Inhibition of Drug Metabolism

III. Inhibition of Hexobarbital Metabolism in the Intact Rat and in the Isolated Perfused Liver by 2-Diethylaminoethyl 2,2-Diphenylvalerate HCl (SKF 525-A) and Its N-Deethylated Derivatives

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SUMMARY

SKF 525-A and its secondary and primary amine analogs, 2-ethylaminoethyl 2,2-diphenylvalerate HCl (SKF 8742-A) and 2-aminoethyl 2,2-diphenylvalerate HBr (AEDV), were compared for their effects on (a) the rate of hexobarbital metabolism in the intact rat, (b) hexobarbital sleeping time, and (c) rate of hexobarbital metabolism by the isolated perfused liver. The three compounds were found to be equipotent inhibitors by all three measurements. However, when the time interval between the injections of the inhibitor and the hexobarbital was increased from 45 min to 5 hr, SKF 525-A prolonged sleeping time longer than SKF 8742-A, and SKF 8742-A was more effective than AEDV. These results are interpreted to support the view that N-dealkylation plays an important role in the inhibition of drug metabolism by SKF 525-A.

INTRODUCTION

In the second publication (1) of this series hepatic microsomal enzymes were shown to N-deethylate SKF 525-A to the secondary amine, SKF 8742-A. SKF 8742-A is also N-deethylated (2). SKF 525-A, SKF 8742-A, and the primary amine analog, AEDV, are all excellent competitive inhibitors of the N-demethylation of ethylmorphine *in vitro* (1, 2). This being the case, it seemed a likely possibility that the relatively long-lasting inhibitory effects of SKF 525-A seen *in vivo* might be due in part to the fact that as this compound is metabolized (N-dealkylated), new, equally potent inhibitors are formed rather than noninhibitory, readily excretable metabolites. A comparison of the ability of these three compounds to inhibit drug metabolism *in vivo* was thus in order. At the time these experiments were in progress, studies were being conducted in this laboratory on the effects of various factors on the rate of metabolism of hexobarbital by the isolated perfused liver. This provided an opportunity to study these inhibitors at a level of biological organization between the current *in vivo* studies and those previously performed *in vitro*.

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METHODS

In vivo experiments. Male Sprague-Dawley rats weighing 90–130 g were used. Varying amounts of SKF 525-A,[4] SKF

8742-A,[4] or AEDV were administered 45 min or 5 hr before hexobarbital sodium (320 μmoles/kg) was given. All drugs were injected intraperitoneally in a volume of 1 ml/100 g of body weight. Either sleeping time was observed or blood barbiturate levels were measured. Sleeping time was defined as the time between the loss and the spontaneous return of the righting reflex. When hexobarbital blood levels were determined (3), the animals were lightly anesthetized with ether and 1.5 ml of blood was removed from the abdominal aorta. Blood samples were taken 30 or 90 min after the injection of hexobarbital or at the time of the recovery of the righting reflex.

Liver perfusion experiments. Isolated rat liver perfusions were performed employing a recirculating system similar to that described by Van Harken *et al.* (4). Male Sprague-Dawley rats weighing 400–500 g served as liver donors. The perfusate was 100 ml of heparinized rat blood diluted to 150 ml with 0.9% NaCl solution. An atmosphere of 5% CO_2:95% O_2 was employed. An equilibration interval of 30 min was allowed between the installation of the liver in the apparatus and the introduction of any substrate or inhibitor. All compounds were introduced directly into the perfusion fluid reservoir. The perfusion pressure (18 cm of H_2O) was maintained constant, and the hepatic blood flow was restricted to 2–3 ml per gram of liver per minute by manipulation of a clamp placed on the portal vein cannula. Oxygen determinations were performed on simultaneously drawn arterial and venous perfusate samples using a Beckman physiological gas analyzer (Model 160). Hexobarbital analyses (3) were performed in duplicate on 1.5 ml samples of the perfusate taken 10, 20, 30, 45, 60, and 90 min after introduction of the barbiturate. Perfusion fluid taken before the addition of hexobarbital was used for blank determinations. None of the inhibitors used in this study interfered with the hexobarbital analysis.

[4] Supplied by Smith Kline & French Laboratories, Philadelphia, Pennsylvania.

Each liver served as its own control. This was accomplished by first determining the rate of hexobarbital disappearance from the perfusate for 90 min. This interval is sufficient to allow the hexobarbital level to decline essentially to zero. A volume of perfusion fluid equal to that removed for hexobarbital analysis was added, and the inhibitor was then introduced. This was followed 1 min later by a second addition of hexobarbital, and the rate of hexobarbital disappearance was again measured for 90 min. The same amount of hexobarbital sodium (96.7 μmoles) was used each time. It was previously established in numerous experiments that, when the inhibitor is excluded, hexobarbital is metabolized at the same rate during the second 90-min period as during the first.

RESULTS

Effects of SKF 525-A, SKF 8742-A, and AEDV on Hexobarbital Metabolism

Figure 1 shows the effects of various equimolar doses of SKF 525-A, SKF 8742-A, and AEDV on the hexobarbital blood levels of rats 30 and 90 min after the administration of hexobarbital sodium. The interval between the injections of inhibitor and hexobarbital was 45 min. All doses of each of the compounds impaired hexobarbital metabolism significantly, but no clear-cut dose-response relationship was discernible at the 30-min interval. At the 90-min interval the distinction between the effects of high and low doses of inhibitor became apparent. Pretreatment with either 65 or 130 μmoles/kg of the inhibitors resulted in hexobarbital blood levels that did not decline significantly between the 30- and 90-min interval. This indicates that enzyme saturation was maintained. At each dose employed, SKF 525-A, SKF 8742-A, and AEDV were equipotent inhibitors of hexobarbital metabolism.

Effects of SKF 525-A, SKF 8742-A, and AEDV on Hexobarbital Sleeping Time

The prolonging effects of increasing doses of SKF 525-A, SKF 8742-A, and AEDV

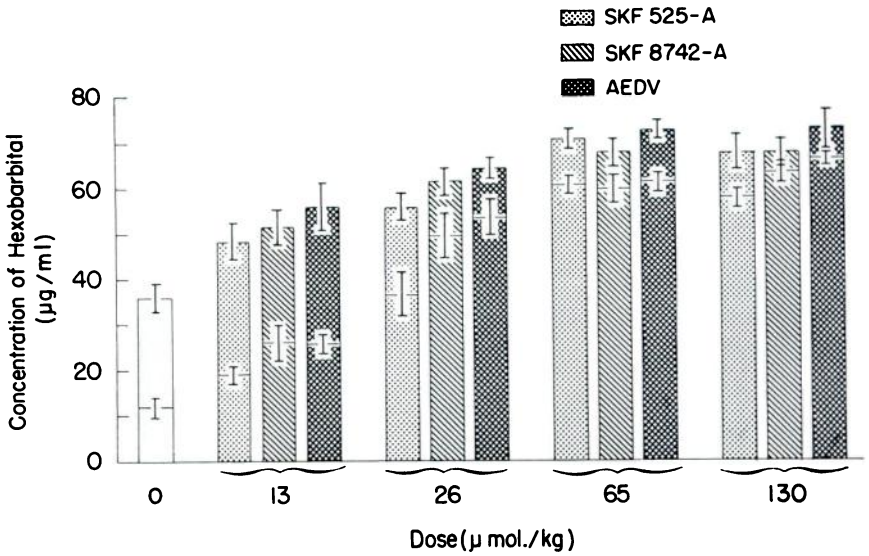

FIG. 1. *Effects of SKF 525-A, SKF 8742-A, and AEDV on hexobarbital blood levels*

Test agents were given 45 minutes prior to hexobarbital sodium (82.5 mg/kg). Bars represent the mean ± standard error for hexobarbital blood levels measured 30 min (upper) and 90 min (lower) after hexobarbital administration. All compounds were administered by the intraperitoneal route. The means are based on the values obtained from 10 rats.

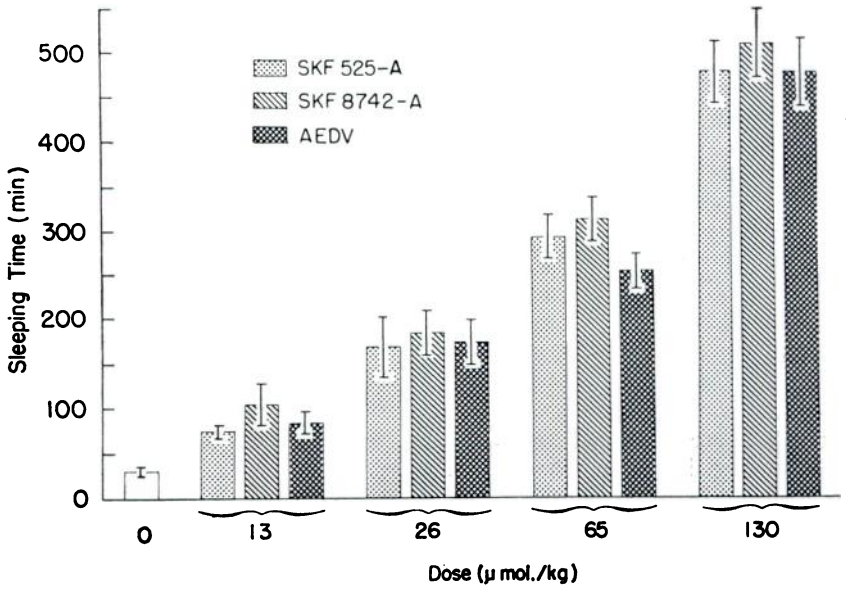

FIG. 2. *Effects of SKF 525-A, SKF 8742-A, and AEDV on hexobarbital sleeping time when the interval between inhibitor and barbiturate injections was 45 min*

Dose of hexobarbital sodium: 82.5 mg/kg. All compounds were administered by the intraperitoneal route. Bars represent the mean ± standard error. Each point is based on values obtained from six rats.

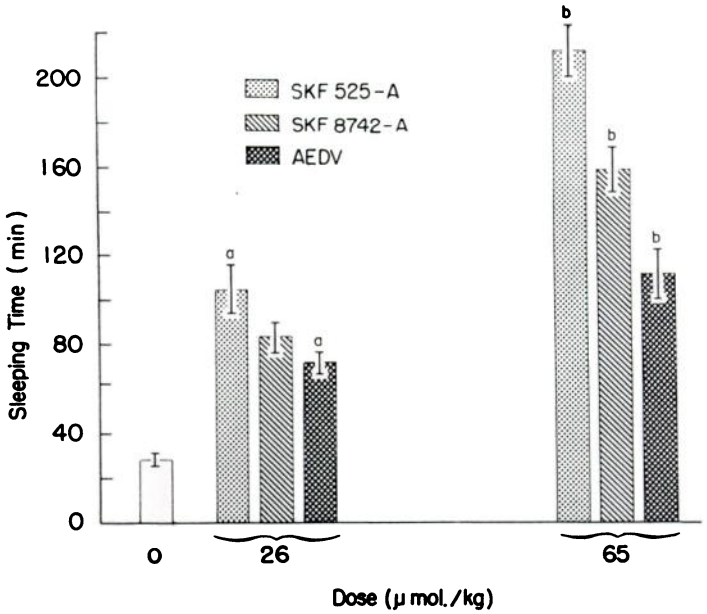

FIG. 3. *Effects of SKF 525-A, SKF 8742-A, and AEDV on hexobarbital sleeping time when the interval between inhibitor and barbiturate injections was 5 hr*

Dose of hexobarbital sodium: 82.5 mg/kg. All compounds were administered by the intraperitoneal route. Bars represent the mean sleeping time ± standard error. Each point is based on values obtained from 12 rats. *a*, Significantly different from each other ($P < 0.01$). *b*, Significantly different from each other ($P < 0.005$).

on hexobarbital sleeping time, where the interval between inhibitor and barbiturate administration was 45 min, are illustrated in Fig. 2. At each dose level the three inhibitors are seen to be about equipotent. Blood was taken when the rats awakened and hexobarbital analyses were performed. Mean blood hexobarbital levels for the various groups ranged between 24.3 and 36.0 μg/ml of blood, and variations bore no relationship either to the dose or the inhibitor used. When the experiment was repeated using a 5-hr interval between the injections of inhibitor and hexobarbital, different results were obtained (Fig. 3). SKF 525-A prolonged sleeping time more effectively than SKF 8742-A, which, in turn, was more effective than AEDV.

Effects of SKF 525-A, SKF 8742-A, and AEDV on Hexobarbital Metabolism by the Isolated, Perfused Liver

The inhibitory effects of SKF 525-A, SKF 8742-A, and AEDV on the rate of disappearance of hexobarbital from the

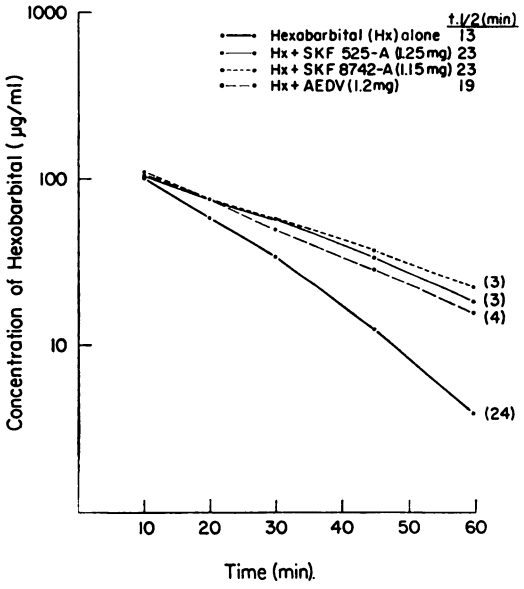

FIG. 4. *Effects of equimolar quantities of SKF 525-A, SKF 8742-A, and AEDV on hexobarbital disappearance from liver perfusates*

Values in parentheses represent the number of experiments.

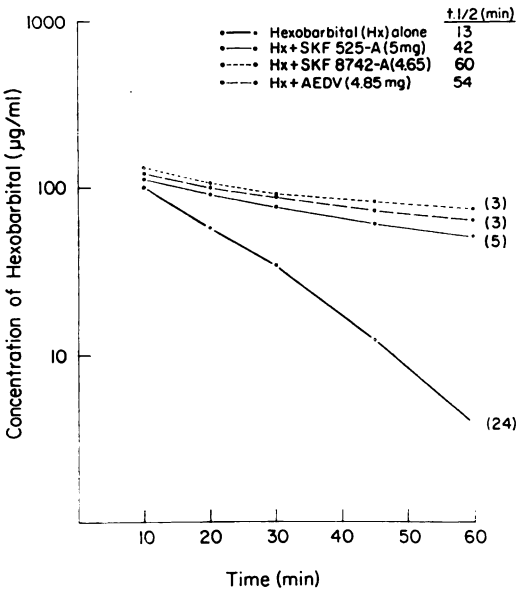

FIG. 5. *Effects of equimolar quantities of SKF 525-A, SKF 8742-A, and AEDV on hexobarbital disappearance from liver perfusates*

Values in parentheses represent the number of experiments.

liver perfusate are shown in Figs. 4 and 5. The three compounds appeared to be about equipotent. The quantities of inhibitors used in this study had no effect on perfusion flow rate. However, when larger quantities (twice the levels shown in Fig. 5) of each of the inhibitors were employed, the outflow was greatly diminished. Differences between the pO_2 values of venous and arterial perfusate samples were not affected by the inhibitors when the inhibitors were employed in quantities which did not impair the flow rate.

DISCUSSION

The inhibitor constants (K_i) for the inhibition of the N-dealkylation of ethylmorphine by SKF 525-A, SKF 8742-A, and AEDV are 6.0, 3.6, and 1.6 $\times$ 10^{-6} M, respectively (1, 2). If these values were to be used to predict inhibitory capacity *in vivo*, the three compounds would be expected to show about equal potency. This proved to be the case when hexobarbital metabolism was studied and a relatively short interval (45 min) was employed between the administration of the inhibitor and of the barbiturate. In the perfusion studies, where the inhibitor and hexobarbital were added almost simultaneously, again the three compounds were about equipotent inhibitors of hexobarbital metabolism. When the time interval between the injection of the inhibitor and hexobarbital was extended from 45 min to 5 hr, thereby permitting more extensive metabolism of the inhibitors before the introduction of the barbiturate, the three inhibitors differed in their relative degrees of potency and the changes were those expected if N-dealkylation plays an important role in the overall inhibitory effect of SKF 525-A. From the moment of association of SKF 525-A with the enzyme and throughout the two deethylations, the active site will have been continuously occupied by an effective inhibitor. However, if AEDV is metabolized to a product which is not a good inhibitor, the overall effectiveness of AEDV will be less than either SKF 525-A or SKF 8742-A even though it is equally potent in an *in vitro* system. SKF 525-A undergoes two N-dealkylations before it becomes AEDV and it is seen to have the longest duration of action with respect to the prolongation of sleeping time. SKF 8742-A, which undergoes only one N-dealkylation to AEDV, has a lesser duration of action, and finally, AEDV, which probably forms a metabolite possessing poor inhibitory properties, or which is more readily excreted than its precursors, has the shortest duration of action.

In the development of this concept, results obtained *in vitro* using ethylmorphine as a substrate were used to explain data obtained *in vivo* using hexobarbital as a substrate. This is justified if a single enzyme is responsible for both the N-demethylation of ethylmorphine and the side-chain oxidation of hexobarbital. Evidence that this is the case has been presented previously (5), and the observation that SKF 525-A is a competitive inhibitor of hexobarbital oxidation (6) supports this view.

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