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Concentration and pH dependency of α-methyldopa absorption in rat intestine

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The intestinal absorption of α-methyldopa from perfused segments of rat intestine was determined. Jejunal and ileal segments were studied at pH 4·5, 6·0 and 7·4 at an α-methyldopa concentration of 0·01 mM, and at pH 6·0 at concentrations of 0·01, 0·10 and 10 mM. Intestinal absorption was found to depend on both concentration and pH but not on segment. A weak positive correlation (r = 0·5) was observed between net water absorption and α-methyldopa absorption, similar to that observed for amino acids. The low intestinal wall permeability of α-methyldopa under normal intestinal pH conditions (pH 7·4) is consistent with the incomplete oral bioavailability of this drug.

α-Methyldopa, a commonly prescribed antihypertensive drug, exhibits incomplete and widely variable oral bioavailability in man. To date, the underlying causes of α-methyldopa's bioavailability behaviour have not been clearly established. The main purpose of this study was to test the hypothesis that the oral absorption of α-methyldopa is pH- and concentration-dependent. A second objective was to determine whether these factors could account for the drug's limited bioavailability characteristics. The animal model used was the in-situ perfused intestine of the rat.

The rat was chosen because of strong similarities between it and man in the oral absorption of α-methyldopa. Oral administration of α-methyldopa in both species results in incomplete and variable absorption. In man, about 40% of the dose is absorbed (Kwan et al 1976; Stenbaek et al 1977). However, approximately half this amount is converted to the sulphate conjugate in the intestinal wall. As a result net absorption of intact methyldopa has been reported at 9–18% (Kwan et al 1976) of the dose administered. Oral bioavailability in man also varies greatly. Kwan et al (1976) found that oral absorption ranged from 8–62% of dose, while Stenbaek et al (1982) found that the percentage absorbed varied by a factor of at least 3. By comparison, oral bioavailability in the rat appears to range from 12–25% using the amount absorbed after an intraperitoneal dose as the standard (Young & Edwards 1964). Poor oral availability is not thought to be due to intraluminal metabolism in either species. In their human study, Au et al (1972) were able to recover all unabsorbed α-methyldopa as intact drug from the faeces. Young & Edwards (1964) subjected α-methyldopa to *Strep. faecelis*, a rich source of tyrosine decarboxylase, and found no evidence of metabolism. A further similarity of α-methyldopa absorption in rat and man is that the L-isomer is absorbed to a significantly greater extent than the D-isomer (Young & Edwards 1964; Au et al 1972).

The hypothesis that the absorption of α-methyldopa is pH- and concentration-dependent is suggested by the structural similarity of α-methyldopa to amino acids which exhibit these properties. Of the most commonly studied amino acids, α-methyldopa is closest structurally to tyrosine and phenylalanine. These and most other amino acids are absorbed from the intestine by concentration-dependent mechanisms (Munck 1981). There is also considerable evidence in the literature that the transport of certain amino acids, dipeptides (Adibi et al 1972; Fogel & Adibi 1972; Burston et al 1982) and other related compounds (Russell et al 1979) is pH-dependent.

The in-situ perfusion method was employed to allow precise pH control, to maximize the period during which the intestinal segments remain viable and because the hydrodynamics are reasonably well defined (Elliot et al 1980; Amidon et al 1980). While the analysis of this transport model pertains strictly only to a linear (as opposed to a saturable) boundary condition at the wall, a wall permeability that decreases with increasing concentration of drug in the perfusate would be strong evidence for a saturable transport process.

MATERIALS AND METHODS

Materials

Buffered, iso-osmotic solutions of L-α-methyldopa (Merck & Co.) were prepared at several pHs and

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concentrations for perfusion studies. Solutions of 0·067 M disodium phosphate and 0·067 M monosodium phosphate were used to prepare pH 6·0 and pH 7·4 buffers. 3·0% Sodium citrate and 2·2% citric acid were used to obtain a pH 4·5 buffer. The α-methyldopa concentrations used in the perfusing solutions were 0·01, 0·1 and 10 mM. Osmolarity was adjusted with sodium chloride.

[1,2-^{14}C]Polyethylene glycol 4000 (New England Nuclear, 1·4 μCi litre^{-1}) was included as a volume marker. In addition, cold polyethylene glycol (0·01%) (Matheson Coleman & Bell) was added to prevent labelled marker binding to the perfusion tubing. The osmolalities of all perfusing solutions were checked on an osmometer (Wescar Model 5100) and fell in the range of 295–300 mmol kg^{-1}. To retard the oxidation of α-methyldopa the following measures were taken: (1) ascorbic acid (0·02%) was included in all the perfusing solutions, (2) nitrogen was bubbled through the solutions at a flow rate of 50 ml min^{-1} for 10 min, (3) all tubing and syringes used were covered with aluminum foil, and (4) α-methyldopa solutions were prepared immediately before use.

Methods

The experimental technique was adapted from that of Smithson & Gray (1977) for the perfusion of the rat small intestine. Male Sprague Dawley rats, 200–300 g, were fasted for 48 h but water was freely available. After the rat was anaesthetized (i.m.) with 30% urethane (0·5 ml/100 g), the peritoneal cavity was opened and 10 cm segments of upper jejunum and ileum were both cannulated with Teflon tubing (Water Associates, Inc. PTFE tubing) (3·2 mm o.d. × 3·1 mm i.d.). The intra-abdominal temperature was maintained by laying the animal on a slide warmer (Clinical Scientific Equipment Co.) with the temperature set to 37 °C.

The intestinal segments were 'pre-perfused' with a solution buffered to the appropriate pH containing ascorbic acid, PEG 4000 and [^{14}C]PEG 4000 but no α-methyldopa. The flow rate used was 0·1 ml min^{-1}, maintained by an infusion pump (Harvard Quadruple Syringe Series 930). After 20–30 min a post-segment perfusate sample was taken to serve as a blank in the α-methyldopa assay. The segments were then perfused at the same flow rate with buffered α-methyldopa solution for 90 min. Fifteen minute collections of the perfusate were made into 1500 μl polypropylene (VWR Scientific Inc.) tubes over the 30–45, 45–60, 60–75 and 75–90 min intervals. The pH of each sample was checked then the sample was stored on ice. At the conclusion of the experiment a sample was taken from the inlet cannula to check for α-methyldopa degradation. A 1 ml aliquot of each sample was acidified to pH 2 with 1 M HCl and vortexed to ensure homogeneity. Each aliquot was then divided into two volumes. 500 μl was mixed with 15 ml scintillation cocktail (Aquasol [New England Nuclear] or Scinti Verse Bio-HP [Fisher Scientific]) for determination of radioactivity by liquid scintillation counting (after quench correction). The rest of the aliquot was stored at −20 °C until analysed by HPLC for α-methyldopa. At the conclusion of the experiment the animal was killed. The jejunal and ileal segments were excised and the length of intestine from cannula to cannula measured.

HPLC analysis

The following types of sample were assayed by HPLC after each experiment: (i) buffer, minus drug, sampled at the end of the pre-perfusion period from the exit cannula. This sample was used as the blank; (ii) drug solution, freshly prepared; (iii) drug solution, from the inlet cannula, before and after the perfusion, to check for decomposition, and (iv) drug in perfusate at 45, 60, 75 and 90 min after starting the drug perfusion.

The 10 mM samples were diluted one in ten with 0·5% phosphoric acid before injection. All other samples were injected without any further workup in addition to acidification at the time of collection. α-Methyldopa standard solutions were prepared in 0·1% phosphoric acid at the same concentration as the initial concentration in the samples being analysed. Standards were injected before analysing the samples and after every fourth sample. For all three concentration ranges, the linearity of peak height/peak area with concentration was validated using at least 3 α-methyldopa concentrations. Sample volumes of 10–20 μl were injected with either a Hamilton syringe or an automatic sample injector (Waters Associates, Inc. WISP 710 B). The chromatography system consisted of a chromatography pump (Water Associates, Inc. Model 6000 A pump), a precolumn loop (Brownlee RP18-5 μM (2·1 mm i.d.) × 3 cm), two columns ((Brownlee RP18-5 μm (4·6 mm i.d.) × 10 cm) in series and a variable wavelength ultraviolet detector (Kratos, Model 773 detector). The mobile phase was composed of 0·1% phosphoric acid and 5% acetonitrile in water, adjusted to pH 2·15. At the operating flow rate of 2 ml min^{-1}, 3500 psi of pressure was generated. The wavelength of detection was either 200 or 254 nm. Under these conditions the limit of detection was found to be 0·02 μg ml^{-1}.

RESULTS

α-Methyldopa absorption

The effects of three parameters on α-methyldopa absorption were studied. These were the location of the intestinal segment (jejunum vs ileum), pH of the perfusing solution and concentration of α-methyldopa in the perfusing solution. Results were expressed two ways. First, the percentage of α-methyldopa absorbed was calculated from the inlet and outlet concentrations in the perfusate. Second, the apparent wall permeability of α-methyldopa was calculated for each pH/concentration condition using the method of Elliot et al (1980). This method normalizes the results with respect to the perfusion hydrodynamics as well as the segment length used enabling the calculated wall permeability to be directly compared with results obtained for other drugs under different experimental conditions. The dimensionless wall permeability $P_w^* = P_w R/D$, where R = intestinal radius, D = diffusivity and P_w the wall permeability is a measure of the rate at which the compounds can penetrate the intestinal mucosal cell. The absorption rate constant using a mixing tank model for the intestinal compartment would be (A/V) P_{eff}, where $P_{eff} = P_a P_w/(P_a + P_w)$, P_a = aqueous permeability, A = surface area and V = volume of intestinal compartment. For a passively absorbed compound P_w (or k_a) would be independent of concentration. A statistically significant concentration dependence of P_w is strongly suggestive of a carrier or active transport process in the mucosal cell. Table 1 summarizes the results obtained for all experiments. Table 2 contains statistical analysis of the data.

Table 1. Effect of pH, concentration and location on α-methyldopa absorption in perfused in-situ rat intestine.

pH	Concn mM	Location	No. of exp.	% absorbed per 10 cm mean (s.e.m.)	Wall permeability P_w^*[a] mean (s.e.m.)
4·5	0·01	Jejunum	4	9·1 (1·4)	0·28 (0·05)
		Ileum	4	9·5 (0·7)	0·29 (0·03)
6·0	0·01	Jejunum	5	12·2 (3·0)	0·43 (0·14)
		Ileum	5	13·7 (2·2)	0·46 (0·09)
	0·10	Jejunum	3	11·8 (5·3)	0·40 (0·22)
		Ileum	3	8·7 (0·2)	0·26 (0·01)
	10	Jejunum	4	7·7 (1·5)	0·24 (0·06)
		Ileum	4	7·6 (0·8)	0·22 (0·03)
7·4	0·01	Jejunum	3	4·4 (0·6)	0·12 (0·02)
		Ileum	3	2·3 (1·5)	0·06 (0·04)

[a] Dimensionless wall permeability, calculated according to method of Elliot et al (1980).

A paired *t*-test was used to determine the effect of location on wall permeability, comparing jejunal and ileal values obtained for each rat. In no case was there a significant difference between the wall permeabilities calculated for jejunal and ileal segments at a given pH and concentration. The lowest *P* value (see Table 2) for jejunal/ileal comparison was 0·257 at pH 7·4, 0·01 mM. Results for jejunum and ileum at each pH and concentration were subsequently pooled to determine the effect of pH and concentration on α-methyldopa absorption.

Table 2. Statistical analysis of effect of pH, concentration and location on α-methyldopa absorption in perfused in-situ rat intestine.

pH	Segment location	Concn mM	Effect tested	ANOVA signif. level *P*
4·5	Jejunum Ileum	0·01	Location	0·82
6·0	Jejunum Ileum	0·01	Location	0·79
6·0	Jejunum Ileum	0·10	Location	0·55
6·0	Jejunum Ileum	10·0	Location	0·81
7·4	Jejunum Ileum	0·01	Location	0·26
4·5 6·0 7·4	Pooled	0·01	pH	0·0025**
6·0	Pooled	0·01 0·10 10·00	Concn	0·12
6·0	Pooled	0·01 10·0	Concn	0·03*

* Significant at a 95% confidence level.
** Significant at a 99% confidence level.

Fig. 1A shows the effect of pH on the apparent wall permeability at an α-methyldopa concentration of 0·01 mM. There appears to be a maximum in the absorption of α-methyldopa near pH 6·0. Analysis of variance of data at all three pH values indicates that the effect of pH on absorption is highly significant ($P = 0·0025$).

Fig. 1B shows the effect of concentration on the apparent wall permeability of α-methyldopa at pH 6. There is a trend for the wall permeability (and hence, percentage absorption) to decrease as the concentration increases. The mean wall permeability at 0·01 mM is twice as high as the wall permeability at 10 mM. Application of analysis of variance to the data for lowest and highest concentrations indicates the trend is significant ($P = 0·03$). When the 0·1 mM data is included in the statistical analysis, the smaller sample size and higher variance results in a less significant trend ($P = 0·12$).

Water flux

Net water absorption occurred in all experiments but one, with values ranging from 0·09–2·2% cm^{-1} of

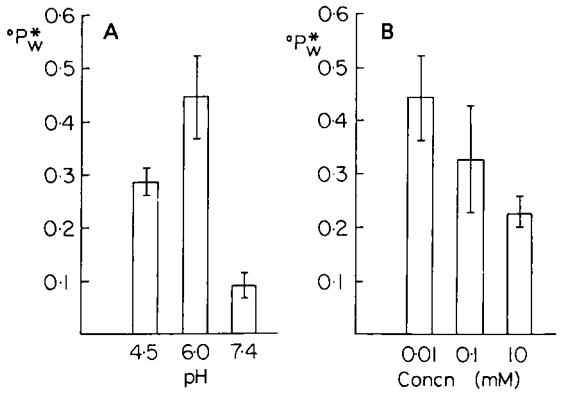

FIG. 1. A. Effect of pH on wall permeability ($°P_w^*$) of α-methyldopa in perfused in-situ rat intestine. Concentration of α-methyldopa was 0·01 mM. Bars represent mean values ± s.e.m. B. Effect of α-methyldopa concentration on wall permeability ($°P_w^*$) in perfused in-situ rat intestine at pH 6·0. Bars represent mean values ± s.e.m.

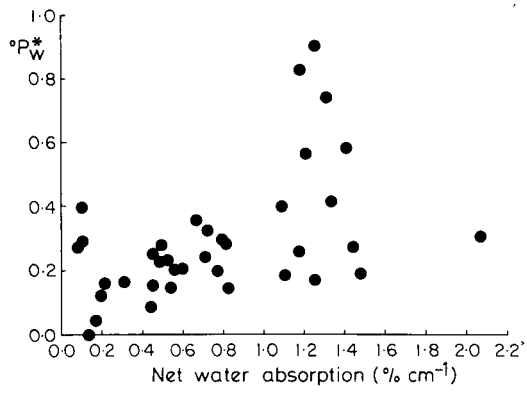

FIG. 2. Apparent wall peremability ($°P_w^*$) of α-methyldopa versus net water flux per cm of intetine. Results for all pHs and concentrations plotted individually.

intestine. The relationship between water flux and experimental parameters was examined by analysis of variance. Results in Table 3 show that water absorption was significantly greater at pH 6 then at either pH 4·5 or 7·4, in both jejunum and ileum. However, the concentration of α-methyldopa in solution did not appear to affect the water flux (see Table 4). Segment location appeared to have no effect on water flux. At pH 4·5, water absorption was greater from the ileum than from the jejunum, but this trend was reversed at pH 7·4. Water absorption was similar in both locations at pH 6·0. Fig. 2 shows individual experimental results for water flux plotted against apparent wall permeability of α-methyldopa. There is a weak positive correlation between these variables ($r = 0·5$) but the relationship is not statistically significant.

Table 3. Effect of pH on mean net water absorption at 0·01 mM α-methyldopa.

pH % Water uptake per cm	4·5	6·0	7·4	ANOVA signif. level P
(a) Jejunum	0·13 ± 0·025[a]	0·86 ± 0·20	0·39 ± 0·10	0·017
(b) Ileum	0·69 ± 0·074	0·97 ± 0·15	0·15 ± 0·01	0·004

[a] Standard error of the mean.

Table 4. Effect of concentration of α-methyldopa on mean net water absorption at pH 6.

% water uptake per cm	Concentration mM			ANOVA signif. level P
	0·01	0·1	10	
(a) Jejunum	0·86 ± 0·20[a]	0·69 ± 0·26	1·01 ± 0·13	0·58
(b) Ileum	0·97 ± 0·15	1·02 ± 0·30	1·35 ± 0·31	0·50

[a] Standard error of the mean.

DISCUSSION

Evidence for facilitated transport of α-methyldopa

The strongest evidence for a carrier-mediated, transport mechanism for α-methyldopa is that the apparent wall permeability decreases as the concentration of α-methyldopa in the perfusion solution increases. The calculation of wall permeability is based on a first order absorption assumption. When absorption is first order over the concentration range studied, this will be reflected by a constant wall permeability value, independent of concentration of the drug in the perfusion solution. A decreasing wall permeability indicates that the transport rate of the drug is not increasing linearly with concentration. This observation is consistent with a saturable transport mechanism. In the case of α-methyldopa, the mean wall permeability decreases by a factor of two over the concentration range studied ($P = 0·03$), strongly suggesting that a carrier-mediated transport mechanism is involved in α-methyldopa absorption.

A second argument for carrier-mediated α-methyldopa transport can be given on the basis of the water flux data. The water absorption characteristics of the α-methyldopa perfusion experiments are similar to those observed for amino acid absorption in two respects. First, net water absorption occurs in most experiments even though the perfusing solution is iso-osmotic. This is also observed, for example, with leucine (Adibi et al 1972) except under extremely acidic (pH 3 or lower) conditions. Water uptake is usually associated with the transport of small, actively transported water-soluble compounds such as glucose and the amino acids (Field

1981). Second, there appears to be a weak positive correlation between the degree of α-methyldopa absorption and the net water flux (Fig. 2, r = 0·5). A similar relationship has also been found for several amino acids (Matthews & Laster 1965). However, the observed water flux might also be secondary to ions secreted by the small intestine in order to return the luminal pH to the customary value.

Although it has been stated that a methyl group in the α position precludes specific uptake by the large neutral amino acid carrier mechanism (Munck 1981), Lin et al (1962) demonstrated that the α-methylated compounds corresponding to L-alanine, L-methionine and L-tyrosine were transported, albeit at a rate reduced by a factor of between 3 and 5. The possibility of a carrier-mediated transport mechanism for α-methyldopa cannot therefore be rejected on the basis of the presence of an α-methyl group.

Several other pieces of evidence supporting a carrier-mediated transport mechanism for α-methyldopa can be found in the literature. It has been demonstrated in both rat and human studies that the L-form of α-methyldopa is absorbed to a much greater extent than the D-form (Young & Edwards 1964; Au et al 1972), suggesting a selective transport process. It has also been shown that α-methyldopa competes for uptake with amino acids (Young & Edwards 1966). Furthermore, the L-form is a better inhibitor than the D-form. The inhibitory effect appears to be rather general, since inhibition has been demonstrated for histidine, glutamic acid, glycine, lysine, taurine and phenylalanine.

Wass & Evererd (1971) were unable to demonstrate α-methyldopa uptake against a concentration gradient in everted rat sacs after a 1 h incubation period. This may have been due to the method, which compared mucosal with serosal fluid levels rather than mucosal fluid with tissue levels. Transport from the mucosal to the serosal fluid involves transfer through the tissue and release into the serosal fluid as well as uptake into the mucosal tissue. Other transport effects may therefore have masked carrier-mediated transport into the tissue.

pH-Dependency of α-methyldopa absorption

There appears to be a strong pH effect on α-methyldopa absorption (see Fig. 1A). The maximum degree of absorption is observed at pH 6, more acidic or basic pHs resulting in a much lower level of absorption. Evidence in the literature suggests that the absorption of amino acids and dipeptides is also pH-dependent. Fogel & Adibi (1972) reported that decreasing the luminal pH in human intestinal perfusions from 6·9 to 3·49, modestly decreased leucine absorption from 7·04 to 4·84 μmol min^{-1} cm^{-1} and severely reduced glycine absorption from 15·63 to 1·10 μmol min^{-1} cm^{-1}. Disappearance of glycylleucine and glycylglycine was also reduced by almost a factor of two. Burston et al (1982) studied pH effect on the uptake of valylvaline in hamster jejunum over a pH range 4 to 8 and observed maximum absorption between pH 6 and 7. Lysyllysine exhibited a much sharper pH dependency, with uptake being optimal at pH 6 and reduced by a factor of 3 at pH 4 and a factor of 2 at pH 8 (Taylor et al 1980). The degree of pH dependency of α-methyldopa absorption is fairly similar to that of leucine in the acidic pH range, and to that of lysyllysine above pH 6. α-Methyldopa, like leucine, exists as a zwitterion over a broad range of pH values since it has pK_as at 2·32 and 9·2. Therefore, the change in the degree of ionization between pH 4·5 and 7·4 is insufficient to account for the strong degree of pH dependency of absorption. A possible explanation is that the carrier molecule undergoes a change in structure at the α-methyldopa interaction site over the pH range of consideration.

Location effect

Under each of the carefully controlled pH and concentration conditions studied, there appeared to be no difference between the wall permeability of methyldopa in the jejunal and ileal segments. Other workers have noted some effect of location on absorption. Adibi (1969) found that the absorption of several neutral amino acids was higher in upper jejunum than in ileum in man. On the other hand, Matthews & Laster (1965) found that the lower midpart of the small intestine was the site of maximum transport for five neutral amino acids in rats. Spencer & Samiy (1961) reported similar findings for phenylalanine in everted hamster intestinal sacs. These differences may be due to species differences in active transport sites for amino acids. An alternative explanation is that the animal segments are usually studied in buffer solution whereas in Adibi's study the pH was adjusted to, rather than buffered at, pH 6·8 and hence the pH of the perfusate was probably controlled by the natural intestinal buffers. As discussed above, pH has a significant effect on absorption and the higher pH in the ileum compared to the upper jejunum may account for the apparent lower absorption seen in that region in the human study.

Permeability of α-methyldopa

The permeability of the rat intestine to α-methyldopa can be compared with permeabilities of compounds which exhibit limited or nearly complete absorption. Several typical values are given, along with (human) bioavailability data where that is available, in Table 5.

Table 5. Comparison of dimensionless wall permeability of α-methyldopa with permeabilities of other compounds.

Compound	P_w^*	Fraction of dose absorbed (oral)	Species
Dexamethasone	8·8[a]	0·83[b]	Man
Hydrocortisone	3·31[c]	0·90[d]	Man
α-Methyldopa	0·46 (pH 6, 0·01 mM) 0·06 (pH 7·4, 0·01 mM)	0·40[e]	Man
Hydrocortisone succinate	0·40	0·17[c]	Dog

[a] Komiya et al (1980). [b] Duggan et al (1975). [c] Fleisher & Amidon (unpublished observation). [d] Toothaker (1981). [e] Kwan et al (1976).

Compounds with wall permeabilities of less than 1 are unlikely to be completely absorbed. α-Methyldopa's permeability of approximately 0·5 under optimal perfusion conditions indicates that it may exhibit incomplete absorption properties even under these circumstances. At higher concentrations or less favourable pH values, where the permeability falls off by as much as a factor of 5, the likelihood of complete oral absorption is reduced even further. This permeability analysis is substantiated by the limited and variable oral bioavailability of α-methyldopa observed in both rat and human studies (Young & Edwards 1964; Kwan et al 1976).

Overall, the data presented in this report combined with information available from the literature strongly suggests that α-methyldopa is transported by a carrier-mediated mechanism similar in many respects to the transport mechanism available to amino acids. The low wall permeability of α-methyldopa under usual intestinal pH conditions (pH 7·4) is consistent with the limited and variable oral bioavailability of this drug.

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