a substrate.

In earlier work, α-methyldopa was found to be a competitive inhibitor of AADC when incubation time was kept short, and a noncompetitive inhibitor upon longer reaction times (*16*). It is also a substrate of AADC, although the rate of decarboxylation is only about 1/100 that of dopa. Upon incubation of AADC with α-methyldopa, the 420-nm absorption band disappears with a half-life of 2 min and the absorption at 330 nm increases. Pyridoxamine is formed during this incubation (*12*). Similar spectral changes were observed upon incubation of AADC with α-methyl-*m*-tyrosine (*15*). The time-dependent inhibition of AADC by these α-methylaminoacids could thus be explained by the same decarboxylation-dependent transamination discussed above for dopa. However, when the products of the reaction of AADC with α-methyldopa were quantitatively analyzed, it was realized that the dihydroxyphenyl acetone formed was 8- to 15-fold in excess of the amount of PLP originally present in the enzyme and very small amounts of α-methyl dopamine were formed (*17*). The authors ruled out oxidation of α-methyl dopamine as well as pyridoxamine as little ammonia was released and no oxygen was consumed. They suggest the existence of ammonia-accepting sites not yet characterized, located on or associated with the enzyme-active site. Except for this puzzling observation, it could be concluded that α-methyl substitution on a substrate of AADC increases the incidence of abnormal protonation by a factor of 100 (*15*). This higher incidence is most certainly due to the additive effects of steric hindrance and the inductive effect of the methyl group. Similar behaviors have been described for bacterial glutamate decarboxylase (*18*) and a bacterial ornithine decarboxylase (*19*).

The relative tolerance of AADC toward substrate modification has led several groups to investigate whether amines with interesting pharmacological properties can be generated from the corresponding amino acids by this enzyme.

(*a*) *Rigid analogs of α-methyldopa.* Rigid analogs of dopamine were studied for their affinity for dopamine receptors, and it was found that *R*-(+)-amino-4,5-

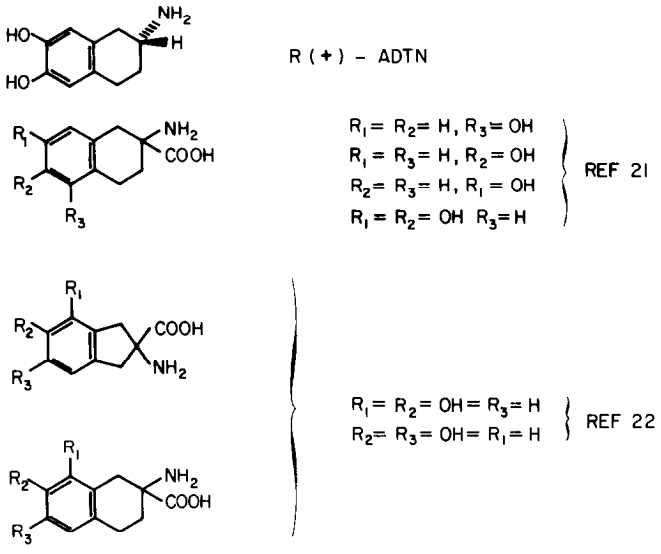


FIG. 2. Rigid analogs of α-methyldopa tested as substrates/inhibitors of AADC.

dihydroxy-1,2,7,8-tetrahydronaphthalene was the most potent dopamine agonist in a series of compounds (*20*). Analogs of dopa linking the α-aminocarbon to one of the ring carbons had been synthesized before (*21, 22*) and tested as substrates or inhibitors of AADC (Fig. 2). These compounds inhibited AADC, the most effective inhibitors being the *o,m*-dihydroxy amino acids as opposed to the *m,p* analogs (*22*). The inhibition is enhanced by preincubation with PLP. Data on amine formation from these compounds has not been reported in the literature to the author's knowledge.

(*b*) *Cyclopropyl analogs of dopa.* 2-Aryl cyclopropylamines are inhibitors of monoamine oxidase and a compound such as tranylcypromine is still in use in certain countries as an antidepressant. Attempts to generate the cyclopropylamine from the corresponding amino acid by AADC have been reported (*23*). To combine chemical stability and structural requirements for an AADC substrate, the 3-hydroxyphenyl cyclopropyl amino acid appeared as the most promising candidate. In an insensitive manometric assay, these compounds were apparently not decarboxylated to a significant extent. They interact with the enzyme, however, in an essentially reversible manner (*24*).

(*c*) *β-Methylene analogs of substrates of AADC.* β-Methylene-*m*-tyrosine was synthesized (*25*) with the hope that it would be an enzyme-activated inhibitor of AADC. This was found not to be the case (*26*). Ring-substituted β-fluoromethylene phenethylamines are extremely potent enzyme-activated inhibitors of monoamine oxidase (*27*). As in the preceding paragraph but this time with success, it was reasoned that the corresponding amino acid could be a substrate of AADC, thus allowing to direct the MAO inhibitor to nerve terminals. Indeed, racemic β-fluoromethylene-*m*-tyrosine upon incubation with AADC is decarboxylated to the extent of 50% and the product has been characterized as being the corresponding

m-tyramine, a potent MAO inhibitor. No inhibition of AADC was observed (*28*). In animal studies, it could be demonstrated that provided the animals are pretreated with a peripheral AADC inhibitor such as carbidopa, the inhibition of MAO is restricted to the brain. This should trigger a new interest in MAO inhibitors as antidepressants (*29*). In a subsequent study (*30*), these authors investigated the substrate activity of other substituted β-methylene-*m*-tyrosines. Rates of decarboxylation were compared with that of dopa. At a concentration of 5 mM, the *E*-fluoro compound is decarboxylated at half the rate of dopa, the *Z*-fluoro at only 6%, *E*-chloro at 22%, and the unsubstituted compound of Chari and Wemple (*25*) at 20%. This unique set of new substrates of AADC could bring useful information on the active sites of AADC and the mechanism of the decarboxylation process.

(*B*) *Interaction of AADC with* D-*Amino Acids and Amines*

AADC decarboxylates only the L-enantiomer of aromatic amino acids to the corresponding amines. The D-forms, however, bind to the enzyme. For instance, D-dopa competitively inhibits the decarboxylation of L-dopa (*31*). This inhibition is accompanied by an increase of the 420-nm absorption of the native enzyme, similar to that described during L-dopa binding at low temperatures, and can be attributed to the formation of a protonated Schiff base. With D-dopa or D-*m*-tyrosine, no further changes occur. However, when AADC is incubated with D-5-hydroxytryptophan after the initial increase of the 420-nm absorption, there is a decrease of this uv band accompanied by a time-dependent loss of enzyme activity. Exogenous PLP partly prevents the inhibition and some PMP is formed. However, the reaction is more complex than a simple transamination: addition of PLP does not totally restore the enzyme activity and the PMP formed accounts for only one-third of the cofactor originally present.

The inhibition of AADC by serotonin (*16*), which is reversed by exogenous PLP, is also accompanied by time-dependent changes in the uv spectrum (*32*) and formation of 5-hydroxyindol acetaldehyde. Other product amines do not show this reaction. It clearly appears that AADC is able to abstract the α-hydrogen of D-5-hydroxytryptophan and one of the α-hydrogens (stereochemistry not determined) of serotonin. It is not presently known whether each hydrogen abstraction results in transamination or whether exchange with solvent protons takes place. This hydrogen abstraction could be inherent to the microscopic reversibility of enzyme reactions (see below also) as implicated for the inhibition of bacterial glutamic acid decarboxylase by *R*-4-aminohex-5-ynoic acid (*33*). The difference in behavior of the D-enantiomers of dopa and 5-HTp and of phenethylamines and serotonin cannot be readily explained without more knowledge of the active site.

(*C*) *Inhibition of AADC by Bisubstrate Adducts*

AADC tightly binds the pyridoxal cofactor which dissociates only in its pyridoxamine form (see above). Upon binding of the substrate to the enzyme-active site, the cofactor which is normally present as an aldimine with the ε-amino group of a lysine residue undergoes transaldimination. For a number of PLP enzymes,

stable analogs of this Schiff base have been prepared by sodium borohydride reduction. These compounds are normally good inhibitors of their respective enzymes. For instance, *N*-pyridoxylalanine binds more tightly than pyridoxamine to pyridoxamine-pyruvate transaminase (*34*), and *N*-5′-phosphopyridoxyl 4-amino butryate inhibits 4-aminobutyrate aminotransferase (*35*); the pyridoxyl derivative at the α-amino group of L-ornithine is a good inhibitor of L-ornithine decarboxylase (*36*). Rudd and Thanassi (*8, 37*) explored the interaction of stable PLP–AADC substrate adducts with AADC. The isoquinolines formed by the Pictet–Spengler reaction of PLP with dopa had no inhibitory effect even at millimolar concentrations. By contrast, *N*-5′-phosphopyridoxyl-L-dopa, which because of the above-named Pictet–Spengler reaction had to be prepared in a multistep synthesis, is a powerful inhibitor ($IC_{50} \sim 10^{-5}$ M). The phosphate group in 5′ is apparently important for binding as the 5′-deoxy compound has a nearly 100 times lower affinity. This last compound, however, causes time-dependent inhibition of AADC (*8*), which can be prevented by the presence of an exogenous cofactor. The decrease of activity is accompanied by a decrease of the pyridoxal content of the enzyme. Despite a higher affinity, the 5′-phospho derivatives inactivate the enzyme more slowly. After incubation of the apo-enzyme with the pyridoxal derivative of *m*-aminotyrosine, exogenous PLP is no longer able to restore all of the enzyme activity, i.e., to displace totally the inhibitor from the active site. Upon short-term incubation of the holoenzyme with this compound, enzyme activity is enhanced. The authors suggest that these PLP-substrate adducts may bind to the second PLP-binding site previously mentioned.

The 5′-pyridoxal phosphate derivative of D-5-hydroxytryptophan binds more strongly to the enzyme-active site than the derivative of the L-amino acid or that of serotonin (*38*) (i.e., enzyme activity is regenerated less readily by exogenous PLP). The additional binding energy could be due to the interaction of the carboxylate group of D-tryptophan with a group of the enzyme's active site.

(*D*) *Interaction of AADC with PLP Scavengers*

Carbonyl trapping agents such as hydrazines and hydroxylamines inhibit AADC by binding to the pyridoxal cofactor. The older literature was reviewed in 1966 (*9*). The most potent of these compounds are those most closely resembling the substrate, i.e., having an aromatic ring with at least one hydroxyl group in the *meta* position. Two inhibitors of this class are marketed drugs: α-methylhydrazino dopa (Carbidopa) and N_1-seryl N_2-(2,3,4-trihydroxybenzyl) hydrazine (benserazide). A third inhibitor is a widely used pharmacological tool to study the metabolism of biogenic amines, i.e., 4-bromo-3-hydroxy-benzyloxyamine.

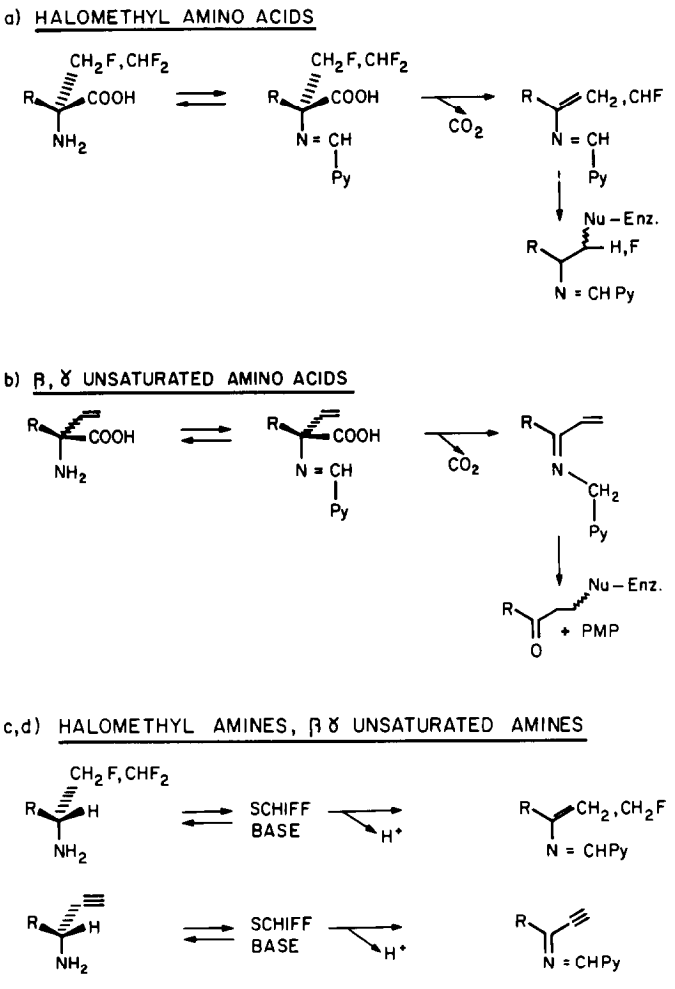
α-Methylhydrazinodopa is an extremely powerful inhibitor of AADC since it reacts with the enzyme in a nearly stoichiometric manner (*39*). For instance, at an inhibitor to enzyme ratio of 1, decarboxylation of dopa (at a concentration of $2 \times K_m$) is inhibited by 75%, and that of 5-HTp (at $4 \times K_m$) by 90%. It must be noted that when the ratio of inhibitor/enzyme is increased to a value of 2–2.5, there is still a residual activity of 5–15% for both substrates. The interaction of hydrazinodopa with AADC is characterized by the appearance of an intense uv absorption

band at 395 nm, which does not correspond to the absorption of the hydrazone formed between PLP and carbidopa in the absence of enzyme. Similarly, the rate of carbidopa binding to enzyme-bound PLP is much faster ($t1/2$ = 0.5 s) than in model studies under similar conditions ($t1/2$ = 1.2 min). Carbidopa falls clearly in the category of tight-binding inhibitors which have an extremely slow dissociation. The uv spectrum described above does not change upon extensive dialysis and while the presence of substrate slows the rate of complex formation, substrate is not able to displace the inhibitor once the complex if formed.

In earlier studies, it had been observed that benserazide caused a slow time-dependent decrease of enzyme activity upon incubation with a crude preparation of AADC. Again, the inhibition could not be reversed by dialysis or coenzyme addition (*9*). The type of inhibition is totally different, however, when a purified preparation of AADC is used (*40*). Benserazide is a pure competitive inhibitor of dopa decarboxylation and a noncompetitive inhibitor of 5-HTp, *m*-, or *o*-tryosine decarboxylation. This difference in behavior against different substrates suggests that there might be different overlapping binding sites. The difference in the type of inhibition of the crude vs the pure preparation of enzyme strongly suggests that benserazide is not the active species *in vivo* and that it is only a pro-drug form of the tight-binding hydrazine, (2,3,4-trihydroxy)benzyl hydrazine.

(*E*) *Enzyme-Activated Inhibitors and AADC*

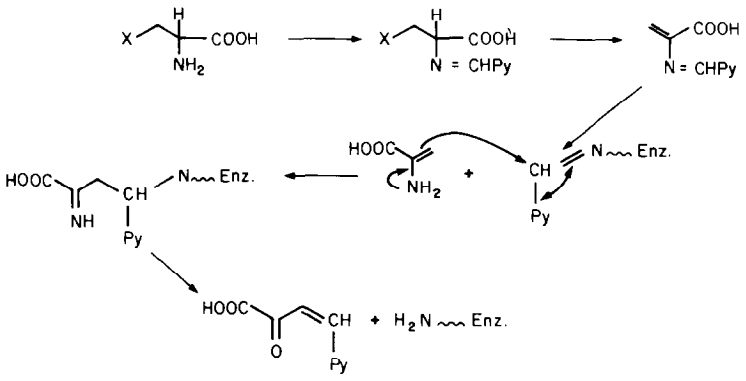
We have seen in previous sections that dopa and α-methyldopa can lead to enzyme inactivation in the absence of exogenous cofactor by the transamination-dependent decarboxylation mechanism. In addition, D-tryptophan and serotonin lead to enzyme inactivation by a transamination mechanism. These natural substrates or products of AADC could be considered enzyme-activated inhibitors of this enzyme. However, before the mechanistic details of how these compounds interact with AADC were known, several groups had engaged in designing enzyme-activated inhibitors. The different possibilities of designing such inhibitors of enzymes which catalyze the decarboxylation of α-amino acids have been discussed in previous reviews (*41, 42*). Potential inhibitors can be derived from either the substrate or the product of a decarboxylase by replacing the α-hydrogen of a substrate amino acid, or one of the α-hydrogen atoms of the product amine, either by a halomethyl group or by an unsaturated group such as vinyl or acetylene. When these compounds were conceived, it was expected that decarboxylation or hydrogen abstraction (microscopic reversibility principle) could generate an α,β-unsaturated imine by elimination of a halide anion in the case of halomethyl-substituted compounds, or a Michael acceptor in the case of α-vinyl or acetylenic compounds. Irreversible inhibition of the enzyme would then result from the alkylation of an active site nucelophile by these electrophilic species (Scheme 1). In recent years, however, an alternative mechanism has been demonstrated for the inhibition of aspartate amino transferase and glutamate decarboxylase by L-serine-*O*-sulfate (*43, 44*) and for the inhibition of alanine racemase by D-fluoroalanine (*45*). This second mechanism in its generalized form is described in Scheme 2. The α,β-unsaturated imine is generated as previously shown (so far this



SCHEME 1. Summary of the various possibilities to design suicide inhibition of AADC.

mechanism has been demonstrated only in the case of hydrogen abstraction, i.e., aminoacrylates are formed), and the imine released reacts in a nucleophilic manner with the Schiff base reformed between the newly liberated PLP and the ε-amino group of lysine, linking the cofactor–enzyme complex to the modified inhibitor. Subsequent chemical modifications may be artifacts of the workup.

We now review which compounds have been described for AADC, and what experimental evidence is available to allow one to decide on a mechanism of inhibition. It must first be stressed that for all of the compounds reported, the time-dependent loss of AADC activity markedly deviates from pseudo-first-order kinetics. (For the kinetic treatment of enzyme-activated inhibitors, the reader is referred to the review on ornithine decarboxylase inhibitors by Bey *et al.* (*46*).) This behavior may mean that, as previously suggested, AADC even in its purest form is a mixture of isoenzymes or more likely that the mechanisms are far more



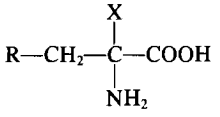
SCHEME 2. Alternative mechanism for the compounds of Scheme 1.

complex than anticipated. The practical consequence of this fact is that potencies of compounds cannot be compared in terms of K_I and $T_{1/2}$ as in (*46*).

(1) *α-Halomethyl amino acids*. The different compounds reported are listed in Table 1; potencies can be compared by the amount of time required to achieve 50% inhibition at the given inhibitor concentration. With the exception of chloromethyldopa, all other compounds are mono- and difluoromethyl derivatives of known substrates of AADC (*50*). Two conclusions can be drawn: (1) the mono-

TABLE 1

α-HALOMETHYL DERIVATIVES OF AROMATIC AMINO ACIDS AS TIME-DEPENDENT INHIBITORS OF AADC



R	X	$t_{1/2}$ (min)	[I] (μM)	Reference
3,4-Dihydroxyphenyl	CH_2CL	2	40	41
3,4-Dihydroxyphenyl	CH_2F	1–2	5	47,48
3,4-Dihydroxyphenyl	CHF_2	4	20	49
2,3-Dihydroxyphenyl	CH_2F	1	10	50
2,3-Dihydroxyphenyl	CHF_2	1	100	50
2,5-Dihydroxyphenyl	CH_2F	2–3	10	50
2,5-Dihydroxyphenyl	CHF_2	2	100	50
2-Hydroxyphenyl	CH_2F	<1	100	51
3-Hydroxyphenyl	CH_2F	5	100	51
4-Hydroxyphenyl	CH_2F	<50	100	51
4-Hydroxyphenyl	CHF_2	No effect	100	50
5-Hydroxyindole	CH_2F	>60	10	51
5-Hydroxyindole	CH_2F	2	100	51
5-Hydroxyindole	CHF_2	>60	10	51
5-Hydroxyindole	CHF_2	2	100	51
Indole	CH_2F	No effect	100	51
Indole	CHF_2	No effect	100	51

fluoromethyl derivatives are more potent than the difluoromethyl analogs, and (2) regarding the nature of the aromatic ring, the potency as inhibitors follows the structure–activity requirements of the parent aminoacids as substrates, i.e., 3,4-dihydroxyphenyl, 2,3- or 2,5-dihydroxyphenyl, *m*- or *o*-hydroxyphenyl > 5-hydroxyindole > *p*-hydroxyphenyl (*52*).

The most detailed study of the mechanism of inactivation has been conducted with monofluoromethldopa (*47*). As already indicated, the time course of inactivation does not follow pseudo-first-order kinetics. The inhibition is independent of exogenous PLP and is not reversed by extensive dialysis. Incubation with α-methyldopa slows down the rate of inhibition. As we have seen before, in this experiment, the holoenzyme is at least partly in the pyridoxamine form, which cannot bind the inhibitor. The inhibition by racemic monofluoromethyldopa can be accounted for by one enantiometer (called S by these authors) in agreement with the preferred stereospecificity of the enzyme. As shown by the absence of incorporation of radioactivity into the enzyme, when [1-^{14}C]monofluoromethyldopa is employed, the inhibitor undergoes decarboxylation and the radioactivity is released as CO_2. On the contrary when ring-labeled ^{3}H inhibitor is used, there is a stoichiometric labeling of the enzyme. One atom-gram of fluoride is released per mole of inhibited enzyme. Insignificant amounts of α-fluoromethyldopamine are formed during inhibition. In these experiments, incorporation of radioactivity into the inhibited enzyme was determined after removal of the excess inhibitor under nondenaturing conditions. The fate of the cofactor was not studied. In a similar study, using ring-labeled difluoromethyldopa (*49*), if was found that incorporation of radioactivity into the acid-precipitated protein mirrored the loss of enzyme activity. Spectral changes observed during inhibition were not very informative concerning the fate of the cofactor: a decrease of the 420-nm absorption band accompanied by a slight increase of the 330-nm band was observed. The fact that the radioactivity associated with the difluoromethyldopa-inhibited enzyme cannot be washed out in the denatured protein, plus the efficiency of enzyme inhibition by monofluoromethyldopa (partition ratio close to 1), is considered by the author of this review as evidence for the mechanism of Fig. 1.

(2) *Unsaturated amino acids.* The syntheses of α-vinyl- and α-ethynyldopa were published almost simultaneously by two groups (*53, 54*). Studies on the mechanism of interaction of these compounds with AADC were reported by two separate groups (*55–56* and *57–58*). The conclusions drawn by the two groups may seem at variance. Maycock *et al.* (*55, 56*) reported a rapid inactivation of the enzyme in the absence of exogenous PLP followed by a slower phase. The second group (*57, 58*) detected only the slow phase of inactivation in the presence of PLP in the incubation medium. The two results can be reconciled since the enzyme slowly reactivates upon dilution into a pyridoxal-containing medium. Using carboxyl and ring-labeled inhibitor, it was observed that both compounds are decarboxylated and that a stoichiometric amount of ring-labeled inhibitor is incorporated into the inhibited enzyme (*55, 56*). The radioactive label is lost during dialysis while some of the enzyme activity is restored. The amines which should be formed by the normal decarboxylation could not be detected, although both

compounds are catalytically decarboxylated by the enzyme. For instance, α-ethynyldopa after an initial burst of CO_2 release corresponding to 20 times the amount of enzyme present releases CO_2 at a rate of 10 pmol/min/pmol enzyme (*55*). In the second study, using cold substrates, no decrease of substrate concentration could be detected over long periods of incubation, and as before, there was no amine formation (*58*). The HPLC method used, however, may not have been sensitive enough to detect a change of a few percentage points in substrate concentration.

The mechanism is certainly a complex one. One can tentatively suggest at this point that the rapid inactivation seen in the absence of PLP may be due to the decarboxylation-dependent transamination. The slower phase could be due to decarboxylation associated with double-bond rearrangement, which could partition between hydrolysis to enamine and PLP (active enzyme), and a mechanism similar to that described in Fig. 2. A definitive explanation will require further work.

More recently, a series of α-allenyl derivatives of amines and amino acids was described (*59*). For instance, α-allenyldopa was claimed to rapidly inactivate AADC: $t1/2 = 6$ min at 0.1 mM concentration of inhibitor. No further details of the mechanism of action are available presently.

(3) *Amine derivatives*. During the evaluation of the enzyme specificity of *R*- and *S*-4-aminohex-5-ynoic acid, it was realized that the *R*-enantiomer is a good time-dependent inhibitor of bacterial glutamic acid decarboxylase (*33*). This finding was rationalized on the basis of the microscopic reversibility principle of reactions catalyzed by enzymes and was found to be applicable to design inhibitors of other decarboxylases. The most prominent examples of the approach are α-acetylenic putrescine (*60*) and *R,R*-,2,-5-diaminoheptyne [see (*46*) for review]. The microreversibility principle may apply also to halmomethylamines. Fluoromethyl putrescines are time-dependent inhibitors of ornithine decarboxylase (*46*). However, fluoromethyl analogs of β-alanine or 4-aminobutyric acid do not inhibit glutamic acid decarboxylase (unpublished).

The various possibilities have been applied to dopamine, and the corresponding compounds were examined as inhibitors of AADC (*55*). Vinyl and ethynyl dopamine are time-dependent inhibitors of AADC; however, the reaction is very slow and was not further investigated. Fluoromethyl dopamine, on the other hand, is a potent irreversible inhibitor of AADC (*47, 55*). The inhibition is associated with the R-isomer in agreement with the stereochemistry expected from hydrogen abstraction via the microreversibility principle. There is a stoichiometric incorporation of ring-labeled fluoromethyldopamine into the inhibited enzyme. The rate of inhibition by the dopamine analog is much slower than by the corresponding amino acid analog (monofluoromethyldopa), which suggests that hydrogen abstraction is slower than decarboxylation.

As AADC transaminates serotonin more efficiently than dopamine (*32*), it would be interesting to see whether the ethynyl, vinyl, or fluoromethyl analogs of serotonin are more potent inhibitors of AADC than the corresponding dopamine derivatives.

(F) Inhibitors of AADC with Unspecified Mode of Action

Over 200 compounds of various classes were systematically tested as AADC inhibitors by Hartmann *et al.* (*61*). The best inhibitors were hydroxycinnamic acid or chalcone derivatives. Interest in these compounds has vanished in recent literature. There have been a number of reports of endogenous inhibitors of AADC. For instance, AADC activity is measurable in submaxillary glands of rats at birth and decreases after 2 weeks of age. It has been claimed that this decrease of activity is due to the age-dependent synthesis of an inhibitor (*62*). Later it was demonstrated that this inhibitor is a protein belonging to the family of trypsin proteases (*63*). On the other hand, a small-molecular-weight, heat-stable inhibitor of AADC was identified in human semen (*64*), while a macromolecule (MW > 300,000) inhibits AADC in postmortem human and primate brains (*65*). The physiological relevance of these endogenous inhibitors is unknown.

(G) Biology and Pharmacology of AADC Inhibitors

We are concerned with three classes of compounds: α-methyldopa, the pyridoxal scavengers, and the irreversible inhibitors.

(1) α-Methyldopa. This compound is a marketed drug for lowering blood pressure in man. The mechanism of action is certainly not due to its inhibitory effect on AADC but rather to its decarboxylation by this enzyme to α-methyldopamine. This compound is further metabolized to α-methylnoradrenaline, a weak sympathomimetic agent (*66*).

(2) Pyridoxal scavengers. α-Methylhydrazinodopa and N_1-seryl, N_2 (2,3,4-trihydroxybenzyl) hydrazine are used clinically in combination with dopa for the treatment of parkinsonism. This disease is characterized by a degeneration of dopaminergic neurons in *substantia nigra*. The associated motor disorders can be alleviated by supplementing with dopamine or dopamine agonists. As dopamine itself is too rapidly metabolized and does not readily cross the blood brain barrier, it has to be given in its bioprecursor form, dopa, in combination with a peripheral dopa decarboxylase inhibitor. Carbidopa and benserazide are very effective in this respect. They totally block the peripheral decarboxylation of exogenous dopa and have little or no effect on the biosynthesis of biogenic amines from endogenous precursors.

(3) Enzyme-activated irreversible inhibitors. Difluoromethyldopa is similar to carbidopa in its biological effects in animals. After intraperitoneal or oral administration, it effectively blocks AADC in peripheral organs. However, it has an effect on brain AADC activity at doses only above 500 mg/kg (*67*). It has no effect on catecholamine levels, but effectively protects exogenous dopa from peripheral decarboxylation.

Monofluoromethyldopa has a totally different profile. This compound blocks AADC activity almost as well in the brain as in peripheral organs. In addition, AADC can be inhibited to an extent where decarboxylation becomes rate limiting, for example, as in the synthesis of catechol- and indole amines. Therefore one can achieve selective depletion of biogenic amines utilizing this compound (*48, 68*). This results in antihypertensive effects which are reversed by iv infusion of

dopamine (converted to norepinephrine in sympathetic nerve terminals) (*69*). The compound can also be used to measure changes in amine turnover by detecting simultaneously accumulation of the precursor amino acids (dopa and 5-HTp) and decrease of the amines and their acidic metabolites (*70*). We have recently demonstrated that fluoromethyldopa can be generated from fluoromethyltyrosine by the action of tyrosine hydrolase *in vitro* and *in vivo* (*71*). This permits a selective action on catecholamine synthesis as opposed to serotonin and furthermore modulates the inhibitory effects by taking advantage of the delicate regulation of tyrosine hydroxylase (*51, 71*).

The counterpart for the serotoninergic neurons has not yet been achieved, although α-fluoromethyltryptophan is converted to the 5-hydroxyl analog by partially purified tryptophan hydroxylase. However, *in vivo* fluoromethyltryptophan has only a marginal effect on serotonin biosynthesis, presumably due to the poor potency of 5-hydroxy-α-fluoromethyltryptophan as an AADC inhibitor (*51*, Table 1).

CONCLUSION

AADC belongs to a class of enzymes which was thought to be well understood. However, there are still a number of unanswered questions such as, are there different iso-enzymes, are the sites of dopa and 5-HTp overlapping, what is the nature of the ammonia-accepting site(s) described by Borri-Voltattorni *et al.* (*31*), and how do the irreversible inhibitors (vinyl and acetylenic dopa essentially) interact with the enzyme?

These questions are not only of pure academic interest. AADC is present in neurons of rat spinal cord which do not contain any of the classical biogenic amines (*10*). A potential role of AADC in this location could be the synthesis of other aminergic compounds. It may some day be important to be able to selectively control their synthesis.

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