Pharmaceutical Research, Vol. 6, No. 4, 1989

Report

Nutrient Influences on Rat Intestinal Phenytoin Uptake

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Received July 18, 1988; accepted November 16, 1988

The intestinal uptake of phenytoin was studied as a function of concentration, intestinal region, coperfused glucose, and calcium chloride in rat intestinal perfusions and everted intestinal rings. Steady-state intestinal membrane permeabilities were obtained in an *in situ* perfusion system and initial rates of intestinal tissue uptake were obtained in an *in vitro* everted ring system as rate of absorption parameters. Steady-state membrane permeabilities were independent of phenytoin perfusion concentration and decreased from duodenum to ileum. Coperfusion of glucose increased, and high calcium chloride concentrations decreased phenytoin permeabilities. While phenytoin uptake in the *in vitro* ring system was also concentration-independent and depressed by high calcium concentrations, regional variations and glucose enhancement were not observed. Thus, drug–nutrient interactions involved in intestinal absorption from phenytoin solutions are a function of the isolation procedure.

KEY WORDS: phenytoin; intestinal absorption; membrane transport; drug–nutrient interactions.

INTRODUCTION

Phenytoin (PHT; 5,5-diphenylhydantoin) was introduced as an anticonvulsant agent in 1938 and is currently the drug of choice for generalized major motor and focal seizures. In addition, PHT has been used clinically to correct cardiac arrhythmias, myokymia, and excessive secretion of insulin and antidiuretic hormone. It is a weak acid with pK_a 8.1, intrinsic aqueous solubility 0.1 m*M*, and log membrane–water partition coefficient 2.4 (1,2). There have been several clinical reports in which PHT plasma levels were affected by nutrient coadministration with oral phenytoin (3–5). The fact that phenytoin possesses a narrow therapeutic index warrants investigation of nutrient effects on this drug's absorption variability.

Drug absorption is a function of drug dissolution, gastric emptying, and gastrointestinal (GI) residence time, as well as membrane transport. Dissolution and GI transit time effects on PHT absorption are the focus of another report (6). In this report, nutrient effects on phenytoin intestinal membrane transport are studied in rats using steady-state *in situ* intestinal perfusions and *in vitro* everted intestinal ring incubations. Each technique offers particular advantages for isolating the role of membrane transport in intestinal drug absorption. The strengths of the perfusion system include the availability of an intact blood supply and the potential for control of input and hydrodynamic conditions. However, non-steady-state drug absorption data are complicated by system response (water absorption or secretion) and only one steady-state data point can be obtained per experimental animal. In the ring system, a tissue accumulation technique, many initial rate data points can be obtained from the same animal but rapid loss of tissue viability and lack of drug removal by the blood compartment present transport limitations. Control of perfused length and flow rate (in perfusion) and shaking rate and incubation time (in rings) permit factoring of intestinal residence time and drug aqueous lumenal resistance from drug membrane resistance (7).

MATERIALS AND METHODS

Male Sprague Dawley rats, 50–75 days old, weighing 200–300 g, and maintained on Ralston Purina Rat Chow A-5012, were fasted 12 hr prior to surgery, which began at 10 AM. The rat was anesthetized with an intramuscular injection of 150 mg ethyl carbamate/100 g body weight (urethane, Sigma Chemical Co.). This anesthetic produces minimal effects on gastrointestinal function by this route of administration (8). Baseline perfusion and incubation solutions contained 100 m*M* sodium chloride, 5 m*M* potassium chloride and 10 m*M* Pipes or Mes buffer. Osmolality, as measured by Wescor vapor pressure osmometry, was adjusted to 300 ± 5 mOsm/kg with choline chloride or mannitol, while pH was adjusted with HCl or NH_4OH according to compatibility with the intestinal region.

Perfusions. The rat was secured on its back to a slide warmer maintained at body temperature and the abdomen was opened by a midline longitudinal incision. The segment of intestine chosen for perfusion was ligated with 1/16-in.-inner diameter tygon tubing at the inlet and 4–5 cm distal at the outlet. The inlet tubing was water jacketed at 37°C to maintain the inlet perfusate at body temperature and connected to a 50-ml syringe containing perfusion solution which was placed on a Harvard perfusion apparatus. The flow rate was set at 0.5 ml/min, which over 4–5 cm of intes-

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0724-8741/89/0400-0332$06.00/0

tinal length, resulted in output-to-input concentration ratios of 0.9 ± 0.05 for phenytoin when corrected for water transport. Output samples were collected over 5-min intervals out to 80 min. Steady-state conditions were achieved by 30–35 min, while reduced tissue viability effects on permeability and water transport were not observed until 2 hr had elapsed in baseline experiments. Output concentrations were corrected for water transport by measuring changes in the levels of a nonabsorbable marker, 1,2-[^{14}C]polyethylene glycol 4000 (New England Nuclear; sp act, 4.4 × 10^5 dpm/μmol), in the perfusate. Phenytoin concentrations (0.3–100 μ*M*) were measured by a high-performance liquid chromatographic (HPLC) assay (9) in the input and output perfusate as well as in mesenteric and systemic blood obtained during collection of the last steady-state sample.

Rings. Intestinal segments were isolated and excised from the anesthetized rat, then everted on a glass rod while floating in a tray of oxygenated buffer. The rat was sacrificed by anoxis. Rings were cut in a size range of 10–30 mg wet weight and placed in a petri dish under a gentle stream of 95% oxygen, 5% carbon dioxide. Incubations were carried out in a shaking water bath in which the shaking rate and incubation temperature could be varied. Incubation solutions were freshly prepared and equilibrated 20–24 hr prior to experiment. Viability, as monitored by uptake of [^{14}C]polyethylene glycol 4000 as an extracellular water marker, suggested that incubations must be completed within 10 min of removal of the intestine. Phenytoin uptake was linear with time up to 5 min and all experiments were performed using 3-min incubation times at 37°C. The tissue incubation was stopped by emptying the incubated rings onto a cheesecloth-covered beaker, rinsing with ice-cold buffer, and blotting the tissue dry. Tissues were transferred to preweighed scintillation vials to obtain tissue weights to either freeze-dried and ground for HPLC analysis or digested by Scintigest (Fisher) before scintillation counting of [4-^{14}C]phenytoin (Amersham; sp act, 2.2 × 10^6 dpm/μmol).

Data Analyses. In order to obtain intrinsic membrane transport rate parameters, aqueous resistance to drug transport must be factored out. In the perfusion system, drug output-to-input concentration ratios were used to calculate an effective resistance to transport. The flow conditions were adjusted so that aqueous resistance (a function of perfusion flow rate, intestinal length, and drug aqueous diffusivity) was not dominant. By laminar flow analysis (7), aqueous resistance could be calculated in this system. Membrane resistance was obtained by subtracting calculated aqueous resistance from experimentally determined effective resistance. The inverse of the membrane resistance is the membrane permeability (p_m, with units of cm/hr). In the ring system, aqueous resistance was factored out by performing uptake experiments at various shaking rates. In a plot of uptake versus reciprocal shaking rate, the tissue resistance to uptake was obtained by extrapolating to infinite shaking rate (Fig. 1).

Data are reported as dimensionless membrane permeabilities (P_m*) after normalizing permeability for aqueous resistance to drug transport (intestinal radius over phenytoin aqueous diffusivity at 37°C). Tissue uptake is reported as nanomoles phenytoin uptake per minute per gram tissue weight. Correlations and statistical significance were evaluated using Statgraphics software routines on an IBM XT computer. Results are presented as means ± standard error. Differences between treatments were evaluated using a paired *t* test in which a value of $P \leq 0.05$ was considered statistically significant.

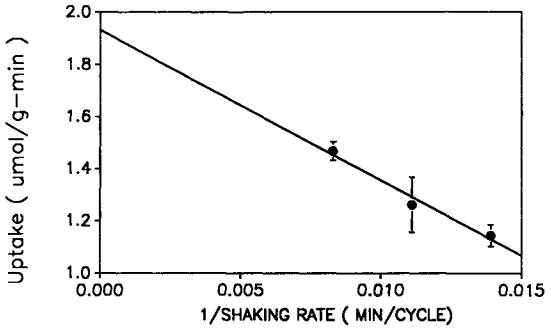

Fig. 1. Phenytoin uptake by rat jejunal rings as a function of reciprocal shaking rate at 37°C. Extrapolation to infinite shaking yields tissue uptake unbiased by aqueous resistance.

RESULTS

Several baseline experiments were performed with each experimental system. In the perfusion system, water transport was studied as a function of inlet pH and osmolality. Minimal variation in these parameters from outlet to inlet correlated with minimal steady-state water transport. Initially, phosphate-citrate buffers were compared with Pipes and Mes (Sigma Chemical) buffers and it appeared that permeabilities varied with sodium concentration. As a result of this observation, the perfusion concentration of sodium was maintained constant with 100 m*M* NaCl. Osmolalities were adjusted using either mannitol or choline chloride. These baseline solutions were used in all perfusion and ring experiments (Table I).

Table I. Perfusate and Incubation Solutions

pH	
Duodenum[a]	6.0
Upper jejunum	6.5
Upper ileum with 10 m*M* Pipes or Mes adjusted with HCl or NH_4OH	7.2
Osmolality	300 ± 5 mOsm/kg, adjusted with choline chloride or mannitol
Sodium	100 m*M* as sodium chloride
Potassium	5 m*M* as potassium chloride
Perfusion flow rate	0.5 ml/min
Perfusion intestinal lengths	4–6 cm
Intestinal radius at this flow rate	0.2–0.25 cm
Water transport at this flow rate	≤0.5%/cm[b]
Steady-state perfusion time	35–80 min
Incubation time	3 min
Shaking rate	72, 90, 120 cycles/min

[a] Duodenal perfusions bypassed the pancreatic-bile duct.
[b] As much as 2%/cm water absorption was observed at 100 m*M* glucose. Water secretion was observed when glucose was not present.

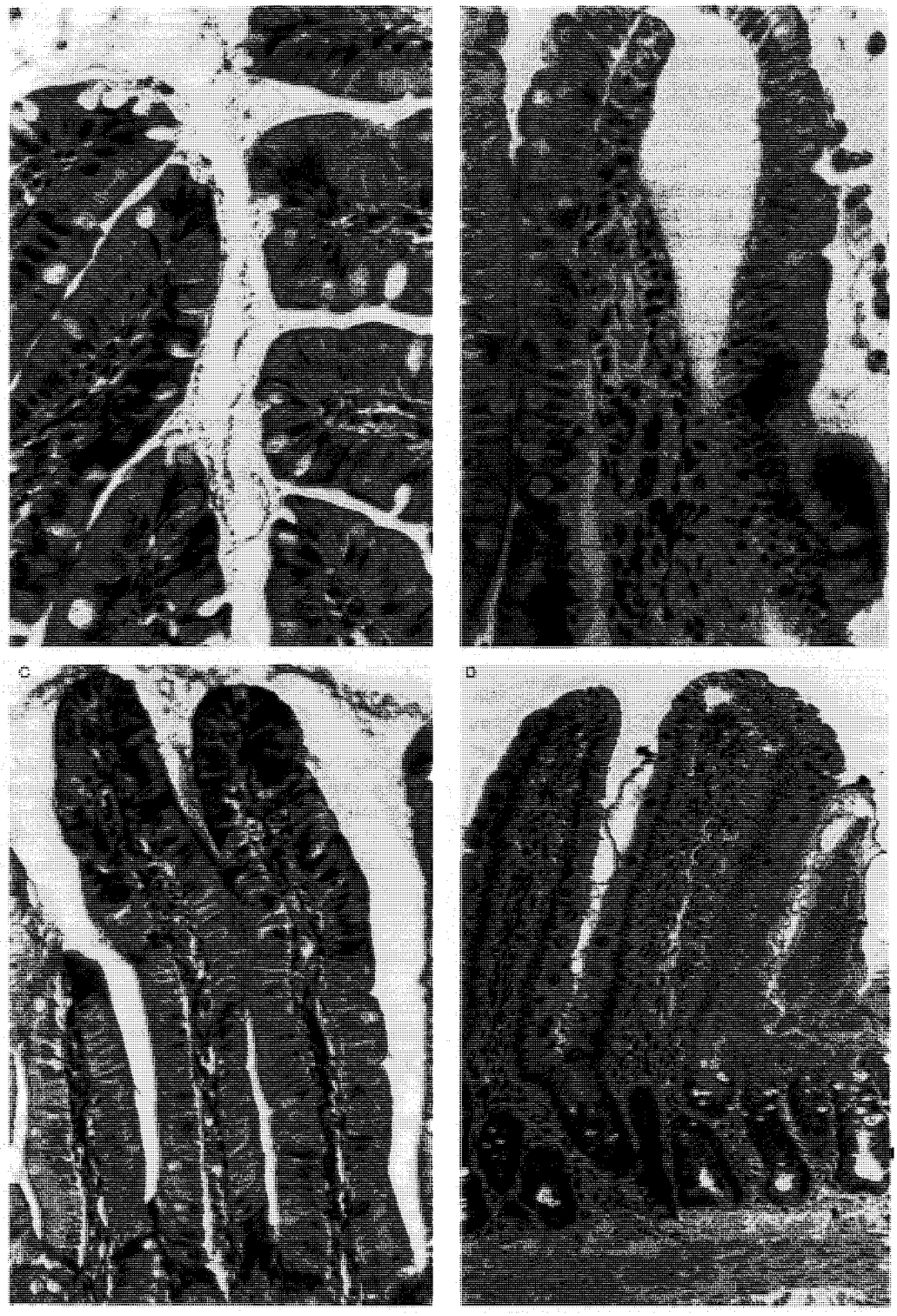

Fig. 2. Jejunal perfusion and ring morphology. Hematoxylin and eosin stain. (A) perfusion control; (B) perfusion at steady state (60 min); (C) ring control; (D) 5-min ring incubation. 100–400×; reduced 40% for reproduction.

Intestinal tissue was not analyzed for drug levels following perfusion and mesenteric and systemic blood was sampled at the end of the perfusion experiments in only a few cases. Mass balance was not obtained when comparing mesenteric and systemic drug levels to drug mass as measured by loss from the intestine. Steady-state drug blood levels did, however, correlate in rank order with membrane permeability. In addition, ^{14}C-labeled water marker did not appear in the plasma even with the high water absorption observed in the presence of glucose.

Water transport, usually seen as water secretion in the perfusions, was less than 0.5%/cm of perfused intestine in all studies except those with 20 and 100 m*M* glucose. In the presence of 100 m*M* glucose, as much as 2%/cm water absorption was observed (Table I). No significant changes in sodium or potassium were seen as monitored by flame photometry. There was no significant uptake of [^{14}C]PEG 4000 in the ring experiments at 3 min but considerable marker was associated with the ring tissue if incubations were carried out as long as 10 min.

Morphology showed intact villi and epithelial cells at 3-min ring incubation and 80-min perfusion times (Fig. 2). Damage to membranes was observed at 10-min incubation times and at 120-min perfusion times. The villi showed readily discernible intervillus space in the 3-min ring incubations (Fig. 2D), while steady-state perfusion samples showed edematous tissue such that the villi were crowded together and intervillus spacing could not be observed (Fig. 2B).

Phenytoin membrane permeabilities from perfusions and uptake from everted rings were seen to be independent of PHT concentrations over a range of 10–100 μ*M* (Figs. 3 and 4). PHT permeabilities were significantly higher in the duodenum than the jejunum in the same rat in all experiments, while ring uptakes were region independent (Fig. 5). Permeabilities of PHT in the perfusion system were significantly higher in the presence of both 20 and 100 m*M* glucose, with less variability at the higher glucose concentration (Fig. 6). In addition, inclusion of 500 μ*M* phlorizin, an inhibitor of active glucose transport in the mucosal membrane, resulted in phenytoin permeabilities similar to those obtained in the absence of glucose. These effects were demonstrable in both the duodenum and the jejunum. Glucose enhancement of phenytoin uptake was not observed in the everted ring experiments (Fig. 7). Calcium chloride decreased PHT perfusion permeabilities and ring uptake at concentrations above physiological extracellular calcium concentration (greater than 2 m*M*) (Figs. 6 and 8).

DISCUSSION

There have been several *in vivo* studies on the influence of coadministered drugs and nutrients on PHT plasma levels from oral phenytoin (3–5). *In situ* and *in vitro* studies concerned with the influence of phenytoin on glucose (10) and calcium (11) intestinal transport have also been reported. Phenytoin is known to influence sodium and calcium gradients across cell membranes in other tissues by its action on ion channels (12,13). It is possible that similar influences in epithelia affect sodium–glucose cotransport and active calcium transport. This report, however, focuses on the significant effect of glucose and calcium on phenytoin transport.

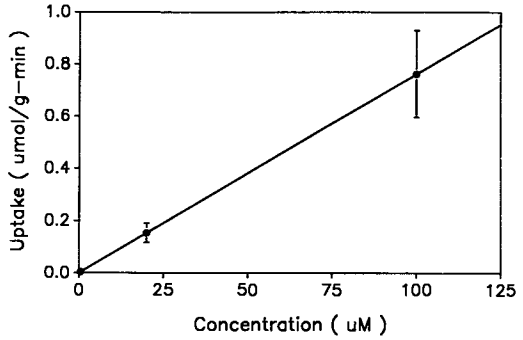

Fig. 4. Phenytoin jejunal ring uptake at 90 cycles/min at 37°C as a function of phenytoin incubation solution concentration (0.5, 20, and 100 μ*M*).

Permeability and uptake results are independent of phenytoin concentration, indicating a passive transport process. Since there is no physicochemical interaction between either calcium and phenytoin (14,15) or glucose and phenytoin (16) at this pH, the implication from the effects of these solutes on PHT absorption is that the membrane transport pathway for PHT is being altered. Both paracellular and transcellular routes through the intestinal membrane may be altered by the presence of actively transported species in the intestinal lumen. In a recent study (17), it was shown that sodium cotransported monosaccharides and amino acids stimulate cytoskeleton contraction to open paracellular spaces. It was felt that this may be an energy conserving mechanism for nutrient transport at high nutrient concentrations (the K_m for intestinal glucose transport is 2–3 m*M*). Dimensions of these openings were sufficient to pass molecules of molecular weight up to 5500 by solvent drag. This is consistent with the increased water absorption observed in the presence of 100 m*M* glucose in these studies. However, [^{14}C]polyethylene

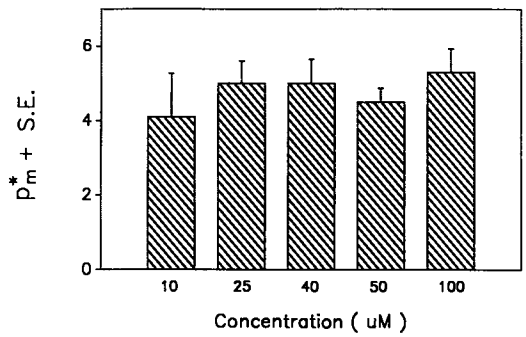

Fig. 3. Phenytoin dimensionless membrane permeabilities + standard error at steady-state rat jejunal perfusion as a function of phenytoin perfusate concentration. Permeabilities were not significantly different at a *t*-test *P* value ≤0.05.

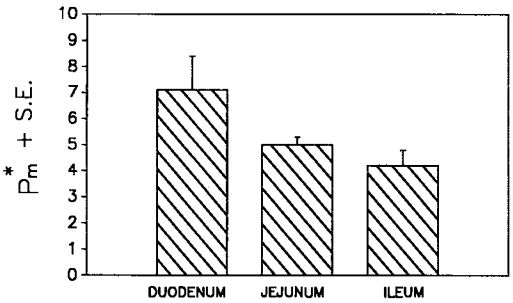

Fig. 5. Phenytoin dimensionless membrane permeabilities + standard error as a function of perfused rat intestinal region at steady state. Duodenal permeability was significantly different from jejunal and ileal permeabilities at a *t*-test *P* value ≤0.05. Duodenal/jejunal ring uptake of phenytoin was 1.00 ± 0.038 in 30 rings from three rats.

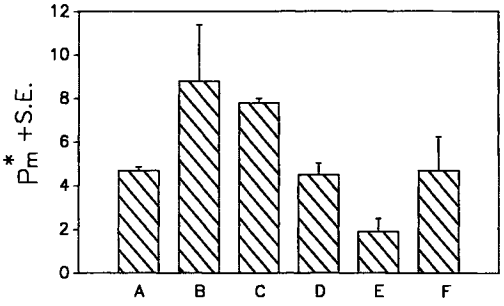

Fig. 6. Phenytoin dimensionless membrane permeabilities + standard error for (A) phenytoin, (B) + 20 m*M* glucose, (C) + 100 m*M* glucose, (D) + 20 m*M* glucose + 500 μ*M* phlorizin, (E) + 15–100 m*M* calcium chloride, and (F) + 2–5 m*M* calcium chloride. Treatments B, C, and E were significantly different from treatment A at a *t*-test *P* value ≤0.05.

glycol 4000 did not appear in the mesenteric blood for the short intestinal lengths used in these perfusions. Alternatively, it has been suggested (18) that changes in ion gradients across epithelial membranes influence membrane fluidity. A change in membrane fluidity would alter transcellular membrane diffusion, thus affecting membrane permeability or uptake of water and solutes.

Previous reports of glucose effects on passive intestinal absorption of drugs are conflicting (19,20). This is likely to be a function of both the drug studied and, as demonstrated here, the extent of experimental isolation. In the perfusion experiments, flow rate and intestinal length are chosen to minimize aqueous resistance to drug diffusion so as to optimize determination of membrane resistance (7). The stimulation of water absorption by lumenal iso-osmolar glucose results in convective flow sufficient to carry drug to the membrane, further reducing aqueous resistance. In this way, solvent drag may enhance drug uptake from perfusion.

In the ring tissue accumulation experiments, the fact that glucose did not enhance uptake may be the result of too great an aqueous diffusional resistance or too small a tissue capacity to generate solvent drag. The latter may be a function of the total tissue mass and surface area, viability and cellular glucose metabolism, and lack of availability of a transporting blood compartment. The villus swelling and edema seen in perfusion and not in short-term ring incubation are consistent with this conjecture (Fig. 2).

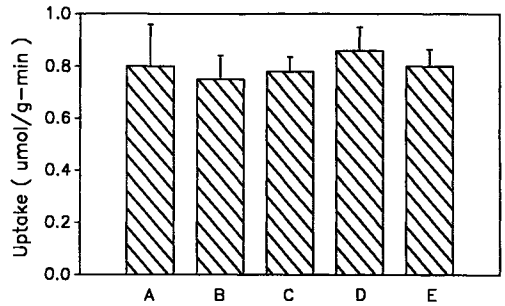

Fig. 7. Phenytoin jejunal ring uptake at 90 cycles/min at 37°C + standard error for (A) phenytoin, (B) + 5 m*M* glucose, (C) + 20 m*M* glucose, (D) + 100 m*M* glucose, and (E) + 100 m*M* alpha-methyl glucose (a nonmetabolizable sugar). There were no significant differences between treatments at a *t*-test *P* value ≤0.05.

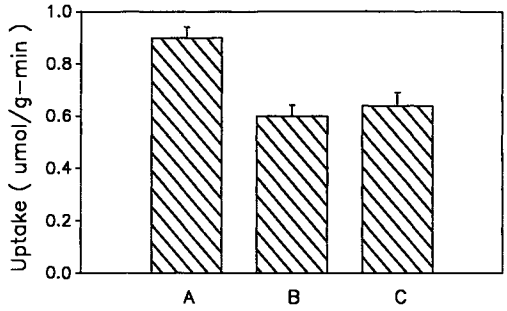

Fig. 8. Phenytoin duodenal ring uptake at 90 cycles/min at 37°C + standard error for (A) phenytoin, (B) + 10 m*M* calcium chloride, and (C) + 50 m*M* calcium chloride. Treatments B and C were significantly different from treatment A at a *t*-test *P* value ≤0.05.

In this regard, the paracellular pathway is a candidate for nutrient control of drug membrane uptake since its extent of availability is influenced favorably by low calcium (21) and high glucose levels (17). Variability in this pathway with respect to drug transport is a function of drug molecular size and polarity. Since phenytoin is a small molecule (molecular weight 252) and nonionized at perfusion pH, this pathway may vary with nutrient input for phenytoin uptake from solution.

The membrane permeability of phenytoin is quite high suggesting that PHT membrane transport is not rate limiting for its absorption *in vivo* (22). Furthermore, its high partition coefficient suggests that villus tip surface area provides adequate capacity for uptake of PHT reaching the membrane by aqueous diffusion and/or solvent drag. Nutrient effects on those drugs where passive absorption is controlled by membrane transport (low partition coefficient) or aqueous resistance as a function of gastrointestinal residence time and availability from dissolution (6) (i.e., effective resistance to uptake from solution) may be of special clinical significance.

ACKNOWLEDGMENTS

This research was supported by University of Michigan Rackham Graduate School Grant 386043 and a National Institutes of Health Grant 1 R29 NS24616-01.

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