the bioassay of samples containing multiple drugs or abnormal levels of normally occurring metabolic products.

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Absorption of Sodium γ-Hydroxybutyrate and Its Prodrug γ-Butyrolactone: Relationship between *In Vitro* Transport and *In Vivo* Absorption

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Received August 20, 1979, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, NY 14260.* Accepted for publication October 11, 1979.

Abstract □ A qualitative relationship between *in vitro* transport and *in vivo* absorption of sodium γ-hydroxybutyrate and γ-butyrolactone was demonstrated. As with other short-chain acids, sodium γ-hydroxybutyrate showed capacity-limited transport *in vitro*, consistent with the previous observation that this drug exhibited slower *in vivo* absorption with increasing dose. The prodrug lactone, on the other hand, showed a higher intestinal flux than the acid in the everted gut, and *in vivo* absorption also was more rapid. Capacity-limited transport and absorption of the lactone appeared less evident. Thus, the increased oral hypnotic activity of the lactone over that of the acid most likely is a result of its more favorable intestinal transport characteristics.

Keyphrases □ Sodium γ-hydroxybutyrate—relationship between *in vitro* transport and *in vivo* absorption □ γ-Butyrolactone—prodrug for sodium γ-hydroxybutyrate, relationship between *in vitro* transport and *in vivo* absorption □ Hypnotic agents—sodium γ-hydroxybutyrate and γ-butyrolactone, relationship between *in vitro* transport and *in vivo* absorption

γ-Hydroxybutyrate (I), a metabolite of γ-aminobutyric acid, is found endogenously in the human brain (1). When introduced intravenously, I is a useful anesthetic (2) and is beneficial in Parkinson's disease (3). However, oral administration of this compound results in decreased and variable pharmacological activity (4–6). Recently, oral doses of I totaling 50 mg/kg were shown to be useful in the treatment of narcolepsy and cataplexy in patients, but the duration of sleep induction after each oral dose lasted only for ~2 hr (7).

BACKGROUND

In previous animal studies in these laboratories (8–10), orally administered I was shown to be subject to first-pass metabolism at low doses (≤200 mg/kg) in rats. At higher doses (400–1600 mg/kg), systemic availability approached 100%, presumably due to saturation of first-pass metabolism, but the relative absorption rate appeared to decrease with increasing dose. Thus, although the extent of drug absorption was almost complete, peak plasma I concentrations were relatively insensitive to increases in the oral dose and, in most animals, threshold hypnotic concentrations in plasma were not reached in spite of high oral doses.

The lactone analog of I, γ-butyrolactone (II), is hydrolyzed rapidly and exclusively *in vivo* to I (11, 12) and, therefore, can be classified as a prodrug. Compound II is rapidly and completely absorbed *in vivo* after oral administration over a wide dose range. In contrast to I, the peak drug concentration after oral dosing of II was proportional to the dose, and II was equally effective as a hypnotic whether given orally or intravenously (9).

The reason for the apparent difference in *in vivo* absorption characteristics between I and II has not been delineated. In this paper, *in vitro* experiments that compared the transport properties of these two compounds across the everted rat gut are described.

EXPERIMENTAL

Reagents—Compound I, obtained as the sodium salt[1], and II[1] were used without purification. The buffer and assay reagents[1–3] were all reagent or analytical grade.

Everted Rat Gut Preparation—Male Sprague–Dawley rats, 260–310 g, were sacrificed by decapitation. An intestinal segment, ~12 cm long, was taken from a region 20 cm from the pylorus sphincter; it was everted and mounted according to the technique originally devised by Wilson and Wiseman (13) and modified by Crane and Wilson (14).

Flux Experiment—The everted gut was placed inside a test tube with the mucosal side exposed to 90 ml of a 0.05 *M* physiological tromethamine buffer (pH 7.4) containing the appropriate drug concentration. All flux studies were carried out at 37°. At 5-min intervals up to 25 min, the serosal solution (~1 ml) was removed for the assay and replaced with an equal volume of fresh buffer. Three or four replicate flux experiments were conducted at each initial mucosal concentration.

Spectrophotometric Analysis—The Hestrin (15) assay for short-chain *O*-acyl derivatives as adopted for I and II by Guidotti and Ballotti (16) was employed. Conversion of I to II was effected by reaction with two parts of concentrated sulfuric acid[2] and subsequent neutralization with 10 parts of 6 *N* NaOH[2].

[1] Eastman Kodak Co., Rochester, NY 14650.
[2] Fisher Scientific Co., Fair Lawn, NJ 07410.
[3] J. T. Baker Chemical Co., Phillipsburg, NJ 08865.

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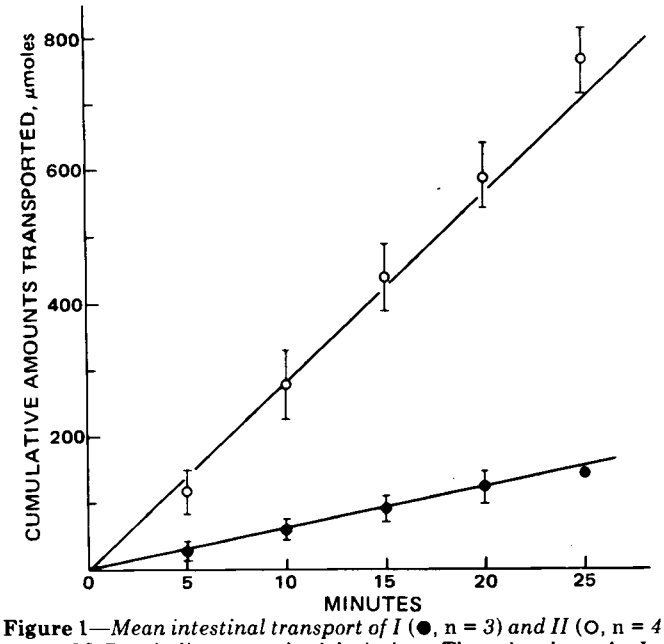

Figure 1—*Mean intestinal transport of I (●, n = 3) and II (○, n = 4) at 0.40* M. *Bars indicate standard deviations. The point shown for I at 25 min represents the mean value of two measurements.*

RESULTS AND DISCUSSION

Transport of I and II through the everted rat gut was examined at various initial mucosal drug concentrations. Intestinal flux was determined for each animal preparation by linear regression of a plot of cumulative amount transported to the serosal side *versus* time. Representative plots showing intestinal transport of I and II at 0.40 *M* are given in Fig. 1. At low mucosal concentrations, linearity of flux was maintained throughout the experiment. However, at high I concentrations, positive deviations (increased flux) occurred at the later time points, suggesting possible tissue damage with prolonged drug exposure. In these instances, initial rates of transport restricted to the linear portion of the curve (usually 0–20 min) were used to calculate flux. In all experiments, the total amounts transported to the serosal side were small (<0.4% for I and <2.5% for II) compared to the total drug available from the mucosal pool. Thus, the initial mucosal concentration remained essentially unchanged throughout each experiment.

Figure 2 shows the relationships between intestinal flux of I and II and their respective mucosal concentrations. Over the concentration range studied, intestinal transport of II was considerably more rapid than that of I. At equimolar mucosal concentrations, the differences in flux between I and II were statistically significant at $p < 0.001$ using the Student t test. Compound II fluxes were ~5, 7, and 10 times higher than I fluxes at 0.40, 0.79, and 1.19 *M*, respectively. In addition, I transport leveled off at concentrations above 0.40 *M*. If nonspecific effects on intestinal permeability could be ruled out, this flux behavior suggested the presence of a capacity-limited transport system for I in the rat intestine. In comparison, concentration-dependent transport of II was less evident.

In these experiments, the ionic strength in the mucosal solution was not constant over the concentration range studied. Although the mucosal solution was prepared with buffer, high I concentrations also could affect the pH slightly because I, as its sodium salt, is mildly basic. The leveling in I flux could, in principle, have been partially contributed to by nonspecific ionic strength and/or pH effects created by increasing mucosal concentrations of the ionic drug. The possibility of this artifact was ruled out by the following experiment.

Flux studies were carried out at 0.08 *M* I under two sets of conditions. In one case, no pH or salt adjustments were made (Condition A: pH 7.4, $\mu = 0.23$ *M*); in the other case, sodium chloride and sodium hydroxide were added so that the pH and ionic conditions were equivalent to those present when flux was studied at 1.19 *M* I (Condition B: pH 8.1, $\mu = 1.34$ *M*). If ionic strength and pH affected flux significantly, then the observed fluxes under Conditions A and B would be different, with the flux of B similar to that observed at 1.19 *M* I. In fact, the flux of I was identical whether or not additional salt or alkalinizing agents were added.

In duplicate determinations, the fluxes obtained under Condition A were 2.2 and 2.3 μ*M*/min; those under Condition B both were 2.4 μ*M*/min. Thus, minor differences in pH and ionic strength contributed by changes

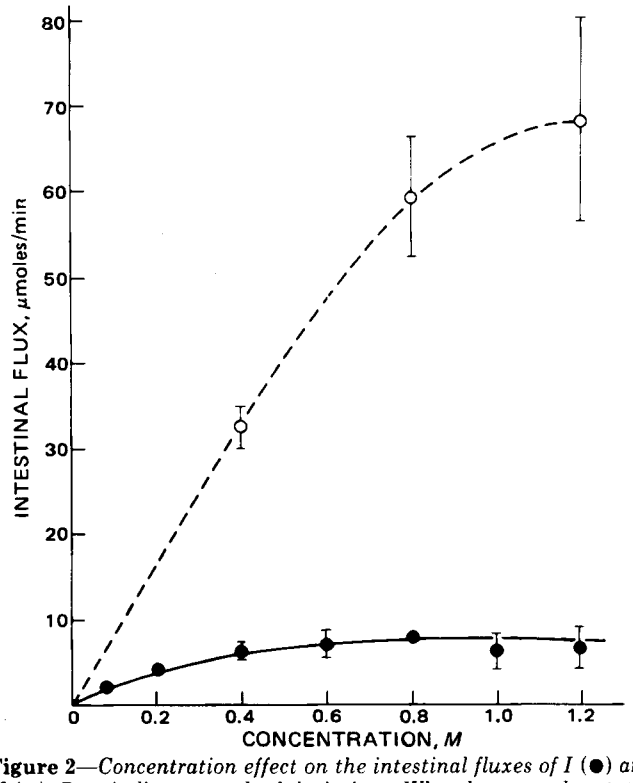

Figure 2—*Concentration effect on the intestinal fluxes of I (●) and II (○). Bars indicate standard deviations. When bars are absent, the standard deviations were too small to be shown.*

in the mucosal I concentration did not affect flux significantly. Since II is nonionic, ionic strength and pH effects produced by increasing II concentrations were presumed to be negligible. Bender *et al.* (17) found the second-order alkaline hydrolytic constant of II to be ~0.2 liter/mole-sec at 25°. At pH 7.4, the hydrolysis half-life would be about 1000 days. Thus, conversion of II to I in the buffered mucosal solution was insignificant during the experiment.

The *in vitro* transport characteristics of I and II are consistent with their *in vivo* absorption properties reported previously (8–10). Compound I, which showed capacity-limited transport *in vitro*, also exhibited relatively slower *in vivo* absorption rates with increasing oral dose (10). Other short-chain acids, such as acetic and butyric acids, also have been shown to be transported *via* an active system (18, 19). Therefore, a capacity-limited absorption mechanism might be a reason for the decreased and variable activity of I when given in high oral doses to humans (4–7). The prodrug lactone II, on the other hand, showed a much higher intestinal flux than I in the everted gut and was almost instantaneously absorbed when orally administered (9). Capacity-limited transport of II, if existent, appeared to occur at much higher drug concentrations.

The present study demonstrated a qualitative relationship between *in vitro* transport and *in vivo* absorption of the two compounds studied. Thus, the increased oral activity of the lactone over that of its open-chain hydroxy acid is most likely a result of its more favorable intestinal transport characteristics. The usefulness of II has not been investigated in humans.

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Temporal Variations in Trough Serum Theophylline Concentrations at Steady State

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Received June 21, 1979, from the **Drug Concentration Laboratory, University of Massachusetts Medical Center, Worcester, MA 01605*, and ‡*Fisons Corporation, Bedford, MA 01730.* Accepted for publication October 11, 1979.

Abstract □ Temporal variations in serum theophylline concentrations were observed in 14 healthy volunteers receiving multiple doses of theophylline. After repeated oral doses (6.9–18.2 mg/kg/day) of theophylline as either a nonalcoholic aminophylline solution or a controlled-release capsule, trough theophylline levels at steady state were significantly higher ($p < 0.05$) in the morning than in the afternoon or evening. With the solution, the mean ($\pm SE$) trough serum level at 7 am was 11.1 ± 0.9 μg/ml, and at 1 pm it was 9.6 ± 0.8 μg/ml. With the capsule, the mean ($\pm SE$) trough serum level at 8 am was 13.8 ± 0.9 μg/ml, and at 8 pm it was 10.7 ± 0.9 μg/ml. Temporal variations in serum theophylline concentrations have not been reported previously and may be important in therapeutic monitoring.

Keyphrases □ Theophylline—trough serum concentrations at steady state, temporal variations □ Bronchodilators—theophylline, trough serum concentrations at steady state, temporal variations □ Pharmacokinetics—theophylline, trough serum concentrations at steady state, temporal variations

Temporal variation in the absorption and disposition of drugs is an area of pharmacokinetics about which relatively little is known. In the few studies performed, the findings have not been consistent. For example, Shirley and Vesell (1) reported that temporal variations in the disposition of acetaminophen and phenacetin occur. However, Vesell *et al.* (2) observed no temporal variations in the pharmacokinetics of antipyrine (2), and Nakano and Hollister (3) reported no time-related changes in the disposition of nortriptyline. The causes of temporal variations in drug pharmacokinetics may be varied. Circadian rhythm apparently influences the distribution of potassium between body compartments (4), while changes in body posture alter the absorption of cephradine (5) and erythromycin (6) from the GI tract.

One mechanism suggested to account for the temporal variations in the disposition of phenacetin and acetaminophen was the occurrence of diurnal changes in the amount and activity of hepatic microsomal oxidases (1). Theophylline is a drug whose disposition also is determined by microsomal oxidases, so it seemed possible that temporal variations in theophylline disposition may occur. Since this aspect of theophylline kinetics had not been reported previously, one objective of this study was to determine if temporal variations exist.

EXPERIMENTAL

Subjects—The seven male and seven female volunteers were 21–40 years old, and their average weight was 67.5 kg. All volunteers were nonsmokers and were in good physical health with no history of alcoholism or cardiovascular disease.

Drug Administration and Blood Sampling—The volunteers randomly received either a nonalcoholic aminophylline solution or a controlled-release theophylline capsule. The oral theophylline dose was individualized for each volunteer, based on single-dose kinetics, to produce peak serum theophylline concentrations no larger than 18 μg/ml after repeated dosing. The daily doses ranged from 6.9 to 18.2 mg/kg. The solution was administered at 7 am, 1 pm, 7 pm, and 1 am, and the capsule was given at 8 am and 8 pm. Dosing was continued for 6 days prior to each study day. The study days were separated by 1 week during which the volunteers took the alternate formulation.

On each study day, 1 ml of serum was obtained immediately before the morning dose of each dosage form and 6 or 12 hr after administration of the solution or capsule, respectively.

Theophylline Assay—Serum theophylline determinations were made by high-pressure liquid chromatography using a method described previously (7).

Data Analysis—A paired t test was used to analyze within-subject differences between the am and pm trough theophylline concentrations observed for each dosage form.

RESULTS AND DISCUSSION

The am and pm trough serum theophylline concentrations determined for each dosage form are listed in Table I. The percentage changes in trough level are noted for each volunteer. The mean ($\pm SE$) serum theophylline concentration at 7 am for the solution was 11.1 ± 0.9 μg/ml, while at 1 pm the serum theophylline concentration was 9.6 ± 0.8 μg/ml, representing a change of 13%. For the capsule, the mean ($\pm SE$) serum theophylline concentration at 8 am was 13.8 ± 0.9 μg/ml, and at 8 pm it was 10.7 ± 0.9 μg/ml, reflecting a decrease of 24%. The differences between the am and pm serum theophylline concentrations were significant ($p < 0.05$) for each dosage form.

Based on these results, there appear to be temporal variations in theophylline pharmacokinetics. Higher trough levels at 7 or 8 am compared to those at 1 or 8 pm may be related to a shorter plasma half-life at the latter times. Indeed, Shirley and Vesell (1) reported that plasma half-lives of phenacetin and acetaminophen were ~15% shorter at 2 pm than at 6 am. Another possible cause of higher am trough levels may be

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