J. Pharm. Pharmacol. 1987, 39: 357–362
Received October 9, 1986

The effect of molecular size on the nasal absorption of water-soluble compounds in the albino rat

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The nasal absorption of a range of water-soluble compounds with different molecular weights, 4-oxo-4*H*-1-benzopyran-2-carboxylic acid (mol. wt 190), *p*-aminohippuric acid (mol. wt 194), inulin (mol. wt 5200) and dextran (mol. wt 70 000), has been investigated in the male Wistar rat. Compounds were instilled into the nasal cavities of anaesthetized animals and, for comparison, similar doses were administered intravenously. Serial samples of bile and urine were collected for up to 6 h. Nasal absorption, estimated by comparison of the extent of excretion in bile and urine following intranasal and intravenous administration, was 100% for 4-oxo-4*H*-1-benzopyran-2-carboxylic acid (1 mg kg^{-1}), 75% for *p*-aminohippuric acid (1 mg kg^{-1}), 15% for inulin (0·1 mg kg^{-1}) and 2·8% for dextran (0·25 mg kg^{-1}). The log molecular weight gave a good linear correlation with the log per cent intranasally absorbed (correlation coefficient of −0·996). From the molecular weight relationship, these data infer aqueous channel mechanisms for the nasal absorption of water-soluble compounds.

In man the intranasal route has been used for many years to administer a range of compounds including steroids, peptides, decongestants, antiallergics, antibiotics, antihistamines and cocaine. (For reviews see Parr 1983; Chien & Chang 1985). However, only recently has the process of absorption through the nasal mucosa been studied (Hussain et al 1979, 1980a, b; Hirai et al 1981; Kaneo 1983; Huang et al 1985). The use of animal models to quantify the nasal absorption of propranolol has been shown to correlate well with human studies (Hussain et al 1979, 1980a, b).

Hirai et al (1981) have described an in-situ recirculating perfusion technique and an in-vivo technique to study nasal absorption in the rat. These techniques have been used to study the effects on nasal absorption of pH, partition coefficient, dose, rate of perfusion and volume of perfusate (Hirai et al 1981; Kaneo 1983; Ohwaki et al 1985; Hussain et al 1985, Huang et al 1985). However, the mechanisms of nasal absorption are still not fully understood.

Water-soluble compounds such as sodium cromoglycate are well absorbed (Fisher et al 1985). The absorption of such compounds through the nasal mucosa is likely to be dependent on aqueous channel diffusion (pores). The molecular size of the compound will, therefore, be a determinant in the rate of the absorption process as demonstrated for other membranes (Scheler & Blanck 1977; Schanker 1962, 1971, 1978; Schou 1971). There has been speculation about the existence of aqueous channels or pores in the nasal mucosa (Hirai et al 1981; Kaneo 1983). Recently the size of pores in the nasal mucosa, and the flux of water through them, have been estimated (Hayashi et al 1985). There are no published studies on the effect of molecular size on nasal absorption.

This report describes the use of a simplified version of the in-vivo technique of Hirai et al (1981) to study the nasal absorption of a range of water soluble compounds. Previous experiments performed using this technique have demonstrated that absorption of the compounds is exclusively from the nasal cavity (Fisher et al 1985). Absorption was estimated by comparing the extent of excretion in bile and urine over 6 h after intravenous and intranasal administration. Compounds selected were: 4-oxo-4*H*-1-benzopyran-2-carboxylic acid (mol. wt 190), *p*-aminohippuric acid (mol. wt 194), inulin (mol. wt 5200) and dextran (mol. wt 70 000). Data from a previous study with sodium cromoglycate (mol. wt 512) (Fisher et al 1985) are also included.

MATERIALS AND METHODS

Materials

All test compounds were tritium-labelled. 4-Oxo-4*H*-1-benzopyran-2-carboxylic acid (chromone-2-

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carboxylic acid), with a specific activity of 226 μCi mg^{-1}, was synthesized by Dr W. J. S. Lockley of Fisons plc, Pharmaceutical Division (Lockley 1985). *p*-Aminohippuric acid, specific activity 1300 μCi mg^{-1}, inulin, specific activities 465 and 345 μCi mg^{-1}, and dextran, specific activities 183 and 269 μCi mg^{-1}, were purchased from Amersham International (Amersham, Bucks, UK). Additional inulin, specific activity 185 μCi mg^{-1}, was purchased from New England Nuclear (NEN, Southampton, Hants, UK).

Preparation of animals

Male COBS/Wistar rats (Charles River, Manston, Kent, UK), 262 ± 24 g (mean ± s.d.) were used. Animals were anaesthetized with sodium pentobarbitone (Sagatal, May & Baker, Dagenham, Essex, UK) administered via an indwelling needle implanted into a caudal vein. Bile was collected from a cannula implanted into the common bile duct. Urine was collected from a cannula inserted into the urinary bladder. To prevent any urine loss the penis was tied off.

Animals were prepared for intranasal dosing using a simplified version of the technique of Hirai et al (1981). The animal was placed on its back, a cannula was inserted into the trachea to maintain respiration, and the oesophagus was occluded by tying it on to this cannula.

Dosing of animals

Animals were dosed into the nasal cavity (50 μL dose volume), via a nostril, using a microsyringe (100 μL capacity) fitted with approximately 40 mm of pp10 cannula tubing (Portex, Hyde, Kent, UK) attached to the needle. 4-Oxo-4*H*-1-benzopyran-2-carboxylic acid and *p*-aminohippuric acid were administered at 1 mg kg^{-1}. Both compounds were diluted with unlabelled material (Aldrich, Gillingham, Dorset, UK and Sigma, Poole, Dorset, UK, respectively), dissolved in a minimum of 1% sodium bicarbonate solution, and the appropriate volume of 0·9% NaCl (saline) was added. Inulin was administered at 0·1 mg kg^{-1} and dextran at 0·25 mg kg^{-1}, both dissolved in saline. For comparison, groups of animals were also dosed intravenously (0·5 mL), via a caudal vein, at the same dose levels. At least three animals were sampled for each compound and each dose route.

Determination of radioactivity

Samples of bile and urine were adjusted to a volume of 0·5 mL with distilled water, 5 mL of liquid scintillation cocktail (Fisofluor mpc, Fisons plc, Scientific Apparatus, Loughborough, Leics, UK or Optiphase 1, LKB Instruments, Croydon, Surrey, UK) was added, and the radioactivity present determined by liquid scintillation spectrometry. Efficiency of counting was determined by comparison with an external standard.

Metabolic fate of 4-oxo-4H-1-benzopyran-2-carboxylic acid

Representative samples of bile and urine collected after intravenous administration of 4-oxo-4*H*-1-benzopyran-2-carboxylic acid were subjected to TLC. Aliquots were applied to Merck 20 × 20 cm, silica gel, glass backed plates (Camlab, Cambridge, Cambs, UK) together with 4-oxo-4*H*-1-benzopyran-2-carboxylic acid standards. Plates were developed in three solvent systems; toluene–acetic acid (75 : 25 v/v), chloroform–ethyl acetate–acetic acid (50 : 50 : 10 v/v) and chloroform–diethyl ether–formic acid (70 : 20 : 10 v/v). Plates were divided into rectangles (2 × 1·5 cm) which were removed with Stripmix (Applied Science Labs Inc., Alltech Associates, Carnforth, Lancs, UK) and transferred to counting vials. Methanol (1 mL) was added and vials were sonicated (Dawe sonic water bath, Dawe Scientific, London, UK) for at least 30 min. Distilled water (0·5 mL) and liquid scintillation cocktail (10 mL) (Fisons or LKB) were added and the radioactivity present determined by liquid scintillation spectrometry.

Metabolic fate of dextran

Representative samples of bile and urine collected after intranasal and intravenous administration were subjected to Sephadex gel column chromatography. Aliquots were applied to a Sephadex G75 superfine gel column (approximately 20 × 1 cm) (Pharmacia Biotechnology, Milton Keynes, Bucks, UK) in a pH 7·4 phosphate buffer. Dextran blue (mol. wt 2 000 000) (Sigma) and phenol red (mol. wt 354) (Sigma) were used as high and low molecular weight markers, respectively. Fractions were collected (10 drops) (Gilson fraction collector, Anachem, Luton, Beds, UK) and the radioactivity present was determined as described above for bile and urine.

RESULTS AND DISCUSSION

The mean rates of excretion in bile and urine after intranasal and intravenous administration of 4-oxo-4*H*-1-benzopyran-2-carboxylic acid, *p*-aminohippuric acid, inulin and dextran are illustrated in Figs 1–4, respectively. The mean peak and 6 h concentra-

tions of radioactivity in bile and urine are shown in Table 1. The mean total excreted over 6 h and the per cent nasally absorbed, including data for sodium cromoglycate (Fisher et al 1985), are shown in Table 2.

*4-Oxo-4*H*-1-benzopyran-2-carboxylic acid*

Greater than 96% of the radioactivity in representative bile and urine samples co-chromatographed with 4-oxo-4*H*-1-benzopyran-2-carboxylic acid. Therefore this compound is not metabolized to any significant degree and concentrations of radioactivity represent concentrations of 4-oxo-4*H*-1-benzopyran-2-carboxylic acid (Fig. 1, Tables 1, 2). After intravenous administration, the concentrations in bile rapidly peaked then biphasically declined over the period from 0·25 h to 6 h. In urine, concentrations reached a plateau from 0·5 h to 2·5 h before declining over the period from 2·5 to 6 h. However, the early urine flow was low and this would explain the early plateau in concentrations. After intranasal administration, concentrations in bile rose to a peak at 0·5 h followed by a decline in the remaining 5·5 h, which mirrored that seen after intravenous administration. Urine concentrations peaked at 1 h and then steadily declined over the next 5 h. It is apparent that 4-oxo-4*H*-1-benzopyran-2-carboxylic acid is completely absorbed after intranasal administration, the figure of 112% being explained by the low urinary flow after intravenous administration with significant concentrations of material still being excreted at 6 h.

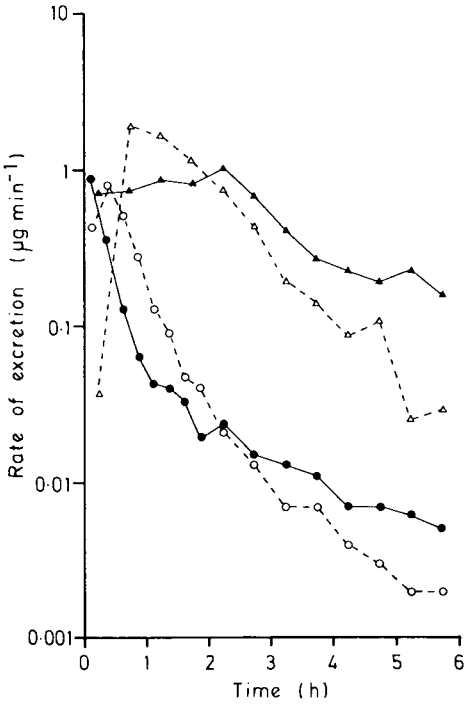

FIG. 1. Mean rate of excretion of radioactivity after administration of ^{3}H-labelled 4-oxo-4*H*-1-benzopyran-2-carboxylic acid at 1 mg kg^{-1}. Bile ● and urine ▲ following intravenous dosing. Bile ○ and urine △ following intranasal dosing.

p-*Aminohippuric acid*

p-Aminohippuric acid is metabolized by the rat and metabolites have been detected in bile (Abou-El-Makarem et al 1967) and urine (Riggs & Christensen 1951; Malyusz et al 1972). In these reports *p*-aminohippuric acid and *p*-acetoamidohippuric acid were the major compounds detected. However, in urine small amounts of glycine and *p*-aminobenzoic acid were detected with subsequent *N*-acetylation of the latter to *p*-acetoamidobenzoic acid (Riggs & Christensen 1951; Malyusz et al 1972). Concentrations of radioactivity therefore represent *p*-aminohippuric acid and metabolites (Fig. 2, Tables 1, 2). After

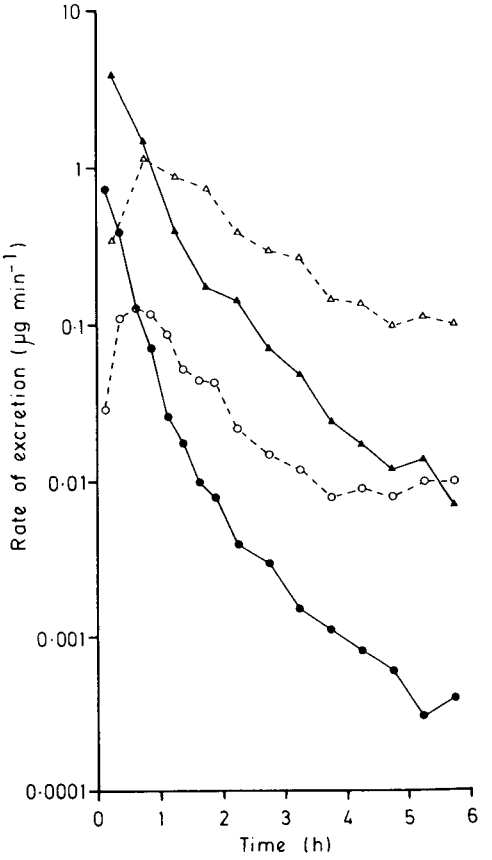

FIG. 2. Mean rate of excretion of radioactivity after administration of ^{3}H-labelled *p*-aminohippuric acid at 1 mg kg^{-1}. Bile ● and urine ▲ following intravenous dosing. Bile ○ and urine △ following intranasal dosing.

intravenous administration, concentrations in both bile and urine showed a steady biphasic decline from the first samples collected to 6 h. Bile concentrations following intranasal administration rose to an early peak at 0·75 h before steadily and biphasically declining to 6 h. Urine concentrations rose to a peak at 1 h before steadily declining to 6 h. *p*-Aminohippuric acid is well absorbed after intranasal administration. As significant concentrations of radioactivity were still being excreted 6 h after intranasal administration, the figure of 75% may be an underestimate.

Inulin

Inulin is not metabolized (Bowman & Rand 1980) and therefore concentrations of radioactivity represent concentrations of inulin (Fig. 3, Tables 1, 2). Only very low concentrations of inulin were excreted in bile following intravenous administration. These fell from 0·25 to 2 h and remained approximately constant thereafter. Urine concentrations steadily, and monophasically, declined from 0·5 h. After intranasal administration only urine was collected. Concentrations rose to a broad peak between 1 and 2·5 h before declining to 6 h. Inulin was significantly absorbed after intranasal administration. This result disagrees with that of Kotani et al (1983) who found, using both in-vivo and in-situ techniques, that inulin was not absorbed by the rat after intranasal administration. However, in their in-vivo experiments inulin was only in contact with the nasal mucosa for 1 h. The present study (Fig. 3, Table 1) shows that this may be too short a time to achieve a significant absorption. Lack of absorption with the in-situ technique has been seen before. Hirai et al (1981) showed that the highly water-soluble compound phenol red was not absorbed when the in-situ technique was used, but it was well absorbed when the in-vivo technique was used.

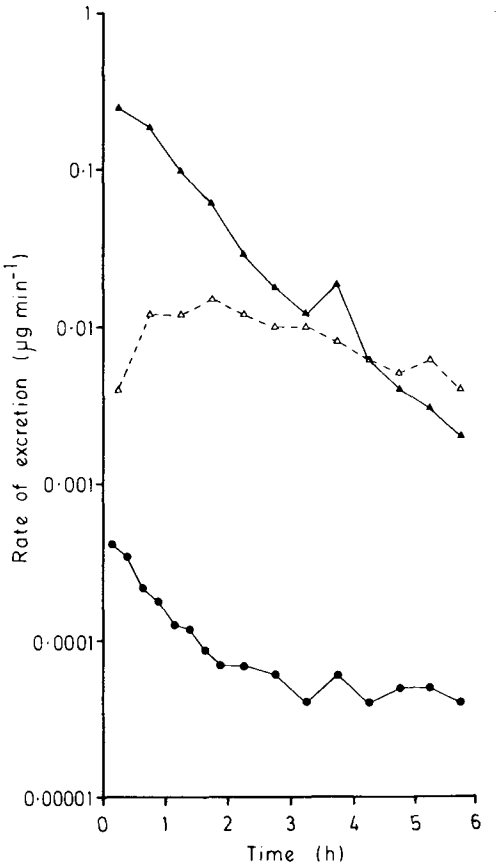

FIG. 3. Mean rate of excretion of radioactivity after administration of ^{3}H-labelled inulin at 0·1 mg kg^{-1}. Bile ● and urine ▲ following intravenous dosing. Urine △ following intranasal dosing.

Dextran

Dextran is slowly hydrolysed in many species (Grönwall 1957; Gruber 1969) including the rat (Vars et al 1952). Eventually glucose is produced and this can be incorporated into various normal body constituents

Table 1. Mean peak and 6 h concentrations of radioactivity (% of administered dose) in bile and urine after intravenous and intranasal administration of various compounds.

	Intravenous						Intranasal					
	Bile			Urine			Bile			Urine		
	Peak concn			Peak concn			Peak concn			Peak concn		
Compound	Time reached (h)	% in sample	6 h % in sample	Time reached (h)	% in sample	6 h % in sample	Time reached (h)	% in sample	6 h % in sample	Time reached (h)	% in sample	6 h % in sample
4-Oxo-4*H*-1-benzopyran-2-carboxylic acid	0·25	4·5	0·05	0·5–2·5	≈9·0	1·6	0·5	4·8	0·02	1	20·0	0·3
p-Aminohippuric acid	0·25	4·4	0·005	0·5	47·0	0·09	0·75	0·8	0·12	1	15·0	1·3
Inulin	0·25	0·02	0·005	0·5	29·0	0·2	—	—	—	1–2·5	≈1·5	0·5
Dextran	0·75	0·5	0·03	0·5	30·0	0·1	0·5–6	≈0·01	0·01	3–6	≈0·2	0·2

Table 2. Comparison of the mean total amounts of radioactivity excreted over 6 h in bile and urine following intravenous and intranasal administration, and the proportion of the intranasal dose absorbed.

Compound	Mol wt	Total excreted over 6 h, % ± s.d.						Nasal absorp. %
		Intravenous			Intranasal			
		Bile	Urine	Total	Bile	Urine	Total	
4-Oxo-4*H*-1-benzopyran-2-carboxylic acid	190	9 ± 4	64 ± 15	73 ± 12	14 ± 4	68 ± 7	82 ± 3	112
p-Aminohippuric acid	194	8 ± 1	76 ± 2	84 ± 2	5 ± 2	58 ± 6	63 ± 7	75
Sodium cromoglycate*	512	56 ± 13	—	56 ± 13	30 ± 8	—	30 ± 8	53
Inulin	5200	0·14 ± 0·03	80 ± 5	80 ± 5	—	12 ± 5	12 ± 5	15
Dextran	70000	3 ± 1	64 ± 15	67 ± 14	0·12 ± 0·01	1·8 ± 0·4	1·9 ± 0·4	2·8

* Fisher et al (1985).

(Grey 1953). Concentrations of radioactivity are assumed to represent concentrations of dextran (Fig. 4), although not necessarily as high a molecular weight as that administered. This assumption was supported by the Sephadex gel chromatography of representative bile and urine samples. After intravenous administration, high molecular weight material (>50 000) was exclusively excreted in bile. In urine, 85% of material in early samples was of high molecular weight, falling to 70% in later samples. Usually the urinary threshold for dextran excretion is a molecular weight of 50 000 (Gruber 1969).

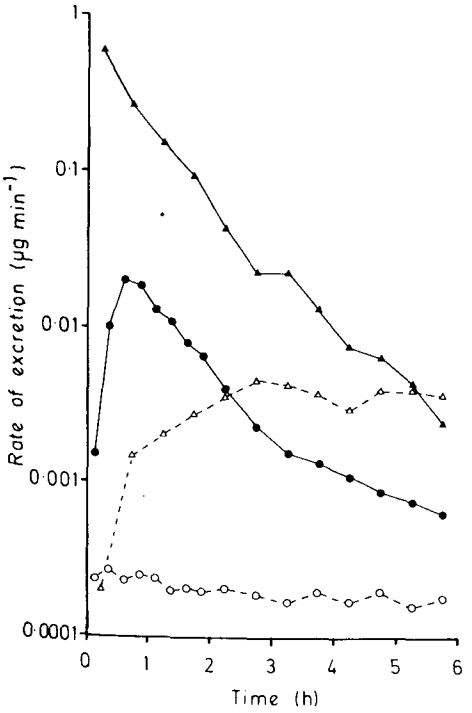

FIG. 4. Mean rate of excretion of radioactivity after administration of ^{3}H-labelled dextran at 0·25 mg kg^{-1}. Bile ● and urine ▲ following intravenous dosing. Bile ○ and urine △ following intranasal dosing.

However, larger molecules are excreted in rat urine than in other species, the mean maximum molecular weight detected being 63 000 (Wallenius 1954). Gel chromatography of samples obtained after intranasal administration indicated that most of the material excreted in the bile was low molecular weight, although traces of higher molecular weight material were detected. In urine a continuous range of material was detected from molecular weights greater than 50 000 to 1000 or less. After intravenous administration (Fig. 4, Tables 1, 2) bile concentrations rose to a peak at 0·75 h and then biphasically declined to 6 h. Urine concentrations fell monophasically from 0·5 to 6 h. After intranasal administration (Fig. 4, Tables 1, 2) radioactivity was excreted at a constant low rate in bile. In urine, concentrations rose rapidly from 0·5 to 1 h, rose more slowly to 3 h and then remained constant until 6 h. There is no doubt that some high molecular weight material is absorbed after intranasal administration of dextran. However, from the gel filtration data, not all of the absorption is of this molecular size.

Molecular weight and absorption

There is a direct correlation between the log of the proportion of the dose nasally absorbed and the log of the molecular weight (Fig. 5). The points have a correlation coefficient of −0·996 and the line drawn is the best fit by linear least squares regression analysis. Therefore, for these water-soluble molecules the proportion of an intranasal dose absorbed is a function of molecular weight. This is an established factor in the absorption of water-soluble compounds through other membranes (Schou 1971; Schanker 1962, 1971, 1978; Scheler & Blanck 1977) but has not been demonstrated previously for the nasal mucosa. It has previously been suggested that some absorption may take place through channels or pores in the membrane (Hirai et al 1981; Kaneo 1983). Recently water influx and sieving coefficient has been estimated in rat jejunal, rectal and nasal

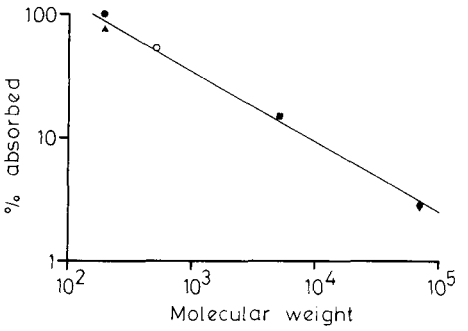

FIG. 5. Correlation between log proportion of the dose nasally absorbed and log molecular weight. 4-Oxo-4*H*-1-benzopyran-2-carboxylic acid ●, *p*-aminohippuric acid ▲, sodium cromoglycate (Fisher et al 1985) ○, inulin ■ and dextran ◆. Line shows best fits r = −0·996.

membranes. Compared with jejunum the nasal membrane has a greater water flux because of more water channels (×4) of a smaller (4–8 Å) pore size (Hayashi et al 1985). While the data presented here strongly support the concept of aqueous channel diffusion they also suggest that the pore size must be large enough to allow the passage of molecules such as inulin and dextran.

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