Biochemical Pharmacology, Vol. 19, pp. 2069–2073. Pergamon Press. 1970. Printed in Great Britain

EFFECT OF PHENOBARBITONE PRETREATMENT ON THE METABOLISM OF NEOSTIGMINE *IN VIVO* AND *IN VITRO*

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(*Received* 3 *November* 1969; *accepted* 9 *January* 1970)

Abstract—Increased excretion of the glucuronide of 3-hydroxyphenyltrimethyl ammonium in urine occurred in rats pretreated with phenobarbitone after a single intramuscular injection of ^{14}C-neostigmine.

No increase in the metabolism of neostigmine to 3-hydroxyphenyltrimethyl ammonium was observed *in vitro* with microsome preparations from the livers of rats pretreated with phenobarbitone. A significant increase in urinary excretion of the glucuronide of 3-hydroxyphenyltrimethyl ammonium occurred in phenobarbitone pretreated rats after a single injection of 3-hydroxyphenyltrimethyl ammonium.

NEOSTIGMINE is metabolised to 3-hydroxyphenyltrimethyl ammonium (3-OH PTMA) and its glucuronide conjugate in the rat.[1, 2] It has also been shown by *in vitro* experiments that the metabolism of neostigmine to 3-OH PTMA is via the oxidative hepatic microsomal pathway.[3] Many drugs are metabolised by this pathway and it has been demonstrated that pre-treatment of animals with several doses of phenobarbitone considerably increases the rate of metabolism of most of these compounds both *in vivo* and *in vitro*.[4–6] In view of the clinical importance of this type of drug interaction it was decided to investigate the effect of phenobarbitone pretreatment on the metabolism of ^{14}C-neostigmine in the rat.

MATERIALS AND METHODS

^{14}C-neostigmine iodide (specific activity 15·2 μc/mg) and ^{14}C-3-hydroxyphenyltrimethyl ammonium (specific activity 36·15 μc/mg) each labelled with ^{14}C in one of the methyl groups of the quaternary nitrogen were supplied by the Radiochemical Centre, Amersham.

Male albino rats weighing 150–200 g were allowed food and water *ad lib.* throughout the experiments. Each rat was placed in a separate metabolism cage. Phenobarbitone 40 mg/kg was administered intraperitoneally as a 2·0% aqueous solution twice a day for 3 days;[7] control animals were given an equivalent volume of saline. A single dose of ^{14}C-neostigmine (25 μg) or of ^{14}C-3-OH PTMA (100 μg) was injected intramuscularly approximately 15 hr after the last dose of phenobarbitone or saline. Urine free from faeces was collected 1, 2, 5 and 24 hr after the intramuscular injection of ^{14}C-neostigmine and estimated for total radioactivity, unchanged neostigmine, 3-OH

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PTMA and its glucuronide conjugate by paper electrophoresis at pH 6·9 and 9·2 as described by Husain *et al.*[2] In the experiments with ^{14}C-3-OH PTMA urine was collected at 1, 2, 4 and 24 hr and estimated for total radioactivity, unchanged 3-OH PTMA and its glucuronide.

For *in vitro* experiments, rats were pretreated with phenobarbitone or saline as described above. Livers were excised and homogenised in groups of three (using a Townson and Mercer Top Drive Macerator) in 0·25 M sucrose and centrifuged at 15,000 *g* for 20 min. The supernatant containing the microsomes was incubated with neostigmine as described by Roberts *et al.*,[3] except that half quantities were used giving a final volume in each flask of 3 ml. The proportion of neostigmine metabolised to 3-OH PTMA was estimated by alkaline paper chromatography (Somani, S.M.*).

RESULTS

Neostigmine administration

Table 1 shows the results obtained from phenobarbitone treated and control rats at each time period. It can be seen that there are no differences between the groups

TABLE 1. EXCRETION OF NEOSTIGMINE, 3-OH PTMA AND GLUCURONIDE AS PERCENTAGE DOSE IN THE URINE OF RATS AFTER A SINGLE INTRAMUSCULAR INJECTION OF ^{14}C-NEOSTIGMINE (25 μg)

Time (hr)	Treatment	Total radioactivity	Neostigmine	3-OH PTMA	Glucuronide
1	Control (6)	33·07 ± 6·9	21·39 ± 4·2	10·11 ± 3·1	1·57 ± 0·8
	Treated (6)	34·94 ± 12·0	21·35 ± 9·3	10·66 ± 3·3	2·93 ± 1·0
	Diff: P = 0·05	n/s	n/s	n/s	Significant
2	Control (6)	45·64 ± 8·3	22·8 ± 3·4	17·42 ± 4·8	5·42 ± 1·7
	Treated (6)	47·7 ± 11·5	22·51 ± 8·7	16·32 ± 3·7	8·87 ± 1·8
	Diff: P = 0·05	n/s	n/s	n/s	Significant
5	Control (6)	56·53 ± 8·1	22·37 ± 3·7	20·74 ± 5·3	12·42 ± 3·0
	Treated (6)	62·07 ± 14·3	23·22 ± 8·7	18·83 ± 3·7	20·02 ± 4·9
	Diff: P = 0·05	n/s	n/s	n/s	Significant
24	Control (6)	73·6 ± 5·7	24·83 ± 3·9	23·72 ± 5·0	25·05 ± 4·5
	Treated (6)	75·99 ± 12·7	24·31 ± 7·8	21·73 ± 3·9	29·95 ± 2·9
	Diff: P = 0·05	n/s	n/s	n/s	Significant

Treated rats were given an intraperitoneal injection of phenobarbitone (40 mg/kg body wt.) twice daily for 3 days before neostigmine.
The figures are means with standard deviations.
Number of rats are shown in parenthesis.

in the total radioactivity and 3-OH PTMA excreted, but that in the phenobarbitone treated rats there is a significant increase in the amount of 3-OH PTMA glucuronide excreted. This suggests that phenobarbitone treatment does not stimulate the oxidative metabolism of neostigmine but that it increases the conjugation of its metabolite, 3-OH PTMA. A series of *in vitro* experiments were designed to confirm the former conclusion.

* to be published.

TABLE 2. EFFECT OF PRETREATMENT WITH PHENOBARBITONE ON THE *IN VITRO* MICROSOMAL METABOLISM OF NEOSTIGMINE

Neostigmine (μg)	Metabolised (%)	
	Pretreated with phenobarbitone	Controls
50	19·2	19·5
	21·2	21·5
	15·7	19·3
	Mean 18·7	20·1
500	3·18	3·26
	3·98	3·87
	Mean 3·63	3·56

^{14}C-neostigmine iodide (50 and 500 μg) was incubated with the equivalent of 0·1 g liver wet wt. in pH 7·4 phosphate buffer as described in the text.

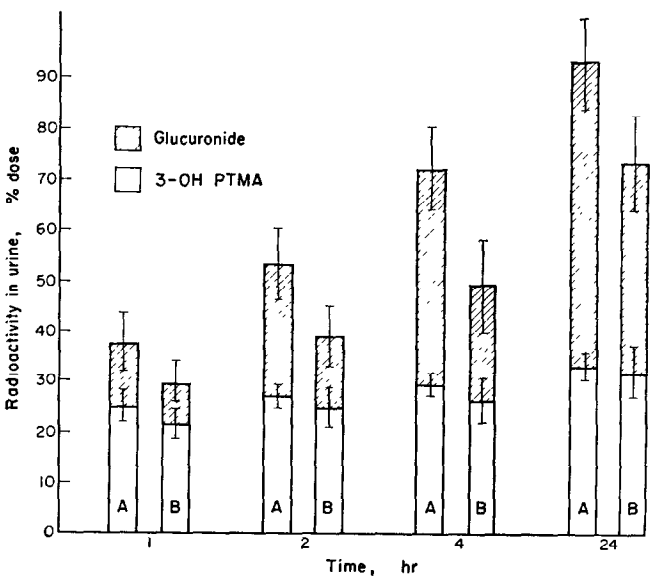

FIG. 1. Excretion of unchanged drug (3-OH PTMA) and its glucuronide conjugate (glucuronide) in urine after a single intramuscular injection of 3-OH PTMA in rats treated with phenobarbitone 40 mg/kg twice daily for 3 days (A) and saline injected control rats (B).

Incubation of neostigmine with liver microsomes

The results obtained from groups of phenobarbitone treated and control animals are shown in Table 2. With both concentrations of neostigmine there are no differences between the two groups of animals in the proportion of neostigmine metabolised to 3-OH PTMA. These results support the conclusion that phenobarbitone pretreatment does not stimulate the oxidative metabolism of neostigmine. To confirm that phenobarbitone pretreatment had been effective in stimulating microsomal metabolism,

similar experiments using *p*-nitroanisole at a concentration of 10^{-4} were performed using the method described by McMahon *et al.*[8] Preparations from phenobarbitone pretreated animals metabolised 46·7 per cent of the *p*-nitroanisole compared with 13·5 per cent from the control preparations.

3-OH PTMA administration

Figure 1 shows that after a single intramuscular injection of ^{14}C-3-OH PTMA the total radioactivity excreted in the urine of phenobarbitone treated animals was greater than that of the controls; it is also apparent that this is mainly due to increased excretion of the glucuronide conjugate. The results indicate therefore, that glucuronide conjugation of 3-OH PTMA occurs more rapidly in phenobarbitone pretreated animals.

DISCUSSION

The experiments in which neostigmine was administered to rats indicate that pretreatment with phenobarbitone has little or no effect on the metabolism of neostigmine, but that it probably increases the glucuronide conjugation of its principal metabolite, 3-OH PTMA. The *in vitro* experiments support the conclusion that the microsomal metabolism of neostigmine is not increased by pretreatment with phenobarbitone. Although Mazel and Henderson[9] have shown that some microsomal S-dealkylation reactions are not stimulated by phenobarbitone pretreatment, the failure of phenobarbitone to increase the *N*-demethylation of neostigmine is unusual, since microsomal reactions of this type are usually considerably increased by barbiturates.[4–6] The present results suggest that in the microsomal metabolism of the water soluble drug neostigmine, the microsomal enzymes are different from those involved in the metabolism of lipid soluble drugs.

Increased glucuronide conjugation of 3-OH PTMA was confirmed by injecting ^{14}C-labelled compound into rats pretreated with phenobarbitone. The results of these experiments are not surprising since Zeidenberg *et al.*[10] have shown that pretreatment of rats with phenobarbitone increases the level of glucuronyl transferase in liver microsomes. The glucuronide conjugation of 3-OH PTMA described by Husain *et al.*[2] is the first report of such a reaction occurring with a quaternary nitrogen compound; it is of further interest to note therefore that this type of reaction is increased by phenobarbitone pretreatment.

If these results in the rat apply also to man, they may have some important clinical relevance since the actions of 3-OH PTMA have been observed in a myasthenic patient and have been reported to be similar to those of the closely related compound edrophonium.[11] If a similar increase in glucuronide conjugation occurs in man after or during phenobarbitone treatment it would be expected to shorten the duration of action of neostigmine by increasing the conjugation and excretion of its chief metabolite 3-OH PTMA.

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