Biochemical Pharmacology, 1966, Vol. 15, pp. 1880–1882. Pergamon Press Ltd., Printed in Great Britain.

Activation of liver dopadecarboxylase by phenobarbital

(*Received* 11 *June* 1966; *accepted* 26 *July* 1966)

THE FACT that the endoplasmatic reticulum of the liver cell synthesizes enzyme protein, and that inducers of microsomal enzymes are also stimulators of protein synthesis,[1] suggested the possibility that cytoplasmatic or mitochondrial enzymes could also be stimulated.

The present studies will show that phenobarbital and α-hexachloro-cyclohexane (α-HCH) both of which are known to stimulate microsomal enzymes in the liver,[2–4] are also capable of causing significant stimulation of dopadecarboxylase, a predominantly cytoplasmatic enzyme.

MATERIALS AND METHODS

Male rats were used in all experiments. Following decapitation the exsanguinated livers were excised, homogenized in 5 vol. of ice cold 0·9% NaCl ('Ultraturrax' homogenizer) and centrifuged at 100,000 *g* for 60 min in a Spinco ultracentrifuge. The supernatant was used to measure the activity of dopadecarboxylase manometrically in a Warburg apparatus.

Incubation

The Warburg vessels contained 1·0 ml supernatant, (equivalent to 200 mg liver wet wt.), 1·0 ml 0·5 M Na-phosphate buffer pH 6·5, 0·2 ml $1{\cdot}88 \times 10^{-3}$ M pyridoxal-5′-phosphate, and 0·3 ml $4{\cdot}3 \times 10^{-2}$ M L-dopa in the side arm. The gas phase was nitrogen. Following temperature equilibrium at 37 °C, the substrate was tipped in from the side arm. After an incubation period of 30 min the reaction was stopped by tipping in 0·2 ml of 1 N H_2SO_4 from a second side arm. All values are expressed as μl CO_2/g wet wt. per min ± S.E.M.; they are corrected for retention of CO_2 in the incubation medium. For pretreatment of animals see text.

RESULTS

Stimulation by phenobarbital and α-HCH

Forty-eight hours after a single injection intraperitoneally of 100 mg/kg of phenobarbital into 14 day-old rats (40–60 g) the activity of the dopadecarboxylase was increased by 150 per cent (Fig. 1),

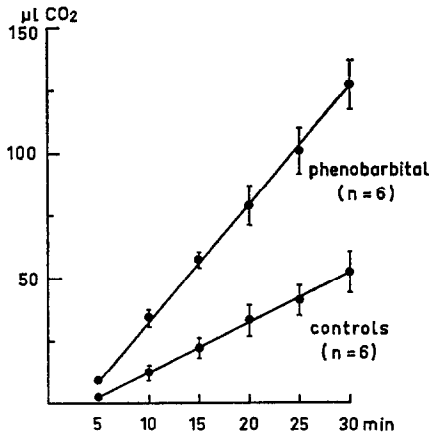

FIG. 1. Activation of liver dopadecarboxylase in rats 48 hr after i.p. injection of 100 mg/kg phenobarbital. n = number of rats (for experimental details see text).

in this experiment from 7·9 to 19·9 μl CO_2/g per min. Since the amount of coenzyme (pyridoxal-5′-phosphate) added to the incubation mixtures was sufficient to ensure maximal enzyme activity in the preparations of both normal and pretreated animals the increase due to the treatment with phenobarbital was apparently caused by an increase in apoenzyme.

While maximal stimulation was obtained 48 hr after the injection of phenobarbital, significant stimulation was still seen after 96 hr. However, in the untreated young animals the activity of dopadecarboxylase rises spontaneously. Thus, with increasing time interval after the injection of phenobarbital, the differences between untreated and pretreated animals became progressively smaller, since the maximum stimulation by phenobarbital after 48 hr did not increase further.

Experiments with adult rats (200 g) have shown that the degree of stimulation of dopadecarboxylase varied with the basal enzyme activity before treatment. If the control values were in the range of 28–30 μl CO_2/g per min stimulation by phenobarbital was not always achieved. With lower control values around 20 μl CO_2/g per min treatment with 100 mg/kg of phenobarbital on 3 consecutive days consistently caused stimulation by more than 100 per cent. The maximum was reached 96 hr after the last phenobarbital injection but stimulation was still demonstrable after 6 days, irrespective whether calculation was based on liver wet wt., dry wt. or mg protein.

Similar results were obtained by treating rats with 200 mg/kg i.p. of α-hexachloro-cyclohexane on 5 consecutive days. Stimulation, however, was less than with phenobarbital.

Tolbutamide which also is known to induce microsomal enzyme activity,[5] was applicated orally in a single dose of 750 mg/kg to 40–60 g rats resp. in daily doses of 100 mg/kg on 5 consecutive days to 200 g rats. However, in both cases dopadecarboxylase activity was not influenced.

Inhibition of stimulation by SKF 525 A

If rats (40–60 g) received SKF 525 A, an inhibitor of microsomal enzymes,[6, 7] at a dose of 50 mg/kg i.p. 30 min before and 24 hr after the i.p. injection of phenobarbital (100 mg/kg) as well as 2 hr before decapitation (48 hr after phenobarbital), the barbiturate-induced stimulation of dopadecarboxylase was not seen. The small rise in enzyme activity was due to SKF 525 A itself (Table 1). The inhibitory

TABLE 1. INHIBITION OF PHENOBARBITAL-INDUCED ACTIVATION OF LIVER DOPADECARBOXYLASE BY PRETREATMENT WITH SKF 525 A

	Controls	Phenobarbital	SKF 525 A	SKF 525 A + phenobarbital
μl CO_2/min per g wet wt.	10·5 ± 0·9	23·6 ± 3·3	14·3 ± 0·9	14·2 ± 1·5
No. of rats	9	8	8	8
Per cent change		+125	+36	+35

For experimental details see text.

action of SKF suggests that the stimulatory effect of phenobarbital on dopadecarboxylase has its primary site of action at the endoplasmatic reticulum.[8]

Inhibition of stimulation by ethionine

Instead of SKF 525 A, rats were pretreated with D,L-ethionine (2 × 200 mg/kg i.p. 30 min before and 20 hr after the injection of phenobarbital; decapitation 48 hr after phenobarbital). While ethionine *per se* had no effect on dopadecarboxylase, phenobarbital-induced stimulation was effectively blocked (Table 2). Ethionine is known to inhibit protein synthesis.[9, 10] Therefore, its blocking effect on the

TABLE 2. INHIBITION OF PHENOBARBITAL INDUCED ACTIVATION OF LIVER DOPADECARBOXYLASE BY PRETREATMENT WITH D,L-ETHIONINE

	Controls	Phenobarbital	D,L-Ethionine	D,L-Ethionine + phenobarbital
μl CO_2/min per g wet wt.	9·2 ± 1·4	22·8 ± 1·6	9·4 ± 1·1	10·4 ± 1·8
No. of rats	7	8	8	7
Per cent change		+148	+2	+13

For experimental details see text.

action of phenobarbital speaks in favour of an increased synthesis of enzyme protein induced by phenobarbital. Such a mechanism underlying the action of phenobarbital might explain, why stimulation of *microsomal* enzymes eventually leads to an increased activity also of *cytoplasmatic* enzymes.

It is not clear why phenobarbital did not stimulate other cytoplasmatic enzymes—lactate dehydrogenase for instance remained unaffected—and why tolbutamide was without any effect on liver dopadecarboxylase.

Pharmakologisches Institut
der Universität,
Frankfurt am Main,
Germany

C. ERNZERHOFF
P. HOLTZ
D. PALM

REFERENCES

1. H. V. GELBOIN and L. SOKOLOFF, *Science, N.Y.* **134**, 611 (1961).
2. H. REMMER, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **237**, 296 (1959).
3. A. H. CONNEY, C. DAVISON, R. GASTEL and J. J. BURNS, *J. Pharmac. exp. Ther.* **130**, 1 (1960).
4. W. KORANSKY, J. PORTIG, H. W. VOHLAND and I. KLEMPAU, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **247**, 61 (1964).
5. H. REMMER, *Proc. Int. Pharmac. Meetings*, Vol. 6, 235 (1962).
6. L. COOK, J. J. TONER and E. J. FELLOWS, *J. Pharmac. exp. Ther.* **111**, 131 (1954).
7. J. AXELROD, J. REICHENTHAL and B. B. BRODIE, *J. Pharmac. exp. Ther.* **112**, 49 (1954).
8. J. B. SCHENKMAN and H. REMMER, *Fedn Proc.* **25**, 343 (1966).
9. M. V. SIMPSON, E. FARBER and H. TARVER, *J. biol. Chem.* **182**, 81 (1950).
10. H. HERKEN, D. MAIBAUER and D. NEUBERT, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **233**, 139 (1958).

Biochemical Pharmacology, 1966, Vol. 15, pp. 1882–1885. Pergamon Press Ltd., Printed in Great Britain.

Excretion of N-hydroxy-2-aminofluorene by guinea pigs injected with 2-acetylaminofluorene

(*Received* 20 *May* 1966; *accepted* 26 *July* 1966)

GUINEA PIGS are refractory to the carcinogenic action of 2-aminofluorene[1, 2] and 2-acetylaminofluorene.[3–5] In contrast to many other species they do not excrete N-hydroxy-2-acetylaminofluorene in the urine after the administration of 2-acetylaminofluorene, at any rate less than could hitherto be detected.[5, 6] Kiese *et al.*[7] found N-hydroxy-2-aminofluorene in the urine of guinea pigs intraperitoneally injected with 2-aminofluorene. Only 0·5 per cent of a dose of 100 mg/kg was recovered as free N-hydroxy derivative in the urine. Since guinea pig liver microsomes have been observed to deacetylate 2-acetylaminofluorene[8] and, even more rapidly, N-hydroxy-2-acetylaminofluorene,[9] we studied whether guinea pigs excrete N-hydroxy-2-aminofluorene after the injection of 2-acetylaminofluorene.

The role of N-hydroxy derivatives of aromatic amines as proximate metabolites in carcinogenic and ferrihaemoglobin-forming action of aromatic amines has been recently reviewed by Miller and Miller[10] and by Kiese.[11]

METHODS

N-Hydroxy-2-aminofluorene was determined in the urine after being oxidized to 2-nitrosofluorene by means of ferricyanide, as described by Kiese *et al.*[7] Silica gel HF 254 + 366 was used for thin-layer chromatography of carbon tetrachloride extracts prepared from urine. The authentic 2-nitrosofluorene used for identifying the compound observed in the urine was the same as used in the experiments of Kiese *et al.*[7] Its preparation is described in a paper by Jagow *et al.*[12]