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ABSORPTION OF NICOTINIC ACID AND NICOTINAMIDE FROM RAT SMALL INTESTINE IN VITRO

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Summary

Intestinal absorption of nicotinic acid and nicotinamide was studied using everted sacs of rat small intestine. Transport down the concentration gradient showed saturation kinetics at low concentrations and linear kinetics at higher concentrations. Addition of ouabain or omission of sodium ions decreased absorption. Neither compound was absorbed against a concentration gradient. The mode of transport was thought to be carrier-mediated facilitated diffusion at lower concentrations masked by passive diffusion at higher concentrations.

Introduction

In the human, nicotinic acid was better absorbed from the upper small intestine than the stomach, but the mechanism of transport was not established [1]. Previous workers have found no evidence for active transport of nicotinic acid from rat small intestine, however they have not confirmed that the mode of transport is passive diffusion [2,3].

Rats are not known to show symptoms of niacin deficiency. However rats appear to have a high nutritional requirement for niacin, namely 1.5 mg per 100 g body weight. This compares with the much lower total requirement of 8 mg per day for man [4].

Hence a carrier-mediated mechanism might be expected to pump niacin across the mucosa of small intestine. Passive diffusion would seem unlikely as

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nicotinic acid is strongly negatively charged and nicotinamide is a fairly strong base. Charged compounds are usually poorly absorbed from intestine.

We have studied the transport kinetics of nicotinic acid and nicotinamide using everted sacs of rat small intestine. The possible inhibitory effects of structural analogues, ouabain and the absence of sodium ions have also been investigated.

Materials and Methods

Animals. Male albino rats of the Wistar strain of body weight 200—250 g were used. The rats were fed diet 86 (Dixon and Sons, Ware, Herts., U.K.). For each experiment the rats were starved overnight but allowed water ad lib. Everted sacs 2—3 cm in length were made from small intestine at 10 cm onwards from the pyloric sphincter. Preparation of these sacs, their incubation and the use of alternate sacs for inhibition experiments were as previously published [5]. All test solutions were prepared in Krebs-Ringer phosphate buffer at pH 7.4 [6], containing 1.28 mM calcium and 0.3% w/v glucose. In each experiment a sac containing buffer with no added vitamin was used as a blank. After gassing with 5% CO_2/95% O_2 the sacs were incubated for 30 min.

Chemicals and assay. Nicotinic acid, isonicotinic acid (British Drug Houses Ltd., Poole, Dorset, U.K.) and nicotinamide (Sigma Chemical Co., London) were assayed by a semispecific spectrophotometric method [7]. In experiments with both forms of the vitamin, since the method assays both nicotinic acid and nicotinamide, they were first separated by thin-layer chromatography and eluted for assay.

Thin-layer chromatography (TLC). TLC plates of aluminium pre-coated with silica gel $60F_{254}$ of 0.2 mm thickness were used (E. Merck, Darmstadt, F.R.G.). The solvent system was 95 : 5 v/v *n*-propanol/10% v/v aqueous ammonia [8]. The spots were detected by ultraviolet light at 254 nm. Separated compounds were measured by first scraping off the appropriate area of the silica gel, extracting with distilled water and using the spectrophotometric assay [7]. Appropriate standards were also subjected to TLC for comparison with unknown solutions.

A portion of the rat diet was extracted with water, the extract centrifuged and then chromatographed as above.

As a recovery experiment, nicotinamide transport across the intestine was measured by the spectrophotometric assay. An aliquot was submitted to TLC and the nicotinamide measured by extraction and spectrophotometry. The second figure was expressed as a percentage of the first value.

High-voltage electrophoresis. Using the Locarte apparatus high-voltage electrophoresis was performed using Whatman 3 MM paper with 6% v/v aqueous acetic acid as the electrolyte [9]. The separated compounds were detected, after drying the paper, by dipping through 1% w/v alcoholic picryl chloride [10].

Results

On the basis of TLC the rat diet contained adequate amounts of nicotinic acid and nicotinamide namely 0.7 and 0.3 μmol/g food, respectively. These

are minimal values as the efficiency of the extraction procedure was not studied. In everted sac experiments neither form of the vitamin moved against the concentration gradient when the same concentration was initially present inside and outside the sac. The final serosal/mucosal concentration ratios were as follows: 5 mM nicotinic acid initially, 0.93 ± 0.07 (number of sacs, $n = 6$); 6 mM nicotinamide, 0.88 ± 0.03 ($n = 6$); 1 mM nicotinamide, 0.96 ± 0.01 ($n = 4$).

Absorption with the concentration gradient, when no vitamin was placed within the sac, showed saturation kinetics at low initial concentrations. At higher initial concentrations linear kinetics were observed (Figs. 1, 2). Gut blanks were negligible.

The presence of ouabain, or the absence of Na^+ (replaced by equimolar K^+), decreased the absorption down the gradient of nicotinic acid and nicotinamide (Table I). The presence of metabolic inhibitors, azide or 2,4-dinitrophenol, had no inhibiting effect on nicotinamide absorption down the gradient (Table II).

Nicotinic acid at four times the concentration of nicotinamide had a significant increasing effect on nicotinamide transport. Thus 5 mM nicotinamide gave transport of 3.6 ± 0.11 (6) μmol/g wet weight per 30 min (number of experiments in parentheses) but in the presence of 20 mM nicotinic acid transport was 4.0 ± 0.13 (8) with a statistical significance $P < 0.005$. 5 mM Nicotinic acid gave transport of 2.7 ± 0.42 (4) but in the presence of 20 mM nicotinamide only 2.1 ± 0.32 (4) with a statistical significance $P < 0.1$. In this case, since nicotinamide absorption was measured spectrophotometrically after separation

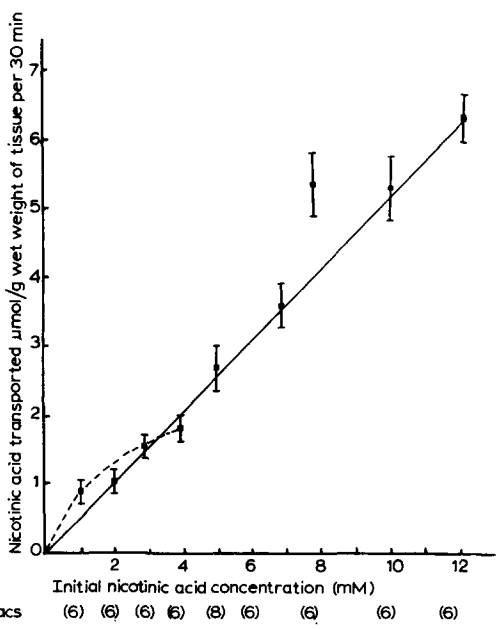

Fig. 1. The effect of concentration (mM) of nicotinic acid on absorption (μmol transported/g wet weight of tissue per 30 min) across the mucosa of rat small intestine. Points represent mean values with their standard errors represented by vertical bars. The number of experiments is given in parentheses. The dotted line indicates saturation kinetics. The full line indicates simple diffusion.

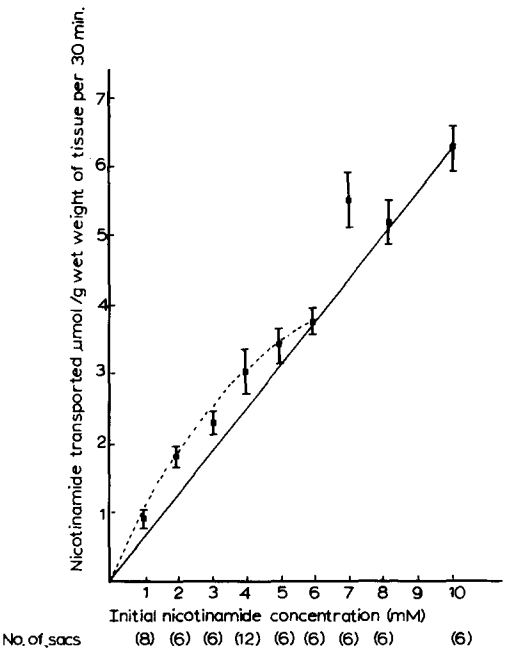

Fig. 2. The effect of concentration (mM) of nicotinamide on absorption (µmol transported/g wet weight of tissue per 30 min) across the mucosa of rat small intestine. Points represent mean values with their standard errors represented by vertical bars. The number of experiments is given in parentheses. The full line indicates simple diffusion and the dotted line indicates saturation kinetics.

by TLC, recovery experiments were necessary. 3.70 ± 0.09 µmol (n = 8) of nicotinamide were recovered after TLC compared with 3.90 ± 0.02 µmol measured spectrophotometrically without TLC, a recovery of 95%.

Nicotinic acid was partially converted into nicotinamide during intestinal transport. At 10 mM from 6.4 µmol nicotinic acid transported down the con-

TABLE I

EFFECT OF OUABAIN AND SODIUM IONS ON THE INTESTINAL ABSORPTION OF NICOTINIC ACID AND NICOTINAMIDE

Mean values with their standard errors; number of experiments in parenthesis. For details of procedures, see text.

Compound (mM)	Amount transported (µmol/g wet weight per 30 min)		Statistical significance of difference between treatments * (P)
	Ouabain absent	(1 mM) ouabain present	
Nicotinic acid (5)	2.9 ± 0.16 (8)	2.5 ± 0.13 (8)	<0.05
Nicotinamide (5)	2.2 ± 0.16 (4)	1.7 ± 0.13 (4)	<0.025
	Na^+ present	Na^+ absent	
Nicotinic acid (5)	2.4 ± 0.15 (8)	1.2 ± 0.17 (8)	<0.005
Nicotinamide (5)	3.6 ± 0.09 (4)	1.7 ± 0.34 (4)	<0.005
Nicotinamide (10)	6.3 ± 0.19 (4)	3.3 ± 0.24 (4)	<0.005

* Student's t-test.

TABLE II

INTESTINAL TRANSPORT OF NICOTINAMIDE DOWN THE CONCENTRATION GRADIENT IN THE PRESENCE OF 1 mM SODIUM AZIDE AND DINITROPHENOL

Mean values with standard errors; number of experiments in parenthesis. For details of procedures, see text. n.s., not significant.

Nicotinamide concentration (mM)	Amount transported (μmol/g wet weight per 30 min)		Statistical significance of difference between treatments *
	Azide absent	Azide present	
6	4.3 ± 0.25 (4)	4.0 ± 0.84 (4)	n.s.
3	2.1 ± 0.24 (4)	2.0 ± 0.2 (4)	n.s.
	DNP absent	DNP present	
1	0.99 ± 0.08 (4)	0.97 ± 0.06 (4)	n.s.

* Student's *t*-test.

centration gradient, 0.35 ± 0.03 (n = 4) μmol nicotinamide were formed. In subsequent experiments the amount aminated was not proportional to the initial concentration of nicotinic acid. Hence the results were not presented as the percentage of nicotinic acid transformed into nicotinamide, as this expression would have been misleading. Qualitative experiments revealed that mucosal cells scraped from everted sacs had a greater capacity for this amination than did serosal tissues.

The analogue, isonicotinic acid, also gave an extra spot on TLC after intestinal transport. It was presumably isonicotinamide. Unfortunately, isonicotinamide has the same R_F value as nicotinamide so that unequivocal identification could not be made. The following results were obtained in comparing the transport of 3 mM nicotinic acid and 3 mM isonicotinic acid down the concentration gradient, 1.70 ± 0.10 (n = 4) and 1.00 ± 0.17 (n = 4) μmol transported per g wet weight of tissue, respectively ($P < 0.005$). At 8 mM the following results were obtained, 5.05 ± 0.33 (n = 4) and 3.2 ± 0.20 (n = 4) ($P < 0.005$).

High-voltage filter paper electrophoresis of samples from intestinal transport experiments confirmed the formation of nicotinamide from nicotinic acid. Two other metabolites were detected but not identified. They were not NAD. Furthermore, transported isonicotinic acid gave a metabolite, presumably isonicotinamide, which had a higher mobility than nicotinamide. Two other metabolites were also derived from isonicotinic acid. They had the same electrophoretic mobility as the unknown products from nicotinic acid.

Discussion

Under the conditions of our experiments neither nicotinic acid nor nicotinamide were transported against a concentration gradient. The serosal/mucosal concentration ratios were below unity, which can be attributed to water transport into the everted sacs exceeding water efflux from the sacs, a well-known phenomenon in such experiments [11].

Saturation kinetics at low concentrations for both nicotinic acid and nico-

tinamide suggests carrier-mediated transport, namely, facilitated diffusion. Saturation of nicotinic acid transport at lower concentrations than nicotinamide implies that nicotinic acid has a higher affinity for the carrier. Inhibition of nicotinic acid transport by nicotinamide confirms that both compounds are utilising the same carrier. The related derivative isonicotinic acid hydrazide, the antitubercular drug isoniazid, does not share this transport pathway. Nicotinic acid did not decrease the transport of isoniazid across everted sacs of rat small intestine [12].

Absorptions of both forms of the vitamin were decreased in the absence of Na^+, and inhibited by the presence of ouabain. The glycoside ouabain is known to inhibit the membrane pump for Na^+ [13]. The lack of inhibition of transport by azide and 2,4-dinitrophenol also excludes active transport as the absorption mechanism. Hence it appears that at low concentrations both nicotinic acid and nicotinamide are transported across the mucosa of rat small intestine by Na^+-mediated facilitated diffusion. At higher concentrations passive diffusion (linear kinetics) overshadows the saturable facilitated diffusion. By contrast, bullfrog small intestine in vitro exhibits active transport of nicotinic acid [14].

Passive diffusion of the vitamin across the intestinal mucosa must be discussed. Many charged molecules, e.g. drugs, exhibit this phenomenon. The greater the proportion of such a compound in the unionised lipid-soluble form then the greater will be the gastrointestinal transport. At the lower pH of the stomach contents, nicotinic acid ($pK_a = 4.84$) would be poorly ionised (0.2%). In our intestinal experiments at pH 7.4, nicotinic acid would be fully ionised (99.8%). Yet nicotinic acid was absorbed better from the upper small intestine than from the stomach [1] although the opposite might have been expected. This is further evidence for carrier-mediated transport across mucosa of the small intestine. Surprisingly, vascular perfusion experiments suggested simple diffusion across rat small intestine [15] for both nicotinic acid and nicotinamide. Unfortunately, the manner in which the results were expressed, using different amounts rather than initial concentrations, precluded detailed comparison with our results.

During transport across rat small intestine some of the nicotinic acid was converted into nicotinamide probably by the brush border cells. The apparent increase in nicotinamide absorption in the presence of nicotinic acid is clearly an artefact due to the metabolic production of nicotinamide from the nicotinic acid. Thus the small intestine also metabolised nicotinic acid to nicotinamide as described in other tissues [16]. Recently intravascular perfusion studies revealed this transformation [17] in small intestine.

The poor transport of isonicotinic acid in relation to nicotinic acid was perhaps surprising. With identical molecular weights and very similar pK_a values, 4.86 and 4.81 [18] it would be expected that these two compounds would be absorbed at the same rate.

Comparison of our results with those of other workers who did not find a carrier-mediated process may reveal the reasons for this discrepancy. Turner and Hughes [2] using a sensitive microbiological assay were seeking specifically transport across everted sacs against the concentration gradient, i.e. active transport. In its absence they assumed passive diffusion and, by not testing possible

inhibitors, they did not reveal facilitated diffusion. Similarly, experiments using the ^{14}C-labelled vitamin [3,19] were designed to seek absorption against the concentration gradient and this was not found. Compared with other B vitamins, [^{14}C]nicotinic acid achieved a high concentration in the intestinal wall [3] which might have suggested a carrier-mediated absorption.

Loss of ^{14}C-labelled nicotinic acid of about 21% [14] could be due to the formation of nicotinamide. In fact there was chromatographic evidence for a metabolite formed with small intestine from the hamster [3] and the bullfrog [14], but in neither study was the compound identified.

The described phenomenon of carrier-mediated transport at a low concentration and passive diffusion at higher concentrations is not an isolated example. It has been described for the transport of the pyrimidines, thymine and uracil, across rat small intestine in vivo [20] and also arachidonic acid in vitro [21]. Similar results have been reported for the following vitamins, cyanocobalamin [22], thiamine [23,25], and retinol [24].

In our experiments nicotinic acid was transported faster than isonicotinic acid both at the lower saturating concentrations and the higher, linear, portion of the graph. Also nicotinamide transport was Na^+-dependent in both concentration ranges. Two possible hypotheses are: (1) The initial carrier-mediated process, though overshadowed by high concentrations of substrate, is still operating at the higher concentrations, or (2) The carrier has at least two binding sites of very different K_m values. The K_m of the second binding site would have to be very high since we had no evidence of saturation up to 10 mM. A third possibility which cannot be excluded by our experiments is co-transport. If Na^+ is co-transported with nicotinic acid and nicotinamide then active transport will be apparent if the Na^+ cannot escape in response to the electrochemical gradients.

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