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EXCRETION AND METABOLISM OF ORAL ^{14}C-NEOSTIGMINE IN THE RAT

J. B. ROBERTS, B. H. THOMAS and A. WILSON

Department of Pharmacology and General Therapeutics, University of Liverpool

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Abstract—Neostigmine labelled with ^{14}C in one of the methyl groups of the quaternary nitrogen has been used to investigate the excretion and metabolism of neostigmine after administration to rats by stomach tube. After administration of 250 μg of ^{14}C-neostigmine very little radioactivity is excreted in the urine in the first hour; about 10 per cent of the dose is excreted 5 hr after administration and about 20 per cent after 24 hr. Approximately 50 per cent of the dose was detected in the intestinal contents and faeces 24 hr after administration. Paper electrophoresis of urine specimens showed that the radioactivity separated into 3 zones, which are attributed to neostigmine, metabolite 1 (*m*-hydroxyphenyltrimethylammonium) and an unidentified metabolite 2. Neostigmine and metabolite 2 each account for about 5 per cent of the radioactivity whilst about 90 per cent is due to metabolite 1.

The peak concentration in the liver occurs about 2 hr after administration and is equivalent to about 2 per cent of the dose. About 96 per cent of the radioactivity is present as metabolite 1 but metabolite 2 was not detected. Traces of neostigmine occurred in all specimens of liver. The concentration of radioactivity in the blood closely parallels and is about one thirtieth of that in the liver. It is concluded that since radioactivity is slowly excreted in the urine the absorption of ^{14}C-neostigmine after oral administration is slow and prolonged, and that most of the drug that is absorbed is metabolised by the liver.

THERE is consistent evidence from studies in the rat, hen and man that neostigmine is rapidly excreted and metabolised.[1–5] Nowell *et al.*[1] first reported that patients with myasthenia gravis who were being treated with neostigmine by intramuscular injection excreted up to 67 per cent of the daily dose in the urine, but less than 5 per cent of the daily dose when the drug was given by mouth. Two metabolic products of neostigmine were detected in the urine of these patients when the drug was given by mouth and Scott *et al.*,[2] identified one of them as *m*-hydroxyphenyltrimethylammonium. This metabolite was also identified by Roberts *et al.*[5] in the urine of rats given ^{14}C-neostigmine by intramuscular injection; they also showed that neostigmine is metabolised to *m*-hydroxyphenyltrimethylammonium in the liver and that the formation of this metabolite is inhibited by prior treatment of the rats with N, N-diethylaminoethyl 1, 1 diphenyl 1, *n*-propylacetate hydrochloride (SKF 525A).

The work described in this paper is an extension of these studies and concerns the excretion and metabolism of ^{14}C-neostigmine after oral administration to the rat.

MATERIALS AND METHODS

Male rats weighing 150 to 200 g were allowed food and water *ad libitum* up to the time of the experiment and were then placed in metabolism cages.[6] They were hydrated by the administration of 5 ml/100 g of warm tap water by stomach tube. This

was repeated 1 hr later. ^{14}C-neostigmine iodide (250 μg) was then administered in 0·5 ml of water by stomach tube. ^{14}C-neostigmine iodide was supplied by the Radiochemical Centre, Amersham and had a specific activity of 15 μc/mg.

Urine free from faeces was collected at intervals of 1, 2, 3, 5 and 24 hr; blood, liver, intestinal contents and faeces were collected and extracted by methods previously described.[4]

Determination of radioactivity

The radioactivity of samples of urine and extracts of tissues was determined by counting scintillations and the proportions of neostigmine and of metabolites by the methods previously described.[4, 5]

RESULTS

Excretion in urine and in intestinal contents and faeces

The radioactivity excreted in the urine of 4 individual rats, expressed as a percentage of the dose, is shown in Fig. 1. Here it will be seen that very little radioactivity is

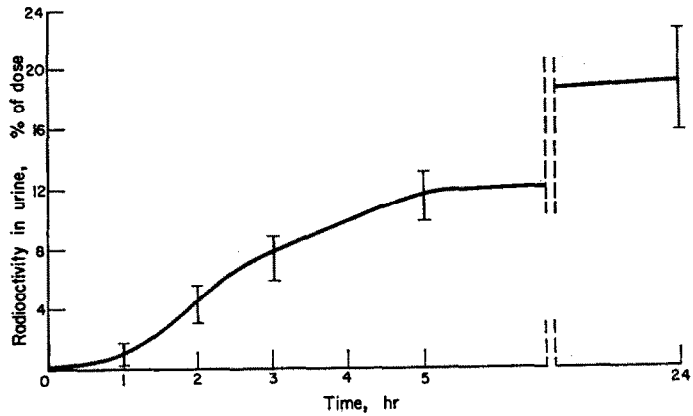

FIG. 1. Radioactivity excreted in the urine of the rat after oral administration of 250 μg of ^{14}C-neostigmine. Each point is the mean of 4 experiments. The standard deviations are represented by the vertical lines.

excreted during the first hour but thereafter there is a slow but steady output so that 5 hr after administration about 11 per cent of the dose is excreted and approximately 20 per cent after 24 hr. These results indicate that at least 50 μg of neostigmine was absorbed from the alimentary tract but throughout the experiment there was little evidence of salivation or muscle twitching.

The radioactivity of intestinal contents and faeces was estimated in 3 of the 4 rats. The output (mean and standard deviation) of radioactivity during 24 hr was 50·8 $\pm$ 6·2 per cent.

The results of these experiments account for about 70% of the dose.

Estimation of Neostigmine and Metabolites in Urine

Specimens of urine collected from another group of rats similarly treated were

subjected to paper electrophoresis. A typical result is illustrated in Fig. 2, which shows three peaks of radioactivity. Two of these occur in the same zones as were observed for concurrently run authentic specimens of neostigmine and *m*-hydroxyphenyltrimethylammonium. The third zone of radioactivity is probably due to a second metabolite with a very low mobility (−1–4 cm) and was observed in all specimens of urine. This had not previously been observed by Roberts *et al.*[5] when the drug was given intramuscularly. For convenience it will be referred to as metabolite 2, whilst *m*-hydroxyphenyltrimethylammonium will be referred to as metabolite 1.

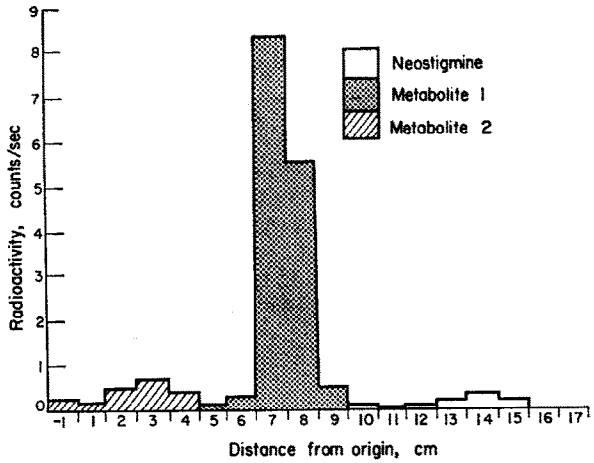

FIG. 2. Separation of metabolites 1 and 2 from neostigmine in rat urine by paper electrophoresis. The hatched area (−1 to 4 cm) represents radioactivity due to metabolite 2, the shaded area (5–9 cm) that due to metabolite 1 and the unshaded area (10–15 cm) that due to neostigmine.

The quantitative estimation of metabolites and unchanged drug in urine collected at different time intervals after oral administration of neostigmine is shown in Table 1. The most characteristic feature of these results is the high proportion (90 per cent) of metabolite 1 which was present in all specimens. By contrast the proportion of radioactivity due to metabolite 2 was small, and like that due to neostigmine accounted for about 5 per cent.

TABLE 1. RADIOACTIVITY DUE TO NEOSTIGMINE AND METABOLITES IN RAT URINE AT DIFFERENT TIME INTERVALS AFTER ORAL ADMINISTRATION OF 250 μg OF ^{14}C-NEOSTIGMINE

Time after administration (hr)	Radioactivity in urine (% dose)			
	Total	Neostigmine	Metabolite 1	Metabolite 2
1	0·98 ± 0·65(9)	0·07 ± 0·04	0·81 ± 0·59	0·10 ± 0·05
2	3·32 ± 1·11(6)	0·16 ± 0·14	2·99 ± 1·00	0·17 ± 0·04
3	4·20 ± 1·36(6)	0·18 ± 0·12	3·87 ± 1·23	0·16 ± 0·03
5	9·87 ± 4·33(6)	0·50 ± 0·58	9·01 ± 3·66	0·35 ± 0·12

Values are means and standard deviations. Numbers of rats are in parentheses. Radioactivity is expressed as a percentage of the dose of ^{14}C-neostigmine.

Estimation of neostigmine and metabolites in liver

The concentration of radioactivity in the liver of groups of 3 rats killed at 1, 2, 3 and 5 hr after oral administration of 250 μg ^{14}C-neostigmine is shown in Table 2. The peak concentration (0·83 μg/g wet wt) calculated as neostigmine occurs 2 hr after administration of the drug and is approximately the same as that seen 30 min after intramuscular injection of 25 μg ^{14}C-neostigmine.[4] Table 2 also shows that the maximum blood level, like that of liver, occurs about 2 hr after administration, after which it falls to a fairly constant level.

TABLE 2. RADIOACTIVITY IN RAT LIVER AND BLOOD AT DIFFERENT TIME INTERVALS AFTER ORAL ADMINISTRATION OF ^{14}C-NEOSTIGMINE (250 μg)

Time after administration (hr)	Liver		Blood concentration (μg/ml)
	concentration (μg/g wet wt)	total content (% dose)	
1	0·57 ± 0·10	1·46 ± 0·23	0·0165 ± 0·0018
2	0·83 ± 0·17	1·92 ± 0·39	0·0280 ± 0·0091
3	0·49 ± 0·27	1·29 ± 0·71	0·0119 ± 0·0032
5	0·36 ± 0·21	0·75 ± 0·51	0·0133 ± 0·0060

Values are means and standard deviations of the results obtained from 3 rats at each time interval.

Extracts of liver taken from 2 rats from each group were estimated for neostigmine and metabolites. The results are remarkably consistent and show that only a trace of neostigmine was present in each extract. Metabolite 2 was not detected in any of the extracts. The concentration (mean and standard deviation) of metabolite 1 in all 8 extracts accounted for 96·3 ± 3·5 per cent of the radioactivity. The rapid occurrence of metabolite 1 in liver was confirmed in a further experiment which showed that 30 min after oral administration of neostigmine 97·7 per cent of the radioactivity was present as metabolite 1.

DISCUSSION

The presence of 50 per cent of the radioactivity in the faeces and intestinal contents 24 hr after an oral dose of ^{14}C-neostigmine suggests that a large proportion of the dose is not absorbed and since about 20 per cent of the dose was excreted in the urine, it is clear that at least one fifth of the dose was absorbed during this period. Some of the radioactivity in the faeces may be due to excretion in the bile; current work in this laboratory (to be published later) supports this assumption.

The wide variations in levels of radioactivity observed in urine, liver, blood and intestinal contents and faeces provide some explanation for the variations in response of myasthenic patients during treatment with oral neostigmine. The evidence that small and variable amounts of neostigmine are excreted in the urine of rats closely resembles the results obtained by Nowell *et al.*[1] in myasthenic patients after oral administration of neostigmine. They were unable to detect in the urine more than 5 per cent of the daily dose, but later showed that some of the neostigmine was metabolised and excreted as two metabolites which could not be estimated by their assay procedure.[2] The present studies in the rat amply confirm this by the presence

of two metabolites of neostigmine in the urine which for convenience we have referred to as metabolite 1 (*m*-hydroxyphenyltrimethylammonium) and as metabolite 2, the chemical nature of which has not yet been established. The importance of the former as the main metabolite of neostigmine is emphasised by the fact that it accounts for 90 per cent of the radioactivity in the urine and for 96 per cent in the liver.

Since there is good evidence in the rat and hen that after intramuscular injection of ^{14}C-neostigmine, radioactivity is rapidly eliminated by renal tubular secretion,[3, 4] we tentatively conclude that the slow but continuous elimination of radioactivity in the urine up to at least 5 hr after oral neostigmine is due to a correspondingly slow and continuous absorption of the drug from the alimentary tract. The fact that the peak concentration of radioactivity in the liver after oral administration was equivalent to that after intramusuclar injection but occurred 1½ hr later, supports this conclusion.

These results also show that the concentration of radioactivity in the liver is about thirty times greater than in the blood and that it is present almost entirely as metabolite 1. This evidence closely resembles the results which we have previously reported after intramuscular injection[5] and supports the conclusion that neostigmine is mainly metabolised in the liver.

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