Biochemical Pharmacology, 1962, Vol. 12, pp. 353–356. Pergamon Press Ltd., Printed in Great Britain.

# A NEW POTENT INHIBITOR OF MICROSOMAL DRUG-METABOLIZING ENZYMES 2:4-DICHLORO-6-PHENYLPHENOXYETHYLAMINE HYDROCHLORIDE (DPEA) (LILLY 32391)

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(*Received* 12 *August* 1962; *accepted* 12 *October* 1962)

**Abstract**—The inhibitory action of 2:4-dichloro-6-phenylphenoxyethylamine (DPEA) on the microsomal drug-metabolizing enzymes has been studied and compared with the action of SKF 525 A and Lilly 18947. DPEA is a primary amine analogue of Lilly 18947, and its inhibitory action of DPEA is about five times greater than that of Lilly 18947. The *in vitro* and *in vivo* experiments show that DPEA is about as potent an inhibitor as SKF 525 A, although it has no N,N-diethylethylamine group.

The mechanism by which DPEA produces the inhibition of drug metabolism is not yet known.

THE important role of drug metabolism in hepatic microsomes for determining the duration and intensity of drug action has been widely recognized by virtue of several investigations carried out in Brodie's laboratory.[1–5]

Inhibition of drug metabolism markedly prolongs the duration and increases the intensity of drug action. For example, SKF 525 A (β-diethylaminoethyl diphenylpropylacetate hydrochloride) and Lilly 18947 (2:4-dichloro-6-phenylphenoxy-N,N-diethylethylamine hydrobromide) prolongs several-fold the duration of hexobarbital hypnosis.[1–2] The present work deals with the inhibitory action of 2:4-dichloro-6-phenylphenoxyethylamine hydrochloride (DPEA) (Lilly 32391) on the metabolism of hexobarbital, pentobarbital, meprobamate and carisoprodol, in comparison with the inhibitory activity of SKF 525 A and Lilly 18947. DPEA was recently synthesized in the Lilly Research Laboratories and supplied by courtesy of Dr. Leighty.

## EXPERIMENTAL

Female and male rats of the Sprague–Dawley strain were used.

The *in vivo* metabolism of pentobarbital, meprobamate and carisoprodol were determined by measuring the drug concentrations in brain and serum 1 hr or 3 hr after administration of the drugs. The *in vitro* metabolism of the drugs was determined by estimating the amount of the drugs in the liver microsomal preparation after an incubation of 1 hr. The incubations were carried out as previously reported.[6–7] The final concentrations of hexobarbital, pentobarbital, meprobamate and carisoprodol were $4 \times 10^{-4}$ M, $2 \times 10^{-4}$ M, $3 \times 10^{-4}$ M, $3 \times 10^{-4}$ M, respectively.

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The determinations of hexobarbital, pentobarbital, meprobamate and carisoprodol were carried out according to the methods of Cooper and Brodie, Brodie *et al.*, Hoffman and Ludwig, and Kato *et al.*, respectively.[8-11] Meprobamate and carisoprodol were suspended in a 1% carboxymethylcellulose solution, while hexobarbital and pentobarbital were dissolved in distilled water, all drugs being injected intraperitoneally.

Pentobarbital hypnosis and carisoprodol paralysis were determined from the duration of loss of the righting reflex. The effect of strychnine was determined from convulsions and mortality.

## RESULTS

### *Inhibition of* in vitro *metabolism of hexobarbital, pentobarbital, meprobamate and carisoprodol*

The concentrations of the inhibitors causing 50 per cent inhibition of the metabolism of pentobarbital, hexobarbital, meprobamate and carisoprodol are given in Table 1.

TABLE 1. INHIBITION OF *in vitro* METABOLISM OF HEXOBARBITAL, PENTOBARBITAL, CARISOPRODOL AND MEPROBAMATE BY DPEA, SKF 525 A AND LILLY 18947

(Female rats weighing about 70 g were used.)

| Substrate | Concentration causing 50 per cent inhibition DPEA | SKF 525 A | Lilly 18947 |
|---|---|---|---|
| (1) Hexobarbital | $1{\cdot}2 \times 10^{-5}$ | $3{\cdot}4 \times 10^{-5}$ | $1{\cdot}9 \times 10^{-4}$ |
| (2) Pentobarbital | $5{\cdot}8 \times 10^{-6}$ | $1{\cdot}8 \times 10^{-5}$ | $4{\cdot}4 \times 10^{-5}$ |
| (3) Meprobamate | $9{\cdot}3 \times 10^{-6}$ | $1{\cdot}2 \times 10^{-5}$ | $4{\cdot}0 \times 10^{-5}$ |
| (4) Carisoprodol | $1{\cdot}5 \times 10^{-5}$ | $1{\cdot}9 \times 10^{-5}$ | $4{\cdot}9 \times 10^{-5}$ |

TABLE 2. INHIBITION OF *in vivo* METABOLISM OF PENTOBARBITAL, CARISOPRODOL AND MEPROBAMATE BY DPEA, SKF 525 A AND LILLY 18947

(Pentobarbital (25 mg/kg) or carisoprodol (150 mg/kg) meprobamate (150 mg/kg) were administered intraperitoneally 30 min after the pretreatment with the inhibitors. The determination of pentobarbital concentration was carried out 1 hr later, while that of carisoprodol meprobamate concentration was carried out 3 hr later. Male rats weighing about 340 g were used. The values given represent averages ± standard errors. The numbers in brackets indicate the numbers of animals used.)

| | Dose (μmole/kg) | Pentobarbital concentration (μg/ml) | Carisoprodol concentration (μg/ml) | Meprobamate concentration (μg/ml) |
|---|---|---|---|---|
| Control | | 9·2 ± 0·3 (10) | 32 ± 3·5 (10) | 63 ± 3·9 (10) |
| DPEA | 50 | 17·6 ± 0·9 (10) | 89 ± 5·3 (10) | 108 ± 4·1 (10) |
| SKF 525 A | 50 | 17·8 ± 0·7 (10) | 103 ± 4·1 (10) | 114 ± 5·7 (10) |
| Lilly 18947 | 50 | 13·9 ± 0·6 (10) | 60 ± 4·2 (10) | 87 ± 4·8 (10) |

These results indicate that DPEA is probably somewhat more potent than SKF 525 A in inhibiting the *in vitro* metabolism of the drugs, and it is from two to ten times more potent than Lilly 18947. Pentobarbital metabolism especially is markedly inhibited by DPEA, the concentration causing 50 per cent inhibition ($IC_{50}$) being $5{\cdot}8 \times 10^{-6}$ M.

These results also show that the enzyme responsible for the metabolism of pentobarbital is markedly more sensitive than that of hexobarbital. In fact, the $IC_{50}$ of DPEA, SKF 525 A and Lilly 18947 for the hexobarbital metabolism is about 2·1, 1·9 and 4·4 times, respectively, higher than that for the pentobarbital metabolism.

*Inhibition of* in vivo *metabolism of pentobarbital, carisoprodol and meprobamate*

The inhibition of the *in vivo* metabolism of pentobarbital, carisoprodol and meprobamate is demonstrated by the concentration of the administered drugs in the serum. Table 2 shows that the pentobarbital concentrations in the serum 1 hr after the injection (25 mg/kg i.p.) are markedly higher in rats pretreated 30 min before with 50 μmole/kg of DPEA (15·9 mg/kg), SKF 525 A (19·9 mg/kg) and Lilly 18947 (20·9 mg/kg). The carisoprodol and meprobamate concentrations in the serum 3 hr after the injection (150 mg/kg, i.p.) were also markedly higher in pretreated rats. Similar results were obtained from the brain concentrations.

These results indicate that the inhibitory action of DPEA on the *in vivo* metabolism of the drugs is the same or somewhat weaker than that of SKF 525 A, but it is markedly more potent than that of Lilly 18947.

TABLE 3. PROLONGATION OF PENTOBARBITAL HYPNOSIS AND CARISOPRODOL PARALYSIS BY DPEA, SKF 525 A AND LILLY 18947

(The inhibitors were intraperitoneally administered 30 min before the injection of pentobarbital (22 mg/kg i.p.) or carisoprodol (170 mg/kg). Female rats, weighing about 200 g were used. The numbers in brackets indicate the number of animals used.)

| | Dose (μmole/kg) | Pentobarbital hypnosis (min) | Carisoprodol paralysis (min) |
|---|---|---|---|
| Controls | | 46 ± 3·1 (16) | 58 ± 5·1 (16) |
| DPEA | 50 | 131 ± 6·9 (16) | 291 ± 17·3 (16) |
| SKF 525 A | 50 | 253 ± 18·3 (16) | 445 ± 31·2 (16) |
| Lilly 18947 | 50 | 142 ± 7·9 (16) | 238 ± 21·0 (16) |

TABLE 4. POTENTIATION OF STRYCHNINE TOXICITY BY DPEA, SKF 525 A AND LILLY 18947

(The inhibitors were administered 30 min before the injection of strychnine (2·4 mg/kg i.p.). Male rats, weighing about 220 g were used.)

| | Dose (μmole/kg) | No. of animals | No. of animals in convulsions | No. of animals dead | Mortality (%) |
|---|---|---|---|---|---|
| Controls | | 24 | 5 | 3 | 13 |
| DPEA | 50 | 24 | 20 | 18 | 75 |
| SKF 525 A | 50 | 24 | 19 | 16 | 66 |
| Lilly 18947 | 50 | 24 | 14 | 12 | 50 |

*Prolongation of pentobarbital hypnosis and carisoprodol paralysis*

Pentobarbital hypnosis and carisoprodol paralysis were markedly prolonged by DPEA, SKF 525 A and Lilly 18947. It can clearly be observed that the effect of SKF 525 A is greater than that of DPEA in pentobarbital hypnosis and carisoprodol paralysis. SKF 525 A prolongs pentobarbital hypnosis about 5·5 times and it prolongs carisoprodol paralysis about 7·7 times, while DPEA prolongs pentobarbital hypnosis and carisoprodol paralysis only about 3·9 times and 5·0 times, respectively.

*Potentiation of strychnine toxicity*

Table 4 shows a marked increase in the strychnine toxicity by pretreatment with

DPEA, SKF 525 A and Lilly 18947. The effect of DPEA on the strychnine toxicity is about the same as that of SKF 525 A.

## DISCUSSION

DPEA is a de-ethyl derivative of Lilly 18947 and it is a primary amine. The well-known potent inhibitors of drug metabolism generally have a diethylamine group[12], such as SKF 525 A (β-diethylaminoethyl-diphenylpropylacetate), Lilly 18947 (4:6-dichloro-6-phenylphenoxy-N,N-diethylethylamine) CFT 1201 (diethylaminoethanol-phenylaillylacetate), but the de-ethylation of Lilly 18947 potentiates in our experiments its inhibitory action about five times.

These results suggest that the diethylaminoethyl group in the well-known inhibitors is completely immaterial to the inhibitory action. DPEA seems to be a somewhat more potent inhibitor than SKF 525 A in *in vitro* metabolism, but the *in vivo* experiments show that there are no significant differences between the actions of the two inhibitors. On the other hand, the prolonging action of SKF 525 A on pentobarbital hypnosis and carisoprodol paralysis are significantly more powerful than that of DPEA.

This discrepancy between the *in vitro*, the *in vivo* and the pharmacological effect is not clear, but a more marked and prolonged accumulation of SKF 525 A in the liver than that of DPEA may be assumed to be the responsible factor.

On the other hand, DPEA itself has no visible pharmacological action, while SKF 525 A, although considered as a pharmacologically inactive drug, produces a weak sedation and some flacidity of the skeletal muscles. To these factors may be attributed the more marked effect of SKF 525 A on pentobarbital hypnosis and carisoprodol paralysis than that of DPEA. In fact, recent works have demonstrated that SKF 525 A has a direct action at the neuromuscular junction, and these results support our observations.[13–14]

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