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NICOTINIC ACID ACTIVE TRANSPORT BY IN VITRO BULLFROG SMALL INTESTINE

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SUMMARY

The fluxes of [7-^{14}C]nicotinic acid across the chamber-mounted, in vitro bullfrog (*Rana catesbieana*) small intestine were measured. The flux of tracer in the direction favoring absorption exceeded the opposite flux to a statistically significant degree ($P < 0.05$) at concentrations of $2.0 \cdot 10^{-3}$ M and below. This process saturated at $2.0 \cdot 10^{-2}$ M. Mannitol 115 mM added to the mucosal salines abolished solvent drag as measured by 10 mM urea fluxes. The transmural potential difference across this preparation is too small (approx. 3 mV) to cause a significant asymmetry in the transfer of any charged solute. At $2.0 \cdot 10^{-4}$ M nicotinic acid the flux ratio was 25.7 ± 7.3 (mean ± S.E.). Studies of the chemical nature of the flux of tracer at this concentration disclosed that at least 79% of the label traversing the preparation appeared as nicotinic acid. Since [7-^{14}C]nicotinic acid absorption is accomplished by a saturable transport system with, at most, minor metabolism of nicotinic acid, we conclude that nicotinic acid itself is actively transported.

INTRODUCTION

In experiments with an in vitro preparation of bullfrog (*Rana catesbieana*) small intestine, we find that nicotinic acid is actively absorbed by a saturable process [1]**. Previous workers, (Turner and Hughes [2], Turner [3], Spencer and Bow [4]) were unable to demonstrate specialized transport of nicotinic acid across in vitro mammalian small intestinal preparations. We believe this to be the first documented report of nicotinic acid active transport in any biological system. Dietrich [5] reported active uptake of nicotinic acid by in vitro Ehrlich–Lettre, Sarcoma 180, and Adenocarcinoma 755 ascites cells in an abstract. However, Dietrich and Ahuja [6] later stated that the nicotinic acid accumulation by these tumor cells appeared to occur by a rapid diffusion process and that the final ratio of intracellular to extracellular nicotinic acid behaved as if determined by pH partitioning alone. Lan and Henderson [7] examined the kinetics of [7-^{14}C]nicotinic acid uptake by and egress from the rat erythrocyte and suggested that there may be a component of facilitated diffusion in its transfer across this membrane.

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METHODS AND RESULTS

Bullfrogs obtained commercially were usually kept in tapwater at 15 °C for at least 1 week. A few were kept at room temperature.

We double pithed the frogs and excised about 15 cm of proximal small intestine and opened it along the mesenteric border, thus isolating the entrance of the common bile duct. We then placed the tissue in saline containing (in mequiv) Na^+, 110; K^+, 2.5; Ca^{2+}, 2.5; Mg^{2+}, 1.0; HPO_4^{2-}, 3.0; SO_4^{2-}, 3.5; HCO_3^{2-}, 30; Cl^-, 79.5 and dextrose 5g/l at about 6 °C gassing gently with O_2–CO_2 (95:5, v/v). Approx. 5 min later the sheet shortened and widened. We cut the sheet transversely and mounted two adjacent segments in separate modified Ussing's flux chambers (round aperture, 0.5 cm^2). The interval between pithing the animals and mounting the tissues averaged less than 15 min.

M→S (mucosal to serosal, lumen to blood) and S→M (serosal to mucosal, blood to lumen) fluxes of [7-^{14}C]nicotinic acid (New England Nuclear NEC-121, between experiments stored as 20 mM aqueous solution at −6 °C) were then measured in separate chambers. Each flux chamber compartment contained 2.5 ml of saline. All salines contained identical chemical concentrations of nicotinic acid.

We discarded all salines initially placed in the chambers after 1 h. Fresh solutions were added to all compartments and changed every 2 h during the ensuing 6 h, thus providing three observation periods. Portions of all saline samples were assayed in a liquid scintillation counter. We computed the mean flux of tracer for each sheet over the final 6-h period based on the label appearing in the initially nonradioactive flux chamber compartments.

Experiments were conducted at ambient room temperature (22–27 °C). The bathing salines were gassed and agitated by O_2–CO_2 (95:5, v/v). Mannitol (115 mM) added to the mucosal salines abolished solvent drag as determined by radio-labeled urea fluxes (urea concentration, 10 mM) both with and without 115 mM mannitol (Table I). We did not electrically short-circuit this preparation, as the spontaneous

TABLE I

ISOTOPIC FLUXES OF UREA

Fluxes and flux ratios are given as means ± S.E. *N*, number of intestinal pairs. Except for mannitol, both surfaces bathed by identical saline with 10 mM urea. The standard errors of the ratios have been calculated by using the approximation $(C_x)^2+(C_y)^2=(C_{x/y})^2$ where C_x is the coefficient of variance of numerator and C_y is the coefficient of variance for the denominator and $C_{x/y}$ is the coefficient of variance for the ratio. The isotopic fluxes of urea represent the transfers of label during 6-h periods following 1-h periods of incubation with labeled and unlabeled saline as in the [7-^{14}C]nicotinic acid experiments.

	Flux ratio	Flux		*N*
		M→S (nmoles·cm^{-2}·h^{-1})	S→M (nmoles·cm^{-2}·h^{-1})	
Mucosal saline without mannitol	1.4 ± 0.2	321 ± 18	227 ± 15	6
Mucosal saline with mannitol (115 mM)	0.9 ± 0.1	172 ± 14	194 ± 18	6

transmural potential difference across the bullfrog small intestine is a few millivolts [8] and thus would not account for significant asymmetry of fluxes of a charged solute.

The nicotinic acid concentrations and isotopic fluxes are given in Table II. The flux ratios (the mean mucosal to serosal flux divided by the mean opposite, serosal to mucosal, flux) of tracer at $2.0 \cdot 10^{-5}$, $2.0 \cdot 10^{-4}$ and $2.0 \cdot 10^{-3}$ M are statistically distinct from unity at the $P < 0.05$ level of confidence as determined with Student's t test.

TABLE II

ISOTOPIC FLUXES OF NICOTINIC ACID

Fluxes and flux ratios given as means ± S.E. N, number of intestinal pairs. The standard errors of the ratios are calculated as for Table I.

Concentration (M)	Flux ratio	Flux		N
		M→S (nmoles·cm^{-2}·h^{-1})	S→M (nmoles·cm^{-2}·h^{-1})	
$2.0 \cdot 10^{-5}$	16.5 ± 5.1	2.48 ± 0.51	0.15 ± 0.035	6
$2.0 \cdot 10^{-4}$	25.7 ± 7.3	31.9 ± 5.8	1.24 ± 0.27	6
$2.0 \cdot 10^{-3}$	9.3 ± 2.5	219 ± 31	23.5 ± 1.3	6
$2.0 \cdot 10^{-2}$	1.6 ± 0.25	358 ± 26	222 ± 30	6

In approx. 5% of the experiments we discarded the results from a preparation because of gross leakage, indicated by permeation of chlorphenol red added after the end of the experiment.

In separate experiments with nicotinic acid $2.0 \cdot 10^{-4}$ M, we studied the chemical nature of the isotope passing through the preparation. The initially nonradioactive salines were not changed during the 7-h interval of the experiments. The initially radioactive salines were replenished after 1 h and either every 2 h subsequently or not all during the 6-h period. 50–100 μl samples of these salines, portions of unincubated, radioactive saline and the aqueous [7-^{14}C]nicotinic acid were placed on Whatman No. 1 chromatography paper and analyzed by ascending paper chromatography overnight with n-butanol saturated with 1.5 M NH_4OH, and one of the following system: n-butanol–acetone (1:1, by vol.) saturated with water or n-butanol–formic acid–water (10:2:15, by vol.). Mannitol was omitted in these experiments as it appeared to interfere with some of the chromatographic assays. In studies of nicotinic acid transport without mannitol added to the mucosal solutions, we found a statistically significant flux ratio (15.2 ± 2.1 (6), mean ± S.E.) favoring nicotinic acid absorption at $2.0 \cdot 10^{-4}$ M.

Low activity strips were cut into smaller segments (usually 20) and assayed in a liquid scintillation counter. We analyzed higher activity strips in a gasflow radiochromatograph strip counter. The R_f of the label appearing in the serosal and mucosal compartments was identical to that of the unincubated labeled saline and the concentrated, aqueous radioactive stock. A second peak (21% of total activity) appeared in chromatographic analyses of the label appearing in the serosal compartment in one experiment. We detected no change in the labeled salines incubated with the tissues for 6 h.

DISCUSSION

We have found that the absorption of nicotinic acid by the in vitro bullfrog small intestine is accomplished by a specialized transport process which saturates at $2.0 \cdot 10^{-2}$ M. Transport at the lower concentrations occurred in the absence of a physical force or electrochemical gradient that would produce an asymmetrical flux of tracer such as we observed at low concentrations of nicotinic acid. The spontaneous potential difference across this preparation is in the order of a few millivolts [8] and thus cannot account for more than a trivial asymmetrical flux of a charged solute on the basis of the Ussing equation [9]. pH partitioning could not have occurred as both compartments of the flux chamber were strongly buffered at an identical pH. Solvent drag played at most a minor role, as it was probably abolished by the addition of 115 mM mannitol. Since only a minor portion, at most, of the compound is metabolically transformed during transport, we conclude that the in vitro bullfrog small intestine actively transports nicotinic acid.

Turner and Hughes [2], Turner [3] and Spencer and Bow [4] studied nicotinic acid absorption by in vitro, everted intestinal sacs prepared from mammals. Spencer and Bow [4] utilized isotopic tracer techniques exclusively in their studies of nicotinic acid transport by everted hamster intestinal sacs. It is possible that the hamster actively absorbs nicotinic acid, yet the 1-h period of Spencer and Bow's experiments may have been too short to permit isotopic equilibrium to occur between the bathing media and tissue. If isotopic equillibrium was not attained, particularly if the transport system was of "low capacity", concentration tracer might not be observed.

Turner and Hughes [2] studied nicotinic acid transport in rat everted jejunal and ileal sacs using microbiological assays as their analytical tool. Their failure to demonstrate specialized absorption of nicotinic acid, if occurring in this animal, may have been a consequence of the relatively less sensitive nature of their microbiologic assays for nicotinic acid as compared to radioactive tracer techniques, or their failure to study transport in the duodenal portion of the small intestine where many transport processes are maximal.

Turner [3] in his dissertation reported a number of studies of radioactive nicotinic acid absorption by everted rat intestinal sacs, and he was unable to demonstrate active transport of nicotinic acid. This approach has the same inherent limitations as does that of Spencer and Bow [4] in that isotopic equilibrium between tissue and bathing medium may not have been achieved in the 1-h study period.

Our preparation is viable for a far longer period than the in vitro everted mammalian intestinal sac as the isolated bullfrog small intestine actively transports nicotinic acid for up to 9 h (Fox, K. R. and Hogben, C. A. M., unpublished). It is possible that this extended period of viability has allowed isotopic equilibrium to occur thus aiding us in exhibiting this biological process by isotopic tracer techniques.

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