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EFFECTS OF AMINOACETONITRILE, AN AMINE OXIDASE INHIBITOR, ON MESCALINE METABOLISM IN THE RABBIT*

LOUIS J. RICEBERG, MARCIA SIMON, HELEN VAN VUNAKIS† and ROBERT H. ABELES

Graduate Department of Biochemistry, Brandeis University, Waltham, Mass. 02154, U.S.A.

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Abstract—Aminoacetonitrile inhibits Cu^{2+}-containing "pyridoxal" amine oxidases but not flavin-containing amine oxidases. Pargyline inhibits only flavin-containing amine oxidases. Propargylamine, on the other hand, inhibits both types of enzymes. We have used these inhibitors to study mescaline metabolism in the rabbit. Mescaline rapidly undergoes oxidative deamination to 3,4,5-trimethoxyphenylacetic acid in the control and pargyline-treated rabbits. In the aminoacetonitrile- and propargylamine-treated animals, mescaline oxidation is inhibited and other metabolites, e.g. *N*-acetylmescaline, are formed. These results indicate that enzymes related to plasma enzymes (Cu^{2+}-containing "pyridoxal" amine oxidases) are primarily responsible for the oxidation of mescaline in the rabbit. Similar enzymes are also present in the liver, lung and kidney of the rabbit.

There are several different mammalian enzymes which oxidize amines [1]. They have been classified according to substrate specificity [2, 3] or according to their susceptibility to various inhibitors [3–5]. It is becoming apparent that these enzymes also have different prosthetic groups. Mitochondrial amine oxidases are flavoproteins [6–10] and, in some cases, e.g. beef liver [11], the flavin is covalently bound to the apoenzyme. Other amine oxidases, such as beef [12, 13], pig [14] and human [15] plasma amine oxidases or hog kidney [2, 16] amine oxidase, are Cu^{2+} proteins and allegedly contain pyridoxal at the active site [12, 17–19]. Although classification according to substrate specificity or inhibitor susceptibility can be useful and is sometimes necessary, it is advantageous to characterize these enzymes further according to the nature of the prosthetic group. Therefore, we will refer to the flavin enzymes at Type I, and to the Cu^{2+}-containing enzymes, which may possibly contain a compound related to pyridoxal, as Type II.

In the course of our studies on the mechanism of action of these enzymes, we found that beef plasma and hog kidney amine oxidases (Type II enzymes) are irreversibly inhibited by aminoacetonitrile. At 2.5×10^{-5} M aminoacetonitrile $T_{\frac{1}{2}}$ for inhibition is approximately 1 min (0·07 M sodium phosphate buffer, pH 7·2). Beef mitochondrial enzyme (Type I) is also inhibited but at a significantly higher concentration and a much slower rate. At 1.1×10^{-2} M, $T_{\frac{1}{2}}$ for inhibition is 5 min under similar conditions.‡ Pargyline, a classical inhibitor of mitochondrial amine oxidase [20, 21], does not inactivate the Type II enzymes. Propargylamine irreversibly inhibits, with approximately equal efficiency, the highly purified mitochondrial amine oxidase as well as beef plasma and hog kidney amine oxidase [22]. It also inhibits mitochondrial amine oxidase *in vivo*.

If these inhibitors function *in vivo* as they do *in vitro*, then they can be used to selectively inhibit either the mitochondrial amine oxidase (Type I) or the plasma enzyme (Type II), and presumably also enzymes in other tissue mechanistically similar to the plasma enzyme. Such selective inhibitors are useful to ascertain metabolic functions for the plasma enzyme and mechanistically related enzymes (Type II).

There are a number of Type II amine oxidase inhibitors available [3–5]. These compounds appear less useful for experiments *in vivo* than aminoacetonitrile, since they are either less selective for Type II amine oxidases or have general toxic effects. Carbonyl reagents such as cyanide, hydroxylamine, semicarbazide, etc., inactivate Type II amine oxidases. The use of cyanide appears unattractive on several grounds, and other carbonyl reagents are nonspecific, since they will inactivate pyridoxal and possibly other enzymes. Copper chelators are inhibitors of Type II enzymes, but

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also interact with other enzymes. Hydrazine derivatives, such as iproniazid, inhibit other types of amine oxidases.

As yet, no definite metabolic role for plasma amine oxidase has been established. Aminoacetonitrile, therefore, is particularly useful in the evaluation of the role of plasma amine oxidase and related enzymes (Type II) in the metabolism of various amines. Initially we chose to study the metabolism of mescaline, since it is an exogenous substrate which is rapidly metabolized to the acid derivative in the rabbit [23, 24]. *In vitro*, mescaline is oxidized by the rabbit serum enzyme but is a poor substrate of rabbit liver mitochondrial amine oxidase [25, 26].

MATERIALS AND METHODS

Materials. Mescaline hydrochloride was purchased from Sigma Chemical Co. Propargylamine hydrochloride and aminoacetonitrile bisulfate were purchased from Aldrich Chemical Co. and recrystallized from ether–ethanol. Pargyline (*N*-methyl-*N*-benzylpropargylamine) was the generous gift of Dr. A. O. Geiszler at Abbott Laboratories, Chicago. 8-^{14}C-mescaline hydrochloride was purchased either from New England Nuclear Corp. (sp. act., 4·5 mCi/m-mole) or from Mallinckrodt (sp. act., 21·9 mCi/m-mole). 1-^{14}C-tyramine hydrochloride (sp. act., 12·4 mCi/m-mole) was obtained from New England Nuclear Corp.

N-acetylmescaline was synthesized by the method of Späth and Bruck [27]. All other chemicals and solvents from commercial sources were reagent grade and used without further purification.

Analytical procedures. The assay used to determine the oxidative deamination of substrate is a modification of a published procedure [28]. Tissues, suspended in 10 vol of buffer (0·1 M sodium potassium phosphate, pH 7·8), were homogenized in a glass homogenizer. The homogenates were used without centrifugation. The assay mixture contained plasma or serum (1·0 ml), brain or kidney homogenate (0·2 ml), or liver homogenate (0·05 ml) and mescaline or tyramine (0·025 ml of a 3×10^{-2} M solution, 4×10^5 dis/min/ml) and sufficient buffer to give a final volume of 3·025 ml. The assay mixture was shaken at 37° for 1 hr. One-half ml of 2 M perchloric acid was added and the solution agitated on a Vortex mixer. Toluene (5·0 ml) was then added and the mixture agitated again. The mixture was centrifuged at room temperature at 1000 *g* for 5 min. The sera and plasma samples had to be centrifuged at 10,000 *g* for 30 min to disperse the emulsion. After freezing the water layer in dry ice–acetone, the toluene layer was then poured into a 5-ml toluene Liquifluor (New England Nuclear) mixture (84 ml Liquifluor diluted to 1 liter with toluene) and the radioactivity was determined. A blank containing no homogenate was carried through the entire procedure and the resulting counts were substracted from all determinations.

For thin-layer chromatography, a known amount of radioactivity (between 500 and 1000 counts/min) was applied to Eastman Silica gel plates with fluorescent indicator (No. 6060). Carrier amounts of mescaline, 3,4,5-trimethoxyphenylacetic acid and *N*-acetylmescaline were added, and the plates were developed with ethyl acetate–methanol–concentrated ammonium hydroxide (65:35:11). The plates were scanned with a u.v. lamp for fluorescence and divided into sections for radioactivity measurements. *N*-acetylmescaline, mescaline and 3,4,5-trimethoxyphenylacetic acid had R_f values of 0·83, 0·72 and 0·46, respectively, in this solvent system.

An aliquot of sample was also analyzed using a Waters Associates liquid chromatograph model 202, which was fitted with a diphenylcorasil column (2 ft × 0·125 in.). Acetonitrile–0·1% ammonium carbonate (30:70), at a flow rate of 1·0 ml/min, was used as solvent. Fractions (0·5 ml) were collected and analyzed for radioactivity in Complete Scintisol (Isolab Inc.). Mescaline had a relative elution volume (V_e/V_0) of 8·0 and both the *N*-acetylmescaline and the 3,4,5-trimethoxyphenylacetic acid metabolites eluted with solvent front ($V_e/V_0 = 1·0$).

For the separation of mescaline and its demethylated metabolites, the paper chromatographic method of Daly *et al.* [29] was used.

Hydrolysis of N-*acetylmescaline.* *N*-acetylmescaline in the plasma was characterized by its chromatographic properties. For further identification, it was quantitatively hydrolyzed to mescaline. An aliquot of

Table 1. Effect of amine oxidase inhibitors on the oxidation *in vitro* of tyramine and mescaline*

Tissue	No inhibitor		Aminoacetonitrile		Propargylamine		Pargyline	
	M	T	M	T	M	T	M	T
Serum	9700†	1800	0	0	0	0	9400	1900
Brain	370	4400	260	3700	320	300	280	316
Liver	2400	11,600	60	7600	0	200	3000	500
Kidney	2100	5000	0	3800	0	100	2500	150

* Reaction mixtures are identical to those used in assay procedure except that inhibitor (10 μl of 0·5 M solution, final concn $1·6 \times 10^{-3}$ M) is added prior to addition of substrate. The reaction mixture is maintained for 5 min at room temperature, mescaline (M) or tyramine (T) is added, and the assay is carried out as described. Values given for brain, liver and kidney correspond to activity of 200 mg tissue. Serum used was 1·0 ml.

† Numbers in the table represent cpm extracted into the organic phase.

Table 2. Effect of amine oxidase inhibitors administered *in vivo* on the oxidation of tyramine and mescaline*

	Control		Aminoacetonitrile		Pargyline	
	M	T	M	T	M	T
Serum						
Before injection	14,000†	2600	8200	1660	10,000	2800
One hr after	13,000	2600	590	70	10,000	2800
Liver	12,000‡	33,000	2200	38,000	7100	840
Brain	500	3840	290	4300	350	280
Kidney	4900	10,000	530	9300	3300	330
Lung	14,000	3100	1300	1560	19,000	1900

* A blood sample was removed from each rabbit prior to the injection to assay for amine oxidase activity with mescaline (M) or tyramine (T). One hr after injection another blood sample was removed for assay and animals were sacrificed.

† Numbers in the table represent cpm extracted into the organic phase.

‡ The source of mescaline oxidation in the liver cannot be accounted for by the plasma amine oxidase activity in residual blood. The activities of liver and plasma amine oxidase toward mescaline were approximately the same. For the enzyme activity in the liver to represent only plasma, there would have to be 1·0 ml plasma in 200 mg tissue. In general, 200 mg liver tissue contains approximately 20 μl residual blood [30].

plasma (100 μl) was mixed with an equal volume of 10 N sodium hydroxide and incubated at 60° overnight. The pH was adjusted to 10 with hydrochloric acid and the mixture was extracted with 5 vol of chloroform. The organic layer was then concentrated under a stream of nitrogen and chromatographed.

Effect of inhibitors in vivo. Prior to injection of inhibitor, blood was collected from the right marginal ear vein of rabbits to determine baseline levels of enzyme activity. The animals were then injected in the left marginal ear vein with 12 mg/kg of pargyline, aminoacetonitrile or propargylamine. After 1 hr, another blood sample was taken from the right ear for enzyme assay and the animals were injected with 8-^{14}C-mescaline hydrochloride (sp. act., 280 μCi/mmole, 5 mg/kg) in 0·15 M sodium chloride. Blood (3 ml) was collected from the right ear, allowed to clot in the cold, and was centrifuged to obtain the serum, which was stored at −20° until analyzed. An aliquot (0·1 ml) of serum was counted in Complete Scintisol on a Packard scintillation counter. For chromatography, proteins which would interfere with the analyses were removed by precipitation. One-half ml serum was treated with 1·0 ml absolute ethanol, mixed, allowed to stand at room temperature for 5 min, and centrifuged. The supernatant was removed and concentrated under a stream of nitrogen. An aliquot of the sample was counted in modified Bray's solution.

RESULTS

Preliminary to experiments *in vivo*, the metabolism of mescaline and tyramine, as well as the effects of several inhibitors on the metabolism of these amines, were examined in various tissues. Table 1 shows the effect of the several inhibitors on the metabolism of mescaline and tyramine in homogenates of various tissues. In serum, both mescaline and tyramine are oxidized. Aminoacetonitrile and propargylamine completely inhibit the oxidation of both substrates; pargyline has no effect. This pattern of inhibition is similar to that obtained with purified plasma enzyme (Type II enzyme) and differs from the inhibition pattern with mitochondrial amine oxidase.* These results, therefore, suggest that in rabbit serum metabolism of both amines is due exclusively to a Type II amine oxidase. Liver and kidney also metabolize tyramine and mescaline. Here aminoacetonitrile completely inhibits mescaline metabolism and reduces tyramine metabolism.

The inhibitory characteristics observed with purified enzymes indicate that mescaline metabolism is due entirely to a Type II enzyme and tyramine metabolism is partially due to a Type II enzyme, but predominantly to Type I enzyme. Consistent with this interpretation is the fact that pargyline, a known inhibitor of flavin-containing amine oxidases (Type I) [20, 21], has no effect on mescaline oxidation and almost completely abolishes tyramine oxidation. As expected from results with purified enzymes [22], propargylamine inhibits tyramine and mescaline oxidation.

In the brain, tyramine oxidation is more than 90 per cent abolished by pargyline and propargylamine, indicating that this compound is metabolized by a Type I enzyme. None of the compounds tested had a substantial effect on mescaline metabolism. The level of mescaline metabolism is low compared to other tissues and the significance of this small amount of oxidation is difficult to evaluate.

Table 2 shows the effects of inhibitors *in vivo*. Tissues were removed for enzyme assay 1 hr after injection of inhibitor. The results parallel the results *in vitro* (Table 1) in that aminoacetonitrile almost completely inhibits mescaline oxidation in all tissues, and has at most a small effect on tyramine oxidation in liver, brain and kidney. Pargyline inhibits tyramine oxidation in liver, brain and kidney and has less of an effect on mescaline

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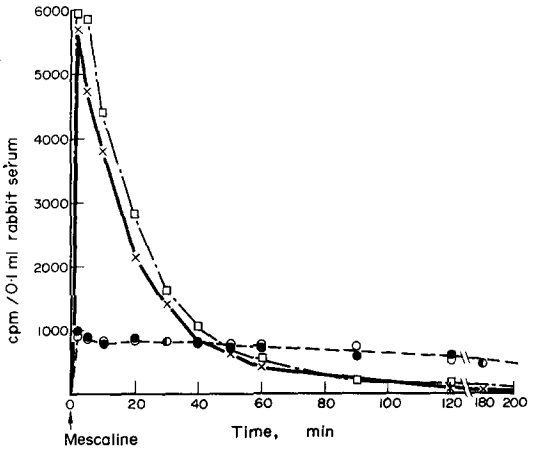

Fig. 1. Effect of amine oxidase inhibitors on the clearance of mescaline from rabbit serum. Rabbits were injected i.v. with aminoacetonitrile (●), propargylamine (○) or pargyline (□). The control animal (×) received no inhibitor. After 1 hr ($t = 0$), animals were given 8-^{14}C-mescaline hydrochloride. Blood samples were collected and serum was analyzed for radioactivity as described in the Methods section.

metabolism in tissues.* These results, therefore, establish that either of the two types of amine oxidases can be selectively inactivated in rabbit tissues *in vivo* with the use of aminoacetonitrile or pargyline.

Experiments were then done to determine the effect of these inhibitors upon mescaline oxidation. The inhibitors were injected intravenously into the ear of the rabbit, and 1 hr later blood samples were taken for enzyme assay. The animals were then injected with mescaline-8-^{14}C hydrochloride and bled periodically from the other ear.

The rapid clearance of radioactivity from the serum of control rabbits is shown in Fig. 1. After 1 hr, over 90 per cent of the injected radioactivity had been dispersed systemically. The rabbit which received pargyline (an inhibitor of the Type I mitochondrial but not the Type II plasma enzyme) gave a clearance pattern identical to the control. Animals treated with inhibitors of Type II enzymes, i.e. aminoacetonitrile or propargylamine, gave distinctly different clearance patterns. The level of radioactivity at 10 min was approximately 15 per cent of that found in the sera of the control and the pargyline-treated animals. At 50 min the level of radioactivity in all animals was the same. At times greater than 1 hr the level of radioactivity in the sera of the control and pargyline-treated animals was less than in the aminoacetonitrile- and propargylamine-treated rabbits.

* The apparent effect of pargyline on mescaline oxidation may not actually represent inhibition but could be due to variation in amine oxidase activity in different rabbits. The data in Table 1 show that pargyline has essentially no effect on mescaline metabolism.

Aliquots of the sera were analyzed by thin-layer chromatography. In the control and pargyline-treated rabbits, over 90 per cent of the radioactivity co-chromatographed with 3,4,5-trimethoxyphenylacetic acid at all times studied. At the earliest times in the aminoacetonitrile- and propargylamine-treated animals, 70 per cent of the radioactivity co-chromatographed with mescaline while only 10 per cent was the acid metabolite. In the serum from a propargylamine-treated animal, the amount of radioactivity representing *N*-acetylmescaline increased with time (Fig. 2), while that representing mescaline declined. At 180 min, the *N*-acetyl derivative accounts for 37 per cent of the radioactivity in the blood. The acid derivative and demethylated products account for the remaining activity. An animal treated with aminoacetonitrile gave qualitatively similar results.

For the tissue distribution studies, the control animals and those which had been pretreated with inhibitor were injected with mescaline-8-^{14}C hydrochloride and sacrificed after 5 min. Table 3 shows the total radioactivity as well as the nature of the products which accumulate in the serum and several tissues. Except for serum, liver and spleen, all tissues of the control and pargyline-treated animals contained 60–70 per cent of the counts as the acid metabolite, while 15–30 per cent of the radioactivity migrated with mescaline. Sera contained only acid metabolite, while the

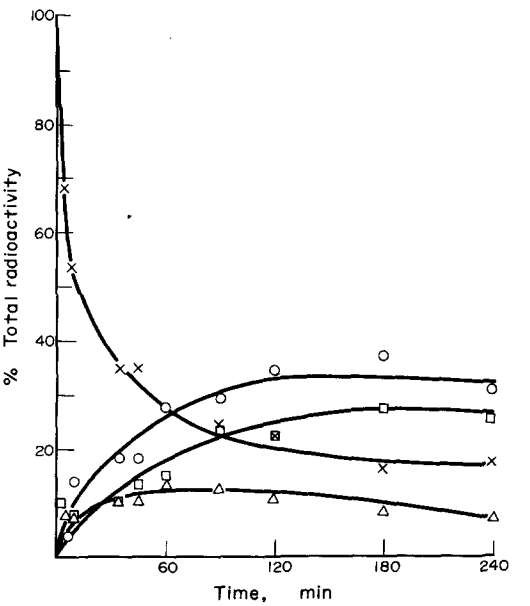

Fig. 2. Metabolites of mescaline in the serum of a propargylamine-treated rabbit. The rabbit was pretreated with propargylamine injected with 8-^{14}C-mescaline and blood samples were collected and analyzed for mescaline (×), *N*-acetylmescaline (○), 3,4,5-trimethoxyphenylacetic acid (△) and demethylated products (□) as described in the Methods section.

Table 3. Effect of amine oxidase inhibitors on the distribution of mescaline and 3,4,5-trimethoxyphenylacetic acid in rabbit tissues*

Tissue	Treatment	Radioactivity (cpm per 100 mg tissue)	% 3,4,5-Trimethoxyphenylacetic acid	% Mescaline
	Control	7877	27	59
Liver	Aminoacetonitrile	22,730	1	79
	Pargyline	5050	28	48
	Control	101,228	72	20
Kidney	Aminoacetonitrile	31,170	4	89
	Pargyline	76,130	70	18
	Control	1150	60	31
Brain	Aminoacetonitrile	1180	4	80
	Pargyline	890	52	35
	Control	16,760	72	19
Lung	Aminoacetonitrile	34,830	0	90
	Pargyline	14,340	67	17
	Control	5936	75	15
Heart	Aminoacetonitrile	6270	2	92
	Pargyline	5590	76	16
	Control	12,764	20	73
Spleen	Aminoacetonitrile	19,420	0	96
	Pargyline	6550	31	65
	Control	3936	70	15
Uterus	Aminoacetonitrile	4950	2	89
	Pargyline	2240	75	10
	Control	2433	58	27
Skeletal muscle	Aminoacetonitrile	5640	1	96
	Pargyline	1310	68	17
	Control	18,473	98	0
Serum	Aminoacetonitrile	3877	17	74
	Pargyline	11,471	93	0

* Rabbits were pretreated with pargyline, aminoacetonitrile or saline. After 1 hr, mescaline-8-^{14}C hydrochloride (sp. act., 1·4 mCi/m-mole, 5 mg/kg) was administered intravenously, and after 5 min the animal was sacrificed by a blow on the back of the neck and decapitated. The organs were immediately removed, rinsed in 0·15 M sodium chloride, and placed in liquid nitrogen. Tissues were weighed and homogenized for 5 min in a Waring blendor with 10 times the weight of 95% ethanol. The homogenates were centrifuged at 12,000 *g* for 30 min. The supernatant was removed and an aliquot was counted in modified Bray's solution.

liver and spleen had more mescaline than acid derivative. The aminoacetonitrile-treated rabbit had lower levels of radioactivity in the plasma and kidneys, while the levels in the liver, lung and spleen were significantly higher. The fraction of radioactivity due to mescaline was greatly increased in all tissues. Except for the serum, all tissues contained at least 80 per cent mescaline and less than 5 per cent of the acid metabolite. In the serum, there were 74 per cent mescaline and 17 per cent of the acid present.

DISCUSSION

Studies with purified enzymes have shown that aminoacetonitrile preferentially inhibits the Type II amine oxidases,* pargyline inhibits Type I amine oxidases [20, 21], while propargylamine inhibits both types of enzyme [22]*. At least two types of enzymes were found in unfractionated homogenates from various tissues: those which preferentially act on mescaline as compared to tyramine and are inhibited by aminoacetonitrile and propargylamine (Type II enzymes), and those which act preferentially on tyramine and are inhibited by pargyline and propargylamine (Type I enzymes) (Table 1). The data obtained with brain homogenates are more complex, but suggest that there may be some enzyme activity which is not affected by any of these inhibitors.

The amine oxidase from the sera of several animals has been characterized and extensively studied [13–15, 31, 32]. Serum contains exclusively Type II enzyme. The presence of a Type II amine oxidase (sometimes

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referred to as diamine oxidase) in the kidney is known and the enzyme has been isolated [33]. The data in Tables 1 and 2 show that both types of enzymes are present in the liver, lung and kidney of the rabbit. As far as we know, Type II amine oxidase has not been isolated from liver or lung, although in the course of a study of mescaline metabolism, evidence indicating that two types of amine oxidases are present in rabbit liver was obtained [25]. Our results confirm this conclusion. Preliminary evidence indicates the presence of Type II enzymes in monkey liver,* and it will be of interest to establish whether Type II enzymes are also present in the liver and lung of other species.

Mescaline is rapidly metabolized in the rabbit to 3,4,5-trimethoxyphenylacetic acid [23, 24], first by its conversion to 3,4,5-trimethoxyphenylacetaldehyde by monoamine oxidase and then by further oxidation to 3,4,5-trimethoxyphenylacetic acid by aldehyde dehydrogenase [1]. Administration of aminoacetonitrile to the rabbit prior to injection of mescaline caused an accumulation of unmetabolized drug. On the other hand, the inhibitor of Type I enzyme(s), pargyline, has little or no effect on mescaline metabolism. We, therefore, conclude that the Type II enzyme(s) is involved in the catabolism of mescaline and that the Type I enzyme(s) plays an insignificant role in its metabolism.

A striking difference was observed in the clearance of radioactivity from the sera of animals treated with aminoacetonitrile or propargylamine prior to injection with ^{14}C-mescaline when compared to control or pargyline-treated animals. The radioactivity is rapidly removed from the blood stream and dispersed in the tissues (Fig. 1). In the sera of the control and pargyline-treated animals, the radioactivity can be accounted for almost exclusively by 3,4,5-trimethoxyphenylacetic acid, while in the tissues some mescaline is found. When mescaline metabolism is inhibited by propargylamine or aminoacetonitrile, the level of radioactivity which appears in the serum is lower than in the control or pargyline-treated animals, and 75 per cent of the radioactivity represents mescaline. A possible explanation is that mescaline is preferentially retained in the tissues and not as readily released as trimethoxyphenylacetic acid. When mescaline oxidation is inhibited, other metabolites such as the non-hallucinogenic derivative, *N*-acetylmescaline [34], accumulate. This is consistent with evidence that *N*-acetylation represents an important alternate metabolic pathway in mice [35] and rats [36] treated with amine oxidase inhibitors. The data show that Type II amine oxidases are primarily, possibly exclusively, responsible for mescaline oxidation *in vivo*.

In addition to tissues mentioned, amine oxidases have been identified in dental pulp, skin, bone and aorta [1]. The amine oxidases responsible for peptidyl lysine oxidation involved in the cross-linking of collagen and elastin are probably Type II amine oxidases [37]. We would, therefore, expect aminoacetonitrile to inhibit collagen formation. This is in fact so; aminoacetonitrile is an even more effective lathyrogenic agent than β-aminopropionitrile [38]. Preliminary results indicate that propargylamine, another inhibitor of Type II amine oxidases, may also be lathyrogenic.*

The availability of highly specific Type II amine oxidase inhibitors which can be used in intact animals opens further possibilities for physiological studies. It will be of particular interest to establish whether they play a significant role in the control of levels of biogenic amines and whether they would give results similar to nonspecific inhibitors [39, 40].

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