J. Pharm. Pharmac., 1973, **25**, 371–375 *Received November* 27, 1972

Intestinal absorption, intestinal distribution, and excretion of [^{14}C] labelled hyoscine *N*-butylbromide (butylscopolamine) in the rat

P. PENTIKÄINEN, ANERI PENTTILÄ, H. VAPAATALO AND R. HACKMAN

Research Laboratories, Medica Ltd, SF-00101 Helsinki 10, Finland, and Department of Pharmacology, University of Helsinki, SF-00170 Helsinki 17, Finland

Butylscopolamine was labelled with ^{14}C and its gastrointestinal absorption, biliary and urinary excretion, enterohepatic circulation and gastrointestinal distribution were examined in anaesthetized rats. Biliary excretion was the main elimination route of intraportally administered [^{14}C]butylscopolamine, with 42% of the dose recovered in the bile during 12 h. About 6% of the radioactivity administered orally as [^{14}C]butylscopolamine was excreted in the bile and 1·2% in the urine during 24 h, which indicates poor gastrointestinal absorption of butylscopolamine in the rat. When collected radioactive bile was readministered intrajejunally, only about 7% of the radioactivity was recovered in bile and urine during 12 h, which suggests that only a small fraction of butylscopolamine and its metabolites engage in an enterohepatic circulation. After oral administration of [^{14}C]butylscopolamine, radioactivity was found to accumulate in the wall of the distal small intestine, and about 20% of the dose was found in this tissue 24 h after drug administration. As a result, local anti-acetylcholine effects of butylscopolamine might be expected.

Hyoscine *N*-butyl bromide, or butylscopolamine, is an anti-acetylcholine drug which has been widely used, especially in the treatment of spastic gastrointestinal disorders. Whereas the spasmolytic effect after parenteral administration is well documented (Kewenter & Kock, 1971), there is some controversy about its effects after oral administration. Several clinical observations indicate that the drug is helpful in the treatment of various gastrointestinal disorders. However, Herxheimer & Haefeli (1966) reported that oral doses of up to 600 mg failed to produce the anti-acetylcholine effects seen after parenteral injection in man, and they concluded that the drug was not absorbed from the gastrointestinal tract. Guignard, Herxheimer & Greenwood, (1968) and Brömster, Carlberger & others, (1969) have reported similar findings. After studies with tritium-labelled butylscopolamine Hellström, Rosén & Söderlund (1970) assumed that intestinal absorption of butylscopolamine was very low, reaching a maximum of 10% in man. In contrast to this, animal studies with several species showed that butylscopolamine was relatively well absorbed (Pennefather, McCulloch & Rand, 1968; Pomeroy & Rand, 1969). In the rat the toxicity was even higher after intraduodenal than after subcutaneous administration (Wick, 1967).

Little is known about the enterohepatic circulation of butylscopolamine and its metabolites. High hepatic uptake and biliary excretion could cause the low urinary excretion and the lack of systemic effects of orally administered butylscopolamine. We have labelled butylscopolamine with carbon-14 and studied the gastrointestinal absorption by measuring biliary and urinary excretion of radioactivity. Use of the

labelled drug also allowed us to study its enterohepatic circulation and distribution in the gastrointestinal tract.

MATERIAL AND METHODS

Synthesis of [^{14}C]*butylscopolamine bromide.* n-Butylbromide-1-^{14}C (Philips-Duphar, spec. activity 4·3 mCi mmol^{-1}) was reacted with a threefold excess of inactive scopolamine in acetonitrile solution. The *N*-butylscopolamine obtained was treated with ether, diluted with carrier and then recrystallized to constant specific activity (1·3 mCi mmol^{-1}). The radiochemical purity was demonstrated by thin-layer and paper chromatography; more than 98·5% of the radioactivity was found at the R_F value of authentic butylscopolamine.

Experimental procedures. Male Sprague-Dawley rats, 210–320 g, were fasted overnight and then anaesthetized with urethane (1 g kg^{-1}). Cannulae of polyethylene tubing were inserted into the common bile duct and into the fundus of the urinary bladder for quantitative collection of bile and urine. During the experiments the body temperature of the animals was maintained at 37°.

[^{14}C]Butylscopolamine in a dose of 1·0 μCi (330 μg) in 0·5 ml of saline was given orally by stomach tube to five rats. Bile and urine were collected in fractions during 24 h, after which the animals were killed. The stomach and intestine were removed and the contents collected by rinsing with saline, and their radioactivity determined. The rinsed gastrointestinal tract was then divided into four parts: stomach, proximal small intestine (20 cm distal from the pylorus), distal small intestine, and colon together with caecum. The mucosa from each of these portions was removed by scraping. The samples of mucosa and intestinal wall were homogenized with water and analysed for radioactivity.

To simulate the fate of absorbed butylscopolamine, the same dose as that given orally was injected into the portal vein of five rats. After administration of the drug, bile and urine were collected in fractions for 12 h. The volume of the fractions was measured, their radioactivity determined, and the most active bile and urine samples were subjected to chromatography.

To determine the extent of the enterohepatic circulation of [^{14}C]butylscopolamine, the bile samples collected during the first 2 h after intraportal administration were pooled and the radioactivity measured. Aliquots of the pooled bile were then injected into the proximal jejunum of four rats. The bile and urine of these rats were collected for 12 h and analysed for radioactivity.

Radioactivity measurements. Aliquots of the bile and urine samples were pipetted into counting vials containing 10 ml of scintillation fluid (Aquasol, NEN), and counted in a Wallac DECEM NTL 314 liquid scintillation counter. The values obtained were corrected for quenching, using an internal standard. The radioactivity excreted in bile and urine was calculated as per cent of administered dose. Aliquots of tissue homogenates were solubilized with Protosuol (NEN) before determination of radioactivity, and the results expressed as per cent of dose.

Chromatography. Some of the bile and urine samples were subjected to ascending two-dimensional paper chromatography on Whatman No. 1 paper. The solvent mixtures used were n-butanol–pyridine–water (1 : 1 : 1) and n-butanol–acetic acid–water (12 : 3 : 5). Autoradiograms were prepared by exposing the chromatograms against X-ray film (Kodirex, Kodak), for one month. Pure [^{14}C]butylscopolamine was run as reference. No further attempts were made to identify the metabolites observed.

RESULTS

Oral administration of [^{14}C]*butylscopolamine.* Mean rates of biliary and urinary excretion of radioactivity after oral administration were slow. During the 24 h after administration of the drug, about 6% of the dose was excreted into the bile and only about 2% in the urine (Fig. 1a). Biliary and urinary flows remained constant and were 29·6 ± 1·9 and 14·3 ± 1·7 μl kg^{-1} min^{-1}, respectively (mean ± s.e.).

The distribution of radioactivity in the alimentary tract 24 h after oral administration of [^{14}C]butylscopolamine is shown in Table 1. A total of 56·6% of the dose was

Table 1. *Distribution of radioactivity in the gastrointestinal tract of rats 24 h after administration of* 1·0 μCi (330 μg) [^{14}C]*butylscopolamine by stomach tube. The figures represent mean ± s.e. of five rats.*

Sample investigated	% of radioactivity administered
Contents of gastrointestinal tract	29·7±7·0
Stomach	
Mucosa	0·2±0·1
Muscle and serous layer	0·8±0·1
Proximal small intestine	
Mucosa	2·5–1·4
Muscle and serous layer	1·9±0·9
Distal small intestine	
Mucosa	10·2±3·4
Muscle and serous layer	9·2±3·4
Colon and caecum	
Mucosa	1·0±0·5
Muscle and serous layer	0·9±0·1
Total in gastrointestinal tract	56·6±5·2

found in the intestinal tract, with approximately one half of this amount in the intestinal contents. About 20% of the dose was recovered from the wall of the distal small intestine, this activity was almost equally divided between the mucosa and the outer layers. The total recovery of radioactivity in bile, urine and intestinal tract was 64·5%.

Intraportal injection of [^{14}C]*butylscopolamine.* The excretion of radioactivity in the bile after intraportal injection of butylscopolamine was rapid. During the 12 h after injection, 42% of the dose was excreted in the bile and 36% in the urine (Fig. 1b). Throughout the experiment the bile flow was stable being 29·6 ± 1·4 μl kg^{-1} min^{-1}, whereas the mean urinary flow was 17·3 ± 3·9 μl kg^{-1} min^{-1}, with a tendency to increase with time.

Intrajejunal administration of radioactive bile. During 12 h after intrajejunal administration of pooled radioactive bile 1·5% of the radioactivity was excreted in the bile and 6·0% in the urine (Fig. 1c). The average bile flow in this group, 37·1 ± 3·2 μl kg^{-1} min^{-1}, was higher than after oral or intraportal administration, probably owing to the choleretic effect of the bile salts (Klaassen, 1972). The urinary flow was 14·9 ± 3·5 μl kg^{-1} min^{-1}.

Metabolites of [^{14}C]*butylscopolamine in bile and urine.* Four compounds were detected as radioactive spots by autoradiography in the bile after intraportal administration of [^{14}C]butylscopolamine. Approximately one half of the biliary radioactivity was present in a spot with the same R_F value as [^{14}C]butylscopolamine standard in both solvent systems. After similar chromatography of the urine, only one radioactive spot, corresponding to [^{14}C]butylscopolamine, was found.

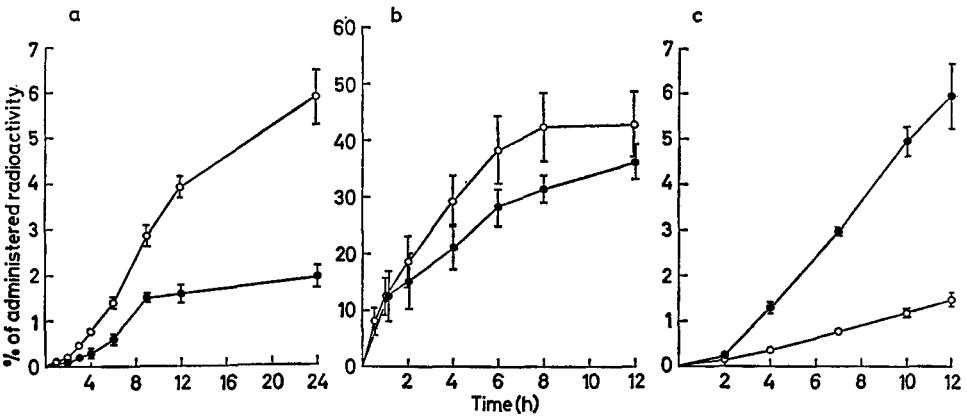

FIG. 1. Cumulative excretion of radioactivity in the bile (○) and urine (●) in five rats after (a) oral (b) intraportal administration of [^{14}C]butylscopolamine, (c) in four rats after intrajejunal administration of radioactive bile, which was obtained after intraportal injection of [^{14}C]butylscopolamine, means ± s.e.

DISCUSSION

In earlier studies, the gastrointestinal absorption of butylscopolamine has been measured exclusively by the presence or absence of systemic anti-acetylcholine effects. Lack of systemic effects does not totally exclude the possibility that the drug is absorbed. Theoretically, after absorption the drug could be efficiently taken up by the liver, excreted into the bile and consequently bypass the systemic circulation. Thus the drug could have a spasmolytic effect on the biliary and intestinal tract without having systemic effects.

Our results indicate that butylscopolamine reaching the liver by the portal vein is eliminated mainly by biliary excretion. Considerable biliary excretion of butylscopolamine could be expected according to Ryrfeld & Hansson (1970), who showed that quaternary ammonium compounds with molecular weights above 200 are excreted mainly in the bile. In the present study, three metabolites of [^{14}C]butylscopolamine were found in the bile. However, Hellström & others (1970) administered [^{3}H]butylscopolamine intravenously to man and found only one metabolite, probably an hydrolysis product of butylscopolamine, in the duodenal contents. The metabolites of butylscopolamine formed in the rat liver are obviously effectively cleared in the bile, since no metabolites could be found in the urine.

The sum of biliary and urinary excretion has been previously used as a measure of gastrointestinal absorption of butylscopolamine, since other elimination pathways are obviously of minor importance (Hellström & others, 1970). Our recovery of 78% of intraportally administered butylscopolamine in the bile and the urine in 12 h supports this concept. Of orally given [^{14}C]butylscopolamine, we recovered only 8% from bile and urine, which indicates a rather poor gastrointestinal absorption. This contrasts with the observation of Waldek (1969), who found that butylscopolamine was absorbed from isolated intestinal loops of rats to an average of 36% within 3 h. A high absorption is also suggested by the fact that butylscopolamine given into the duodenum of rats was 2·8 times more toxic than the same dose given subcutaneously (Wick, 1967). Evidently there are considerable species differences in the alimentary absorption of butylscopolamine. Contrary to the low urinary excretion

found in rats, mice excreted about 30% of orally administered [^{14}C]butylscopolamine in the urine during 24 h and only 6% in the faeces (Duchene-Marullaz, Constantin & others, 1969). In man about 2% of the oral dose was excreted in the urine (Hellström & others, 1970; Beerman, Hellström & Rosén, 1971) and up to 5·7% in the bile (Hellström & others, 1970), which in the light of the present results suggests that the oral absorption of butylscopolamine is very similar in man and rat.

In this study, the total recovery of radioactivity in the bile, urine and gastrointestinal tract after oral administration of [^{14}C]butylscopolamine was about 65%. The fate of the rest of the administered radioactivity is not known. Faecal losses during the experiment are possible. Absorption and retention in body depots cannot be excluded but is unlikely in the light of the high recovery after intraportal administration.

The main part of the butylscopolamine absorbed from the alimentary tract is excreted in the bile and re-enters the intestinal lumen. The enterohepatic circulation is, however, of minor importance in the distribution of butylscopolamine, since only about 7% of the radioactivity that was administered intrajejunally as radioactive bile, was recovered in bile and urine.

It is interesting that a considerable incorporation of radioactivity, about 20% of the dose, was found in the intestinal wall 24 h after oral administration. Earlier, Waldek (1969) showed that this drug is accumulated in the mucosa of the small intestine of rats. However, he found a very low concentration in the muscle layer. In our study, the activity was evenly distributed between the mucosa and the rest of the intestinal wall. Our conclusions that local effects could result from the accumulation of butylscopolamine in the wall of the small intestine agree with those of Pomeroy & Rand (1969) from their work on butylscopolamine. It is not known whether a similar incorporation occurs in man. If this were the case, it would explain the discrepancy between the favourable clinical results and the poor gastrointestinal absorption observed.

REFERENCES

BEERMAN, B., HELLSTRÖM, K. & ROSÉN, A. (1971). *Acta pharmac. tox.*, **29**, 98–109.

BRÖMSTER, D., CARLBERGER, G., LUNDH, G., MÖLLER, J. & ROSÉN, A. (1969). *Scand. J. Gastroent.*, **4**, 185–192.

DUCHENE-MARULLAZ, P., CONSTANTIN, M., TALVARD, J. & BEAU, G. (1969). *Thérapie*, **24**, 621–625.

GUIGNARD, J-P., HERXHEIMER, A. & GREENWOOD, R. M. (1968). *Clin. Pharmac. Ther.*, **9**, 745–748.

HELLSTRÖM, K., ROSÉN, A. & SÖDERLUND, K. (1970). *Scand. J. Gastroent.*, **5**, 585–592.

HELLSTRÖM, K., ROSÉN, A. SÖDERLUND, K. (1970). *Ibid.*, **5**, 585–592. (Reference to personal communication by Deckers, W. & Wahl, D.).

HERXHEIMER, A. & HAEFELI, L. (1966). *Lancet*, **2**, 418–421.

KEWENTER, J. & KOCK, N. G. (1971). *Scand. J. Gastroent.*, **6**, 401–405.

KLAASSEN, C. D. (1972). *J. Physiol.*, **224**, 259–269.

PENNEFATHER, J. N., MCCULLOCH, M. W. & RAND, M. J. (1968). *J. Pharm. Pharmac.*, **20**, 867–872.

POMEROY, A. R. & RAND, M. J. (1969). *Ibid.*, **21**, 180–187.

RYRFELD, A. & HANSSON, E. (1970). *Acta pharmac. tox.*, **28**, Suppl. 1, 78.

WALDEK, F. (1969). *Eur. J. Pharmac.*, **8**, 108–113.

WICK, H. (1967). *J. Pharm. Pharmac.*, **19**, 779.