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BIOACTIVATION OF CATHA EDULIS ALKALOIDS: ENZYMATIC KETONIZATION OF NORPSEUDOEPHEDRINE

Sheldon W. May*, Robert S. Phillips†, Heath H. Herman and Patricia W. Mueller

School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia 30332

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SUMMARY: Among the phenylalkylamines which have been shown to have potent central nervous system stimulatory activity are (+)-norpseudoephedrine and its ketone analog, cathinone, and recent work has suggested that cathinone is the more potent agent of the two. We have found that (+)-norpseudoephedrine is oxygenated by dopamine-β-hydroxylase, producing cathinone with the expected stoichiometry of electrons to O_2 to product of 2:1:1 diagnostic of a monooxygenase-catalyzed reaction. A k_{cat} value of 10 sec^{-1} and K_m of 18.8 mM were obtained, indicating that this reaction proceeds readily enough to be of physiological significance. The delayed onset and long duration of action of (+)-norpseudoephedrine is consistent with a requirement for enzyme activation and thus it is possible that dopamine-β-hydroxylase-catalyzed ketonization mediates the potent stimulatory effect of this and related compounds.

INTRODUCTION: The leaves of Catha edulis (Khat) have been chewed in the Middle East for centuries as a central nervous system stimulant since they produce euphoria, and combat mental fatigue and hunger (1). Although (+)-norpseudoephedrine (cathine) was thought to be the active principle (2), later work suggested that other phenylalkylamines were also highly active and particular attention has focused recently on cathinone, the ketone analog of cathine (3). Very recently, Peterson, et al. (3), comparing the behavioral effects of cathine and cathinone in rats, demonstrated that the latter was 7-10 times more potent and had a more rapid onset and shorter duration of action. On this basis, these authors concluded that cathinone may be the major central nervous system-active component of Khat in man, and the reduced cathinone content in older Khat leaves may be responsible for their reduced

* To whom correspondence should be addressed.

† Present Address: NIMH, Bethesda, MD 20250

Abbreviations: Khat, Catha edulis; DβH, dopamine-beta-hydroxylase; EDTA, ethylenediaminetetra-acetic acid; HPLC, high pressure liquid chromatography; MES, 2-(N-morpholino)ethanesulfonic acid.

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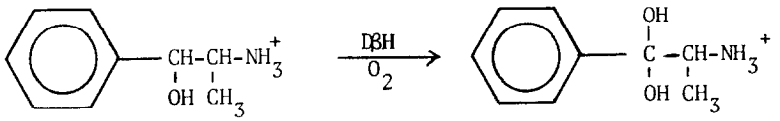
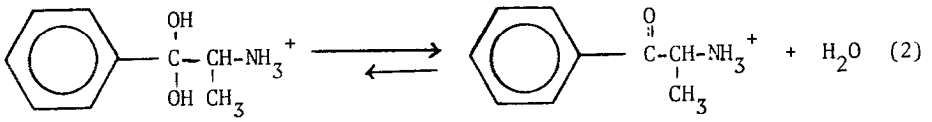
potency. Nevertheless, both cathione and (+)-norpseudoephedrine exhibit qualitatively similar effects.

The configuration at the β-carbon in the naturally occurring (+)-enantiomers of norpseudoephedrine and pseudoephedrine is S, and is thus opposite to the R configuration found in the functional phenylalkylamine neurotransmitters and hormones, such as norepinephrine, epinephrine, and their cognates (4). One possible pathway of bioactivation for such compounds is for dopamine-β-hydroxylase (DβH), the enzyme responsible for stereospecific R-hydroxylation at the β-carbon in phenylethylamines, to convert (S)-β-phenylethanolamines to the corresponding ketones via hydroxylation at the β C-H bond, stereotopically equivalent to the pro-R hydrogen of dopamine (see Eqn. 1). Such a process would presumably generate a gem-diol (see Eqn. 2) as the immediate enzyme product, which exists in solution predominantly as the keto form (5). In addition to cathinone, the ketone analogs of



(1)

S-(+)-Norpseudoephedrine



(2)

Cathinone

epinephrine and norepinephrine have been reported to exhibit a number of potent pharmacological effects (6-9), and thus ketonization could well represent a novel bioactivation pathway for such S-β- phenylethanolamines. In this report we demonstrate the existence of such a dopamine-β-hydroxylase-catalyzed ketonization pathway.

EXPERIMENTAL SECTION: Dopamine-β-Hydroxylase (EC 1. 14. 17.1) was isolated from bovine adrenals as previously described (10,11,13) and exhibited a specific activity of 12-15 units/mg (ascorbate-supported). The enzymatic reaction was followed using either the polarographic O_2 monitor assay or the spectrophotometric assay as previously described (11,12). HPLC analyses were performed using a Laboratory Data Control C-18

reverse phase column (10 cm, 5 A pore, low load) as previously described (11). Tyramine hydrochloride, ascorbic acid, sodium fumarate, disodium EDTA, and 2-(N-morpholino)ethanesulfonic acid were obtained from Sigma Chemical Co. $K_4Fe(CN)_6 \cdot 3H_2O$ was a product of Fisher Scientific. (+)-Norpseudoephedrine was obtained from Knoll Fine Chemicals, and an authentic sample of 2-aminopropiophenone (cathinone) was kindly supplied by Dr. Sheldon Sparber of the University of Minnesota. Other chemicals and solvents were obtained from commercial sources and were of the highest quality obtainable.

RESULTS AND DISCUSSION: Addition of highly purified adrenal dopamine-β-hydroxylase (10) to solutions of (+)-norpseudoephedrine, with either ascorbic acid or potassium ferrocyanide as reductant, resulted in enzyme-dependent consumption of O_2 and electrons, as measured using either a polarographic O_2 electrode (11), or by following the change in absorbance at 420 nm upon oxidation of ferrocyanide (12). High pressure liquid chromatography (HPLC) analysis of reaction mixtures before and after incubation with DβH revealed a new peak with retention time longer than the norpseudoephedrine (Figure 1a and 1c), and which corresponded exactly to the migration of an authentic sample of the expected ketone product, cathinone (Figure 1b and 1c). Furthermore, the stoichiometry of the formation of the DβH product with norpseudoephedrine was determined by quenching reaction mixtures with EDTA after a measured amount of O_2 or $Fe(CN)_6^{4-}$ had been consumed, and by quantitating the product by HPLC analysis. As shown in Table I, the expected stoichiometry of electrons to O_2 to product of 2:1:1 was obtained, which is diagnostic of monooxygenase-catalyzed reactions. Also shown in Table I are the results of a similar experiment with (S)-octopamine, which has been unequivocally established as a substrate for DβH (11) and for which the structure of the corresponding ketone product was established by HPLC, UV, IR and mass spectral analysis. Taken together, these results provide strong evidence that the product of DβH-catalyzed oxygenation of (+)-norpseudoephedrine is indeed the ketone, cathinone.

The kinetics of the DβH reaction with norpseudoephedrine were also examined, with the results shown in Table II. As we have previously shown with other substrates (13) the k_{cat} and K_m values are both reduced with $Fe(CN)_6^{4-}$ as the electron donor, relative to those obtained with the physiological reductant, ascorbate. The k_{cat} value of $10s^{-1}$ obtained for norpseudoephedrine with ascorbate is less than the value of $33s^{-1}$ obtained with S-octopamine; however, norpseudoephedrine is still a respectable substrate for DβH, and its kinetic characteristics are sufficient to suggest that the

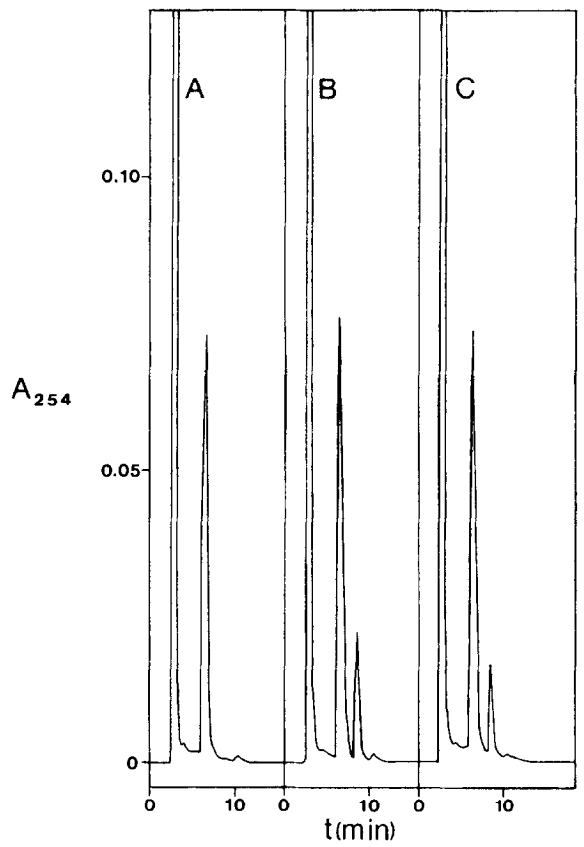


FIGURE 1
Identification of Cathinone as the Product of DβH-Catalyzed Oxygenation of Cathinone. A) Chromatogram of 25 mM cathine in 0.1 M MES, pH 6.1, 2m M $Fe(CN)_6^{4-}$, 5 μ M $CuSO_4$, and 10m M sodium fumarate, using a 25 cm C_{18} reverse phase column, eluting at 1.00 ml/min with 0.05M $NH_4H_2PO_4$/MeOH (3:2). Cathine elutes as the sharp peak after about 7 minutes; B) Chromatogram of the solution used to run A, after addition of an authentic cathinone standard (45 μ M final concentration). Cathinone elutes as the small peak after about 9 minutes; C) Chromatogram of the solution used to run A, 15 minutes after addition of DβH. The cathinone product is readily apparent as the small sharp peak eluting after the cathine.

conversion of norpseudoephedrine to cathinone may readily occur in vivo, through the action of DβH.

This finding of the in vitro conversion of (+)-norpseudoephedrine to cathinone is consistent with the in vivo observations of Peterson, et al. (3), who have compared the behavioral effects of these compounds in rats. These investigators found that cathinone was approximately 10 times more active than (+)norpseudoephedrine and had an immediate onset and short duration of activity. (+)-Norpseudoephedrine, on the

TABLE I

STOICHIOMETRY OF DOPAMINE-β-HYDROXYLASE CATALYZED KETONIZATION REACTIONS

Substrate	$\frac{[O_2]^a}{\text{product}}$	$\frac{[Fe(CN)_6^{3-}]^b}{\text{product}}$	$\frac{\Delta\,[Fe(CN)_6^{3-}]}{\Delta\,O_2}$
(+)-Norpseudoephedrine	0.89 ± 0.04[c]	1.81 ± 0.12[c]	2.03 ± 0.22
S-octopamine	1.01 ± 0.08[c]	2.16 ± 0.12[c]	2.14 ± 0.27

[a] O_2 consumption was measured with a YSI model 53 O_2 monitor equipped with a Clark-type polarographic electrode at 37°C (12). The reactions were carried out in 0.1 M MES, pH 6.1, containing 5 μM $CuSO_4$, 10 mM sodium fumarate, and 250 μM O_2. The reactions were quenched by adding EDTA to a final concentration of 100 μM, and the product formed was measured by HPLC, as shown in Figure 1.

[b] $Fe(CN)_6^{3-}$ production was measured spectrophotometrically at 420 nm as reported previously (12), using the buffer system described in a. The product formed was quantitated by HPLC analysis.

[c] Average of at least 3 determinations.

other hand, exhibited a delay before the onset of activity and a much longer duration. Both effects are consistent with the requirment for enzymatic activation.

Compounds of the ephedrine and norephedrine series possess weak direct sympathomimetic activity as well as a more important indirect (norepinephrine-

TABLE II

KINETIC CONSTANTS FOR DOPAMINE-β-HYDROXYLASE CATALYZED KETONIZATION REACTIONS

Substrate	Ascorbate[a]			Ferrocyanide[b]		
	k_{cat}	K_m	k_{cat}/K_m	k_{cat}	K_m	k_{cat}/Km
(+)-Norpseudoephedrine	$10s^{-1}$	18.8 m M	5.4×10^2	$0.9s^{-1}$	5.4 m M	1.7×10^2
S-octopamine	$33s^{-1}$	13.9 m M	2.4×10^3	$2.8s^{-1}$	4.4 m M	6.4×10^2

[a] Rates were measured by following O_2 consumption with a YSI model 53 O_2 monitor equipped with a Clark-type polarographic electrode. The reactions were carried out in 0.1 M acetate, pH 5.0, containing 5 μM $CuSO_4$, 10 m M sodium fumarate, 300 g/ml catalase, and atmoshperic O_2 saturation (250 μM) at 37°, as previously described (12).

[b] Rates were measured spectrophotometrically by following the production of ferricyanide at 420 nm, as previously described (12). Reactions were carried out using the buffer system described in Table Ia.

releasing) sympathomimetic activity. Similarly, it has been noted that most, if not all, of the central nervous system activity of the amphetamines is related to their ability to act as indirect sympathomimetics (17). It is interesting to note that when all of the stereoisomers of ephedrine and norephedrine were examined for central nervous system stimulating activity under identical experimental conditions, Fairchild and Alles (14) found that the activity of (+)-norpseudoephedrine was only exceeded by that of the amphetamines, which have been demonstrated to be excellent DβH substrates (18). It is tempting to theorize that the central nervous system stimulating potency of (+)-norpseudoephedrine is mediated both by its indirect sympathiomimetic activity and by the herein demonstrated DβH-catalyzed ketonization which produces the potent product cathinone. On the other hand, we have observed that (+)-pseudoephedrine and (+)-ephedrine (which are less active sympathomimetics than (+)-norpseudoephedrine) are very weak DβH substrates since they lack primary amines, and thus this DβH-catalyzed ketonization pathway contributes little to their activity.

Our results clearly establish the conversion of S-phenylethanolamines to the corresponding ketones by DβH. These observations suggest that the sympathomimetic activity of (+)-norpseudoephedrine and related compounds should be modified by specific DβH inhibitors. Also, this work suggests that DβH inhibitors could interfere with the therapeutic effects of (+)-norpseudoephedrine as an anorexic agent (15). Finally, the ketonization activity of DβH demonstrated here may well be exploitable for in vivo activation of appropriately designed pro-drugs.

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4. There is often confusion in the literature regarding the absolute configurations of various phenlethanolamine derivatives due to incorrect interchangeable use of the (d,l) and (D,L) designations. The naturally occurring enantiomers of ephedrine and norephedrine possess the R-configuration at the β-carbon, the S-confi-

guration at the α-carbon, and exhibit (-)-rotation. The naturally occurring enantiomers of pseudoephedrine and norpseudoephedrine posses the S-configuration at the α-carbon, the S-configuration at the β-carbon and exhibit (-)-rotation. In amphetamine, where the β-carbon is not hydroxylated, the enantiomer with the S-configuration at the α-carbon exhibits (+)-rotation.

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