Inhibition of Drug Metabolism

I. Kinetics of the Inhibition of the N-Demethylation of Ethylmorphine by 2-Diethylaminoethyl 2,2-Diphenylvalerate HCl (SKF 525-A) and Related Compounds

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SUMMARY

SKF 525-A and ten congeners were incubated with hepatic microsomes, and all were found to be N-dealkylated. The kinetics of the inhibition of the N-demethylation of ethylmorphine by the eleven compounds was studied and found to be competitive in all cases. These results strongly suggest that SKF 525-A type compounds produce their effects by combining with the active site of the N-demethylase, not by altering the permeability of the lipoid membrane of the microsome or by uncoupling an oxidative mechanism as has been postulated by others. Kinetic data were presented which indicated that many of these compounds produce their inhibitory effects by serving as alternative substrates. Kinetic evidence is also presented to support the view that the activity of SKF 525-A may be due in part to one or more of its metabolites.

INTRODUCTION

Brodie (1, 2) has suggested two possible ways in which 2-diethylaminoethyl 2,2-diphenylvalerate HCl (SKF 525-A) might exert its inhibitory action on the biotransformation of drugs and other foreign compounds: (a) inhibition of a common component of the microsomal enzyme systems, which he considered unlikely, and (b) a physiological effect on microsomes caused by its interaction with the membrane to change its permeability to drugs. Netter (3) has speculated that SKF 525-A might act in a manner analogous to that of an uncoupling agent on oxidative phosphorylation. He proposed that the product of the TPNH oxidase reaction is an "active peroxide" or an "active hydroxyl" and that the drug oxidizing step is coupled to the oxidation of TPNH. SKF 525-A would act in some way to "uncouple" the TPNH oxidative step from the drug oxidizing step. None of these hypotheses has been substantiated experimentally.

Previous work from this laboratory (4) showed that many chemically unrelated drugs inhibit the microsomal metabolism of each other competitively. This was explained on the basis that the "hydroxylases" responsible for drug metabolism are of very limited substrate specificity and that in certain cases the various drugs may be exerting their inhibitory effects by serving as alternative substrates. From these studies the idea was developed that SKF 525-A type compounds may produce their inhibitory effects by acting as alternative substrates for drug-metabolizing microsomal enzymes and that they differ from other drugs only in that their immediate pharmacologic effects are generally quite minimal (4). It is of course implicit in this concept that SKF 525-A and its congeners be metabolized by the microsomal enzymes

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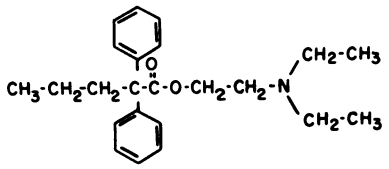

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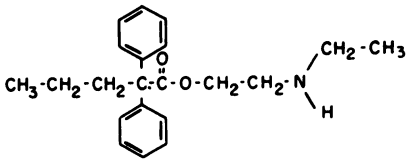

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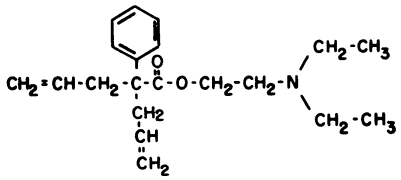

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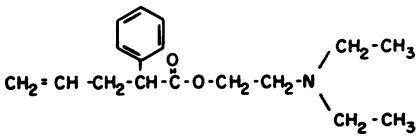

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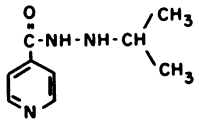

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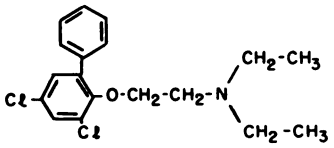

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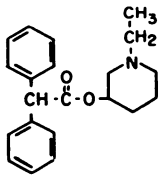
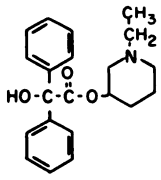
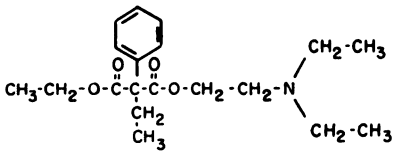


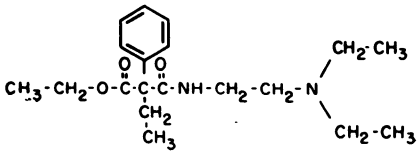
JB 305



JB 318



Sch 5705



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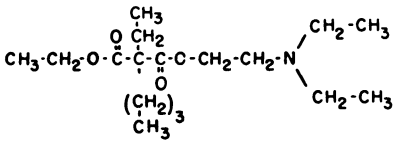

Sch 5712

FIG. 1. *SKF 525-A type inhibitors*

they are known to inhibit. The structure of SKF 525-A (Fig. 1) suggests numerous ways in which the compound might be metabolized: N-dealkylation, aromatic hydroxylation, penultimate and terminal oxidation of the propyl side chain, and hydrolysis. While not all these reactions can occur with the other inhibitors shown in Fig. 1, the potential for N-dealkylation is always present. It was largely for this reason that this reaction was chosen for the current study. Two of these inhibitors, SKF 525-A and iproniazid, have been shown to be N-dealkylated by microsomal enzymes (5, 6).

MATERIALS AND METHODS

Chemicals. Ethylmorphine hydrochloride was employed as the substrate for studying the inhibition of microsomal N-dealkylation. The inhibitors of drug metabolism used in these experiments were selected so that their N-dealkylation would yield either acetaldehyde or acetone and thus not interfere with the determination of the formaldehyde formed when ethylmorphine is N-demethylated. The complete chemical names, code names, sources of these compounds and references relating to their action as inhibitors are summarized in Table 1. Their structures are given in Fig. 1. All chemicals were used as obtained from the suppliers without further purification. Water used in these studies was distilled from glass and boiled just before use.

TABLE 1
Inhibitors of drug metabolism

Code	Chemical name	Reference
SKF 525-A	2-Diethylaminoethyl 2,2-diphenylvalerate HCl[a]	25, 26
SKF 8742-A	2-Ethylaminoethyl 2,2-diphenylvalerate HCl[a]	This paper
CFT 1201	2-Diethylaminoethyl 2-phenyl-2-(2-propene)-4-penten-1-oate HCl[b]	27
CFT 1208	2-Diethylaminoethyl 2-phenyl-4-penten-1-oate HCl[b]	27
Iproniazid	1-Isopropyl-2-isonicotinyl hydrazine PO_4[c]	24
Lilly 18947	2,4-Dichloro-6-phenylphenoxyethyldiethylamine HBr[d]	28
JB 305	*N*-Ethyl-3-piperidyl diphenylacetate HCl[e]	29
JB 318	*N*-Ethyl-3-piperidyl benzilate HCl[e]	29
Sch 5705	Ethyl 2-diethylaminoethyl 2-phenyl-2-ethylmalonate HBr[f]	30
Sch 5706	Ethyl *N*-(2-diethylaminoethyl) 2-phenyl-2-ethylmalonamate HBr[f]	30
Sch 5712	Ethyl 2-diethylaminoethyl 2-ethyl-2-butylmalonate HBr[f]	30

[a] Supplied by Smith Kline & French Laboratories, Philadelphia, Pennsylvania.
[b] Supplied by Chemische Fabrik Tempelhof, Preuss and Temmler, Berlin, Germany.
[c] Supplied by Hoffman-La Roche, Inc., Nutley, New Jersey.
[d] Supplied by Eli Lilly and Co., Indianapolis, Indiana.
[e] Supplied by Lakeside Laboratories, Inc., Milwaukee, Wisconsin.
[f] Supplied by Soc. Italiana Prodotti Schering, Milan, Italy.

For the SKF 525-A type compounds to inhibit drug metabolism by acting as alternative substrates two criteria must be met: (a) the inhibition must be competitive, and (b) the inhibition constant must not differ from the Michaelis constant for the metabolism of the inhibitor. If a compound is to function as a potent inhibitor, the Michaelis constant for the metabolism of the inhibitor should be at least as low as that for the drug being metabolized, and preferably lower. With this in mind, studies of the kinetics of the inhibition of microsomal drug metabolism by SKF 525-A and several of its congeners were undertaken.

Tissue preparation. Livers from male, albino Holtzman rats, weighing 70–100 g were employed as the source of the microsomal enzymes. The animals were stunned by a blow on the head, decapitated, and allowed to exsanguinate. The livers were removed rapidly and placed in ice-cold 1.15% potassium chloride solution. All further procedures for the preparation of the enzyme were carried out in the cold (0–4°).

A 25% homogenate in the potassium chloride solution was prepared using 15 strokes with a Dounce homogenizer fitted with a loose plunger. The homogenate was centrifuged in a Lourdes Model LRA refrigerated centrifuge at 9000 g_{max} (rotor no. 9RA) for 20 min. The supernatant, containing microsomes plus soluble fraction, was centrifuged in a Spinco Model L ultracentrifuge at 105,000 g_{ave} (rotor no. 50) for 60 min. The supernatant was discarded, and the pellet was resuspended in the potassium chloride solution and centrifuged at 105,000 g_{ave} for 30 min. The supernatant was discarded, and the microsomal pellet was resuspended in the potassium chloride solution and diluted so that 1 ml of solution contained the equivalent of 250 mg of liver, except when Sch 5705 and 5706 were used as substrates; in these cases, the equivalent of 125 mg of liver was employed. This microsomal preparation was stored at —25° until used, but not for more than 10 days.

Incubation mixture. The reaction mixture contained TPN[2] (2 μmoles), phosphate buffer, pH 7.4 (2 mmoles), semicarbazide hydrochloride (37.5 μmoles), magnesium chloride (10 μmoles), nicotinamide (20 μmoles), a TPNH generating system consisting of either glucose 6-phosphate[2] (20 μmoles) plus 2 enzyme units (EU)[3] of yeast glucose 6-phosphate dehydrogenase[2] or *d,l*-isocitrate[2] (100 μmoles) plus 4 EU[4] of pork heart isocitrate dehydrogenase,[2] varying amounts of substrate and inhibitor, 1 ml of the microsomal enzyme preparation, and 1.15% potassium chloride solution to a final volume of 5 ml. Incubations were conducted at 37° in an atmosphere of air. Incubation times were 10 min in the cases of SKF 8742-A, CFT 1201, CFT 1208 and JB 305; 15 min in the cases of SKF 525-A, Lilly 18947, JB 318, Sch 5705, Sch 5706 and Sch 5712, and 20 min in the case of iproniazid.

Determination of enzyme activity. Formaldehyde produced by the oxidative N-demethylation of ethylmorphine was measured by the chromotropic acid method as described previously (7) or by an adaptation of the method of Nash (8). When Nash's method was employed, the reaction was stopped by the addition of 2 ml of a 5% $ZnSO_4 \cdot 7\ H_2O$ solution to the reaction mixture followed by 2 ml of a 4.5% $Ba(OH)_2 \cdot 8\ H_2O$ solution. After centrifugation, 5 ml of the supernatant was mixed with 3 ml of double strength Nash's reagent, color was allowed to develop for 15 min at 60°, and readings were made at 412 mμ in a Model B Beckman spectrophotometer.

For the determination of the acetaldehyde resulting from N-deethylation, 4 ml of the incubation mixture was mixed with 2 ml of 30% trichloroacetic acid solution and the mixture was distilled. The distillation procedure and the acetaldehyde analysis were performed as described by Stotz (9).

Acetone formed by the oxidative N-dealkylation of iproniazid was determined by the method of Hansen (10). To reduce blank readings the carbon tetrachloride used in this determination was purified by shaking for 5 min with one-half its volume or 5% sodium bisulfite solution.

Data processing and statistics. For the analysis of enzyme kinetic data, reciprocal velocities were plotted against reciprocal substrate concentrations (11). The data were then analyzed by the method of Wilkinson (12). All calculations were performed with a digital computer using FORTRAN programs written by Cleland (13). These programs provided values of the Michaelis constant (K_m), maximal velocity (V_{max}), slope, $1/v$ intercept, inhibition constant (K_i), and the standard errors of their estimates. Inhibition was interpreted as being competitive when the $1/v$ intercepts (no inhibitor versus inhibitor) were not significantly different ($P > 0.05$).

At the lower substrate concentrations the amount of substrate disappearing during

[2] Obtained from the California Corporation for Biochemical Research, Los Angeles, California, or the Sigma Chemical Co., St. Louis, Missouri.

[3] One enzyme unit reduces 1 μmole of TPN per minute at pH 7.4 at 25°.

[4] One enzyme unit converts 1 μmole of isocitrate to α-ketoglutarate per minute at pH 7.4 at 37°.

TABLE 2

Inhibition constants (K_i), Michaelis constants (K_m) and maximal velocities (V_{max}) of inhibitors of the N-demethylation of ethylmorphine

Inhibitor	K_m($M \times 10^5$)	K_i($M \times 10^5$)[a]	P[b]	V_{max}[c]
SKF 525-A[f,k]	3.64 ± 0.80 (5)[d,e]	0.60 ± 0.13 (9)[d,e]	<0.001	3.73 ± 0.64[d]
SKF 8742-A[g,l]	18.4 ± 2.98 (4)	0.36 ± 0.08 (9)	<0.001	4.69 ± 0.44
CFT 1201[h,m]	2.51 ± 0.43 (3)	0.77 ± 0.08 (6)	<0.001	2.76 ± 0.41
CFT 1208[h,n]	18.2 ± 0.41 (2)	7.28 ± 4.94 (4)	>0.1	3.57 ± 0.03
Iproniazid[i,o]	822 ± 137 (4)	15.5 ± 1.86 (4)	<0.01	5.13 ± 0.94
Lilly 18947[f,m]	4.22 ± 1.15 (6)	1.85 ± 0.24 (5)	>0.05	2.37 ± 0.22
JB 305[j,m]	3.82 ± 0.56 (3)	1.53 ± 0.51 (5)	<0.05	2.62 ± 0.28
JB 318[j,m]	3.70 ± 1.68 (4)	1.13 ± 0.13 (4)	>0.1	1.97 ± 0.09
Sch 5705[j,m]	2.13 ± 0.46 (3)	2.18 ± 0.33 (4)	>0.1	2.45 ± 1.11
Sch 5706[j,m]	1.01 ± 0.13 (3)	1.59 ± 0.46 (4)	>0.1	3.57 ± 0.28
Sch 5712[j,m]	7.32 ± 2.17 (3)	6.63 ± 1.73 (4)	>0.1	3.40 ± 0.22

[a] Concentrations of ethylmorphine used: 0.8, 1.2, 1.6 and 2.0 $\times 10^{-3}$ M.

[b] K_m versus K_i.

[c] Maximal velocities expressed as micromoles of acetaldehyde per gram of liver per hour except for iproniazid, where acetone is the product.

[d] Mean ± SE.

[e] Numbers in parentheses refer to the number of experiments.

Concentration when used as a substrate (M):

[f] 0.6 to 4 $\times 10^{-4}$;

[g] 0.5 to 3 $\times 10^{-4}$;

[h] 3 to 8 $\times 10^{-5}$;

[i] 1 to 10 $\times 10^{-3}$;

[j] 2 to 8 $\times 10^{-5}$.

Concentration when used as an inhibitor (M):

[k] 1 and 2 $\times 10^{-5}$;

[l] 1 and 5 $\times 10^{-5}$;

[m] 0.5 and 1 $\times 10^{-4}$;

[n] 1 and 5 $\times 10^{-4}$;

[o] 1 and 5 $\times 10^{-3}$.

the 10–20 min incubation periods was sometimes great enough to materially alter the initial substrate concentration. The declining reaction rate that could occur with time under such conditions was corrected by recalculating the K_m and V_{max} values using the integrated Michaelis equation described by Dixon and Webb (14). The calculations were performed on the digital computer. No significant differences ($P > 0.05$ with Sch 5706 and $P > 0.1$ with the other 10 inhibitors) were found between the integrated and nonintegrated K_m values. This is due to (a) the low weighting of values obtained with low substrate concentrations when the Wilkinson method of analysis is employed, and (b) the relatively small changes in reaction velocities effected by rather large changes in substrate concentration. The K_m and V_{max} values given in Table 2 are those obtained by the integrated Michaelis equation.

The statistical analyses have been described by Steel and Torrie (15). The Student's t distribution was used as a test of the null hypothesis using a level of significance of P equal to or less than 0.05. Computation of the various statistical parameters was performed with a digital computer using FORTRAN programs written in this laboratory.

RESULTS

Kinetics of the N-dealkylation of Inhibitors

As can be seen in Table 2, all the inhibitors studied were N-dealkylated with V_{max} values falling within a relatively narrow

range (1.97–5.13 μmoles of product per gram of liver per hour). Considerably more variation was found in K_m values, but if iproniazid is excluded, they fall approximately within an order of one magnitude. Thus, all the inhibitors under investigation satisfy the first requirement of an alternative substrate hypothesis of inhibition: they are metabolized.

Kinetics of the Inhibition of the N-demethylation of Ethylmorphine

Kinetic values for the inhibition of the N-demethylation of ethylmorphine by the SKF 525-A type inhibitors are also given in Table 2. In all cases inhibition was competitive, which is compatible with the alternative substrate concept of inhibition. It can be seen that the K_m values of CFT 1208, Lilly 18947, JB 318, Sch 5705, Sch 5706 and Sch 5712, when serving as substrates, were not significantly different from corresponding K_i values when these compounds served as inhibitors. This also suggests the alternative substrate concept of inhibition. On the other hand, the K_m values of SKF 525-A, SKF 8742-A, CFT 1201, iproniazide, and JB 305 were significantly different from their K_i values.

Effect of Incubation Time on the Inhibition Constant

Gillette and Sesame (16) have postulated that in certain cases SKF 525-A may be producing its inhibitory effect through the formation of a metabolite. In this case, any observed K_i value would represent a mean of the inhibitory effects produced by SKF 525-A and the metabolite. If the metabolite is a more potent inhibitor than its parent, then, as the metabolite accumulates during the course of the incubation period, the observed K_i should decrease with time. If the inhibitory properties of the metabolite are sufficiently greater than those of its parent, the shift in K_i with time should be measurable. Accordingly, K_i values for the inhibition of the N-demethylation of ethylmorphine by SKF 525-A and SKF 8742-A were compared at incubation times of 7.5 and 15 min (Table 3). Inhibition was competitive at both time intervals. The K_i of SKF 525-A decreased significantly with increasing incubation time while no such decrease was observed with SKF 8742-A. This supports the view that a metabolite is being formed which is a more potent inhibitor than SKF 525-A itself and, also, that such an inhibitor is not formed from SKF 8742-A.

Effect of Cofactors on the Inhibition of N-dealkylation by SKF 525-A

The possibility existed that SKF 525-A might produce its inhibitory effect through a reduction in the amount of TPNH available for N-dealkylation. Doubling the concentrations of TPN, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase in the incubation mixture did not alter the inhibitory effect of SKF 525-A on the N-demethylation of ethylmorphine, nor did the addition of DPNH (2 μmoles).

TABLE 3

Inhibition of the N-demethylation of ethylmorphine[a]; effect of incubation time on the inhibition constants (K_i) of SKF 525-A and SKF 8742-A

Inhibitor	7.5 min incubation time K_i(M × 10^5)[b]	15 min incubation time K_i(M × 10^5)[b]	P[c]
SKF 525-A[d]	1.24 ± 0.26 (9)[f]	0.60 ± 0.13 (9)	<0.05
SKF 8742-A[e]	0.50 ± 0.08 (9)	0.36 ± 0.07 (9)	>0.1

[a] Concentrations of ethylmorphine used: 0.8, 1.2, 1.6, and 2.0 × 10^{-3} M.

[b] Mean ± SE.

[c] K_i (7.5 min) versus K_i (15 min).

[d] 1 and 2 × 10^{-5} M concentrations were used.

[e] 1 and 5 × 10^{-5} M concentrations were used.

[f] Numbers in parentheses refer to the number of experiments.

DISCUSSION

The competitive inhibition of the N-demethylation of ethylmorphine by SKF 525-A and its congeners suggests that these compounds exert their effects by combining with the active site of the N-demethylase. This is in agreement with McMahon and Mills (17) and McMahon (18), who showed that the N-demethylation of butynamine is inhibited competitively by SKF 525-A and a number of other inhibitors. With the exception of iproniazid, all the inhibitors used in the current series were N-dealkylated with Michaelis constants (10^{-4} to 10^{-5} M) that would permit them to compete effectively with most drugs for microsomal enzymes. For example, the Michaelis constants for the N-demethylation of ethylmorphine and the oxidation of hexobarbital are 5.8×10^{-4} M and 1.2×10^{-3} M, respectively (4). These experiments would tend to eliminate the need to explain the action of SKF 525-A as being due to its ability to alter the permeability of the microsomal membrane or to function as an uncoupling agent.

As Gillette (19) has pointed out, it is difficult to explain the action of SKF 525-A on the basis of altered microsomal membrane permeability when SKF 525-A blocks the metabolism of lipid soluble substrates such as nicotine, codeine, hexobarbital, and aminopyrine, but does not effect the oxidation of other lipid soluble compounds such as monoethyl-4-aminoantipyrine, methylaniline, and acetanilide. On the other hand, this selectivity of SKF 525-A is readily explained by the alternative substrate mechanism if one merely assumes that more than one microsomal enzyme is responsible for the oxidation of drugs; the metabolism of drugs which are oxidized by the same microsomal enzyme(s) responsible for the oxidation of SKF 525-A will be inhibited competitively by SKF 525-A, whereas the metabolism of drugs which employ other microsomal enzymes for their oxidation will not be inhibited by SKF 525-A, or if inhibition occurs, it need not be competitive.

The altered permeability concept of SKF 525-A action was developed largely to explain the inhibition of nonoxidative reactions such as the hydrolysis of procaine, the formation of morphine glucuronide, and the reduction of nitro compounds (1). The mechanism by which SKF 525-A inhibits procaine esterase has been elucidated by Netter (20), who showed that the enzyme is blocked competitively because SKF 525-A is also an ester. It is quite conceivable that some metabolite of SKF 525-A, such as a phenolic derivative or the acid product of SKF 525-A hydrolysis, might interfere with the conjugation of morphine by competing for UDP-glucuronyl transferase. At the high concentration of 10^{-3} M, SKF 525-A inhibited the reduction of *p*-nitrobenzoate by microsomal enzymes by only 20% (21), which led Netter (3) to suggest that the inhibition might be due to an unspecific reaction of SKF 525-A with the enzyme. Gillette and Sesame (16) presented evidence to show that a metabolite of SKF 525-A, produced under aerobic conditions, inhibits the anaerobic reduction of *p*-nitrobenzoate and that SKF 525-A itself does not inhibit the anaerobic reaction.

The hypothesis of Netter (3) that SKF 525-A may function by uncoupling the TPNH oxidative step from the drug oxidizing step in the overall oxidation of drugs was conceived in part because of the need to explain the noncompetitive nature of the inhibition of the O-demethylation of *o*-nitroanisole by SKF 525-A. This concept, although lacking in experimental verification, may prove valid for O-demethylases and certain other drug-metabolizing enzymes, but, in view of the current results and those of McMahon (17, 18), it would not apply to the N-demethylation of ethylmorphine or butynamine.

In order to reconcile the competitive inhibition of the oxidation of drugs in certain cases with the noncompetitive nature of the inhibition in other cases, it [redacted] Many more kinetic data must be forthcoming on the known inhibitory effects of SKF 525-A before conclusive interpretations can be made, but with information already avail-

able some speculation is possible. La Du *et al.* (22, 23) showed that SKF 525-A inhibited the dealkylation of monomethyl-4-aminoantipyrine noncompetitively and that 2,2-diphenylvaleric acid (SKF 2314) was as active as the ester. In our laboratory, the effect of SKF 2314 on the N-demethylation of ethylmorphine was studied. Results were quite erratic, and thus it was not possible to determine whether the inhibition was noncompetitive or uncompetitive, but it was not competitive. It was also much less effective than SKF 525-A. These results and those of La Du and coworkers suggest the possibility that the hydrocarbon portion of the molecule may be responsible for one type of inhibition (noncompetitive) and that the amine portion of the molecule may be responsible for another type of inhibition (competitive). One might further propose that where an enzyme is inhibited competitively, the hydrocarbon moiety attaches to the enzyme at one site (binding site) and the amine moiety falls into juxtaposition with the active site (catalytic site) of the enzyme, where it is N-dealkylated. It is not imperative that all inhibitors be metabolized, and, in fact, the inhibitor would be that much more effective *in vivo,* if indeed it were not (i.e., $V_{max} = 0$). Oxidation of the drug being inhibited would take place at this same catalytic site. Where the enzyme is inhibited noncompetitively, the hydrocarbon portion attaches to a binding site on the enzyme where it interferes in some way with the active site, but for this enzyme, the amine group does not align with the catalytic site. This is not to insist that SKF 525-A type compounds may not exert their inhibitory effects in other ways in some cases, as, for example, by inhibiting reactions at the cofactor level.

A strong case can be built for the importance of N-dealkylation in explaining the mode of action of SKF 525-A type inhibitors. In the present study all eleven inhibitors under consideration were N-dealkylated. It is of interest in this connection that iproniazid, which is N-dealkylated to form acetone, is an inhibitor of drug metabolism, whereas isonicotinic acid hydrazide, which does not offer the possibility for oxidative N-dealkylation, is not (24). Ethylmorphine and codeine, which are N-demethylated, inhibit the oxidation of hexobarbital in the intact rat (4). At first glance, primary amines such as 2,4-dichloro-6-phenylphenoxyamine (Lilly 32391), which is known to inhibit drug metabolism (17), would not appear to offer the possibility of N-dealkylation. However, oxidative deamination can be visualized as an example of N-dealkylation.

The K_i of the inhibition of the N-demethylation of ethylmorphine differed significantly from the K_m of the N-dealkylation of the inhibitor with five of the eleven inhibitors studied. This does not necessarily weigh heavily against the alternative substrate concept of inhibition. If the enzyme responsible for the N-dealkylation of the inhibitor in question is simultaneously engaged in the biotransformation of the inhibitor at some other site on the molecule, as well might be the case with certain of the inhibitors employed in this study, the overall effect would be equivalent to that resulting from the introduction of a second alternative substrate, namely, the observed K_m would be greater than the true K_m. It will be noted in Table 2, that with but two exceptions, K_m values exceed K_i values. The picture is complicated further by the probability that the product of the primary N-dealkylation of the inhibitor undergoes another N-dealkylation to the primary amine and that this second dealkylation utilizes the same enzyme that was responsible for the initial dealkylation. In recognition of such complex kinetics, it is perhaps wise to emphasize the qualitative rather than the quantitative aspects of these kinetic studies.

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