Biochemical Pharmacology, Vol. 21, pp. 1393–1402. Pergamon Press, 1972. Printed in Great Britain.

MICROSOMAL ENZYMES AND POTENTIATION OF TYRAMINE PRESSOR RESPONSE*

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(*Received* 11 *April* 1971; *accepted* 29 *October* 1971)

Abstract—Therapy with monoamine oxidase (MAO) inhibitors has produced hypertensive crises in patients who have ingested tyramine in food. This effect has been attributed to the inability of inhibited MAO to degrade tyramine, but recent work suggests that inhibition of hepatic microsomal oxidative enzymes may also be involved. In our experiments, SKF-525A was more potent (1000 times) than phenelzine as an inhibitor of microsomal tyramine hydroxylase, but less potent than phenelzine in potentiating the pressor response of tyramine. As SKF-525A was also shown to inhibit MAO (10 times less potent than phenelzine), it is suggested that inhibition of tyramine hydroxylase is not a major factor in potentiating the pressor response. In animals in which the microsomal enzymes were induced with phenobarbital, the pressor response to tyramine was not reduced, as would be expected if microsomal enzymes were regulating tyramine levels. These experiments suggest that tyramine potentiation is probably not a problem in therapy with drugs known to be microsomal enzyme inhibitors if these drugs have no MAO inhibitory activity.

A HAZARD associated with the use of monoamine oxidase (MAO) inhibitors is the occurrence of severe hypertensive crises on ingestion of indirectly acting sympathomimetic amines such as tyramine[1] in food, amphetamine[2] or phenylpropanolamine.[3] Potentiation of tyramine during MAO inhibition therapy has generally been attributed to the inhibition of MAO in the liver or intestine, resulting in elevated levels of tyramine reaching the circulation where it exerts its pressor effects via the release of noradrenaline.[4,5] However, amphetamine and phenylpropanolamine are not substrates for MAO, but do produce hypertensive reactions with MAO inhibitors; therefore the explanation offered for tyramine potentiation cannot apply to these amines.[6]

Several theories have been postulated in an effort to explain the potentiation of all of the indirectly acting sympathomimetic amines by MAO inhibitors, by a common mechanism. It has frequently been suggested that, since the indirectly acting sympathomimetic amines act by releasing noradrenaline, MAO inhibition could result in increased noradrenaline levels because of reduced degradation by MAO.[7] However, Trinker *et al.*[8] showed that the pressor response of injected noradrenaline was not potentiated by MAO inhibitors, probably because noradrenaline is mainly inactivated by catechol-*O*-methyl transferase.[9] It therefore seems unlikely that interference with the metabolism of released noradrenaline can account for the potentiation seen.

* This work was supported by Grant No. MA 2339 from the Medical Research Council of Canada and by the Canadian Foundation for the Advancement of Therapeutics.

Sjöqvist[4] postulated an alternative explanation based on the proposition that the noradrenaline released from an intraneuronal depot may be deaminated by MAO as it leaves the neuron. Inhibition of MAO at this site could therefore increase the amount of noradrenaline released with a resultant increase in pressor effect. More recently another explanation has been offered by Pettinger and Oates,[10] who suggested that inhibition of tyramine metabolism by MAO within the neuron, could potentiate the effects of tyramine by allowing greater amounts of tyramine to reach the site of noradrenaline release. They found however, that amphetamine, which is not a substrate for MAO, was potentiated in the same degree as tyramine and therefore suggested that inhibition of the metabolism of tyramine within the neuron was probably not responsible for the increased pressor responses observed. They suggested that the effect observed might be due to an increased amount of noradrenaline being released due to some unspecified reaction involving the MAO inhibitor and the indirectly acting sympathomimetic amine and affecting the release mechanism or noradrenaline at a neuronal site.

The most interesting recent contribution to the explanation of this problem is contained in the work of Rand and Trinker.[11] They showed that in normal animals the pressor responses of tyramine and amphetamine were potentiated by MAO inhibitors. In eviscerated animals pressor responses to these agents were greater than in intact animals, but were not further enhanced by MAO inhibitors. They concluded that potentiation of the pressor response of such indirectly acting amines occurred because MAO inhibitors decreased the rate of their degradation by enzymes present in the liver and intestine. Such experiments made it unlikely that the major cause of the potentiation observed was due to interference with the metabolism of endogenous noradrenaline by MAO inhibitors or due to an effect at the level of the neuron. These authors also reported that the pressor effects of tyramine and amphetamine were potentiated by SKF-525A. This is a potent inhibitor of microsomal enzymes[12] and they concluded that the observed potentiation was due to the inhibition of such enzymes and the accumulation of unchanged amine. Such an explanation is tenable for amphetamine, which is mainly degraded by the hepatic microsomal system in the rat and dog.[13] It could also apply to tyramine potentiation, since tyramine is a substrate for microsomal oxidative enzymes[14,15] as well as MAO,[16] and tyramine hydroxylase has been shown to be inhibited by SKF-525A.[15] The interpretation of these results by Rand and Trinker[11] with respect to tyramine is dependent on the support of an observation by Dubnick *et al.*[17] that SKF-525A has no inhibitory effect on MAO. This observation has since been disproved by Carrano and Malone,[18] Pfeffer *et al.*[19] and Renton and Eade,[20] who showed that this compound is as potent an inhibitor of MAO *in vitro* as some commonly used MAO inhibitors.

Additional support for the role of inhibition of microsomal degradation of tyramine is also suggested by the work of D'Mello,[21] who showed that the pressor effects of phenylpropanolamine were potentiated by MAO inhibitors and reported that this potentiation was due to inhibition of an enzyme other than MAO because phenylpropanolamine is not a substrate for MAO. A similar suggestion has been made by Cuthbert *et al.*,[3] who showed that phenylpropanolamine and ephedrine are potentiated in humans previously treated with an MAO inhibitor. They also concluded that inhibition of MAO is unlikely to be the explanation for the hypertensive episodes noted in their experiments.

In our experiments, we have investigated the role of microsomal tyramine hydroxylase and the role of MAO with respect to the potentiation of tyramine by MAO inhibitors. The action of these drugs on the isolated enzyme systems *in vitro* was related to their effect on the pressor responses of tyramine *in vivo*.

METHODS

Male Sprague–Dawley rats (250–300 g) obtained from Canadian Breeding Farms, St. Constant, Quebec, were used in these experiments. All drugs given *in vivo* were made up in 0·9% NaCl solution (saline).

Blood pressure studies. Rats were anesthetized with 800 mg/kg urethane i.p. After dissection and insertion of the arterial cannula, they received 2·5 mg/kg of heparin i.v. Arterial blood pressure was recorded from the carotid artery by a Statham pressure transducer coupled to a Grass polygraph. The results were recorded in millimeters of mercury. Mean blood pressure as used in these experiments was taken as diastolic blood pressure plus one-third the pulse pressure. Single injections of 50 μg tyramine in 0·1 ml saline were given by way of the jugular vein and the cannula was flushed with 0·2 ml saline after injection. The blood pressure was allowed to return to normal and the animal was killed. Single injections were used to prevent the development of tachyphylaxis to tyramine.[22]

Alteration of the tyramine response by inhibition of MAO or microsomal enzymes using phenelzine, SKF-525A and isoniazid was studied. These inhibitors were given as a single injection i.p. (40 mg/kg) 2 hr before eliciting the tyramine response. Control animals received an equal volume of saline. Alteration of the tyramine response by induction of microsomal enzymes by phenobarbital was also studied. Induced animals were prepared by treating them with 100 mg/kg of phenobarbital i.p. on day 1, followed by 50 mg/kg of phenobarbital i.p. on each of 4 succeeding days.[23] Controls received an equal volume of saline daily for 5 days. This method was also used to prepare animals for a direct measurement of induced hepatic enzyme activity *in vitro*. In an attempt to determine the effect of phenobarbital on the tyramine blood pressure response separately from the effects mediated by enzyme induction, animals were pretreated with a single dose i.p. of 40 mg/kg of phenobarbital 2 hr before eliciting the tyramine response.

Monoamine oxidase activity. A 25 per cent homogenate of rat liver was prepared in 1·15% KCl using a Sorval Omnimix homogenizer. The homogenate was centrifuged at 1000 *g* for 10 min at 4° to remove cell debris. The supernatant was used to estimate MAO enzyme activity according to the method of Weissbach *et al.*[24] in which kynuramine is oxidized to 4-hydroxyquinoline. The oxidation rate of this reaction is almost identical to that seen in the oxidation of tyramine.[25] Kynuramine was used preferentially over tyramine as a substrate because of the ease of assay of the product and the fact that the product of the initial oxidation is not further metabolized. The incubation mixture contained 0·1 ml supernatant, 1·0 ml phosphate buffer (0·1 M, pH 7·4), 1·0 ml kynuramine (0·66 mM) and varying amounts of inhibitor in 0·5 ml water. Control reactions did not contain any inhibitor. The reaction was terminated after 20 min by the addition of 0·5 ml of 5 per cent zinc sulfate solution followed by 0·5 ml of 0·3 N barium hydroxide solution. This mixture was centrifuged and the extinction of the supernatant was determined in a spectrophotometer at 315 nm. The reaction remained

linear for 30 min, and the activity of the enzyme was expressed as micrograms of 4-hydroxyquinoline formed per hour per milligram of protein.

Microsomes. The microsomal fraction of rat liver was prepared by the method of Kato *et al.*[26] using a homogenizer with a Teflon plunger. The livers were homogenized in 4 vol. by weight of 1·15 per cent potassium chloride solution. The homogenate was centrifuged at 9000 *g* for 10 min at 4° in a refrigerated centrifuge and the supernatant (microsomal plus soluble cytoplasmic fraction) was removed and the residue discarded.

Tyramine hydroxylase activity. Tyramine hydroxylase activity was determined using a modification of the method of Lemberger *et al.*,[15] which is based on the formation of dopamine from tyramine by microsomal ring hydroxylation. The incubation mixture contained 1 mM phosphate buffer (pH 7·4), 2 μM NADP, 10 μM magnesium chloride, 20 μM nicotinamide, 20 μM glucose 6-phosphate, 0·2 mg tyramine, a variable concentration of the inhibitor being tested, and 1 ml of microsomal enzyme fraction. The reaction remained linear for 20 min and in all experiments the incubation time was 15 min. The reaction was terminated by the addition of 0·5 ml perchloric acid (70 per cent). The mixture was centrifuged and the clear supernatant assayed for dopamine formed using the method of Drujan *et al.*[27] The activity of the enzyme was expressed as micrograms of dopamine formed per hour per milligram of protein.

Protein determination. Protein concentration in both enzyme preparations was determined by the biuret method.[28]

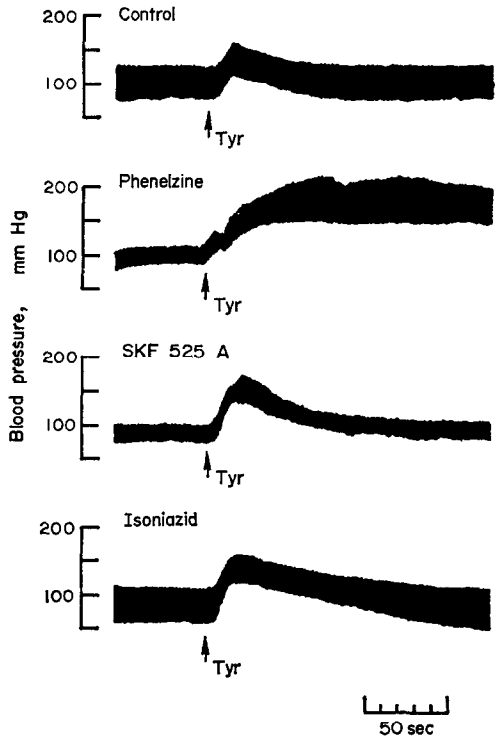

FIG. 1. Effect of phenelzine, SKF-525A and isoniazid on the pressor response of 50 μg tyramine i.v in the rat.

RESULTS

Pressor response. Pretreatment of rats with phenelzine, SKF-525A and isoniazid significantly increased the blood pressure response to an injection of 50 μg tyramine. A typical pressor response for each of these compounds is shown in Fig. 1. The results for each group of six experimental animals are recorded as mean values in Table 1. The maximum mean blood pressure rise for the control animals that received tyramine only was 31·3 ± 2·0 mm Hg and the time required for the response to return to normal was 95·1 ± 12·5 sec. Pretreatment of rats with phenelzine enhanced the pressor response to 72·3 ± 8·9 mm Hg and greatly extended the duration of the response to 225·6 ± 19·0 sec. Pretreatment with SKF-525A and isoniazid increased the blood pressure response to 48·5 ± 8·0 and 46·9 ± 4·7 mm Hg, respectively, but the duration of the response was not significantly different from the control value.

TABLE 1. DRUG-INDUCED POTENTIATION OF THE PRESSOR RESPONSE OF 50 μg OF TYRAMINE I.V. IN THE RAT*

Pretreatment	Pressor response (mm Hg)	Duration of pressor response (sec)
Control	31·3 ± 2·0	95·1 ± 12·5
Phenelzine (40 mg/kg)	72·3 ± 8·9†	225·6 ± 19·0†
SKF-525A (40 mg/kg)	48·5 ± 8·0†	120·7 ± 17·8
Isoniazid (40 mg/kg)	46·9 ± 4·7†	116·3 ± 18·8
Phenobarbital (50 mg/kg)	43·7 ± 9·1	93·7 ± 12·3
Phenobarbital (induced)	54·1 ± 3·6†	89·2 ± 6·2

* Results are expressed as mean ± S.E.; N = 6.
† Student's *t*-test; significantly different from control at $P < 0.05$.

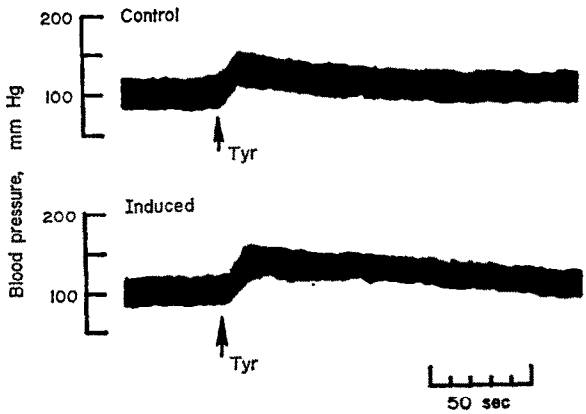

FIG. 2. Effect of acute and chronic pretreatment (induction) with phenobarbital on the pressor response of 50 μg tyramine i.v. in the rat.

A typical pressor response to an injection of 50 μg tyramine after acute and chronic pretreatment (induction) with phenobarbital is shown in Fig. 2. The results of six experimental animals in each group are summarized in Table 1. In rats in which the microsomal enzymes were induced by pretreatment for 5 days with phenobarbital, the pressor responses to tyramine were increased from a mean control value of 31·3 $\pm$ 2·0 to 54·1 $\pm$ 3·6 mm Hg, but the duration of the response was unchanged. A single injection of 50 mg/kg of phenobarbital did not alter the pressor response or its duration.

Monoamine oxidase inhibition. The effect of phenelzine, SKF-525A, isoniazid and phenobarbital on the oxidative activity of liver MAO was determined using kynuramine as substrate. The mean specific activity of this enzyme from 14 individually studied rat livers was 10·575 $\pm$ 0·790 μg 4-hydroxyquinoline formed/hr/mg of protein. The results are shown in Fig. 3. Phenelzine and SKF-525A were potent inhibitors of this enzyme. Phenelzine had a K_i of 4×10^{-5} M and SKF-525A was five times less potent with a K_i of 2×10^{-4} M. Isoniazid and phenobarbital had no inhibitory effect on this enzyme.

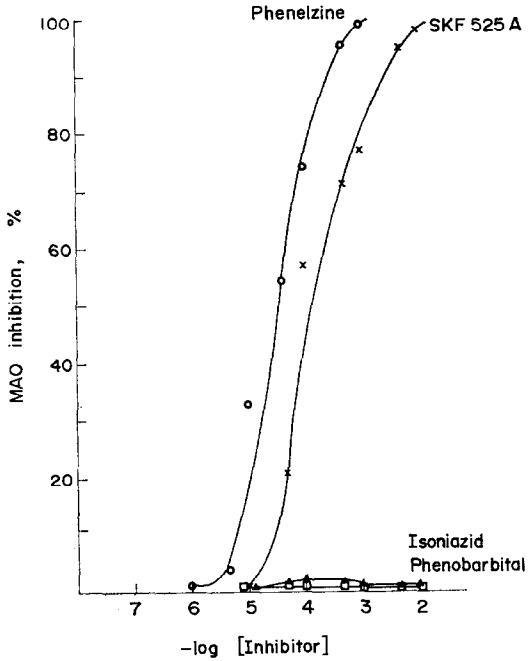

FIG. 3. Inhibition of oxidative activity of liver MAO using kynuramine as a substrate.

Tyramine hydroxylase inhibition. The effect of phenelzine, SKF-525A, isoniazid and phenobarbital on the hydroxylation of tyramine to dopamine was studied using a rat liver microsomal preparation. The results are shown in Fig. 4. The mean specific activity of this enzyme from 16 individually studied rat livers was 0·0841 $\pm$ 0·0068 μg dopamine formed/hr/mg of protein. SKF-525A was a potent inhibitor with a K_i of 7×10^{-8} M. Phenelzine was about 1000 times less potent with a K_i of 8×10^{-5} M.

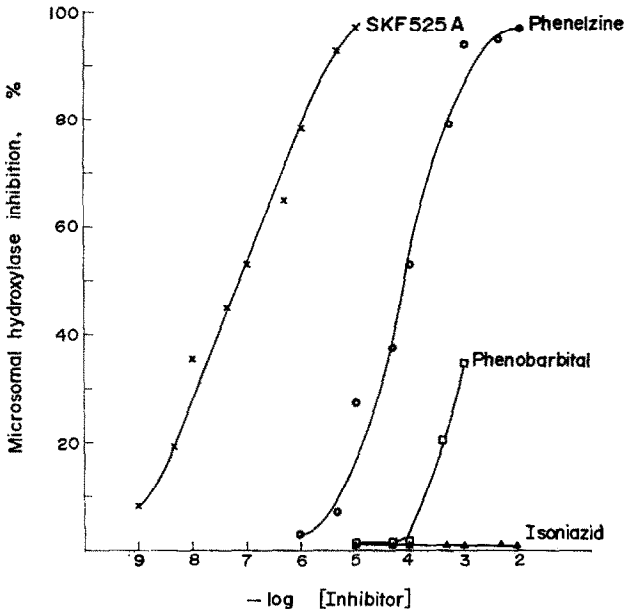

FIG. 4. Inhibition of microsomal hydroxylation of tyramine to dopamine.

Phenobarbital had inhibitory activity at high concentrations, but as the drug precipitated out of the reaction mixture at concentrations greater than 5×10^{-3} M, no K_i could be estimated. Isoniazid, which is known to inhibit other microsomal oxidation reactions, had no effect on the hydroxylation of tyramine.

Monoamine oxidase activity in induced rats. Treatment of rats for 5 days with phenobarbital to induce liver microsomal enzymes had no effect on the activity of MAO. As shown in Table 2, MAO activity in phenobarbital-treated rats was 7·327 ± 0·386 μg 4-hydroxyquinoline formed/hr/mg of protein. Activity in controls was 8·084 ± 0·281 μg 4-hydroxyquinoline formed/hr/mg of protein. The difference was not significant.

TABLE 2. MAO ACTIVITY IN PHENOBARBITAL-INDUCED RATS*

Pretreatment	4-Hydroxyquinoline (μg formed/hr/mg protein)
Control (saline)	8·084 ± 0·281
Phenobarbital (induced)	7·327 ± 0·386†

* Results are expressed as mean ± S.E.; N = 6.
† Student's *t*-test; not significantly different from control at $P < 0{\cdot}05$.

Tyramine hydroxylase in induced rats. Treatment of rats for 5 days with phenobarbital to induce liver microsomal enzymes significantly increased microsomal tyramine hydroxylase activity as compared to the controls. As shown in Table 3, the activity of this enzyme in phenobarbital-treated rats was 0·142 ± 0·004 μg dopamine formed/hr/mg of protein as compared to 0·100 ± 0·007 μg dopamine formed/hr/mg of protein in the saline-treated controls.

TABLE 3. MICROSOMAL TYRAMINE HYDROXYLASE ACTIVITY IN PHENOBARBITAL-INDUCED RATS*

Pretreatment	Dopamine (μg formed/hr/mg protein)
Control (saline)	0·100 ± 0·007
Phenobarbital (induced)	0·142 ± 0·004†

* Results are expressed as mean ± S.E.; N = 6.
† Student's *t*-test; significantly different from control at $P < 0·05$.

DISCUSSION

Potentiation of tyramine and other indirectly acting amines by MAO inhibitors has been attributed to increased levels of these amines in the circulation due to inhibition of enzymes responsible for the degradation of these amines in the liver or intestine.[4] The enzymes which have been implicated in the regulation of the amount of these amines reaching the circulation are MAO[1] and oxidative microsomal enzymes.[11,21]

In confirmation of the work of Rand and Trinker,[11] these experiments have shown that the MAO inhibitor, phenelzine, and the microsomal enzyme inhibitor, SKF-525A, potentiated the pressor response of tyramine, an indirectly acting amine, in the rat. These authors concluded that potentiation of indirectly acting amines such as tyramine and amphetamine was due to the MAO inhibitors "retarding the binding and/or breakdown of these amines within the liver microsomal system". Such inhibition of degradation of these amines within the liver would result in higher levels of amines in the circulation and an increased pressor effect from the noradrenaline released by them from intraneuronal stores. This explanation is probably correct for amphetamine, as this amine is degraded mainly by hepatic microsomal oxidation[29] and MAO inhibitors are known to be potent inhibitors of these enzymes.[30,31] In potentiation of the tyramine response, inhibition of MAO or of microsomal enzymes may be involved, as tyramine is a substrate for both enzymes.[14–16]

Rand and Trinker[11] believed that microsomal enzymes played a role in the potentiation of the tyramine pressor response because Dubnick *et al.*[17] had reported that SKF-525A, a compound which potentiated this response and is known to inhibit microsomal enzyme systems,[12,30,31] was not an inhibitor of MAO, the other enzyme implicated in this potentiation. The experiments reported here show that SKF-525A is a potent inhibitor of MAO. This observation is confirmed by Pfeffer *et al.*[19] and by Carrano and Malone[18] both *in vivo* and *in vitro*, and is in contradiction to the work of Dubnick *et al.*[17] on which Rand and Trinker[11] based their conclusions. Therefore the

potentiation of the tyramine response by SKF-525A or by phenelzine could result from inhibition of MAO, inhibition of microsomal enzymes, or some combination of both types of inhibition.

The studies *in vitro* on the isolated enzyme systems, MAO and microsomal tyramine hydroxylase, reported here showed that SKF-525A was 10 times less potent than phenelzine, with respect to MAO, but was 1000 times more potent than phenelzine with respect to tyramine hydroxlase. Therefore if inhibition of microsomal hydroxylation were the primary cause of increased levels of tyramine in the circulation, it would be expected that SKF-525A would be more potent than phenelzine as a potentiator of the pressor responses of tyramine. However, in the present experiments SKF-525A was less potent than phenelzine as a potentiator of tyramine pressor responses and did not increase the time duration of the responses to the same extent as did phenelzine. It seems likely therefore that inhibition of microsomal tyramine hydroxylase is not a major factor in the potentiation of the pressor responses of tyramine by phenelzine and SKF-525A in the rat. The longer action of phenelzine can be explained by the fact that phenelzine is a more potent inhibitor of MAO, and tyramine therefore circulates for a longer period of time.

Phenobarbital had no inhibitory effect on MAO and a minimal inhibitory effect on tyramine hydroxylase at the relatively high concentration of 10^{-3} M. Isoniazid had no inhibitory effect on either enzyme, but both phenobarbital and isoniazid potentiated the tyramine pressor response. Potentiation of the pressor response was similar to that seen with SKF-525A and may be due to factors other than the inhibition of the two enzymes studied. This unknown factor may also minimally contribute to the potentiation of tyramine by phenelzine and SKF-525A.

Conney[32] has shown that treatment of rats with phenobarbital will induce microsomal enzymes in the liver. If increased tyramine levels were produced by microsomal enzyme inhibition, and were mainly responsible for the increased pressor responses, then in induced rats decreased pressor responses would probably result from an increased rate of tyramine degradation. In these experiments the level of MAO remained unchanged in induced rats, while microsomal hydroxylase was increased by 50 per cent. However, the blood pressure responses in induced animals were increased with no increase in the time duration of the responses.

It could be suggested that microsomal metabolites of tyramine such as dopamine contribute to the pressor responses; however, we believe this to be an insignificant factor, since a single dose of phenobarbital which would not be expected to produce induction also tended to increase blood pressure. We believe, as already noted, that these increased pressor responses are due to unknown factors. These results add support to the idea that microsomal enzymes are minimally involved in regulating the level of tyramine that reaches the circulation. Additional support for this idea may also be derived by comparing the specific activities of the two enzymes. For MAO, the specific activity is 1000 times greater than for tyramine hydroxylase. If one does not consider other factors such as availability or total enzyme titer outside the liver, it would be unlikely that inhibition of the less active tyramine hydroxylase could explain the potentiation of the pressor response.

From these studies it appears that the potentiation of tyramine by phenelzine and SKF-525A is almost totally due to inhibition of MAO in the liver and intestine producing higher levels of tyramine in the circulation. Microsomal oxidation of tyramine

in the liver can have little effect on the regulation of tyramine levels in the circulation, and inhibition of this enzyme system minimally affects the pressor responses of this amine. It is probable, although we have not studied the phenomenon, that other indirectly acting amines such as amphetamine and norephedrine, which are not substrates of MAO but are substrates of microsomal enzymes, are potentiated by inhibition of microsomal oxidation in the liver. Ingestion of tyramine-containing foods such as cheese would therefore not appear to be contra-indicated during therapy with any drug which is known to be a microsomal enzyme inhibitor, as long as that drug had no inhibitory activity on MAO. Microsomal enzyme inhibitors, which include isoniazid,[33] steroids,[34] chloramphenicol[35] and metyrapone,[36] might, however, precipitate an adverse reaction if used concomitantly with indirectly acting amines that are degraded by the hepatic microsomal enzyme system.

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