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THE PHARMACOKINETICS OF PYRIDOSTIGMINE AND 3-HYDROXY-*N*-METHYLPYRIDINIUM IN THE RAT: DOSE-DEPENDENT EFFECTS AFTER PORTAL VEIN ADMINISTRATION

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1 The elimination kinetics of [^{14}C]-pyridostigmine iodide and [^{14}C-methyl]-3-hydroxypyridinium bromide (3-OH NMP) have been studied in the rat.

2 For pyridostigmine, at a given dose level, the fraction of the dose eliminated unchanged was reduced and the metabolite fraction was increased after portal vein administration when compared to jugular vein administration. This indicates that pyridostigmine is subject to metabolism during the first passage through the liver.

3 When doses of pyridostigmine 1.25 μmol/kg and higher were injected via the portal vein, the proportion excreted in urine as unchanged drug remained constant; in contrast, the percentage of the dose eliminated as the metabolite was significantly reduced. This indicates that a dose-dependent process is involved in the urinary excretion of 3-OH NMP.

4 This conclusion was supported by studies involving the portal and systemic venous injection of 3-OH NMP at different dose levels. After 4 h, approximately 85% of the lowest dose was eliminated unchanged in urine; in contrast, only 63% of the higher dose was excreted during this period. The proportion of the dose eliminated in urine was not related to the route of administration.

5 After the injection of pyridostigmine into the jugular vein, the initial rate of drug excretion fell rapidly for approximately 10 min; in contrast, after injection into the portal vein, the rate of excretion of the drug rose to a maximum at 30 minutes. This suggests that the hepatoportal system behaves as a distinct region during the distribution of this drug.

Introduction

Previous pharmacokinetic studies with pyridostigmine have been mainly concerned with its metabolism and renal excretion. In the rat and in man, the quaternary amine is mainly metabolized to 3-hydroxy-*N*-methylpyridinium (3-OH NMP), and both unchanged pyridostigmine and the metabolite are subsequently excreted in urine (Birtley, Roberts, Thomas & Wilson. 1966; Kornfeld, Samuels, Wolf & Osserman, 1970; Somani Roberts & Wilson, 1972). Small amounts of other unidentified metabolites have also been isolated from human urine (Kornfeld *et al.*, 1970; Somani *et al.*, 1972).

The present paper is concerned with the pharmacokinetics of pyridostigmine and 3-OH NMP in the rat. When drugs are cleared from plasma by first-order kinetics, both the biological half-life and the fractional composition of excretion products are independent of the dose. Alternatively, drugs may be eliminated by dose-dependent processes; in these conditions deviations from apparent first-order kinetics may be detected by changes in the fractional composition of excretion products, as long as renal clearance is constant (Levy, 1968). Recent evidence suggests that many drugs may be eliminated in this manner (Wagner, 1973). Pharmacokinetic studies of the quaternary amines neostigmine and 3-hydroxyphenyltrimethylammonium have demonstrated that both these compounds are eliminated by dose-dependent processes and subject to 'first pass' metabolic effects by the liver (Barber & Bourne, 1974).

The aim of this investigation was to characterize the elimination kinetics of pyridostigmine and 3-OH NMP in terms of either first-order or dose-dependent processes. The effect of the dose and the route of administration of both these drugs on the fractional composition of excretion products in urine has been studied.

Part of this work has already been communicated to the British Pharmacological Society (Barber & Bourne, 1973b).

Methods

Experimental procedure

Male Wistar rats (body weight: 250-350 g) were anaesthetized with ethyl carbamate (14% w/v in distilled water; 1.4 g/kg, i.p.) and the trachea was cannulated. A jugular vein and both ureters were also cannulated with polyethylene tubing (Portex pp 50). Mannitol (6% w/v in 0.9% w/v NaCl solution; 75 μl/min) was continuously infused via the jugular vein to maintain a constant diuresis (Barber & Bourne, 1971).

In experiments in which drugs were administered via the portal vein, a 22 gauge needle attached to a length of fine polyethylene tubing (Portex pp 10) was inserted into the portal vein near the hilum of the liver (Back & Calvey, 1972). The other end of the polyethylene tubing was attached to a needle and syringe.

Pharmacokinetic studies

[^{14}C]-pyridostigmine iodide (N-[^{14}C methyl]-3-(*N,N*-dimethyl-carbamato) pyridinium iodide (The Radiochemical Centre, Amersham; sp. act. = 16.1 mCi/mmol) was administered alone or with non-labelled compound. The drug was rapidly injected via the jugular vein (dose: 0.10 or 0.63 μmol/kg) or via the portal vein (dose: 0.10, 0.63, 1.25, 3.00, or 6.0 μmol/kg). Urine was collected in 10, 15, 20 or 30 min periods for up to 4 hours.

[^{14}C]-3-OH NMP (N-[^{14}C-methyl]-3-hydroxy-pyridinium bromide; Roche Products Limited; sp. act. = 3.9 mCi/mmol) was rapidly administered via the jugular vein (dose: 0.10, 1.64 or 16.4 μmol/kg) or via the portal vein (dose: 0.10 or 1.64 μmol/kg). Samples of urine were collected at 15, 30, 45, 60, 90, 120, 180 and 240 minutes. In all experiments, urine volume was indirectly determined by dividing the weight of each sample by its relative density (assuming that $d = 1.000$).

The lower dose used in this study is comparable with that routinely used in the treatment of myasthenia gravis; amounts in the higher doses are similar to those sometimes used to reverse the effects of tubocurarine in anaesthetic practice.

Liquid scintillation counting and assay procedures

Samples of urine (50 μl) were analysed for total radioactivity by liquid scintillation counting, using 10 ml of a homogenous Triton-X-100 solution (6 g butyl P.B.D. (2(4′-*t*-butylphenyl)-5-(4″-biphenylyl)-1,3,4-oxadiazole) in 667 ml toluene, 333 ml Triton-X-100 and 75 ml water) (Barber & Bourne, 1973a). All samples were counted in a Nuclear Chicago Unilux II scintillation spectrometer. Quench corrections were made by the channels ratio method.

Samples of urine were usually analysed for pyridostigmine and its metabolites by electrophoresis in borate buffer (0.1 M, pH = 9.2, 2 h, 300 V) (Somani *et al.*, 1972). The paper electrophoretogram was counted on a 4π scanner (Tracerlab) to determine the relative proportions of the unchanged drug and its metabolites. Most urine samples were resolved by ion exchange chromatography using Amberlite IRC-50 resin. The eluate was then assayed for the parent drug and its metabolites by liquid scintillation spectrometry.

Results

After injection of pyridostigmine via the jugular vein, an average of 52% was eliminated unchanged in urine in 4 hours. A further 22% was excreted as 3-OH NMP during this time. The proportion of the dose eliminated in urine as pyridostigmine and its metabolite was independent of the dose administered. When the same doses of pyridostigmine were injected into the portal vein, an average of 47% was excreted as the unchanged drug in 4 hours. An additional 40% was eliminated as the metabolite during this period (Table 1).

When doses of pyridostigmine 1.25 μmol/kg and higher were injected via the portal vein, the proportion excreted in urine as unchanged drug remained constant; in contrast, the percentage of the dose eliminated as the metabolite was significantly reduced (Table 2). Thus these results show that a dose-dependent process is involved in the urinary excretion of 3-OH NMP.

This conclusion was supported by studies involving the portal and systemic venous injection of 3-OH NMP at different dose levels.

After 4 h, approximately 85% of the lowest dose was eliminated unchanged in urine; in contrast, only 63% of the higher doses was excreted during this period. The proportion of the dose eliminated in urine was not related to the route of administration (Table 3).

After intravenous injection of pyridostigmine, the rate of excretion of the unchanged drug (expressed as the percentage of the dose eliminated per min) was determined for each urinary collection period. This value was plotted semi-logarithmically against the time at the mid-point

Table 1 Fraction of the dose eliminated in urine after administration of pyridostigmine to rats via the jugular or portal vein at the same dose level

Dose and Route	*No. of animals*	*% dose eliminated 0-2 h*	*0-4 h*
Via jugular vein 0.10 μmol/kg			
Pyridostigmine	4	48 ± 2*	53 ± 1**
Metabolite		13 ± 2†	22 ± 3††
0.63 μmol/kg			
Pyridostigmine	4	43 ± 4	51 ± 3
Metabolite		10 ± 1	21 ± 3
Via portal vein 0.10 μmol/kg			
Pyridostigmine	3	40 ± 3*	43 ± 3**
Metabolite		27 ± 3†	40 ± 2††
0.63 μmol/kg			
Pyridostigmine	2	38	42
		54	57
Metabolite		23	38
		35	44

Results are mean ± s.e. mean.
* and ** significantly different from each other ($P = 0.05$) † and †† significantly different from each other ($P = 0.01$).
Probability was determined by Student's *t* test.

Table 2 Fraction of the dose eliminated in urine after administration of pyridostigmine to rats via the portal vein. Doses are greater than 1 μmol/kg.

Time (min)	*1.25 μmol/kg (a)*	*3.00 μmol/kg (b)*	*6.00 μmol/kg (c)*	P
Pyridostigmine				
60	21 ± 2	27 ± 6	21 ± 3	a to b > 0.3** a to c > 0.9**
100	29 ± 2	36 ± 5	28 ± 3	a to b > 0.2** a to c > 0.8**
130	33 ± 5	41 ± 4	34 ± 2	a to b > 0.2* a to c > 0.8*
Metabolite				
60	11 ± 2	7 ± 1	6 ± 1	a to b = 0.05** a to c = 0.05**
100	23 ± 2	15 ± 1	14 ± 3	a to b < 0.02** a to c < 0.05**
140	34 ± 2	22 ± 1	22 ± 4	a to b < 0.01* a to c < 0.05*

Results are mean ± s.e. mean.
Probability was determined by Student's *t* test.
* d.f. = 4; ** d.f. = 6.

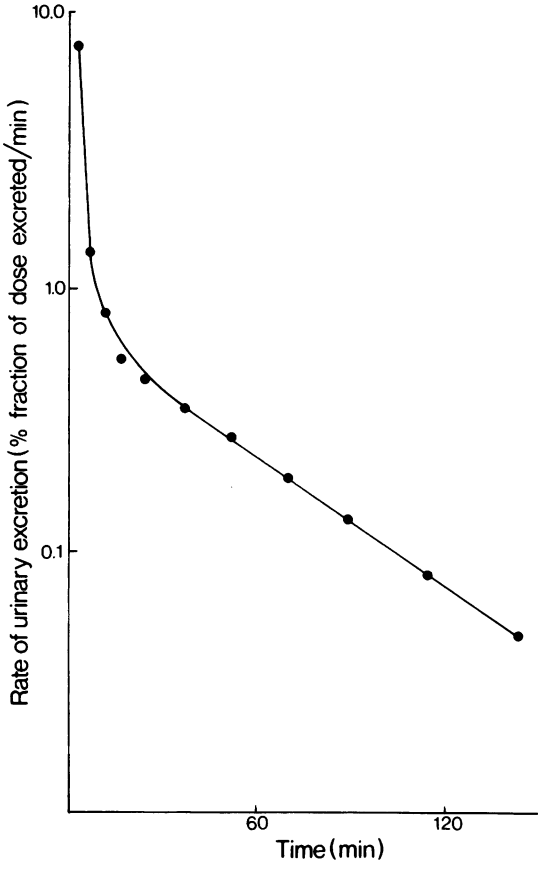

Figure 1 An example of the rate of urinary excretion of pyridostigmine after jugular vein administration. Dose 0.63 μmol/kg.

of each urine collection, after allowing a delay of 4 min between filtration at the glomerulus and the appearance of the drug in the collecting vessel (Bourne & Barber, 1972). After the injection of pyridostigmine into the jugular vein, the initial rate of drug excretion fell rapidly for approximately 10 min; this phase was followed by a less rapid logarithmic decline in the rate of urinary elimination (Figure 1). In contrast, after injection of pyridostigmine into the portal vein, the rate of excretion of the drug rose to a maximum at 30 min, before declining in a logarithmic manner (Figure 2). Thus the urinary excretion profile of pyridostigmine is clearly dependent on the route of administration of the drug (cp. Figures 1 & 2). Similar differences were not observed when 3-OH NMP was injected into the systemic and the portal circulation. In these conditions, the rate of urinary excretion of 3-OH NMP was maximal initially and then fell exponentially, irrespective of the route of administration. Thus, the results resembled the urinary profile observed after injection of pyridostigmine into the systemic venous system (Figure 1).

Paper electrophoresis and ion exchange chromatography (Somani *et al.*, 1972) failed to provide any evidence for the subsequent metabolism of 3-OH NMP. A single peak of radioactivity with an electrophoretic mobility similar to authentic 3-OH NMP was identified. The duration of all the experiments was relatively short, and total recovery of the radioactivity administered was not achieved. Nevertheless, it is considered that the results adequately reflect the pharmacokinetics of pyridostigmine and 3-OH NMP, since the relative

Table 3 Fraction of the dose eliminated in urine after administration of 3-hydroxy-*N*-methyl pyridinium to rats via the jugular or portal vein

Dose and route	*No. of animals*	*% dose eliminated* 0-2 h	0-4 h
0.10 μmol/kg			
via jugular vein	7	73 ± 4	84 ± 3
via portal vein	7	71 ± 1	87 ± 2
1.64 μmol/kg			
via jugular vein	7	52 ± 8	63 ± 8
via portal vein	7	58 ± 3	64 ± 3
16.4 μmol/kg			
via jugular vein	7	54 ± 6	63 ± 5

Results are mean ± s.e. mean.
The fraction eliminated at the higher doses is significantly lower ($P < 0.01$; Student's t test) than the fraction eliminated after the dose of 0.1 μmol/kg by either route of administration.

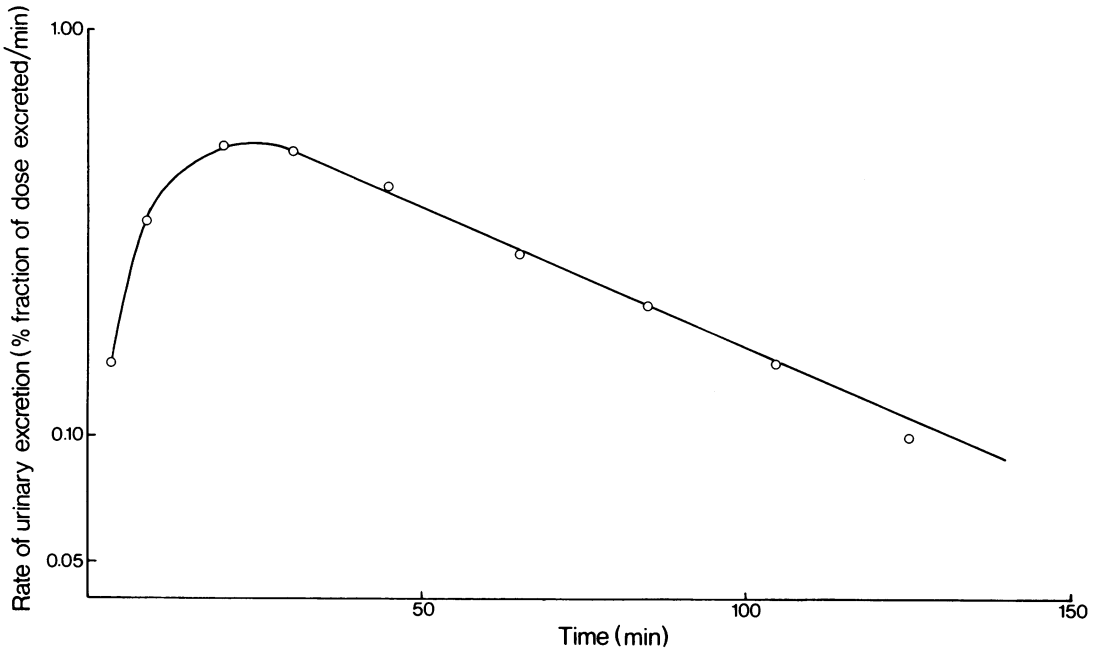

Figure 2 An example of the rate of urinary excretion of pyridostigmine after portal vein administration. Dose 6.0 μmol/kg.

proportions of both compounds in urine invariably approached an asymptote.

Discussion

The present experiments suggest that there are qualitative differences between the elimination kinetics of pyridostigmine and 3-OH NMP. Thus, after injection of pyridostigmine into the portal vein, the proportion eliminated as unchanged drug was independent of the dose in the range studied. In contrast, the urinary excretion of 3-OH NMP was dose-dependent; with small doses of pyridostigmine, a larger fraction was eliminated as the metabolite, 3-OH NMP. After large doses of pyridostigmine, enzymatic hydrolysis to 3-OH NMP was not rate-limiting, since the decrease in the fraction eliminated as the metabolite was not accompanied by an increase in the proportion excreted as the parent drug. This conclusion was consistent with the results obtained after administration of 3-OH NMP via the jugular or the portal vein. In these conditions, the quantitative excretion of the drug in urine was dependent on the dose; after high doses of 3-OH NMP, the proportion eliminated was significantly decreased. These results may reflect the excretion of 3-OH NMP by carrier transport in the proximal renal tubule. Many quaternary amines are eliminated in urine by a tubular transport system with a finite and saturable secretory capacity. The present experimental findings are consistent with the excretion of 3-OH NMP by renal tubular transport, with a secretory capacity that is saturated within the dose range of 0.1-1.64 μmol/kg.

When a drug is eliminated in urine from the region in which it is first distributed, then a semilogarithmic plot of urinary excretion rate with time should give, assuming first order rate processes, a characteristic biexponential curve with a maximum at zero time (Janku & Krebs, 1971). However, if the drug is first distributed into a body compartment from which it is not eliminated, then the rate of excretion curve with time should increase to a maximum before falling, as the drug moves from the initial region of distribution to the compartment from which elimination takes place (see Figure 3).

Pharmacokinetic studies with other quaternary amines (Barber & Bourne, 1974) have not established unequivocally whether these compounds are eliminated from the region in which they are first distributed. On the one hand, both neostigmine

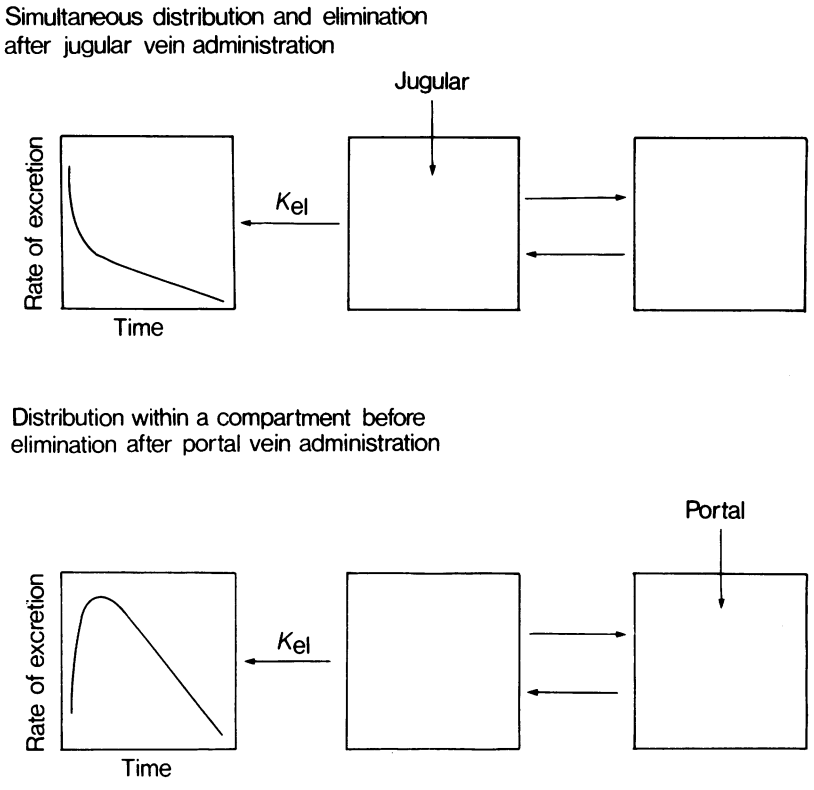

Figure 3

and 3-hydroxy-phenyltrimethylammonium (3-OH PTMA) are subject to a first pass effect. This strongly suggests that the hepatoportal system acts as a distinct region for the distribution of these compounds. On the other hand, the character of the urinary excretion rate plots after portal vein administration and jugular vein administration was closely similar, indicating that elimination takes place from the region in which the drug is first distributed. If the hepatoportal system is a separate region of distribution for these compounds, then the pattern of the rate of excretion should be different after portal and jugular venous injection (Figure 3). The failure to observe this phenomenon may be due to the rapid and extensive metabolism of neostigmine and 3-OH PTMA. Less than 8% of a dose of 3-OH PTMA, and only 5% of a dose of neostigmine are eliminated unchanged, and it may not be possible to detect the distributive phase of such a small fraction of the dose.

Different conditions clearly apply to the distribution of pyridostigmine. After the injection of the drug into the systemic circulation, the initial rate of drug excretion fell rapidly, and was followed by a less rapid linear decline in the rate of urinary elimination. In contrast, after intraportal injection of the drug, the rate of excretion rose to a maximum at 30 min before falling. Thus, the hepatoportal system behaves as a distinct region during the distribution of the drug (Figures 2 & 3).

The fraction of the dose eliminated as pyridostigmine was reduced and the metabolite fraction greatly increased after portal vein administration of a given dose when compared to jugular vein administration of the same dose (Table 1); thus pyridostigmine is subject to metabolism during the first passage through the liver. These results are similar to those reported for the related quaternary amines neostigmine and its metabolite 3-OH PTMA (Barber & Bourne, 1974). It is also possible that the lower fraction of the metabolite excreted after jugular venous administration may be indicative of competition for a renal secretory site by the initial high concentration of pyridostigmine attained by this route of administration.

Differences in the excretion pattern of neo-

stigmine and pyridostigmine may be related to the time of onset of their pharmacological effects. After portal venous administration of neostigmine and pyridostigmine in doses which are equipotent at the time of their maximal plasma concentration, the latent period before the onset of their actions will differ. Thus, neostigmine will immediately reach its maximum level in plasma, while the concentration of pyridostigmine will slowly rise to a maximum at 30 minutes. In the rat, the onset of muscle fasciculation is rapid after intraportal neostigmine; with pyridostigmine a slower onset of action is observed.

Similar effects may occur after oral administration, irrespective of any differences in intestinal absorption.

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