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Effect of phenobarbitone on the elimination of neostigmine and its metabolites in bile

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Drugs such as phenobarbitone, that induce hepatic microsomal enzymes, may enhance the biliary excretion of other foreign compounds [1–3]. This phenomenon may be related to the effects of phenobarbitone on drug metabolism [1]. bile flow [2], ligandin formation [3], canalicular transport [3] or hepatic blood flow [4], but the role of these different processes has not been defined precisely.

In the present experiments, we have studied the effects of phenobarbitone on the biliary excretion of neostigmine and its metabolites because this quaternary amine provides a novel opportunity to investigate the various hepatic mechanisms affected by enzyme induction. In the rat, neostigmine is mainly hydrolyzed by hepatic enzymes to 3-hydroxyphenyltrimethylammonium, which is subsequently conjugated to a 3-oxyglucuronide [5–7]. Although both of these metabolic steps are dependent on microsomal enzyme systems [5, 6], only glucuronide conjugation is induced by pretreatment with phenobarbitone [8]. By contrast, biliary excretion of unchanged neostigmine (and related quaternary amines) is probably due to direct transfer from hepatic arterial blood, via the peribiliary vascular plexus [9]. This study of the effects of phenobarbitone on the biliary elimination of neostigmine and its metabolites was undertaken to provide some insight into the various processes involved.

Male Sprague–Dawley rats (230–370 g) were pretreated with either saline (0.9%, 1.0 ml, i.p., daily for 5 days; control group) or phenobarbitone sodium (50 mg/kg, i.p., twice daily for 5 days; phenobarbitone-treated group). Animals were anesthetized with urethane (1.4 g/kg, i.p.), the trachea was exposed by a midline incision, and a polyethylene cannula (PE 50) was inserted into an external jugular vein. Respiration was assisted, when necessary, by means of an endotracheal tube. The bile duct was exposed through an upper abdominal incision and cannulated with polyethylene tubing (PE 10) approximately 1 cm above its entry into the duodenum. Rectal temperature was maintained at 37° by a heat lamp, in order to prevent the reduction in biliary flow induced by hypothermia.

After the collection of a control (15 min) sample of bile, [^{14}C]neostigmine iodide (120 μg/kg; sp. act. 5.33 mCi/mmole) was injected into the jugular vein. Samples of bile were collected at 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210 and 240 min. The volumes of the bile samples were calculated by dividing the weight of each specimen by a previously determined value for the specific gravity of bile (1.011). The total radioactivity in samples of bile was measured with an efficiency of approximately 50 per cent by liquid scintillation spectrometry. The samples of bile were combined at hourly intervals, and [^{14}C]neostigmine was separated from [^{14}C]hydroxyphenyltrimethylammonium and its glucuronide conjugate by chromatographic techniques, as described in detail elsewhere [7]. The relative elimination of the unchanged drug and its metabolites in control and phenobarbitone-treated rats was calculated from the total radioactivity in bile and the results of chromatography.

Bile flow rates in control rats ranged from 44 ± 8 to 53 ± 7 $\mu l \cdot kg^{-1} \cdot min^{-1}$ (mean ± S.E.M.) at the various time intervals; in animals pretreated with phenobarbitone, bile secretion rates were approximately double (range = 73 ± 2 to 102 ± 9 $\mu l \cdot kg^{-1} \cdot min^{-1}$; mean ± S.E.M.). The proportion of the total radioactivity that was eliminated in the bile at succeeding time intervals after intravenous [^{14}C]neostigmine was increased by pretreatment with phenobarbitone (Table 1). In the control animals, the proportion increased from 0.48 ± 0.05 per cent at 1 hr to 2.30 ± 0.38 per cent at 4 hr; by contrast, the percentage eliminated in phenobarbitone-treated rats increased from 1.09 ± 0.08 per

Table 1. Elimination of [^{14}C]neostigmine and its metabolites in bile of control and phenobarbitone-treated rats*

Treatment	Time (hr)	Proportion of dose (%) eliminated in bile as: Total radioactivity	[^{14}C]Neostigmine	[^{14}C]Hydroxyphenyltrimethylammonium	[^{14}C]Hydroxyphenyltrimethylammonium glucuronide
Control	1	0.48 ± 0.05	0.072 ± 0.008	0.010 ± 0.001	0.349 ± 0.036
	2	1.23 ± 0.16	0.124 ± 0.016	0.025 ± 0.003	1.024 ± 0.133
	3	1.81 ± 0.27	0.159 ± 0.024	0.036 ± 0.005	1.546 ± 0.231
	4	2.30 ± 0.38	0.208 ± 0.034	0.051 ± 0.008	1.932 ± 0.319
Phenobarbitone	1	1.09 ± 0.08 (127)	0.186 ± 0.014 (158)	0.022 ± 0.002 (120)	0.732 ± 0.054 (110)
	2	2.53 ± 0.11 (105)	0.315 ± 0.043 (154)	0.051 ± 0.004 (104)	1.940 ± 0.142 (89)
	3	3.56 ± 0.13 (197)	0.408 ± 0.037 (157)	0.061 ± 0.004 (69)	2.750 ± 0.202 (78)
	4	4.19 ± 0.14 (182)	0.464 ± 0.016 (123)	0.080 ± 0.003 (57)	3.248 ± 0.109 (68)

* Each value of the proportion of the total radioactive dose of [^{14}C]neostigmine and of its metabolites in bile is the mean ± S.E.M. of six experiments. Values in parentheses are the mean percentage increases in excretion in phenobarbitone-treated animals when compared with the control rats.

cent to 4.19 ± 0.14 per cent during this period. In both groups of animals, more than 90 per cent of the radioactivity in the bile was invariably eliminated as the 3-oxyglucuronide of 3-hydroxyphenyltrimethylammonium; minor amounts were excreted as the unconjugated phenol, other unidentified metabolites, or unchanged neostigmine (Table 1). Nevertheless, pretreatment with phenobarbitone had differential effects on the excretion of these compounds in bile. Phenobarbitone pretreatment increased the elimination of unchanged neostigmine by 123–158 per cent and more than doubled the proportion detected in bile. Pretreatment with phenobarbitone enhanced the excretion of neostigmine metabolites in bile to a lesser extent. Thus, the elimination of 3-hydroxyphenyltrimethylammonium was increased by 57–120 per cent, and that of its glucuronide conjugate by 68–110 per cent, when compared with the results in control animals (Table 1). Increased excretion of neostigmine and its metabolites was most apparent during the first 1–2 hr of the experiments and then declined progressively (Table 1). Nevertheless, these results show that the elimination of each of the identified radiolabeled compounds was enhanced by pretreatment with phenobarbitone and that the relative increase in excretion of the metabolites of [^{14}C]neostigmine was considerably less than of the parent drug.

Although it is generally accepted that treatment with phenobarbitone can increase the elimination of some drugs and their metabolites in bile [1–3], the mechanisms involved are obscure and may vary from compound to compound. In the case of drugs that are largely or completely metabolized by the liver, increased biliary excretion has usually been attributed to the induction of microsomal enzymes [1]. Although neostigmine is metabolized extensively by hepatic microsomes [5, 6], it is unlikely that the increased biliary excretion of the drug and its metabolites after pretreatment with phenobarbitone is related to enzyme induction. Previous studies suggest that phenobarbitone only enhances the conjugation of 3-hydroxyphenyltrimethylammonium by microsomal glucuronyltransferase, and has little or no effect on the hydrolysis of neostigmine [8]. Since the proportional increase in the elimination of the glucuronide conjugate was usually less than that of either unchanged neostigmine or hydroxyphenyltrimethylammonium, it is difficult to explain the present results in terms of induction of microsomal enzymes.

In the present experiments, bile flow rate increased by 70–100 per cent in animals pretreated with phenobarbitone, thus augmenting the effective gradient between the liver cell and the canaliculus. When choleresis is induced by sodium dehydrocholate, a 2- to 3-fold increase in bile flow enhances the elimination of neostigmine and its metabolites by approximately 50 per cent [10]. The increased excretion of neostigmine and its metabolites may be partially related to the effects of phenobarbitone on bile flow, but indirect evidence suggests that other factors may have been involved. Most of the radioactivity in bile was eliminated as hydroxyphenyltrimethylammonium glucuronide; in the rat, canalicular transport may be the limiting step governing the elimination of this and other glucuronides in bile [11]. In these conditions, enhanced canalicular transport induced by phenobarbitone, as proposed on different grounds by other authors [3], may be responsible for the increased elimination of the conjugate in bile.

Treatment with phenobarbitone more than doubled the small proportion of the dose of neostigmine that was eliminated in bile. The percentage increase in the biliary excretion of the unchanged drug at different time intervals (123–158 per cent) was greater than either of its metabolites, and is difficult to explain in terms of the known effects of phenobarbitone on hepatic function. Experimental evidence suggests that the biliary excretion of neostigmine and related quaternary amines is not dependent on canalicular transport, but is mediated by direct transfer from blood to bile via the peribiliary vascular plexus [9]. Although phenobarbitone increases hepato-portal blood flow in the rat by approximately 30 per cent, it has little or no effect on hepatic arterial flow [12]; thus the increased amounts of unchanged neostigmine eliminated in bile are unlikely to be derived from the peribiliary plexus. It is possible that tributaries of the portal vein supply the ducts downstream from the canaliculus, or that the increased amounts of neostigmine in bile are due to its enhanced transport from the liver cell.

In summary, treatment of male rats with phenobarbitone doubled the excretion of [^{14}C]neostigmine and its metabolites in bile. Elimination of both neostigmine and its two main metabolites in bile was invariably increased; the proportional rise in the excretion of the unchanged drug was greater than that of either 3-hydroxyphenyltrimethylammonium or its 3-oxyglucuronide. It is proposed that enzymne induction plays little or no part in the increased elimination of neostigmine and its metabolites in bile. The explanation for the enhanced excretion of unchanged neostigmine is a matter of conjecture.

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