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THE TRANSPORT OF ^{14}C-NEOSTIGMINE FROM PLASMA TO BILE

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Abstract—The transport of ^{14}C-neostigmine from plasma to bile was investigated in the rat. After i.v. injection of ^{14}C-neostigmine, approximately 0·8 per cent of the administered radioactivity was recovered from bile within 4 hr. Concurrent infusion of glycopyrronium or oxyphenonium did not modify the biliary excretion of radioactivity. However choleresis, induced by infusion of sodium dehydrocholate, significantly increased the excretion of radioactivity; approximately 1·3 per cent of the dose was detected in bile within 4 hr.

In other experiments the concentration of radioactivity in plasma, liver cells, and bile was determined 30 min after intravenous ^{14}C-neostigmine. The concentration of radioactivity in liver cell water was 3–15 times that in bile and 23–59 times that of plasma. Although neostigmine was concentrated 5-fold during transfer from plasma to bile, it was concluded that passive mechanisms were responsible for both the entry of the drug into liver cells and for its excretion in bile.

DRUGS and their metabolites may be transferred from plasma to bile in various ways. Many diffusable constituents of plasma are present in similar concentrations in bile, and it has been suggested that equilibration between plasma and bile may occur in the peribiliary vascular plexus.[1] Trace amounts of many drugs are excreted in bile, probably due to direct diffusion from periductular plasma.[2]

Alternatively, foreign compounds may pass indirectly from plasma to bile via the liver cell. Carrier transport across the excretory membrane between hepatic cells and bile has been reported for both anions and cations,[3] and the process appears to be qualitatively similar to renal tubular secretion.[4] Passive diffusion may also occur down the concentration gradient between liver cells and bile.

In previous studies,[5] it was shown that less than 3 per cent of a dose of neostigmine iodide was excreted in bile within 6 hr, and that the low concentration gradient between plasma and bile was consistent with either active or passive elimination of the drug. The present paper is primarily concerned with the method of transfer of neostigmine and its metabolic products from plasma to bile. Three different types of experiment were carried out. In the first place, the influence of other quaternary amines on the biliary excretion of neostigmine was studied. Later experiments were concerned with the effect of biliary flow rate on excretion, since there is circumstantial evidence that the elimination of solutes by active and passive mechanisms is modified differently by choleresis.[6] Finally, the concentration gradient of neostigmine and its metabolites between plasma, liver cell, and bile was investigated.

MATERIALS AND METHODS

Experimental procedure

Nonfasted male Wistar rats (140–250 g) were anaesthetized with urethane (20% w/v in distilled water; 7·0 ml/kg, i.p.). A polyethylene cannula was inserted in the common bile duct through an abdominal incision, and a similar cannula was placed in a femoral vein. ^{14}C-neostigmine iodide (trimethyl-^{14}C(*N*,*N*-dimethyl-*m*-carbamatophenyl)ammonium iodide; sp. act. 5·27 μc/μmole) was injected i.v. over a 5-min period. The dose injected (50 μg/kg) was contained in approximately 0·2 ml saline and washed in with 0·2 ml saline.

Effect of quaternary amines and choleresis on biliary excretion

In some experiments oxyphenonium bromide (25·5 μg/kg/min) or glycopyrronium bromide (23·7 μg/kg/min) was given by continuous i.v. infusion immediately after ^{14}C-neostigmine iodide. Each drug was dissolved in isotonic saline and infused throughout the duration of the 4-hr experiment; the total amount infused was equivalent to 100 times the molar dose of neostigmine (0·143 μmole/kg). In control studies, equivalent volumes of isotonic saline were infused.

Other experiments were concerned with the effect of choleresis on the biliary excretion of neostigmine. Sodium dehydrocholate (2 mg/kg/min; Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.) was infused i.v. after the injection of ^{14}C-neostigmine iodide.

In all experiments, bile was collected at hourly intervals for 4 hr. The volume of bile was measured to the nearest 0·05 ml, and radioactivity was estimated by methods previously described.[5]

Radioactivity in plasma, liver cell, and bile after ^{14}C-neostigmine

In other studies collection of bile was started 15 min after ^{14}C-neostigmine injection; 10 min later, the animal was heparinized (1000 units, i.v.) and a common carotid artery was cannulated. 30 Min after ^{14}C-neostigmine, arterial blood was collected under liquid paraffin and the supernatant plasma was removed after centrifugation. The liver was then removed, washed momentarily in cold water, blotted dry between filter paper, and weighed. Part of the median lobe was weighed separately and placed in a hot air oven; the loss of weight after drying (110° $\pm$ 10° for 24 hr) was assumed to be the weight of water in the sample. The remainder of the liver was homogenized with a solution of ethanol in distilled water (80% v/v; 20 ml). The homogenate was centrifuged, the supernatant decanted, and the final volume adjusted to 25 ml.

Measurements of the radioactivity in plasma, liver extract, and bile were made in a series 3002 Packard Tri-Carb liquid scintillation spectrometer. Samples were counted at an efficiency of 69–70 per cent, and all results were corrected for quenching by the use of an internal standard. Chlorides in plasma and liver extract were determined by the method of Schales and Schales,[7] and the extracellular space of liver was calculated from the chloride space by a modification of the method of Truax.[8] The concentration of radioactivity in liver cell water (dpm/ml) was calculated from the expression

$$\frac{C - C_1 \,.\, V_1}{V - V_1},$$

where C is the concentration of radioactivity in liver (dpm/g), V is the water content of the liver (ml/g), and C_1 and V_1 are the concentration of radioactivity (dpm/ml) and the volume (ml/g) of hepatic extracellular fluid. C_1 was assumed to be equal to the concentration of radioactivity in plasma. Since the protein content of hepatic interstitial fluid is closely related to that of plasma,[9] the error involved is probably insignificant.

RESULTS

Excretion of radioactivity in bile

Fig. 1 shows the effect of oxyphenonium, glycopyrronium, and dehydrocholate on the excretion of radioactivity in bile after ^{14}C-neostigmine iodide. Results are expressed

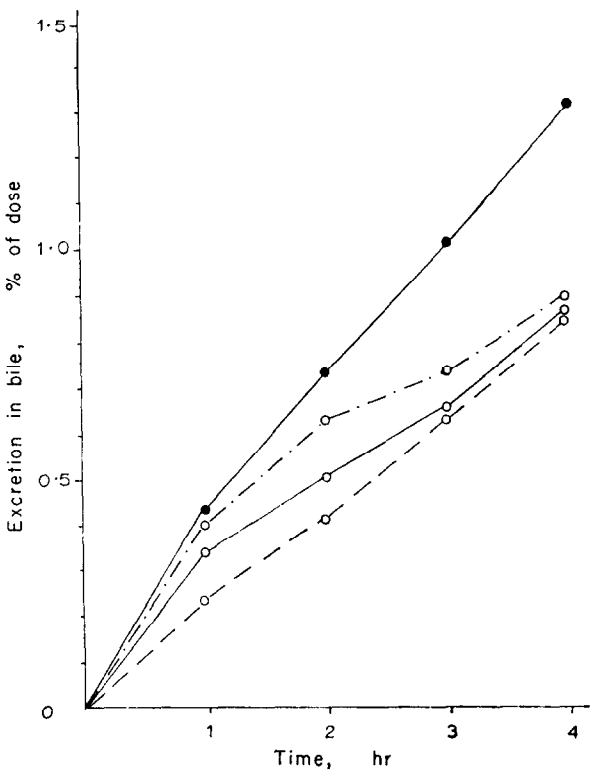

FIG. 1. The influence of oxyphenonium, glycopyrronium, and dehydrocholate on the excretion of radioactivity in bile after ^{14}C-neostigmine iodide (50 μg/kg). Intravenous infusion of (i) saline ○———○; (ii) oxyphenonium ○—·—·—○; (iii) glycopyrronium ○ — — — ○; (iv) dehydrocholate ●———●. Each point represents the mean of 5–9 experiments.

as a percentage of the dose of neostigmine, 1–4 hr after injection of the drug. Each point represents the mean of 5–9 experiments. Neither oxyphenonium nor glycopyrronium significantly modified the biliary excretion of neostigmine, when compared with results of control experiments; approximately 0·8 per cent of the dose was recovered from the bile within 4 hr. Comparable results have been reported in normal animals under similar experimental conditions.[5]

In contrast, choleresis induced by sodium dehydrocholate infusion increased the excretion of radioactivity in bile (Fig. 1). Increased excretion was most apparent during the latter half of the experiment. Within 4 hr, 1·32 ± 0·32 per cent (mean ± S.D.) of the dose was recovered from bile; when compared with the results in saline-infused controls (0·84 ± 0·08), the difference between the means was statistically significant ($t = \pm 2{\cdot}94$, $0{\cdot}05 > P > 0{\cdot}01$). The rate of biliary secretion was increased 2–3 times by sodium dehydrocholate infusion (Table 1).

TABLE 1. THE EFFECT OF SODIUM DEHYDROCHOLATE ON BILIARY FLOW

Time after neostigmine injection (hr)	Rate of bile secretion (μl/kg/min)	
	saline (2·8 μl/min)	sodium dehydrocholate (2 mg/kg/min)
1	40 ± 5	99 ± 29
2	32 ± 5	89 ± 30
3	27 ± 7	72 ± 22
4	25 ± 5	53 ± 22

Values represent the mean and S.D. of 5–9 experiments. Saline (2·8 μl/min) or sodium dehydrocholate (2 mg/kg/min) was infused i.v. throughout the duration of each experiment.

Radioactivity in plasma, liver, and bile

The concentration of radioactivity in plasma, liver, and bile 30 min after ^{14}C-neostigmine is shown in Table 2. Radioactivity was counted at an efficiency of approximately 70 per cent. The proportion of radioactivity detected in liver (23·3 ± 4·3 per cent of the dose) was comparable to previous determinations in different experimental conditions.[10] Similarly, estimates of the water content and extracellular space of liver were almost identical with figures quoted by other authors.[8] The values for the concentration of radioactivity in liver (6110–13,700 dpm/g) and liver cell water (12,100–29,100 dpm/ml) were invariably greater than the concentration in either bile (1450–6290 dpm/ml) or plasma (371–1230 dpm/ml). In some experiments the concentration of radioactivity in liver cell water was extremely high; for example, in experiments 2 and 4 the concentration was equivalent to 1 μg/ml of ^{14}C-neostigmine iodide.

Fig. 2 shows the gradient between liver cells and bile (abscissa) and liver cells and plasma (ordinate) in the nine experiments. One feature of the results is the marked variation in the gradient of radioactivity from experiment to experiment. Values for the liver cell: plasma ratio ranged from 22·8 to 59·4, while estimates of the liver cell: bile gradient varied from 3·2 to 15·0; the correlation coefficient between the variables was not statistically significant ($r = +0{\cdot}19$, $P > 0{\cdot}05$). In confirmation of previous results,[5] the mean bile: plasma gradient was low (4·6 ± 2·3); the coefficient of variation (50%) is an indication of the variability of the results.

DISCUSSION

Previous studies in this laboratory have established that neostigmine is rapidly broken down in the liver. Within 10 min of intramuscular injection, more than 98 per cent of the drug in liver had been metabolized to hydroxyphenyltrimethylammonium.[10]

TABLE 2. THE CONCENTRATION OF RADIOACTIVITY IN PLASMA, LIVER, AND BILE

Experiment number	Plasma		Liver				Bile
	concentration	% of dose	concentration	water content	extracellular space	concentration in cell water	concentration
	(dpm/ml)		(dpm/g)	(ml/g)	(ml/g)	(dpm/ml)	(dpm/ml)
1	825	24·7	10100	0·712	0·254	21600	1450
2	482	30·3	13000	0·727	0·276	28600	2060
3	456	25·8	10600	0·713	0·254	22700	1850
4	1230	26·5	13700	0·717	0·259	29100	3060
5	406	17·5	6140	0·726	0·227	12100	1700
6	616	25·9	9800	0·728	0·242	19900	6290
7	371	18·5	6110	0·715	0·221	12200	1700
8	864	20·5	10000	0·712	0·212	19700	4540
9	404	20·3	10300	0·728	0·230	20600	1970

Similarly, most of the radioactivity excreted in bile after ^{14}C-neostigmine iodide was identified as this compound or a possible demethylated derivative.[5] The quantitative data presented in this paper is therefore mainly related to the biliary excretion of hydroxyphenyltrimethylammonium, rather than unchanged neostigmine.

In the present experiments, both oxyphenonium and glycopyrronium failed to modify the biliary excretion of radioactivity after ^{14}C-neostigmine. In contrast, both compounds competitively inhibit the biliary excretion of procainamide ethobromide (an actively transported quaternary compound).[3] Although the non-saturability of biliary excretion could not be demonstrated due to the toxicity of neostigmine, the absence of competition suggests that radioactivity is eliminated in bile by diffusion rather than carrier transport. Further evidence in support of this concept was provided by experiments in which sodium dehydrocholate was infused. According to Cook *et al.*[6] the excretion of substances in bile by carrier transport is unaltered by choleresis,

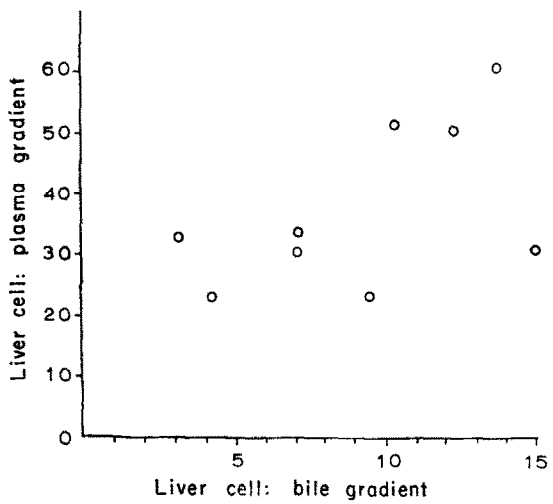

FIG. 2. The concentration gradient of radioactivity between liver cell and bile (abscissa) and liver cell and plasma (ordinate). Each point represents the results of a single experiment.

while in contrast. the elimination of compounds by diffusion is significantly increased by augmented bile flow. The influence of biliary secretion on the elimination of procainamide ethobromide is consistent with this hypothesis.[3] In the present experiments, choleresis increased the biliary excretion of radioactivity 4 hr after ^{14}C-neostigmine injection by more than 50 per cent, suggesting that diffusion from the liver is an important factor in the elimination of the drug in bile.

In confirmation of previous studies,[5] the concentration of radioactivity in bile was approximately 5 times that of plasma. Although collection of bile was not precisely synchronized with that of plasma, and may not entirely reflect the composition of canalicular or periductular bile, it is clear that diffusion from periductular plasma cannot account for the observed blood: bile gradient of radioactivity. Alternatively, biliary excretion of radioactivity may be due to direct diffusion from the liver cell. In the present experiments, a gradient favouring diffusion was invariably present between the liver cells and the bile. The concentration of radioactivity in liver cell water

ranged from 3 to 15 times the simultaneous concentration in bile, suggesting that the appearance of metabolites of neostigmine in bile may be due to direct diffusion from the liver cell.

Passive factors may also be responsible for the uptake of neostigmine by the hepatic parenchyma in spite of the adverse concentration gradient. Many plasma proteins are synthesized in liver,[11] and subsequently enter the blood stream via capillary walls or lymphatic vessels.[8] Cellular membranes that must readily permit the extrusion of molecules of plasma protein are correspondingly unlikely to prevent the inward diffusion of neostigmine. Thus in the liver, in contrast to most other tissues, cellular membranes may not present a barrier to the diffusion of quaternary amines. Possibly the rapid hepatic metabolism of neostigmine[10] provides a downhill gradient for the continual diffusion of the drug, and accounts for the high amounts of radioactivity in the liver cell in relation to plasma. Other evidence suggests that most of the breakdown products of neostigmine in plasma are derived from hepatic metabolism, although a certain amount of the drug may be slowly split by plasma cholinesterase.[5, 12]

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