Mechanism-based Inhibition of Dopamine $\beta$-Monoxygenase by Aldehydes and Amides*

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A mechanism for $\beta$-chlorophenethylamine inhibition of dopamine $\beta$-monoxygenase has been postulated in which enzyme-bound $\alpha$-aminoacetophenone is generated, followed by an intramolecular redox reaction to yield a ketone-derived radical cation as the enzyme inhibitory species (Mangold, J. B., and Klinman, J. P. (1984) J. Biol. Chem. 259, 7772–7779). If correct, additional compounds capable of producing enzyme-bound reductant should inhibit dopamine $\beta$-monoxygenase. Phenylacetaldehyde was chosen to test this model, since $\beta$-hydroxyphenylacetaldehyde is expected to function as a reductant in a manner analogous to $\alpha$-aminoacetophenone.

Phenylacetaldehyde exhibits the properties of a mechanism-based inhibitor. Kinetic parameters are comparable to $\beta$-chlorophenethylamine under both initial velocity and inactivation conditions. Since phenylacetaldehyde bears little resemblance to $\beta$-chlorophenethylamine, its analogous inhibitory action provides support for an intramolecular redox reaction (via $\beta$-hydroxyphenylacetaldehyde oxidation to a radical cation) in dopamine $\beta$-monoxygenase inactivation. $\beta$-Hydroxyphenylacetaldehyde was identified as the enzymatic product of phenylacetaldehyde turnover. As predicted, this product behaves both as a time-dependent inhibitor of dopamine $\beta$-monoxygenase and as an electron donor in enzyme-catalyzed hydroxylation of tyramine to octopamine.

Phenylacetamide and $p$-hydroxyphenylacetamide are also found to be mechanism-based inhibitors of dopamine $\beta$-monoxygenase. In this case the product of hydroxylation ($\beta$-hydroxyphenylacetamide) is redox inactive and, therefore, is unable to function as either a reductant or an inhibitor. Thus, mechanism-based inhibitors are divided into two types: type I, which undergoes hydroxylation prior to inactivation, and type II, which only requires hydrogen atom abstraction. A general mechanism for dopamine $\beta$-monoxygenase inactivation is described, in which a common mechanistic radical intermediate is formed from both pathways.

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Dopamine $\beta$-monoxygenase catalyzes the conversion of dopamine to norepinephrine in a net reaction requiring two electrons and two protons (Equation 1). Ascorbic acid is the preferred electron donor, reducing the two copper centers/subunit in one-electron steps to form semidehydroascorbate as a transient intermediate (1, 2, 29, 30).

Although specific for pro-$R$ hydrogen removal, dopamine $\beta$-monoxygenase hydroxylates a range of compounds such as ring-substituted phenethylamines (3–5), $\beta$-substituted phenethylamines (6, 7), phenyl-$\alpha$-aminoethyl sulfide (8) and selendine (9), 1-phenyl-1-(aminomethyl) ethenenes (10, 11), benzylcyanides (12–15), $p$-phenylethenediolamine (11), and $p$-phenylpropanes (16, 26). This lack of substrate specificity can be exploited in the design of mechanism-based inhibitors. Ideally, with the increasing availability of mechanistic information (e.g. Refs. 16–18) the selection of such inhibitors will derive from their ability to form postulated reaction intermediates. Previous work from this laboratory has shown that whereas both $\beta$-hydroxy- and $\beta$-chlorophenethylamines are hydroxylated to generate $\alpha$-aminoacetophenone, only the $\beta$-chloro compound gives a time-dependent inhibition of enzyme (7). Further findings indicate that the gem-diol derived from $\beta$-hydroxyphenethylamine dissociates from enzyme prior to dehydration to $\alpha$-aminoacetophenone and that $\alpha$-aminoacetophenone can replace ascorbate as the external reductant in tyramine hydroxylation (19). These data have led to a model in which the (less stable) chlorohydrin derived from $\beta$-chlorophenethylamine decomposes to enzyme-bound ketone, which upon tautomeration to an enol undergoes an oxidation by active site Cu(II). As described by Equation 2, the resulting one-electron oxidized radical cation is the species responsible for the irreversible inhibition of dopamine $\beta$-monoxygenase.

To test the mechanism in Equation 2, we have examined a class of compounds which, though structurally very different from either dopamine or $\beta$-halophenethylamine, is predicted to form a species analogous to the enol of $\alpha$-aminoacetophenone in the presence of dopamine $\beta$-monoxygenase. As shown herein, phenylacetaldehyde exhibits both substrate and inhibitory activities analogous to $\beta$-chlorophenethylamine. The enzymatic product of phenylacetaldehyde hydroxylation, $\beta$-hydroxyphenylacetaldehyde, is a readily oxidized species (20). Consistent with the mechanism outlined above, product $\beta$-hydroxyphenylacetaldehyde causes time-dependent inhibition of dopamine $\beta$-monoxygenase and is able to serve as an electron donor for the hydroxylation of tyramine.

A second class of compounds, phenylacetamides, was tested for substrate and inhibitory activity. The product of enzymatic turnover, $\beta$-hydroxyphenylacetamide, is not likely to
oxygenize as is required by Equation 2. As expected, $\beta$-hydroxyphenylacetamide did not serve as an electron donor nor did it cause inhibition. The initial phenylacetamide substrates did, however, meet the requirements for mechanism-based inhibitors indicating an alternate pathway for inhibition.

Product inhibition studies have not yet been done on many of the inhibitors of dopamine $\beta$-monooxygenase in the literature, but the majority of these are unlikely to form readily oxidized species. Thus, mechanism-based inhibitors for dopamine $\beta$-monooxygenase are divided into two types: type I, which undergo oxygen insertion prior to inactivation; and type II, which only require hydrogen abstraction. Significantly, both classes of inhibitors appear capable of generating a common intermediate, attributed to a delocalized radical center two atoms removed from the carbon of substrate which undergoes hydroxylation.

**Materials and Methods**

All chemicals were reagent grade unless otherwise noted. Phenethylamine HCl, octopamine HCl, tyramine HCl, disodium fumarate, and phenethyl alcohol were supplied by Sigma. Catalase (specific activity, 39,000 units/mg) and ascorbic acid were obtained from Boehringer Mannheim and BDH Chemicals, respectively. Sodium borohydride, phenylacetic acid, mandelic acid, phenylacetamide, and mandelamide ($\beta$-hydroxyphenylacetamide), and phenylacetaldehyde were purchased from Aldrich. Phenylacetaldehyde was distilled and stored under nitrogen for up to 2 weeks. Dopamine HCl was supplied by either Sigma or Behring Diagnostics who also supplied the methylvanomine used in enzyme isolation. Phenethylcylec alcohol was furnished by CTC Organics.

Chromaffin granules were prepared from fresh bovine adrenal medulla. Dopamine $\beta$-monooxygenase was isolated from frozen granules using concanavalin A, Sephadex G-25 (Pharmacia P-L Biochemicals), and DE52 (Whatman) chromatography resins as previously described (7). Oxygen uptake was measured with a Yellow Springs Instrument Co. model 53 biological oxygen monitor and a model 5331 oxygen electrode. HPLC was accomplished with a Beckman model 6422 high pressure liquid chromatography system, equipped with a Hitachi model 155 variable wavelength detector. Mass spectral analyses were carried out on a A.E.I. model 12 mass spectrometer.

**Standard assay**—Enzyme activity was measured by oxygen uptake at 35 °C. Assay mixtures contained 0.1 M potassium phosphate (pH 6.0), 10 mM ascorbate, 10 mM fumarate, 17 mM potassium chloride, 50 μg/ml catalase, 0.22 mM oxygen, 1 mM dopamine, and ~1 μM free copper.

**Initial Velocity Measurements with Aldehyde and Amide Substrates**—Enzyme activity was measured by oxygen uptake at 35 °C. Assay components included 0.1 M potassium phosphate (pH 6.0), 10 mM fumarate, 10 mM ascorbate, and 50 μg/ml catalase. Substrate concentration varied as indicated in the figures. Dopamine $\beta$-monooxygenase concentration was 0.7 μM. Exogenous copper was added such that the final copper content was 2.4 μM total copper, unless otherwise indicated in the figure legend. Oxygen and substrate levels are given in the legends. At various time intervals, small aliquots were withdrawn and assayed for residual activity by the standard assay described above. Half-times for inactivation were determined using a linear least squares program. Limiting first and second order rate constants for inactivation were obtained from nonlinear least squares analysis of $t_\text{50}$ versus inhibitor concentration (19).

**HPLC Identification of $\beta$-Hydroxyphenylacetaldehyde—**Dopamine $\beta$-monooxygenase was preincubated with 10 mM phenylacetaldehyde and 6.5 mM O$_2$ for 2 h under the conditions described for the initial velocity measurements. Aliquots were withdrawn and centrifuged in an Amicon micropartition system to halt the reaction and remove protein. An Altex C-18 ultrasphere column with a mobile phase of 15% methanol, 85% 10 mM ammonium formate (v/v), flow rate of 1 ml/min, was used for HPLC separation of reaction components. Filtrate was injected directly onto the HPLC (data not shown) or reduced (Fig. 2) with a 14-fold excess of sodium borohydride, pH 7.0-7.5, for 15-20 min. In the latter case, the borohydride reaction was quenched with HCl to pH 5.5-6.0, prior to injection on the HPLC.

**Mass Spectral Characterization of Products**—Incubation mixtures contained 0.1 M potassium phosphate (pH 6.0), 10 mM ascorbate, 10 mM fumarate, 1.2 mg/ml catalase, 0.75 mM oxygen, 10 mM phenylacetaldehyde, 1 mg/ml dopamine $\beta$-monooxygenase, and 1 μM free copper in excess of the 2 g atoms/unit. Substrates were allowed to proceed for 2 h. The reaction was halted by either extraction. The ether was removed under a stream of nitrogen and the residue submitted for mass spectral analysis.

**Synthesis of $\beta$-Hydroxyphenylacetaldehyde—**Monoamine oxidase (specific activity, 130 units/mg with benzylamine as substrate) was centrifuged to remove the glycerol present in the storage buffer. It was resuspended in 0.1 M triethanolamine buffer, pH 7.2, to a concentration of 1 mg/ml. Catalase (100 μg/ml) and solid $\beta$-hydroxyphenethylamine (to a concentration of 116 mM) were added and the reaction incubated at 30 °C for 4 h with frequent vortexing (to ensure maintenance of O$_2$ levels at 1 mM). Conversion of dopamine to aldehyde was monitored by HPLC using a 25% MeOH, 75% 10 mM ammonium formate solvent system. Although retention times varied with concentration, the $\beta$-hydroxyphenethylamine eluted at about 10 min followed by the $\beta$-hydroxyphenylacetaldehyde at 15 min. At >85% conversion, the reaction was halted by adjusting the pH to 5. Aldehyde was separated from unreacted amine by ether extraction. Ether was removed under a stream of nitrogen and the aldehyde product isolated by preparative liquid chromatography.

**RESULTS**

The proposed inhibitory species, $\beta$-hydroxyphenylacetaldehyde, can only occur if dopamine $\beta$-monooxygenase utilizes phenylacetaldehyde as a substrate. As illustrated in Fig. 1, initial velocity studies monitored by oxygen uptake at 0.75 mM O$_2$ indicate $K_m$ and $k_{inact}$ values for phenylacetaldehyde of

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1 The abbreviation used is: HPLC, high pressure liquid chromatography.
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7.4 mM and 1.7 s⁻¹, respectively. Kinetic parameters for β-chlorophenethylamine determined under conditions of O₂ saturation were previously determined to be $K_m = 15$ mM and $k_{cat} = 13$ s⁻¹ (where $K_m (O_2) = 0.4$ mM (19)).

Product identification was carried out by HPLC. Analysis of reaction mixtures showed an enzyme- and time-dependent formation of a new peak characterized by a retention time of 14 min relative to 20 min for phenylacetaldehyde. To establish the identity of the product, the reaction mixture was reduced with sodium borohydride to convert the β-hydroxyphenylacetaldehyde to phenethylamine. These conditions also convert phenylacetaldehyde to phenethyl alcohol. Fig. 2 shows the HPLC analysis of reduced reaction mixtures. An initial time point shows only phenethyl alcohol eluting at 23 min, together with a weakly retained broad peak containing ascorbate and fumarate (panel A). As shown in panel B, a 2-h incubation in the presence of dopamine β-monoxygenase leads to formation of a new peak eluting at 9 min. Adding commercial phenethylene glycol to the reduced reaction mixture indicates coincidence of this species with the product peak (panel C) assigning the original product as β-hydroxyphenylacetaldehyde. Further confirmation of the product was obtained by mass spectrometry (data not shown). Although the control reaction contained a small molecular ion peak at 136 due to nonenzymatic formation of phenylacetic acid, a large peak at 136 corresponding to β-hydroxyphenylacetaldehyde was dopamine β-monoxygenase dependent.

Compounds behaving as suicide inhibitors are expected to require turnover conditions, show saturation kinetics, and give rise to an irreversible loss of enzyme activity. Fig. 3A illustrates the time-dependent inhibition observed with phenylacetaldehyde at 0.46 mM O₂. Substitution of oxygen by nitrogen (△) or omission of ascorbate (■) in the preincubation mixtures prevented inhibition. These results are consistent with mechanism-based inhibition since the loss of activity required all substrates. Compounds which could be generated due to nonenzymatic destruction of phenylacetaldehyde did not interfere. The inhibition was also examined as a function of phenylacetaldehyde concentration at high (0.75 mM) O₂,

1 No evidence of either phenylacetic acid or mandelic acid was found by HPLC analysis of preincubation mixtures with or without enzyme present. Although retention times are less than 5 min under conditions of Fig. 2, in 0.5 M ammonium formate mandelic acid elutes at 5 min, phenylacetic acid at 7 min, and phenethylene glycol at 10 min. Phenylacetic acid (up to 15 mM) did not appear to be a substrate under initial velocity studies nor did 10 mM acid inhibit in the presence of 0.25 mM dopamine.
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Fig. 4. A, time course for dopamine β-monoxygenase activity following preincubation in the presence of 12.5 (•) or 20 (□) mM p-hydroxyphenylacetamide. Time course for dopamine β-monoxygenase control incubations shows no loss of activity when either oxygen (○) or ascorbate (○) are omitted. B, half-time of enzyme inactivation as a function of the reciprocal of p-hydroxyphenylacetamide concentrations.

Table I

Comparison of the kinetic parameters for mechanism-based inhibitors

Determined at 0.75 mM oxygen, with the exception of β-chlorophenethylamine, where kinetic data were extrapolated to infinite oxygen.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$K_a$</th>
<th>$k_{cat}$</th>
<th>$K_i$</th>
<th>$k_i$</th>
<th>$k_{cat}/k_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylacetdehyde</td>
<td>7.4</td>
<td>1.7</td>
<td>7.9</td>
<td>1.1</td>
<td>1600</td>
</tr>
<tr>
<td>p-Hydroxyphenylacetamide</td>
<td>39</td>
<td>1.5</td>
<td>43</td>
<td>2.5</td>
<td>610</td>
</tr>
<tr>
<td>Phenylacetamide</td>
<td>ND</td>
<td>ND</td>
<td>68</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>β-Hydroxyphenylacetaldehyde</td>
<td>ND</td>
<td>ND</td>
<td>27 (0.74)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*From Ref. 19.

Table II

Comparison of the kinetic parameters for mechanism-based inhibitors

Determined at 0.75 mM oxygen, with the exception of β-chlorophenethylamine, where kinetic data were extrapolated to infinite oxygen.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$K_a$</th>
<th>$k_{cat}$</th>
<th>$K_i$</th>
<th>$k_i$</th>
<th>$k_{cat}/k_i$</th>
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</tr>
<tr>
<td>Phenylacetamide</td>
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<td></td>
</tr>
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<td>β-Hydroxyphenylacetaldehyde</td>
<td>ND</td>
<td>ND</td>
<td>27 (0.74)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*From Ref. 19.

A second class of compounds was tested for substrate activity by monitoring oxygen uptake. Fig. 1 compares initial velocity data for p-hydroxyphenylacetamide to phenylacetdehyde. Although the amide is more soluble than the aldehyde, it doesn’t appear to bind as well to the enzyme. Incubation of p-hydroxyphenylacetamide with dopamine β-monoxygenase also generated a new HPLC peak (data not shown), attributed to the hydroxylated species. Both p-hydroxyphenylacetamide and phenylacetamide were tested as mechanism-based inhibitors. In Fig. 4A, the semi-log plot of activity versus time for 12.5 and 20 mM p-hydroxyphenylacetamide is shown. Analogous to phenylacetdehyde, no loss of activity occurs without ascorbate (○) or oxygen (●). Analysis of the dependence of $t_{1/2}$ for enzyme inactivation on amide concentration yielded $K_i = 43$ mM and $k_i = 2.5 \times 10^{-3}$ s⁻¹. Similar inactivation experiments with phenylacetamide led to kinetic values of $K_i = 68$ mM and $k_i = 0.68 \times 10^{-3}$ s⁻¹.

The mechanism outlined in Equation 2 for inactivation by...
As phenylacetaldehyde inactivation follows the same mechanism as the dopa- 
monooxygenase-catalyzed hydroxylation of tyramine and is irreversible, we do not have 
direct evidence for enzyme-bound enols. There are actually two broad peaks in the pheny-
lglyoxal region whose relative heights varied as the solution aged. These double peaks also appear in 
certain samples of phenylglyoxal and may be due to aldehyde oxidation to acid or polymerization. 
Although phenylglyoxal might be expected to inhibit dopamine β-monoxygenase, incubation 
of enzyme with phenylglyoxal was not inhibitory. β-Hydroxyphenylacetamide was also tested 
as a reductant in the dopa-
monooxygenase system and served as an activator. Activity with either phenylacetaldehyde or P-hydroxyphenylacetam-
ide, followed by dialysis to assay for regain of activity. As shown in Table II, both classes of inhibitors lead to an 
irreversible inactivation of enzyme.

**DISCUSSION**

As demonstrated in this study, dopamine β-monoxygenase inhibition by phenylacetaldehyde requires oxygen and ascor-
bate, implicating substrate hydroxylation as a prerequisite for inactivation. A comparison of kinetic parameters in Table I 
for turnover versus inhibition reveals similar $K_m$ and $K_i$ values (7.4 and 7.9 mM, respectively), together with a partition ratio 
for turnover/inactivation of 1600. Phenylacetaldehyde bears little resemblance to dopamine, the natural substrate, or β-
chlophenethylamine, the parent suicide inhibitor. Its selection 
as a candidate for mechanism-based inactivation resulted from the structural similarity of the enol form of β-hydroxyphenylacetaldelyde, 1, to the enol of α-aminoacetonaphone, 2, and hence the expectation that β-hydroxyphenylacetaldelyde 
would undergo oxidation at the enzyme active site to generate an inhibitory radical cation species.

Thus, it is significant first, that phenylacetaldehyde behaves as a suicide inhibitor, and second, that the rate parameters 
for this inactivation are comparable to those for β-chlorophenethylamine where the partition ratio for turnover/inac-
tivation is 2000.

A possible mechanism for phenylacetaldehyde inhibition, presented in Equation 3, is analogous to the mechanism 
postulated for β-chlorophenethylamine, Equation 2. This mechanism shows β-hydroxyphenylacetaldehyde enolizing to 
a form which oxidizes readily. Data for aldehyde enolization have been both limited and indirect, deriving from halogena-

dation and isotope exchange experiments (cf. Ref. 23). Recent direct measurement of the conversion of vinyl alcohol to 
acetaldehyde has led to an estimate for $pK_{enol} = 6.5$ (24). The enol equilibrium for the enediol species shown in 1 is likely to 
be significantly increased relative to acetaldehyde. Further, the actual amount of enol formed on the enzyme may be 
considerably larger than in solution due to possible coordination to an active site electrophile such as copper. Although 
we do not yet have direct evidence for enzyme-bound enols from either α-aminoacetonaphone or β-hydroxyphenylacetal-

delyde, the presence of the former species has been inferred.
from a comparison of deuterium isotope effects in \( \beta \)-chloro-
phenethylamine inactivation versus turnover (22).\(^3\)

\( \beta \)-Hydroxyphenylacetaldehyde was synthesized to test the
validity of the proposed intermediate. Limiting quantities of
\( \beta \)-hydroxyphenylacetaldehyde precluded extensive kinetic
studies, but two important points can be made. First, \( \beta \)-
hydroxyphenylacetaldehyde is a time-dependent inhibitor for
dopamine \( \beta \)-monooxygenase (Fig. 5), and second, \( \beta \)-hydroxy-
phenylacetaldehyde can function as a reductant to support the
hydroxylation of tyramine (Fig. 6).

A key mechanistic feature of the mechanism in Equation 3 is eno-
lization of the turnover product, followed by donation of an
electron to Cu(I\(1\)) to generate a radical cation interme-
diate. This mechanism was tested further with phenylacetam-
ides, since \( \beta \)-hydroxyamides are not expected to enolize and
hence to inhibit dopamine \( \beta \)-monooxygenase. Consistent with
this prediction, phenylacetamides are found to be substrates
for dopamine \( \beta \)-monooxygenase (Fig. 1), but the products, \( \beta \)-
hydroxyphenylacetamides, behave neither as inhibitors nor as
electron donors in tyramine hydroxylation. Somewhat un-
expectedly, both phenyl and \( \beta \)-hydroxyphenylacetamide be-
have as mechanism-based inhibitors (Fig. 4). In the literature
there is an increasing number of mechanism-based inhibitors,
benzylcyanides (12-15), phenylethylenes (10, 11), and
phenylpropenes (16, 26) whose products would not be expected to
undergo oxidation by active site Cu(II). Thus, there are at
least two pathways that can be mechanistically differentiated
to account for mechanism-based inhibition of dopamine \( \beta \)-
monooxygenase.

Miller and Klinman (4) have recently completed a detailed
physical organic chemical analysis of the mechanism of C—
H bond cleavage in the dopamine \( \beta \)-monooxygenase-catalyzed
hydroxylation of phenylethamines. Isotope effect studies and
structure reactivity correlations support a net hydrogen atom
abstraction from the amine substrate to produce a radical
intermediate, which undergoes a rapid recombination with a
metal-bound oxygen radical to yield product. A similar con-
clusion regarding the nature of C—H bond cleavage has be-
en reached by Fitzpatrick et al. (16) in the inactivation of enzyme
by phenylpropenes.

Significantly, substrate-derived radical has a discrete life-
time, which is postulated to be limited by the rate at which
an intervening water molecule can be displaced (4). The
mechanism described by Miller and Klinman (4) argues against
formation of an electron-deficient oxygen species such as an oxene or hydroxyl radical as the hydroxylating agent. The
data are concluded to support a concerted transition
state involving concomitant C—H homolysis (from sub-
strate) and O—O homolysis (from the putative Cu(II)—OOH
species). A concerted mechanism requires that oxygen and
substrate be bound such that maximum overlap of orbitals
occurs at the transition state. It is expected that the nature of
the organic substrate will influence the position of these
orbitals, as well as the stability of the intermediate radical and its
partitioning between normal collapse to products versus oxidative damage to the active site.

The accumulated evidence at the present time implicates
radical intermediates in dopamine \( \beta \)-monooxygenase turnover
and inhibition. As this manuscript describes, at least two
pathways exist for generation of such an intermediate. We
have classified dopamine \( \beta \)-monooxygenase inhibitors as type
I, which undergo hydroxylation and subsequent oxidation by

\[ A \xrightarrow{\text{hydroxylation}} B \xrightarrow{\text{hydroxylation}} C \]

active site Cu(II) versus type II, which only require hydrogen
abstraction. As shown in pathway C, Scheme I, phenylacetam-
ides are proposed to inactivate dopamine \( \beta \)-monooxygenase by
simple abstraction of a hydrogen atom to form a delocalized
radical, extended in space from the carbon normally undergo-
ning hydroxylation. An analogous mechanism has already been
described for phenylpropenes (16, 26). Although full product
analysis has not been reported for many dopamine \( \beta \)-monooxy-
genase inhibitors, the available data suggest that compounds
whose hydroxylated products cannot be oxidized by E-Cu(II)
will inactivate by this mechanism. In Scheme I, pathways A
and B, type I inhibitors are also illustrated. In these cases,
the initial hydroxylated species serves as a reductant to gen-
erate a radical cation with the same general structure as
formed via pathway A. The existing data for dopamine \( \beta \)-
monooxygenase inactivation by \( \beta \)-chlorophenethylamine (7,
20) and phenylacetaldehyde (this paper) provide strong sup-
port for pathways A and B, respectively. Although product
analysis from 1-phenylpropyne inactivation of dopamine \( \beta \)-
monooxygenase is not available (25), it may also undergo
hydroxylation to 1 and hence inactivate by a mechanism
analogous to phenylacetaldehyde. Most significantly, inde-
pendent of the initial substrate analog and pathway, the
mechanism-based inhibitors described in this study and ear-
lier work can be rationalized in terms of a generic radical, 3
in Scheme I.

In closing, it is of value to consider possible differences
between radical intermediates derived from substrates which
do not give rise to a detectable level of enzyme inhibition and
mechanism-based inhibitors. One plausible explanation is the
presence of an easily oxidized active site residue, which is
normally inaccessible to the benzyl radical center of sub-
strates with saturated site chains. The presence of such an
active site residue is supported by the ease with which dopa-
mine \( \beta \)-monooxygenase is known to undergo oxidation by
hydrogen peroxide (27). Alterations in substrate structure
such that a radical center can either be generated or extended
several angstroms from its normal location would provide the
generic proximity required for an oxidative inactivation of
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a mechanistically essential side chain (e.g. the residue of pK, 6–7, seen repeatedly in pH studies of enzyme turnover (18) and inhibition (13)). This explanation is of particular interest in light of the recent findings of Kruse and co-workers (28) that cresols behave as mechanism-based inhibitors toward dopamine β-monooxygenase. For this latter class of compounds, the absence of a side chain may permit the diffusion of a radical intermediate and hence inactivation by the benzylic radical itself.

REFERENCES