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IN VITRO AND IN VIVO INHIBITION OF LYSYL OXIDASE BY AMINOPROPIONITRILES

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Inhibition of lysyl oxidase (protein-lysine 6-oxidase, EC 1.4.3.13) decreases the rate of collagen and elastin cross-link formation and produces osteolathyrism in animals. Organic nitriles, including β-aminopropionitrile (BAPN), have been shown to irreversibly inhibit lysyl oxidase in vitro. Both BAPN and 3,3'-iminodipropionitrile (IDPN) have been shown to produce osteolathyric changes when administered to animals. To date compounds that have been reported to inhibit this enzyme possess a primary amine functional group. In this study a series of primary and substituted aminopropionitriles was studied for their ability to inhibit lysyl oxidase activity both in vitro and in vivo. Our results show that of the compounds tested, BAPN was the most potent inhibitor of the enzyme. Reversible inhibition of lysyl oxidase in vitro was found with two secondary aminonitriles, IDPN and monomethylaminopropionitrile (MMAPN). There was no inhibition of enzyme activity associated with the tertiary compound 3,3'-dimethylaminopropionitrile (DMAPN) or propionitrile, a compound lacking an amine functional group. IDPN was found to produce a slight irreversible inhibition of the enzyme both in vitro and in vivo. Pretreatment of rats with pargyline, an inhibitor of monoamine oxidase, was found to increase the inhibitory potential of BAPN ($p \leq .1$). Pargyline pretreatment did not alter the inhibitory potential for any of the other aminonitriles tested. These results suggest that the presence of a primary amino functional group is not a strict requirement for inhibition of lysyl oxidase. In addition, reversible and irreversible mechanisms of inhibition may be involved in the production of osteolathyric changes associated with IDPN exposure.

INTRODUCTION

Aliphatic aminonitriles are a group of structurally related chemicals recognized to possess neurotoxic and connective tissue damaging properties (Ferris, 1983; Selye, 1957). The naturally occurring asparagine derivatives β-cyanoalanine (β-CLA) and γ-glutamyl-β-cyanoalanine have been shown to produce hyperactivity, convulsions, and rigidity when administered to rats as well as being effective inhibitors of biochemical processes (Ressler, 1962; Ressler and Koga, 1971). Administration of 3,3'-iminodipropionitrile (IDPN) to animals produces giant axonal swellings containing masses of 10-nm neurofilaments at the prenodal sites along

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myelinated axons (Griffen and Price, 1981; Griffen et al., 1983). IDPN administration to animals is also associated with the development of permanent changes in behavior characterized by excitation, circling, and choreiform head and neck movements, referred to as the ECC syndrome (Selye, 1957).

Another synthetic chemical, 3,3'-dimethylaminopropionitrile (DMAPN), has been studied in both animals and humans (Gad et al., 1979; Jaeger et al., 1980; Keogh et al., 1980; Kreiss et al., 1980). Clinical evaluation of workers exposed to DMAPN, the principal component in a polyurethane foam catalyst, found that many workers developed neurogenic bladders including symptoms of sensory loss, urinary hesitancy, and incomplete bladder emptying. A smaller number of affected workers developed behavioral symptoms including insomnia, irritability, and loss of libido (Baker et al., 1981). Administration of DMAPN to animals results in a loss of normal micturition response (Gad et al., 1979) and the development of enlarged urinary bladders as the only consistent gross pathological lesion (Jaeger et al., 1980). Exposure to DMAPN results in distal axonal degeneration in both humans and animals. The neuropathology is characterized by axonal swellings filled with accumulated neurofilaments and nonspecific organelles (Pestronk et al., 1980).

β-Aminopropionitrile (BAPN), the nonneurotoxic primary aminonitrile analog of DMAPN, and IDPN induce osteolathyrogenic changes in animals (Bachhuber et al., 1955; Selye, 1957). Osteolathyrism, a disorder that can be induced in experimental animals but that has not been observed in humans, is characterized by the occurrence of hernia, aneurysm, and skeletal deformity (Selye, 1957). There is an associated decrease in the tensile strength of connective tissue, and an abnormal increase in the salt solubility of collagen and elastin (Page and Benditt, 1967). Osteolathyrogenic activity has been associated with organic nitriles, including BAPN, aminoacetonitrile (AAN), and methylene aminoacetonitrile (MAAN), uridines, hydraides, and hydraine hydrate (Levene, 1961). Agents that produce osteolathyrism have been shown to act by inhibiting lysyl oxidase both in vivo (Chvapil et al., 1981; Levene and Carrington, 1985) and in vitro (Tang et al., 1989; Trackman and Kagan, 1979). Lysyl oxidase is a copper-dependent amine oxidase that oxidatively deaminates lysyl residues in collagen and elastin, resulting in the formation of peptidyl α-aminoadipic-γ-semialdehyde (Pinnell and Martin, 1968). Nonenzymatic cross-linking of these residues between collagen chains by Schiff-base formation or aldol condensation provides the increased tensile strength found in mature collagen (Franzblau et al., 1977).

The structurally similar aminopropionitriles DMAPN and *N*-methyl-β-alaninenitrile (monomethylaminopropionitrile, MMAPN) were tested for their ability to induce osteolathyrism and were found not to produce skeletal deformities or connective tissue damage (Bachhuber et al., 1955; Levene, 1961). These studies were performed before the discovery of

lysyl oxidase and used collagen extractability from treated animals as the measure of toxicity. Subsequently, Pinnell and Martin (1968) identified lysyl oxidase and demonstrated inhibition by BAPN using a sensitive radiometric assay. Since this time there appear to be no reports in the open literature that have studied the inhibition of lysyl oxidase by the substituted aminopropionitriles MMAPN, DMAPN, and IDPN.

Trackman and Kagan (1979) have reported that for a variety of nonpeptidyl aliphatic amines tested for their ability to inhibit lysyl oxidase, the presence of a primary amino group was a requirement for inhibition. IDPN has been shown to produce osteolathyrogenic changes in animals (Rosmus et al., 1965), and metabolism of the substituted neurotoxic aminopropionitriles, IDPN and DMAPN, to the primary amine BAPN could result in inhibition of lysyl oxidase in vivo. BAPN has been reported to be an excretion product in the urine of rats administered both DMAPN (Ahmed and Farooqui, 1984) and IDPN (Williams et al., 1970).

Structural similarities exist between lysyl oxidase and plasma amine oxidase in that both contain copper and pyrroloquinoline as essential cofactors at their active sites (Tang et al., 1989). These researchers have suggested that this commonality of cofactors implies a strong similarity in their mechanism of action. Inhibition of plasma amine oxidase by BAPN has been shown to occur in several species of animals (Raimondi et al., 1985; Tang et al., 1989). The inhibition of bovine plasma amine oxidase by the secondary aminonitrile MMAPN has also been reported (Wilmarth and Froines, 1991). These findings suggest that possession of a primary amino function is not a stringent requirement of inhibition of copper-containing amine oxidases and raises the possibility that aminopropionitriles other than BAPN might be capable of interacting with lysyl oxidase.

The objective of this study was to assess the inhibitory potential of aminopropionitriles toward lysyl oxidase both in vivo and in vitro and to determine if any observed inhibition was the result of parent compound toxicity or formation of an active metabolite such as BAPN.

MATERIALS AND METHODS

Chemicals

L-[4,5-^{3}H]Lysine (60 Ci/mmol) was purchased from ICN Radiochemicals, Irvine, Calif. 3,3′-Dimethylaminopropionitrile (98%) and *N*-methyl-β-alaninenitrile (96%) were obtained from Aldrich Chemical Company, Milwaukee, Wis. β-Aminopropionitrile fumarate and pargyline were products of Sigma Chemical Company, St. Louis, Mo. 3,3′-Iminodipropionitrile (99%) was purchased from Eastman Kodak Company, Rochester, N.Y. All other reagents used were of research or analytical grade and obtained from Fisher Scientific, Irvine, Calif.

Substrate Preparation

The assay of lysyl oxidase activity was determined using as substrate the saline-insoluble elastin pellet of homogenates prepared from 16-d-old chick embryo aortas that had been pulsed in organ culture with L-[4,5-^{3}H]lysine by a modification (Kagan et al., 1974) of the method described by Pinnell and Martin (1968). The aortas from 20 embryos were incubated for 24h at 37°C in 10 ml of lysine-free minimum essential medium containing Earle's salts and sodium bicarbonate (MEM Select-Amine Kit, Grand Island Biological Company, Grand Island, N.Y.). Each 10 ml of medium was supplemented with 250 μCi of L-[4,5-^{3}H]lysine, streptomycin (1 mg), penicillin (1000 units), and BAPN (0.50 mg). Each flask was flushed with CO_2 and O_2 (5 : 95, by volume) and stoppered before incubation with gentle shaking. Following incubation, the aortas were rinsed with water, lyophilized to dryness, and stored at −70°C until used in assays. Aortas were prepared for use as substrate by grinding with a glass homogenizer in 0.16 *M* NaCl followed by centrifugation at 10,000 × g. The resulting pellet was rehomogenized and centrifuged twice more in saline, followed by rinsing one time with 1 *M* HCl and two more times with saline. The washed pellet was then suspended in 0.15 *M* NaCl, 0.1 *M* sodium borate, pH 8.0, before being used in assays.

Preparation of Enzyme

The cartilaginous ends from the tibias and femurs of 16-d chick embryos were homogenized (10 bones/ml) in 0.15 *M* NaCl, 0.1 *M* sodium borate, pH 8.0, using a Virtishear mechanical homogenizer, model 225318 (VirTis Company, Inc., Gardiner, N.Y.). The homogenate was shaken for 4 h at 4°C followed by centrifugation at 100,000 × g for 2 h at 4°C. The supernatant from this centrifugation served as the source of enzyme.

For in vivo inhibitor studies the enzyme activity in rat lung tissue was also investigated. In these experiments the lungs were excised from animals and homogenized in 5 *M* urea/0.1 *M* Tris-HCl, pH 7.6, at tissue concentration of 0.5 g (wet weight)/ml, using a Virtishear homogenizer. The homogenate was extracted with shaking for 4 h at 4°C, followed by centrifugation at 100,000 × g for 2 h at 4°C. The 100,000 × g supernatant was exhaustively dialyzed against saline/borate buffer at 4°C before being used as a source of enzyme. Protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Lysyl oxidase from rat lung was selected as the source of enzyme for the in vivo experiments because of the large amount of enzyme required for the assays. Using enzyme extracted from bone would have involved the use of a much greater number of animals. There are no reports in the literature that indicate that the activity of the enzyme is either species or tissue specific.

Assay of Lysyl Oxidase Activity by Release of Tritium

To each assay tube was added a 0.5-ml aliquot of tritiated aortic pellet containing 250,000 cpm of ^{3}H, and 1.0 ml of enzyme extract, to give a final volume of 1.5 ml. In experiments in which an inhibitor was co-incubated with the enzyme–substrate mixture, 0.2 ml of the inhibitor or buffer was added to the mixture to give a final volume of 1.7 ml. Substrate, enzyme, and inhibitors were prepared in the same 0.15 *M* NaCl, 0.1 *M* sodium borate, pH 8.0, buffer. Tubes were stoppered, vortexed, and incubated in a shaking water bath at 37°C for 4 h. The reaction was stopped by the addition of β-aminopropionitrile fumarate (50 μl) to give a final concentration of 50 μg/ml in the assay mixture. The samples were then distilled under reduced pressure as described by Pinnell and Martin (1968) to isolate released tritium. A 1.0-ml portion of the tritiated water that distilled over from each sample was counted in a liquid scintillation spectrometer, model LS-100C (Beckman Instruments), using ScintiVerse II scintillation fluid (Fisher Scientific). Results of lysyl oxidase assays are expressed as a percentage of control values and corrected for the nonenzymatic release of tritium as determined by the incubation of enzyme-free reaction mixtures.

In Vitro Inhibition Studies

The inhibition of lysyl oxidase by aminonitriles was determined by incubating various concentrations of each test agent with a 1.0-ml aliquot of the 100,000 × g supernatant obtained from chick embryo leg bones, and 250,000 cpm of aorta substrate in a final volume of 1.7 ml. Each reaction tube was vortexed, incubated, and assayed for the release of tritium as described above.

The reversibility of lysyl oxidase inhibition by test compounds was determined by preincubating the 100,000 × g supernatant from chick leg bone homogenates in the presence of BAPN (50 μg/ml), MMAPN, DMAPN, or IDPN (250 μg/ml) prepared in sodium borate/saline buffer for 2 h at 37°C. The enzyme was then dialyzed against the same buffer at 4°C to remove the inhibitor. Following dialysis, 1-ml aliquots from each mixture were removed and assayed for release of tritium as previously described. Solutions containing enzyme and buffer only were prepared as controls to determine the effect of incubation and dialysis on lysyl oxidase activity.

In Vivo Inhibition Experiments

Male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, Calif.) weighing 220–245 g were randomly divided into treatment and control groups of 3 animals each. Each group was administered one of the test compounds, BAPN (400 mg/kg), MMAPN (100 mg/kg), DMAPN (250 mg/kg), IDPN (300 mg/kg), or isotonic saline once a day via intraperitoneal injection for 4 d. The dosage levels for DMAPN and MMAPN were within

25% of levels that resulted in high mortality in previous studies in our laboratory. When expressed in terms of mmol aminonitrile/kg body weight the values are BAPN (1.5 mmol/kg), MMAPN (1.2 mmol/kg), DMAPN (2.6 mmol/kg), and IDPN (2.4 mmol/kg). To test the hypothesis that metabolism of aminonitriles to BAPN is important in the development of osteolathyric changes, animals were pretreated with the MAO inhibitor pargyline prior to administration of test compounds. Pargyline has been shown to have no inhibitory potential toward lysyl oxidase in vitro (Tang et al., 1989) and did not alter the activity of the enzyme measured in vivo in our study. A second set of treatment groups was administered to the test agents using the same dosage regime but had been pretreated with pargyline (15 mg/kg via ip injection) at 24 and 1 h prior to the first injection of a test agent. Pargyline was administered to these animals 1 h prior to injection with a test compound on each subsequent day of the experiment. At 12 h following the cessation of dosing, animals were sacrificed and the lungs were rapidly removed. Lung tissue was rinsed in ice-cold isotonic saline, homogenized, extracted in 5 *M* urea, dialyzed against saline-borate buffer, and extracts were assayed for lysyl oxidase activity.

Statistics

Data were analyzed for statistically significant difference from control values by one-way analysis of variance using SYSTAT statistical analysis software, version 5.0.

RESULTS

As shown in Figure 1, BAPN, IDPN, and MMAPN were found to inhibit the lysyl oxidase-catalyzed release of tritium from labeled aortic substrate. The IC50 values (molar concentration resulting in a 50% inhibition of tritium release) calculated from the data in Figure 1 are <50 μM for BAPN, 0.50 m*M* for IDPN, and 10 m*M* for MMAPN. Propionitrile and DMAPN were much less potent inhibitors of lysyl oxidase, with IC50 values exceeding the maximum 50 m*M* concentrations tested.

Irreversible inhibition of lysyl oxidase by aminonitriles in vitro was observed following preincubation with both BAPN and IDPN. The relative inhibitory potentials as determined from data presented in Table 1 were BAPN ν IDPN. No irreversible inhibition of lysyl oxidase was observed with DMAPN, MMAPN, or propionitrile.

Data presented in Table 2 show that administration of BAPN to animals resulted in a significant decrease in lysyl oxidase activity as compared to controls ($p \leq .05$). BAPN-induced inhibition of lysyl oxidase was increased slightly ($p \leq .1$) by pretreatment of animals with the MAO inhibitor pargyline. There was a small but statistically insignificant decrease in lysyl oxidase activity observed following IDPN administration.

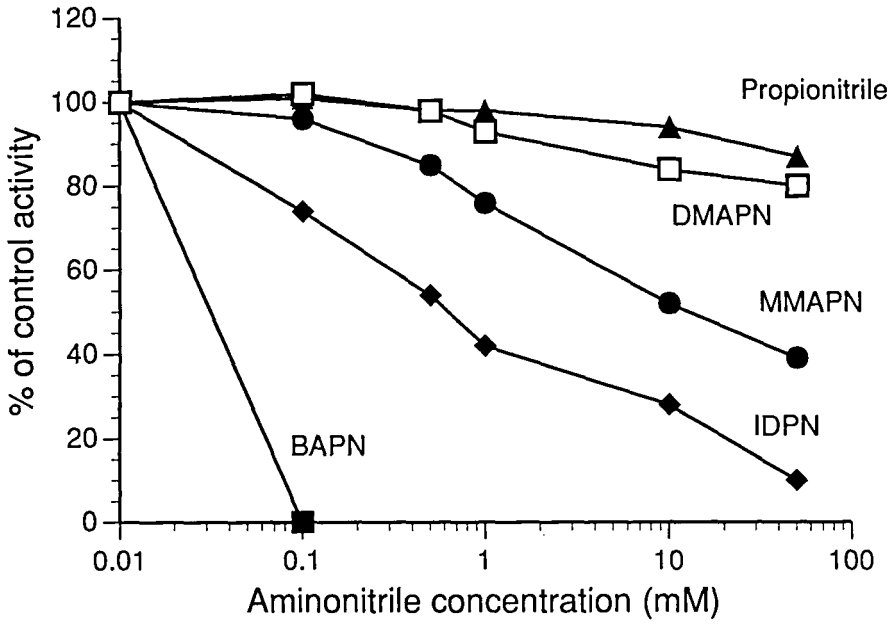

FIGURE 1. Inhibition of lysyl oxidase by aminopropionitriles in vitro. Assay mixtures were prepared as described under Materials and Methods and contained 250,000 cpm tritiated aortic substrate, 1 ml enzyme extract, and the indicated concentration of aminonitrile in a final volume of 1.7 ml. Enzyme activity was determined by measuring the tritium released over a 4-h incubation at 37°C. Values are presented as a percentage of control activity following correction for an enzyme-free control.

TABLE 1. *Effect of Preincubation with Aminonitriles on the Activity of Lysyl Oxidase from Chick Embryo Leg Bones in Vitro*

Test compound[a]	Lysyl oxidase activity[b]
Control	3461 ± 70 (100)
Propionitrile	3510 ± 99 (101)
BAPN	2319 ± 38 (67)[c]
MMAPN	3369 ± 64 (97)
DMAPN	3543 ± 41 (102)
IDPN	3210 ± 83 (93)

[a]Enzyme extract from chick embryo leg bones was incubated in the presence of 10 m*M* concentrations of each nitrile for 2 h at 37°C, followed by dialysis against saline-borate buffer before assay for tritium release.

[b]Values presented as the mean (dpm) ± SE from 5 determinations after correction for enzyme-free control, followed by percent of control activity (in parentheses) for 1 ml of enzyme extract.

[c]$p \leq .05$ Compared to control value.

TABLE 2. Effect of Aminopropionitriles on Lysyl Oxidase Activity in Lung Homogenates

Test compound[b]	Lysyl oxidase activity[a]	
	Group I[c]	Group II
Isotonic saline (1 ml/kg)	2647 ± 72[d] (100)[e]	2695 ± 63 (100)
Propionitrile (100 mg/kg)	2562 ± 63 (97)	2819 ± 56 (104)
BAPN (400 mg/kg)	2027 ± 95 (77)[f]	1761 ± 67 (65)[f,g]
MMAPN (100 mg/kg)	2632 ± 88 (101)	2611 ± 75 (97)
DMAPN (250 mg/kg)	2710 ± 49 (102)	2637 ± 84 (98)
IDPN (300 mg/kg)	2395 ± 81 (90)	2474 ± 55 (92)

[a]Results expressed as dpm/ml of extract.

[b]Five rats per group, received four daily injections of a test agent at the indicated dosage.

[c]Animals in group I received only an aminonitrile: animals in group II were pretreated with pargyline (15 mg/kg) as described under Materials and Methods.

[d]Presented as mean (dpm) ± SE of triplicate samples from five animals after correction for enzyme-free control.

[e]Percent of control activity.

[f]$p \leq .05$ As compared to control value.

[g]$p \leq .10$ As compared to BAPN treatment minus pargyline.

We found no evidence for the inhibition of lysyl oxidase in vivo by any other aminonitriles tested both with and without pargyline pretreatment.

DISCUSSION

In vitro studies in which aminonitriles were incubated with enzyme extract and tritiated aortic substrate (Fig. 1) show that BAPN was by far the most potent inhibitor of lysyl oxidase of the compounds tested. This is in agreement with other studies that indicate BAPN has an IC50 value in the micromolar range (Pinnell and Martin, 1968; Tang et al., 1983). We also observed inhibition of lysyl oxidase activity for both of the secondary amines tested. IDPN was an order of magnitude more potent an inhibitor than MMAPN (IC50 of 0.5 and 10.0 m*M*, respectively). No inhibition was observed for either the tertiary amine, DMAPN, or propionitrile.

There have been no reports prior to the in vitro findings in this study of aliphatic secondary amines acting as inhibitors of lysyl oxidase. Trackman and Kagan (1979) studied a variety of aliphatic primary and substituted mono- and diamines for their ability to inhibit lysyl oxidase and reported that inhibition required the presence of a primary amine function.

Tang et al. (1983) postulated a mechanism for BAPN inhibition of lysyl oxidase involving the formation of a Schiff base between the carbonyl cofactor of the enzyme and the amine group of BAPN, followed by pro-

ton abstraction from the α-carbon by a nucleophilic site on the enzyme. This results in formation of a ketene imine, which undergoes nucleophilic attack at the β-carbon, forming a covalent bond with the enzyme and irreversibly inhibiting enzyme function. A similar mechanism has been postulated to account for the inhibition of lysyl oxidase by aminoacetonitrile (AAN) (Walsh, 1979). This mechanism would appear to preclude inhibition of lysyl oxidase by secondary aminonitriles such as IDPN and MMAPN. However, generation of a Schiff base is not required for formation of a ketene imine from these compounds. An alternative explanation of the observed inhibition could involve reversible association of the nitrile with a nucleophilic site on the enzyme and conversion of the nitrile to free ketene imine by abstraction of a proton from the α-carbon of the nitrile. The ketene imine could react with a nucleophilic site on the enzyme, covalently binding to and irreversibly inhibiting the enzyme. Evidence for ketene imine formation has been reported in studies on enzyme inhibition by α-cyanoglycine and α-cyanoalanine (Ferris, 1983; Miles, 1975).

The extraction and dialysis procedure used for our in vivo experiments would effectively remove any free inhibitor present in the lung homogenate, indicating that any inhibition of enzyme activity observed would be of an irreversible type. Inhibition of lysyl oxidase was observed following administration of either BAPN ($p \leq .05$) or IDPN (Table 2). No inhibition of enzyme activity was found in animals receiving MMAPN, DMAPN, or propionitrile. The significant decrease in lysyl oxidase activity seen with BAPN treatment is consistent with previous work demonstrating irreversible inhibition (Chvapil et al., 1980; Levene and Carrington, 1986).

Our results show that the tertiary aminonitrile DMAPN has negligible inhibitory potential toward lysyl oxidase both in vitro and in vivo. We have previously reported the lack of inhibitory or substrate potential of this compound toward a similar copper-containing amine oxidase, bovine plasma amine oxidase, as well as the flavin-linked mitochondrial monoamine oxidase (Wilmarth and Froines, 1991). It appears that the unique toxicity of this agent does not involve interaction of the parent compound of metabolic products with amine oxidases.

It is unclear if the slight degree of irreversible inhibition we observed with IDPN would be sufficient to account for the osteolathyric changes previously reported to occur in IDPN-treated animals (Bachhuber et al., 1955; Romus et al., 1965). There are no reported studies that correlate levels of lysyl oxidase activity with clinical signs of osteolathyrism in animals treated with IDPN. Osteolathyrism has been associated with an increase in soluble collagen (Martin et al., 1961) and decreased allysine content of the tissue (Miller et al., 1965). One method to determine the osteolathyric effect of IDPN in vivo would be to examine the cross-link patterns and allysine content of collagen extracted from treated animals

as compared to controls. Changes in these parameters should not depend on the mechanism of lysyl oxidase inhibition.[1]

Administration of IDPN at a dosage of 300 mg/kg, if evenly distributed, results in an initial tissue concentration of approximately 2.4 m*M*. Our in vitro results suggest that at this level, IDPN could result in >50% inhibition of lysyl oxidase activity. It is possible that the weak osteolathyric changes associated with IDPN exposure in animals results from reversible inhibition of lysyl oxidase by the parent compound.

BAPN and cyanoacetic acid (CAA), a principal excretion product of BAPN metabolism (Fleisher et al., 1976; Merkow et al., 1959), have been identified in the urine of animals receiving IDPN (Williams et al., 1970) and DMAPN (Ahmed and Farooqui, 1984). It has been suggested that the osteolathyrogenic potential of IDPN is associated with the in vivo production of BAPN as a metabolite (Williams et al., 1970). If metabolism of substituted aminopropionitriles to BAPN were the operative mechanism of osteolathyrism induction, irreversible inhibition of lysyl oxidase activity in vivo following administration of these compounds would be observed, as we have reported with IDPN.

While monoamine oxidation is thought to be an important route of metabolism for BAPN (Fleisher et al., 1976), IDPN, MMAPN, and DMAPN are poor substrates for mitochondrial monoamine oxidase (Wilmarth and Froines, 1991). This finding suggests that biotransformation of these compounds to BAPN would involve degradative pathways other than mitochondrial monoamine oxidation, possibly microsomally mediated *N*-demethylation or α-C hydroxylation. Inhibition of MAO would not affect the production of BAPN as a metabolite via these pathways. Inhibition of MAO function has been shown to increase the biological half-life and osteolathyrogenic potential of BAPN in animals (Fleisher et al., 1979; Roy et al., 1959). If biotransformation of substituted aminonitriles to BAPN were the primary mechanism of osteolathyrism, inhibition of MAO would increase the tissue concentration of metabolically derived BAPN and potentate the irreversible inhibition of lysyl oxidase.

Results presented in Table 2 indicate that inhibition of MAO by pretreatment of animals with pargyline resulted in increased inhibition of lysyl oxidase by BAPN ($p \leq .1$). The inhibitory potential of the substituted aminonitriles tested toward lysyl oxidase was not affected by pargyline administration to test animals. This suggests that monoamine oxidation of BAPN derived from the metabolism of these compounds does not play a significant role in determining their osteolathyric potential. The inability of pargyline to potentiate the inhibition of lysyl oxidase ob-

[1]A reviewer has suggested that effects of repeated dosing such as enzyme induction may account for the slight inhibition of lysyl oxidase we have seen in vivo. An alternative approach, with particular regard to IDPN, could be to use a single higher dose of the compound and assay for lysyl oxidase activity at an earlier time point.

served in IDPN-treated animals suggests that BAPN, derived from the metabolism of IDPN, may not be the only mechanism involved in producing the osteolathyric changes associated with IDPN administration. It is possible that reversible inhibition of the enzyme by the parent compound (as found in our in vitro studies) or another metabolic product is involved in the production of osteolathyric changes seen in treated animals.

While we observed reversible inhibition of lysyl oxidase in vitro with MMAPN we found no evidence for inhibition of the enzyme in vivo. In a study by Bachhuber et al. (1955), MMAPN was present in the diet of treated animals at four times the concentration of IDPN. These investigators report no osteolathyrogenic changes associated with MMAPN but found fibrotic changes in the femurs of IDPN treated animals. This is consistent with our findings of IDPN being a more potent inhibitor of lysyl oxidase than MMAPN.

In conclusion we report that the secondary aliphatic aminonitriles MMAPN and IDPN reversibly inhibit lysyl oxidase in vitro and that IDPN was found to produce a slight irreversible inhibition of enzyme activity both in vivo and in vitro. The potent neurotoxicant DMAPN did not inhibit lysyl oxidase in vivo or in vitro. The inhibitory potential of IDPN, an aminonitrile known to produce osteolathyrism in animals, was greater than that determined for the nonlathrogenic compound MMAPN. The lack of significant irreversible inhibition seen with IDPN and the inability of pargyline to enhance the inhibitory potential of this compound suggest that reversible inhibition of lysyl oxidase by IDPN may play an important role in the osteolathyric changes induced by this compound. Further studies are needed to elucidate the relationship between lysyl oxidase activity, metabolic yield of BAPN, and clinical signs of osteolathyric changes associated with exposure to the substituted aminonitriles.

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