Biochemical Pharmacology, Vol. 34, No. 6, pp. 781–788, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

4-FLUORO-3-NITROPHENYL AZIDE, A SELECTIVE PHOTOAFFINITY LABEL FOR TYPE B MONOAMINE OXIDASE

SHIUAN CHEN, JEAN CHEN SHIH* and QIU-PING XU†
Institute for Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90033, U.S.A.

(*Received* 21 *January* 1984; *accepted* 27 *June* 1984)

Abstract—The effects of 4-fluoro-3-nitrophenyl azide (FNPA) on types A and B monoamine oxidase in rat brain cortex were studied using serotonin and phenylethylamine as substrates respectively. FNPA competitively inhibited the oxidative deamination of both serotonin (K_i = 3 μM) and phenylethylamine (K_i = 0.78 μM) in the dark. Upon photoirradiation in the presence of FNPA, a photodependent inhibition of type B MAO activity resulted. This photodependent inhibition was apparently irreversible since there was no recovery of activity upon washing of the photolyzed FNPA–enzyme mixture. Additional evidence for the photoinduced covalent binding of FNPA to type B MAO is that non-competitive inhibition kinetics resulted after photolysis. The specificity of the photodependent incorporation of FNPA to type B MAO was shown by the protective effect of phenylethylamine and by decreased [^{3}H]pargyline labeling after the enzyme was photolyzed with FNPA. Under the same experimental conditions, only minimal photodependent inhibition of type A MAO by FNPA was found. The observed difference in the efficiencies of the photodependent inactivation of the two types of MAO by FNPA suggests that there is a conformational or a structural difference in the active sites of the two types of MAO. The active site of type B MAO could be characterized by utilizing FNPA as a photoaffinity labeling probe.

Monoamine oxidase (EC 1.4.3.4) (MAO) catalyzes the oxidative deamination of a number of biogenic amines and exists as multiple forms in most mammalian tissues [1–4]. Based on the differences in substrate specificity and inhibitor selectivity, two types of MAO have been described [5–7]. Type A MAO preferentially deaminates serotonin and norepinephrine and is sensitive to inhibition by clorgyline [6] and LY 51641 [5]. Type B MAO preferentially deaminates phenylethylamine and benzylamine and is sensitive to inhibition by deprenyl [7]. The molecular basis for the two catalytic forms of MAO is not yet clear. Recently, immunological studies with MAO have indicated that human type B MAO has an antigenic site that is not present in human type A MAO [8, 9]. Selective inhibitors have also been utilized to investigate the difference between the two types of MAO. Several laboratories have reported that the two enzymes have slightly different molecular weights based on the electrophoretic migration of MAO that had been covalently labeled with [^{3}H]pargyline in the presence of either clorgyline or deprenyl [10–12]. Studies of the proteolytic digests of [^{3}H]pargyline-labeled MAOs by electrophoresis have revealed different distribution of the inhibitor bound peptide fragments for each of the enzyme forms [12–14]. However, it was found that pargyline inhibited both types of MAO by a formation of flavocyanine with FAD, which was linked to the same penta peptide (Ser-Gly-Gly-Cys-Tyr) in both beef liver MAO (a type B MAO) and human placenta MAO (a type A MAO) [15–18]. These results would indicate that the inhibitor selectivity between two enzymes must be attributed to differences at the region(s) other than the flavin binding site. Information on the biochemical basis for this selectivity is not available at the present time.

The use of photoaffinity labels has been particularly successful in obtaining information concerning the structure of specific ligand binding sites on macromolecules [19]. 4-Fluoro-3-nitrophenyl azide (FNPA), a photoaffinity probe, was first synthesized by Fleet *et al.* [20] and utilized to study antibody binding sites. Subsequent investigations by other researchers have found that FNPA inhibits photodependently the mitochondrial ATPase [21], α-chymotrypsin [22, 23], trypsin [22, 23] and several membrane transport systems [24]. In the present paper we report on the effects of FNPA on the two types of MAO. We have found that FNPA is a competitive inhibitor of both types of MAO, yet is an effective photodependent inhibitor of only type B enzyme.

MATERIALS AND METHODS

Chemicals. FNPA was obtained from the Pierce Chemical Co. (Rockford, IL). [Phenyl-3,benzyl-^{3}H]-Pargyline (15.3 Ci/mmole), β-[ethyl-1-^{14}C]phenylethylamine hydrochloride (50 mCi/mmole), and 5-[2-^{14}C]serotonin binoxalate (58.5 mCi/mmole) were from the New England Nuclear Corp. (Boston, MA).

MAO assays. Male Sprague–Dawley rats (140–180 g) were killed by decapitation, the brains were

* To whom reprint requests should be addressed.
† Permanent address: Department of Pharmacology, Beijing College of Traditional Chinese Medicine, Beijing, China.

rapidly removed, and the cerebral cortices were dissected on ice. The cortices were homogenized in 40–50 vol. of 50 mM potassium phosphate buffer, pH 7.4, and the homogenates were used for MAO assays without further purification. MAO activities were measured by a procedure modified from that reported by Otsuka and Kobayashi [25]. The assay was performed in screw cap culture tubes. The 1-ml assay mixture contained 50 mM phosphate buffer, pH 7.4, 100 μM [^{14}C]serotonin (for type A) or 10 μM [^{14}C]phenylethylamine (for type B), and 80 μg cortex homogenate. The reaction was started by addition of the homogenate, and the mixture was incubated at 37° for 20 min. The reaction was terminated by the rapid addition of 0.1 ml of 6 N HCl. Six milliliters of the extraction solvent [benzene–ethylacetate (1/1) for type A and toluene for type B] was then added, and each tube was capped and shaken vigorously. The tubes were briefly centrifuged with a clinical centrifuge to separate the two phases. Four milliliters of organic layer was mixed with 10 ml of scintillation fluid, and the radioactivity was determined by liquid scintillation spectrometry. With each assay a reagent control was performed by adding 0.1 ml of 6 N NCl before initiating the reaction with enzyme.

Photolysis. The photoirradiation experiments were carried out in a manner similar to that described by Chen and Guillory [26]. Sample solutions in a quartz cuvette were irradiated at a distance of 5 cm from a tungsten projection lamp (DVY 120 V, 650 W). Photolysis was performed by allowing 1 min for the lamp to cool after each minute of irradiation.

Detection of [^{3}H]pargyline labeling proteins. The [^{3}H]pargyline labeling experiments were performed as follows. After a 1-hr incubation of the homogenate with [^{3}H]pargyline at 37°, mitochondria were isolated according to Lai and Clark [27]. These mitochondria preparations were then boiled for 3 min in the presence of 1% sodium dodecyl sulfate and 1% β-mercaptoethanol and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis according to the procedure of Weber and Osborn [28]. The gels were stained and sliced according to the staining patterns. A 0.2-ml aliquot of 30% H_2O_2 was used to solubilize the gel slices. The radioactivity was detected by adding 10 ml of scintillation fluid to the solubilized gel fractions.

All other preparations are detailed in the legends of the figures and tables.

RESULTS

Inhibition of MAO by 4-fluoro-3-nitrophenyl azide. Figure 1 shows that FNPA inhibited both types of MAO, and that FNPA was a more potent inhibitor of the type B enzyme than the type A enzyme. At 0.79 μM FNPA, type B MAO activity was inhibited 50%, whereas type A MAO was inhibited 10%. Since dimethylformamide, the solvent used to dissolve FNPA, inhibited both types of MAO at concentrations greater than 10% (v/v), the maximum concentration of FNPA used in this study was 10 μM. At 10 μM FNPA, type B MAO activity was inhibited 80% and type A MAO activity was inhibited 32%. In these experiments, we have chosen the specific concentration for the two substrates

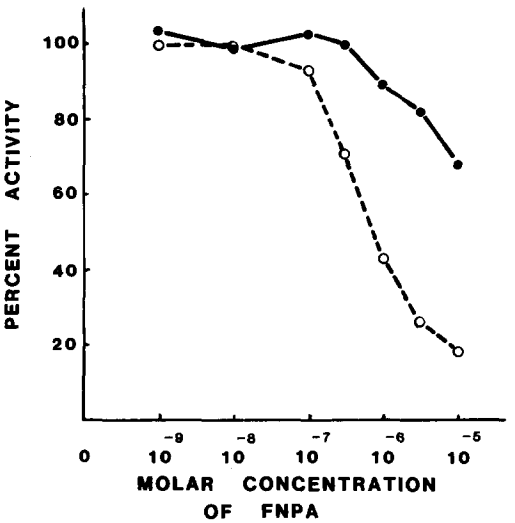

Fig. 1. Inhibition of two types of MAO activities in rat cortex by FNPA. After 20 min of incubation at 37° of a solution (1 ml) containing 50 mM phosphate buffer, pH 7.4, 80 μg rat cortex homogenate, 100 μM [^{14}C]serotonin (●—●) (sp. act. 0.5 mCi/mmole) or 10 μM [^{14}C]phenylethylamine (○—○) (sp. act. 5 mCi/mmole), and FNPA at concentrations indicated in the figure, 0.1 ml of 6 N HCl was added to stop the reaction. The products were extracted as described in Materials and Methods. A 4-ml aliquot of extract was withdrawn and counted for radioactivity. The control activities, which did not contain FNPA, were taken as 100%. Type A activity was 40 nmoles [^{14}C]serotonin oxidized/20 min/mg protein at 100 μM [^{14}C]serotonin and the control type B activity as 30 nmoles [^{14}C]phenylethylamine oxidized/20 min/mg protein at 10 μM [^{14}C]-phenylethylamine. Each point is the average of two determinations.

which has restricted each substrate to react with only one type of MAO—100 μM for serotonin and 10 μM for phenylethylamine. Furthermore, we have found that the serotonin deamination at 100 μM was completely inhibited by clorgyline at 3 nM but only inhibited 15% by deprenyl at the same concentration. Also, phenylethylamine deamination at 10 μM was inhibited 92% by deprenyl at 30 nM but only inhibited 7% by clorgyline at the same concentration. These results indicate that serotonin deamination at 100 μM and phenylethylamine deamination at 10 μM are accurate measurements of type A and type B MAO activities respectively.

Our kinetic studies revealed that FNPA inhibited both activities by competing with the respective substrate. The K_i value for FNPA inhibition of phenylethylamine deamination was determined to be 0.78 μM (Fig. 2) and 3 μM for inhibition of serotonin deamination (Fig. 3). The K_i analysis demonstrates again that FNPA is a slightly better inhibitor of type B MAO than of type A MAO.

The specificity of the FNPA inhibition of the two MAO activities was further indicated in the following experiments. Acetylenic amines such as pargyline, clorgyline, and deprenyl, are potent suicide inhibitors of MAO [29]. The inhibition by these inhibitors is initially competitive and fully reversible. An irreversible inactivation proceeding as a first-order reaction develops upon incubation with the enzyme

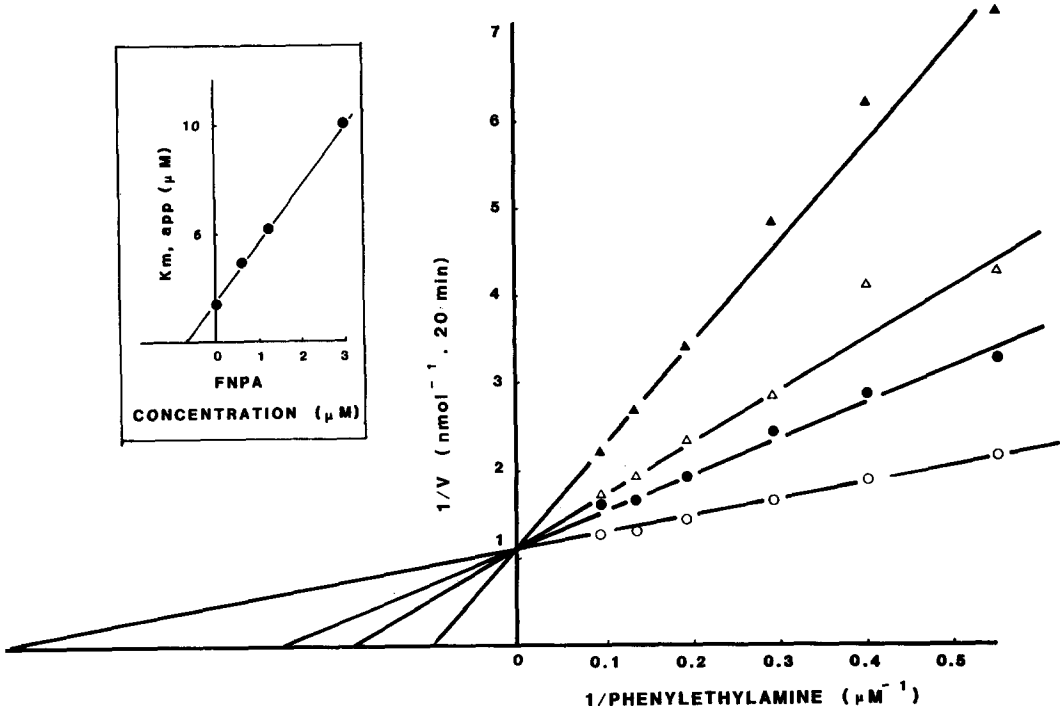

Fig. 2. Competitive inhibition of [^{14}C]phenylethylamine oxidation (type B MAO activity) by FNPA. The assay was performed in the same manner as described in the legend of Fig. 1. The FNPA concentrations were 0 (○), 0.6 (●), 1.2 (△), and 3 μM (▲). K_i determination is shown in the inset.

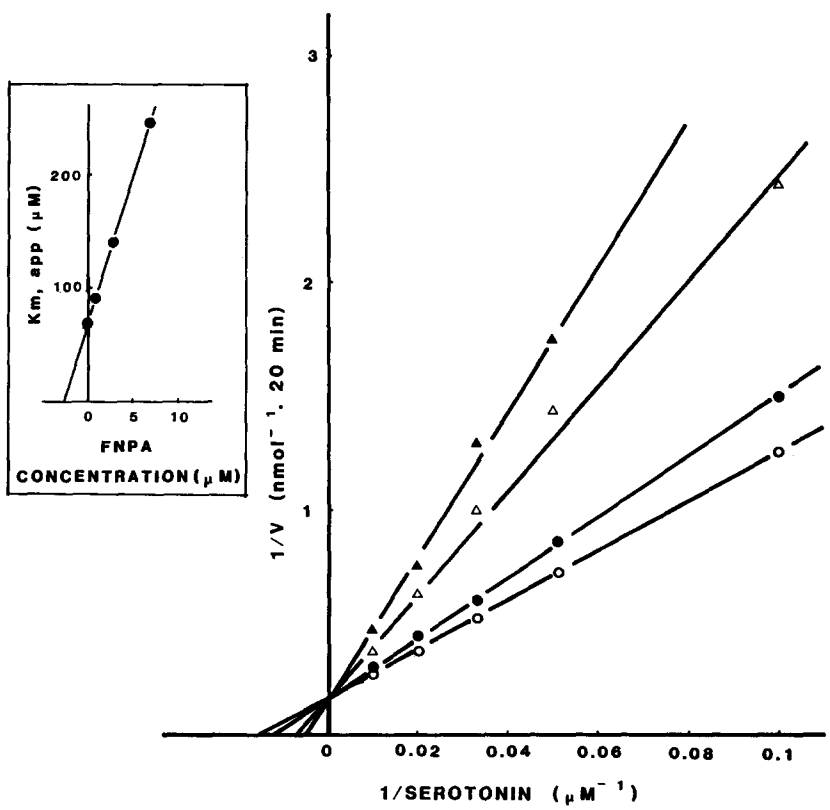

Fig. 3. Kinetic analysis of FNPA inhibition of the [^{14}C]serotonin oxidation (type A MAO activity). The assay procedures followed that described in the legend of Fig. 1. The FNPA concentrations were 0 (○), 1 (●), 3 (△), and 7 μM (▲). K_i determination is shown in the inset.

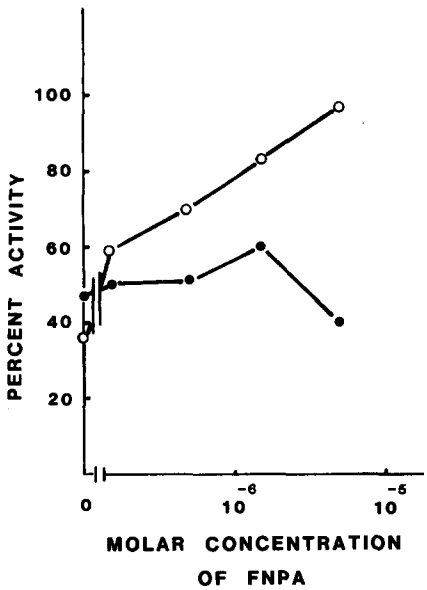

Fig. 4. Protection of clorgyline inhibition of type A MAO activity and deprenyl inhibition of type B MAO activity by FNPA. Solutions (1 ml) containing 0.66 mg cortex homogenate, 15 nM deprenyl (○——○) or 3.5 nM clorgyline (●——●), and FNPA at concentrations from 0 to 5 μM were incubated at 37° for 1 hr in the dark. After centrifugation at 50,000 *g* for 10 min, the pellets were resuspended in 1 ml of 50 mM phosphate buffer, pH 7.4, and the suspensions were washed once by the same centrifugation procedure. The type A MAO activity was measured for those samples treated with clorgyline and type B MAO activity was measured for those treated with deprenyl. The activity of the control sample, containing only cortex homogenate, was taken as 100%.

[30, 31]. Accordingly, the irreversible inactivation can be prevented by adding substrates or competitive inhibitors while acetylenic amines are incubated with the enzyme. We incubated the enzyme preparation with deprenyl at 15 nM (or clorgyline at 3.5 nM) and FNPA (concentrations ranging from 0 to 5 μM) at 37° for 1 hr in the dark. The enzyme preparation was centrifuged at 50,000 *g* for 10 min, washed with buffer, and re-centrifuged. We found that 5 μM FNPA completely prevented the 64% inhibition of type B MAO produced by deprenyl (Fig. 4). Such a result indicates again that FNPA probably binds to the active site of the type B MAO. On the other hand, the 53% inhibition of type A MAO produced by clorgyline at 3.5 nM was only reduced 10–15% by the same concentration of FNPA. It has been reported that deprenyl and clorgyline have K_i values of 1.2 and 0.012 μM, respectively, to type B and A MAO activities present in rat liver [32]. The small degree of protection of clorgyline inhibition of type A MAO by FNPA is expected if the K_i values of deprenyl and clorgyline for the two types of MAO in rat brain cortex are similar to those in rat livers.

Photodependent effect of FNPA on MAO. The photodependent inhibition of type B MAO by FNPA was demonstrated by the fact that the degree of MAO B inhibition increased as the time of irradiation increased (Fig. 5). A 64% inhibition of type B MAO was obtained after 5 min photolysis of 4.4 mg rat cortex homogenate (in 4.6 ml) in the presence of 1 μM FNPA. For the light control, the cortex homogenate was photolyzed in the absence of FNPA; type B MAO activity was only slightly affected as only 18% inhibition occurred after 5 min irradiation (Fig. 5). Since only a 0.2-ml aliquot of the irradiation mixture was used in each assay, the final concentration of FNPA in the assay was 0.2 μM. At this concentration of FNPA, only 13% inhibition was obtained in the dark. Under the same conditions, photodependent inhibition was not clearly observed for type A MAO.

Figure 6 shows the concentration dependence of the FNPA inhibition on two types of MAO activities before and after photoirradiation. In this experiment, at 1 μM FNPA, a 75% inhibition of the type B activity resulted after 5 min irradiation (Fig. 6B). A 68% photodependent inhibition of type B MAO activity was obtained upon irradiation at 0.2 μM FNPA. In comparison, there was no inhibition of type B activity at this FNPA concentration in the dark. Twenty micromolar FNPA (i.e. 4 μM in the final assay) was needed to inhibit type B MAO activity by 60% in the dark (Fig. 6B). Thus, a clear photodependent inhibitory effect on type B MAO by FNPA is demonstrated.

On the other hand, under the same photolysis conditions, only minimal photodependent inhibition of type A MAO by FNPA was found. At 0.2 μM FNPA the photolyzed sample was inhibited 26% which is similar to the inhibition (28%) produced for the sample photolyzed in the absence of FNPA—the light control (Fig. 6A). This phenomenon was observed at all concentrations of FNPA tested as the inhibition curve of FNPA to type A MAO after photolysis paralleled that of the dark controls. These

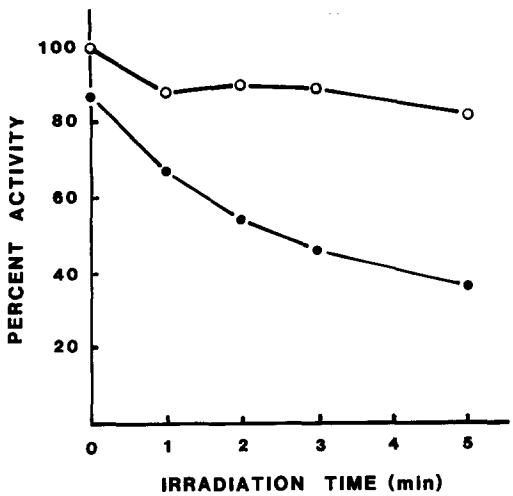

Fig. 5. Photodependent inhibition of type B MAO activity by FNPA. The irradiation mixture, 4.55 ml, contained 1 μM FNPA, 4.4 mg rat cortex homogenate, and 50 mM phosphate buffer, pH 7.4. After 0, 1, 2, 3, or 5 min of irradiation, an aliquot of 0.2 ml was withdrawn for assay of type B activity (●——●). Light control (○——○) was prepared in the same manner except that FNPA was absent from the mixture. The control without light irradiation was 18 nmoles phenylethylamine oxidized/20 min/mg protein at 10 μM phenylethylamine and was taken as 100%.

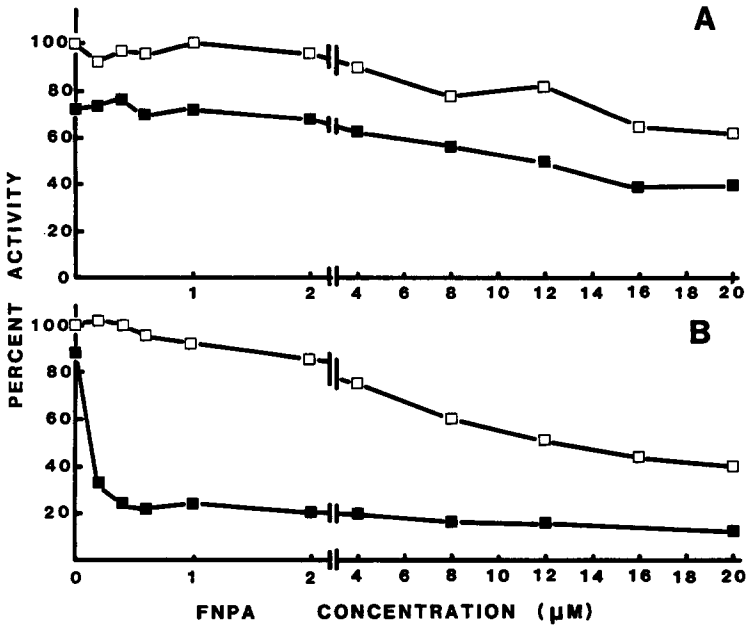

Fig. 6. Concentration dependence of the inhibition of MAO activities by FNPA. The irradiation mixture (2 ml) contained 1.5 mg rat cortex homogenate and FNPA at a concentration as indicated in the figures. Before (□——□) and after (■——■) 5 min of irradiation, 0.2 ml of each mixture was taken and assayed for serotonin oxidation (A) or phenylethylamine oxidation (B). The activity of the sample without FNPA prior to photolysis was taken as representing 100% activity.

results indicate that FNPA does not photodependently inhibit the type A MAO activity. However, the possibility for a weak photodependent inhibition of type A MAO by FNPA may exist and is not easily shown because of the strong light effect to type A MAO.

The specificity of the photodependent inhibition of type B MAO is shown by the protective effect of the substrate, phenylethylamine (Fig. 7). In this experiment, type B MAO activity was inhibited 53% at 1 μM FNPA after 5 min irradiation. This photodependent inhibition could be prevented by including phenylethylamine in the irradiation mixture during the photolysis. Complete protection was observed at 100 μM phenylethylamine. The high concentration of phenylethylamine required for complete protection is not surprising since our kinetic study has shown that FNPA has higher affinity for the enzyme (K_i = 0.78 μM) than does phenylethylamine ($K_{m,app}$ = 1.7 μM). Moreover, FNPA irreversibly bound to the enzyme upon irradiation (see next section), whereas the substrate, phenylethylamine, was deaminated and released from the active site of the enzyme throughout these treatments. Thus, a high concentration of phenylethylamine is necessary to provide complete protection against FNPA inactivation of the enzyme. Type B MAO was slightly inhibited when phenylethylamine itself was irradiated with cortex homogenate (inset, Fig. 7). The enzyme activity of these samples was taken as 100% for calculating the degree of protection of FNPA inhibition by phenylethylamine.

The differential photodependent effect of FNPA was also found toward the two MAO activities in other organs. As shown in Table 1, similar to that in the brain cortices, serotonin deamination-type A

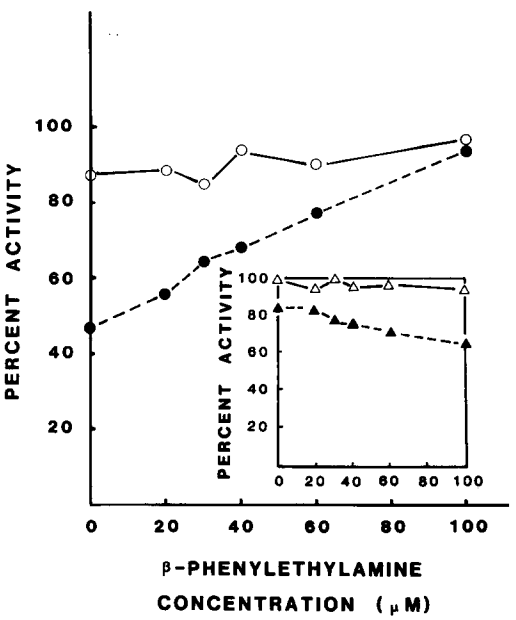

Fig. 7. Phenylethylamine protection of photodependent inhibition of type B MAO activity by FNPA. The mixture (2 ml) contained 1.6 mg cortex homogenate, 1 μM FNPA, and phenylethylamine up to 100 μM as indicated in the figure. An aliquot of 0.2 ml was taken to assay for [^{14}C]-phenylethylamine oxidation before (○——○) and after (●——●) a 5-min photoirradiation. A control containing only enzyme and phenylethylamine was assayed as well before (△——△) and following (▲——▲) photolysis (inset). The latter results were taken as 100% control activity for the photolyzed samples containing FNPA.

Table 1. Effect of FNPA on types A and B MAO activities in different organs of rat*

Organ	Serotonin deamination				Phenylethylamine deamination			
	Activity (nmoles/mg/20 min)	Light control (%)	Dark control (%)	Experimental (%)	Activity (nmoles/mg/20 min)	Light control (%)	Dark control (%)	Experimental (%)
Cortex	19.8	92	104	90	9.0	96	81	32
Heart	14.3	95	98	92	1.2	89	85	66
Liver	28.4	97	101	90	24.5	94	83	46
Lung	5.8	105	110	99	4.6	96	81	41

* The irradiation mixture, 2 ml, contained 6 nmoles FNPA and tissue homogenates (cortex, 2 mg; heart, 2.1 mg; liver, 4.3 mg; lung, 2 mg). Two-tenths milliliter of the mixture was withdrawn before (dark controls) and after 5 min of irradiation (experimentals), and MAO activities were measured as described in Materials and Methods. Light controls are samples irradiated for 5 min in the absence of FNPA.

MAO activity was not affected by 3 μM FNPA, neither in the dark nor after light irradiation using homogenates from rat heart, liver, or lung. On the other hand, phenylethylamine deamination-type B MAO activities from these homogenates were inhibited approximately 20% in the dark and 50–70% upon photolysis. Interestingly, phenylethylamine deamination in heart homogenates was 20–30% less inhibited by FNPA than that in the other organs. This finding may be explained by the previous report that there is very low type B MAO activity in rat heart, and phenylethylamine, nevertheless, serves as a substrate for type A MAO in rat heart [33]. Thus, the phenylethylamine deamination in the heart homogenate may very well be in part catalyzed by type A MAO.

Photodependent irreversible labeling of type B MAO by FNPA. Evidence for photoinduced covalently labeling of type B MAO by FNPA was provided by the following experiments. Four samples were prepared: (1) a control containing only cortex homogenate, (2) a sample containing cortex homogenate and FNPA (dark control), (3) a sample containing cortex homogenate and FNPA, and subjected to photoirradiation, and (4) a final sample containing cortex homogenate together with previously photolyzed FNPA. The type B activity in these preparations before and after removing the unbound FNPA by centrifugation was measured, and the control activity (sample 1) was taken as 100%. As shown in Table 2, only the activity of the preparation which was photoirradiated in the presence of FNPA (sample 3) remained inhibited after extensive washing. This observation suggests a photodependent covalent labeling of FNPA on the type B MAO. The lack of inhibition of MAO activity in the sample treated with prephotolyzed FNPA (sample 4) eliminates the possibility of strong noncovalent interactions between enzyme and light-induced FNPA derivative(s).

Table 2. FNPA inhibition of type B MAO*

Expt.	Condition	Percent type B monoamine oxidase activity	
		Prior to centrifugation	Following centrifugation
1	Control	100	100
2	Dark control with FNPA	64	102
3	Photoirradiated in the presence of FNPA	16	14
4	Prephotoirradiation of FNPA	88	100

* Experiment 1: Cortex homogenate (2.2 mg) was suspended at 4° in 2 ml of 50 mM phosphate buffer, pH 7.4. Experiment 2: Cortex homogenate (2.2 mg) was suspended in 1 ml of the same buffer containing in addition 5 μM FNPA and the mixture was kept in the dark. Experiment 3: Cortex homogenate (2.2 mg) was suspended in 2 ml of the buffer and probe mixture and subjected to a 5-min photoirradiation. Experiment 4 was identical to that of Experiment 3 except that FNPA was photoirradiated in the absence of cortex homogenate which was then subsequently added to the incubation mixture. An 0.2-ml aliquot of each of the mixtures was taken for the determination of type B MAO activity, and the remaining portion of the preparation was subjected to centrifugation at 50,000 g for 10 min. The pellet was washed once more by the same centrifugation procedure and was finally suspended in 1 ml of 50 mM phosphate buffer, pH 7.4, and assayed for type B MAO activity. Control activity (Experiment 1) was assayed at 10 nmoles phenylethylamine oxidized/min/mg protein with a substrate concentration of 10 μM phenylethylamine.

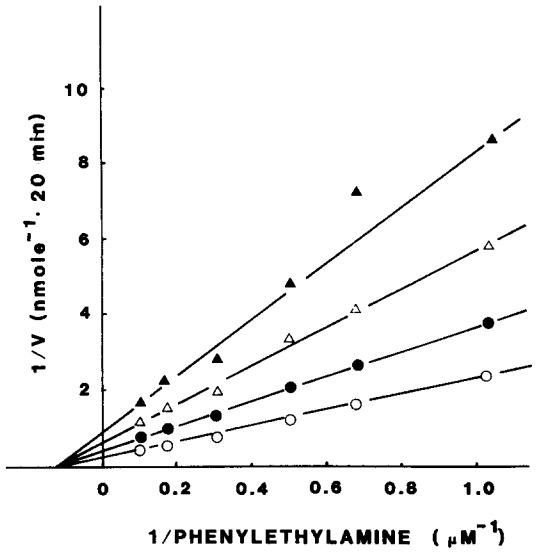

Fig. 8. Non-competitive inhibition of [^{14}C]phenylethylamine oxidation following photolysis in the presence of FNPA. For the photolysis, 4 ml of a mixture containing 3.92 mg cortex homogenate was incubated with 0.15 μM (●——●), 0.5 μM (△——△), or 1.5 μM (▲——▲) FNPA at 5° and subjected to 5 min of irradiation. The assay was initiated by addition of a 0.2-ml aliquot of the mixture. The control sample (○——○) was incubated for the same period of time in the absence of FNPA.

Kinetic studies provided additional evidence for the covalent labeling of type B enzyme by FNPA. In this experiment cortex homogenate was incubated with 0, 0.15, 0.5 or 1.5 μM FNPA and photolyzed. Type B MAO activity was determined with different concentrations of phenylethylamine, as indicated in Fig. 8. A double-reciprocal plot indicated that the K_m remained the same, whereas the V_{max} was decreased by increasing FNPA concentrations, which is typical non-competitive inhibition kinetics. In the dark, FNPA was a competitive inhibitor with respect to phenylethylamine (see Fig. 2). This kinetic change of FNPA inhibition of phenylethylamine deamination after photolysis strongly suggests that a covalent linkage is formed between the enzyme and the nitrene species resulting from the photolysis of FNPA.

The specificity of the photodependent incorporation of FNPA to type B MAO was further demonstrated by the decreased [^{3}H]pargyline labeling after the enzyme was photolyzed with FNPA. In this study, mitochondria were isolated from a rat brain cortex homogenate preparation (3 mg) which was preincubated with 0.21 μM [^{3}H]pargyline at 37° for 1 hr. The subunits of [^{3}H]pargyline labeled type A and B MAO were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis as described by previous investigators [10–12] and in the legend of Table 3. The results show that 449 cpm of radioactivity was associated with the subunit of type A enzyme and 12,769 was associated with the subunit of type B MAO (Table 3). When the enzyme preparation was preirradiated with 1.6 μM FNPA, the [^{3}H]pargyline labeling of the type B MAO was decreased 36%. On the other hand, [^{3}H]pargyline labeling of type A MAO in the sample preirradiated with FNPA was increased 2-fold. The increase in [^{3}H]pargyline labeling of type A MAO cannot be explained at the present time.

DISCUSSION

This paper has provided evidence indicating that FNPA is a potent and selective photoaffinity labeling probe for type B MAO. In the dark, FNPA was a competitive inhibitor with respect to phenylethylamine, the substrate for type B MAO (Fig. 2). It also competed for the binding of deprenyl at the active site of type B enzyme (Fig. 4). Upon photoirradiation a nitrene derivative is generated and

Table 3. Prevention of [^{3}H]pargyline labeling of MAO by FNPA in rat cortex*

FNPA concn (μM)	Type A MAO		Type B MAO	
	Radioactivity (cpm)	Percent of labeling	Radioactivity (cpm)	Percent of labeling
0	449	100	12,769	100
0.7	559	124	9,702	76
1.3	755	168	8,104	63
1.6	990	220	8,201	64

* A mixture of 3 ml solution containing 3 mg cortex homogenate, and FNPA at 0, 0.7, 1.3, or 1.6 μM, was photolyzed for 5 min at 5–10° and 0.21 μM [^{3}H]pargyline (15.3 Ci/mmole) was then added. The mixture was incubated at 37° for 1 hr. Mitochondria were isolated from these treated preparations according to Lai and Clark [27] and applied to sodium dodecyl sulfate polyacrylamide gel electrophoresis by a procedure of Weber and Osborn [28]. After the gels were stained by Coomassie blue R, they were sliced according to the staining pattern. The gel slices were solubilized by incubation at 50–60° for 4 hr in the presence of 0.2 ml of 30% H_2O_2. Scintillation fluid (10 ml) was added to the solubilized fractions and radioactivity was measured. Type A MAO was identified by the ability to block [^{3}H]pargyline labeling by 10 nM clorgyline; type B MAO was identified by the ability to block [^{3}H]pargyline labeling by 10 nM deprenyl.

covalently labels the type B enzyme, resulting in a strong photodependent irreversible inhibition of the enzyme. The specificity of FNPA labeling was demonstrated by the fact that the presence of phenylethylamine during photolysis could effectively prevent the photodependent inhibitory effect of FNPA. Also, the photodependent covalently labeling by FNPA derivative(s) decreased the [^{3}H]pargyline labeling of this enzyme.

For type A MAO, kinetic studies indicated that FNPA competitively inhibited the oxidative deamination of serotonin in the dark, but it did not have a clear photodependent inhibitory effect on this activity. One possible explanation for these different efficiencies in the photoinactivation of the two enzymes is that the active site for type A MAO is more exposed and, therefore, FNPA has a greater opportunity to react with solvent rather than the enzyme upon irradiation. An alternative explanation is that the active site of type A MAO is such that the azide (nitrene) group of FNPA may be located outside the active site when FNPA binds to the type A enzyme. However, the latter explanation is less likely because our recent experiments have indicated that the azide group of FNPA is involved in the FNPA inhibition of both MAO activities (unpublished observation). In any case, these results suggest that there is a basic difference in the active sites of the two types of MAO. Such difference(s) could be either conformational differences or differences in the amino acid sequence of the active sites. The present studies indicate that FNPA has the ability to probe this region of the active site on type B MAO.

Nagy and Salach [17] have suggested that the active sites of MAO are composed of two segments, one bearing the flavin and the other the amino acid residues comprising the substrate binding site. It has been shown that the amino acid sequence around the flavin site (pargyline binding site) is conserved and is the same in both types of MAO [15–18]. Thus, the differences in the sensitivity to inhibitors and specificity to substrates between the two types of MAO must be attributed to the differences in the structure of the substrate binding site.

Our results show that, upon irradiation, FNPA labeled type B MAO at the active site. However, the experiments indicate an incomplete protection of [^{3}H]pargyline labeling by FNPA, i.e. 1.6 μM FNPA would provide 70% photodependent inhibition of type B monoamine activity and only 36% protection of [^{3}H]pargyline labeling of type B MAO. Such results may suggest that FNPA labels at a site not identical to the pargyline labeling site. Therefore, FNPA appears to have the potential for labeling the important region(s) of the active site for type B MAO which may contribute valuable information for the molecular differences of the two types of MAO.

Acknowledgements—This study has been supported by NIMH Grant MH 37020, Department of Health and Human Services, and by BRSG S07 RR05792 awarded by the Biomedical Research Support Grant, Division of Research Resources, NIH.

REFERENCES

1. M. B. H. Youdim, G. G. S. Collins and M. Sandler, *Nature, Lond.* **223**, 626 (1969).
2. J. C. Shih and S. Eiduson, *Nature, Lond.* **224**, 1309 (1969).
3. J. C. Shih and S. Eiduson, *J. Neurochem.* **18**, 1221 (1971).
4. J. C. Shih and S. Eiduson, *J. Neurochem.* **21**, 41 (1973).
5. R. W. Fuller, *Biochem. Pharmac.* **17**, 2097 (1968).
6. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
7. J. Knoll and K. Magyar, *Adv. biochem. Psychopharmac.* **5**, 393 (1972).
8. R. M. Denney, R. R. Fritz, N. T. Patel and C. W. Abell, *Science* **215**, 1400 (1982).
9. R. M. Denney, N. T. Patel, R. R. Fritz and C. W. Abell, *Molec. Pharmac.* **22**, 500 (1982).
10. B. A. Callingham and D. Parkinson, in *Monoamine Oxidase: Structure, Function and Altered Functions* (Eds. T. P. Singer, R. W. Vonkorff and D. L. Murphy), p. 81. Academic Press, New York (1979).
11. G. K. Brown, J. F. Powell and I. W. Craig, *Biochem. Pharmac.* **29**, 2595 (1980).
12. R. M. Cawthon, J. E. Pinter, F. P. Haseltine and X. O. Breakefield, *J. Neurochem.* **37**, 363 (1981).
13. R. M. Cawthon and X. O. Breakefield, *Nature, Lond.* **281**, 692 (1979).
14. R. M. Cawthon and X. O. Breakefield, *Biochem. Pharmac.* **32**, 441 (1983).
15. E. B. Kearney, J. I. Salach, W. H. Walker, R. L. Seng, W. C. Kenney, E. Zeszotek and T. P. Singer, *Eur. J. Biochem.* **24**, 321 (1971).
16. W. H. Walker, E. B. Kearney, R. L. Seng and T. P. Singer, *Eur. J. Biochem.* **24**, 328 (1971).
17. J. Nagy and J. I. Salach, *Archs Biochem. Biophys.* **208**, 388 (1981).
18. P. H. Yu, *Can. J. Biochem.* **59**, 30 (1981).
19. H. Bayley and J. R. Knowles, *Meth. Enzym.* **46**, 69 (1977).
20. G. W. J. Fleet, J. R. Knowles and R. R. Porter, *Biochem. J.* **128**, 499 (1972).
21. A. E. Senior and A. M. Tometsko, in *Election Transfer Chains and Oxidative Phosphorylation* (Eds. E. Quagliariello, S. Papa, F. Palmieri, E. Slater and N. Siliprandi), p. 155. Elsevier, New York (1975).
22. A. M. Tometsko and J. Turula, *Photochem. Photobiol.* **24**, 579 (1976).
23. A. M. Tometsko and J. Turula, *Int. J. Peptide Protein Res.* **8**, 331 (1976).
24. J. P. Hare, G. V. Marinetti, A. I. Meisler and A. M. Tometsko, *Biochim. biophys. Acta* **443**, 485 (1976).
25. S. Otsuka and Y. Kobayashi, *Biochem. Pharmac.* **13**, 995 (1964).
26. S. Chen and R. J. Guillory, *J. biol. Chem.* **252**, 8990 (1977).
27. J. L. K. Lai and J. B. Clark, *Meth. Enzym.* **55**, 51 (1979).
28. K. Weber and M. Osborn, *J. biol. Chem.* **244**, 4406 (1969).
29. T. P. Singer, in *Monoamine Oxidase: Structure, Function, and Altered Functions* (Eds. T. P. Singer, R. W. Vonkorff and D. L. Murphy), p. 7. Academic Press, New York (1979).
30. C. M. McEwen, G. Sasaki and D. C. Jones, *Biochemistry* **8**, 3963 (1969).
31. C. J. Fowler and B. A. Callingham, *J. Pharm. Pharmac.* **30**, 304 (1978).
32. K. F. Tipton and T. J. Mantle, in *Monoamine Oxidase Inhibitors—The State of the Art* (Eds. M. B. H. Youdim and E. G. Paykel), p. 3. John Wiley, Chichester, England (1981).
33. G. A. Lyles and B. A. Callingham, *J. Pharm. Pharmac.* **27**, 682 (1975).