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Factors influencing the transfer of xanthines across the everted rat jejunum

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Summary

A number of factors having the potential to influence the transfer of theophylline across the everted rat jejunum were examined. The transfer of several other xanthine derivatives was also investigated. Strophanthin-K and 2,4-dinitrophenol had no effect on theophylline transfer. The transfer of theophylline from a 2 : 1 complex with phenobarbital was identical to that of theophylline alone. Magnesium ion (120 mM) and hypertonicity (600 mOsm) had no effect on the transfer of theophylline at pH 5.5. With the exception of xanthine, the clearance of all of the xanthine homologs tested was related to their partition coefficients in a chloroform/pH 7.4 phosphate buffer.

Introduction

In a previous report (Perry et al., 1984) the effect of pH on the transfer of theophylline across the everted rat jejunum was examined. The structural integrity and biochemical viability of the intestine were also assessed using light and scanning electron microscopy, and glucose transfer measurements, respectively. In this report these studies are extended to include a discussion of some other factors affecting the transport of theophylline and an examination of how structural alterations in the xanthine nucleus affect the intestinal transfer of several homologs.

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Materials and Methods

Materials

The following reagent grade chemicals were employed: 8-chlorotheophylline, 7-(2-chloroethyl)theophylline, 3-methylxanthine (Aldrich Chemicals, Milwaukee, WI), theophylline, theobromine, sodium phenobarbital, and 2,4-dinitrophenol (Sigma Chemicals, St. Louis, MO), strophanthin-K (Burroughs-Wellcome, Research Triangle Park, NC), monosodium xanthine (Schwarz/Mann Div., Becton, Dickinson and Co., Orangeburg, NY), caffeine (Merck, Rahway, NJ), allopurinol (Calbiochem, San Diego, CA), dichloromethane and acetonitrile (Burdick and Jackson Labs., Muskegon, MI). The 2,4-dinitrophenol was purified by converting it to its sodium salt by reacting it with an excess of 6 N sodium hydroxide, following which it was recrystallized from 95% v/v ethanol. Monobasic sodium phosphate with one water of hydration, dibasic sodium phosphate anhydrous, sodium carbonate, and sodium bicarbonate (all of reagent grade) were used in preparing the isotonic buffers.

Methods

The preparation of the everted rat jejunal segments, the compositions of the buffers used and the measurement of cumulative transfer rates were as reported previously (Perry et al., 1984). The clearances were calculated from the slopes of plots of the cumulative amount of drug transferred per unit mucosal concentration vs time using least-squares linear regression of the 0–30-min data. The use of only the early time points ensured that the data were obtained while the intestine was still viable (Perry et al., 1984). The xanthine derivatives were assayed by a high-performance liquid chromatographic (HPLC) procedure similar to one described earlier for theophylline (Perry et al., 1984).

Determination of the pK_a of 8-chlorotheophylline

This was accomplished by preparing a 0.01 M solution of 8-chlorotheophylline in acetonitrile : water (25 : 75, v/v). Two 20 ml aliquots of this solution were then titrated with 0.1 N sodium hydroxide at 25°C while monitoring the pH with a digital pH meter (Markson Electromark, Markson Science, Del Mar, CA). The pH was recorded after each addition of the titrant and the K_a, determined using the Gran plot program of Seymour and Fernando (1977), was 3.614×10^{-6} ($pK_a = 5.44$). Although measured in a non-aqueous system, this value is in close agreement with values reported elsewhere (Maulding and Zoglio, 1971; Meyer and Guttman, 1968) which were located subsequent to our own experimental determination.

Preparation of a 2 : 1 theophylline–phenobarbital complex

The theophylline–phenobarbital complex was prepared according to a method described by Higgins and Dunker (1944). A solution of 1 g of phenobarbital in 10 ml of water was added to 50 ml of hot ethanol containing 1.55 g of theophylline. The mixture was allowed to cool and the crystals which formed were filtered off. After one recrystallization from ethanol the crystals had an MP of 253°C. This compares favorably with the value of 250.7–251.7°C reported by Higgins and Dunker (1944).

Determination of the apparent partition coefficients of the xanthine derivatives

Solutions of each xanthine (10^{-3} M) were prepared in 0.1 M phosphate buffer, pH 7.4, that was previously saturated with chloroform. Five ml of each solution was then pipetted into 15 ml screw-capped culture tubes. Then 5 ml of chloroform, previously saturated with pH 7.4 phosphate buffer, was added to each tube and the tubes were capped. Triplicate samples of each compound were mixed on a shaker (Catalog No. 6000, Eberbach, Ann Arbor, MI) for 5 h at low speed. After equilibration, the two phases were separated by centrifugation. The concentrations of each compound in the equilibrated and control (non-equilibrated) buffer phases were determined by ultraviolet spectroscopy at their absorption maxima against appropriate blanks, by comparison with previously prepared standard curves. The apparent partition coefficient (APC) for each compound between an aqueous pH 7.4 phosphate buffer and chloroform was calculated using the following expression:

$$\text{APC} = \frac{C_i - C_e}{C_e} \tag{1}$$

where C_i = initial concentration of compound in buffer, and C_e = equilibrium concentration of compound in buffer.

Data treatment

The effects of the biochemical inhibitors (2,4-dinitrophenol and strophanthin-K) and complexation with phenobarbital on the intestinal transfer of theophylline were evaluated using unpaired *t*-tests, with $P < 0.05$ being regarded as the minimum level of significance.

Results and Discussion

Effect of biochemical inhibitors on theophylline transfer

In order to determine whether or not the transport of theophylline across the intestinal membrane was an active process, experiments were performed using inhibitors to disrupt various biochemical functions of the intestine. In one set of experiments the sodium salt of 2,4-dinitrophenol, a potent uncoupler of oxidative phosphorylation, was added to the mucosal bathing solution at a concentration (1 mM) sufficient to inhibit metabolic processes (Berlin and Hawkins, 1968a and b; Coupar and McColl, 1975). The transfer of theophylline in those segments to which 2,4-dinitrophenol was added was not significantly different from that of control segments (mean clearance = 0.0172 ml/min, $P = 0.39$).

There is evidence to suggest that the intestinal transport of some drugs in vitro may be related to sodium-ion-coupled transport (Turner et al., 1970; Benet et al., 1971) such as that known to exist for sugars and amino acids (Coupar and McColl, 1975; Mayersohn and Gibaldi, 1969). In this process nutrients, and possibly drugs, enter the mucosal cell with Na^+ via receptors on the microvillus membrane. They are then transported across the cell and extruded with Na^+. This process is controlled by

a Na^+, K^+-ATPase 'pump' which can be specifically inhibited by the cardiac glycoside strophanthin-K.

To investigate whether this process might be involved in theophylline transfer across the intestine, experiments were performed in which strophanthin-K (1 mM) was added to the serosal side of two intestinal segments and to the mucosal side of another two segments, in order to determine on which side it might exert its greatest effect. Regardless of which side of the intestinal segment was exposed to strophanthin-K, no statistically significant change in theophylline transfer was observed, i.e. mean clearance = 0.0168 ml/min, $P = 0.37$. This observation is in agreement with the work of Mayersohn and Gibaldi (1969) who found no differences in the transfer rates of several water-soluble drugs across the everted rat intestine in the presence of 1 mM ouabain, another cardiac glycoside reported to specifically inhibit Na^+ transport at this concentration.

These combined data indicate that the transfer of theophylline across the everted rat intestine is a passive process. This conclusion is further substantiated by the work of Sanvordeker (1978) who noted that theophylline transfer across the everted rat intestine obeyed Fick's Law over a 10-fold range of concentration.

Effect of complexation with phenobarbital on the transfer of theophylline across the everted rat intestine

Bettis et al. (1973) observed lower and more slowly attained serum theophylline levels in man following the oral administration of a 2 : 1 theophylline–phenobarbital complex than after the administration of theophylline alone. One possible explanation for this is a decreased intestinal absorption resulting from a lower lipid or aqueous solubility of the theophylline–phenobarbital complex. These authors showed that the dissolution rate of the complex was, in fact, slower than that of theophylline or phenobarbital alone. Since the lower serum levels of theophylline observed by these workers could also have been due to a reduced intestinal permeability of the complex, the present experiment was designed to test the effect of complexation with phenobarbital on the transfer of theophylline across the everted rat jejunum.

In these experiments the mucosal bathing solution contained the dissolved complex at a concentration equivalent to 50 μg/ml of theophylline. Control segments were run in which the mucosal solution contained an equivalent concentration of theophylline alone. The results of these experiments indicated that the transfer of theophylline across the everted intestine from the complex was not significantly different from that of theophylline alone (mean clearance = 0.0151 ml/min, $P = 0.14$). No rigorous testing was done to determine whether or not this complex exists in solution. However, these data indicate that the presence of phenobarbital in solution, or as a complex with theophylline, does not affect theophylline transfer across the intestine in vitro. This conclusion is supported by the findings of Bettis et al. (1973) who showed that human serum theophylline levels after the oral administration of a physical mixture of theophylline and phenobarbital (not as a complex) were identical to those following the administration of theophylline alone. Thus it appears that the reduced bioavailability of the theophylline–phenobarbital complex is primarily due to its slower dissolution rather than any effect on the intestinal transfer of the theophylline.

Effect of magnesium ion and hypertonicity on the intestinal transfer of theophylline

Although generally considered innocuous, antacids can affect the absorption and pharmacologic effects of other orally administered drugs. Interactions have been documented wherein antacids chelated, complexed with, or altered the dissolution or ionization of drugs through direct interactions, pH shifts, or changes in gastrointestinal function (Hurwitz and Sheehan, 1971). Magnesium hydroxide, one of the most common ingredients in antacids, has been shown to depress the absorption in vivo of pentobarbital, sulfadiazine, and quinine by altering the pH of the gastrointestinal environment (Hurwitz and Sheehan, 1971; Hurwitz, 1974).

The effect of pH on the transfer of theophylline across the everted intestine has been reported recently (Perry et al., 1984). The present experiments were designed to examine the effect of Mg^{2+} ion on the transfer of theophylline across the everted rat intestine in vitro. All experiments were performed with a mucosal theophylline concentration of 50 μg/ml and a pH of 5.5 in order to keep the Mg^{2+} ion in solution. At pH values above 6.5 the solubility of Mg^{2+} is reduced due to the formation of the relatively insoluble salt, magnesium hydroxide, $Mg(OH)_2$.

Mucosal buffers containing 120 mM magnesium chloride ($MgCl_2$) were used to test the effect of Mg^{2+} ion on the intestinal transfer of theophylline. This concentration of $MgCl_2$ increased the osmolality of the buffer to 600 mOsm, twice that of the normal buffer solution. Therefore, to eliminate the presence of an osmotic gradient the osmolality of the serosal buffer was made equal that of the mucosal buffer by the

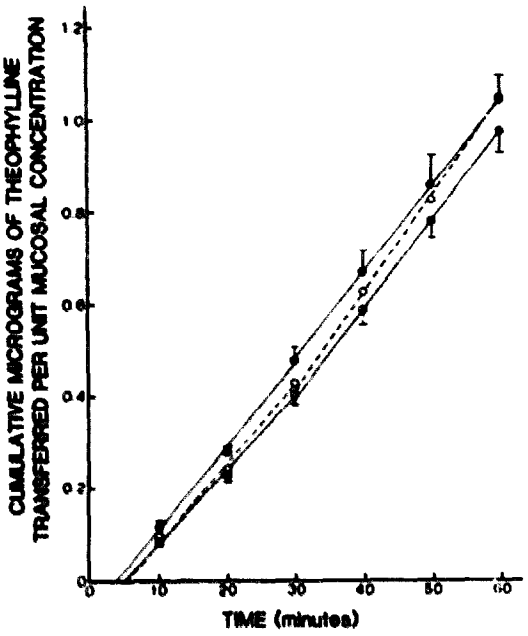


Fig. 1. The effect of Mg^{2+} ion and hypertonicity on theophylline transfer across the everted rat jejunum. All experiments were conducted at pH 5.5 due to the poor solubility of Mg^{2+} at higher pH values. Each data point represents the mean ± 1 S.E.M. of 6 segments. Legend: ●———●, NaCl 300 mOsm mucosal and serosal; O·····O, NaCl 600 mOsm mucosal and serosal; ◑———◑, $MgCl_2$ 600 mOsm mucosal, NaCl 600 mOsm serosal.

addition of NaCl (9.6 g/l). Control experiments were run in which an iso-osmolar amount of NaCl replaced the mucosal $MgCl_2$. Theophylline transfer measured under these conditions was compared to its transfer under isotonic conditions. Fig. 1 summarizes these data. A one-way ANOVA performed on the clearance values detected no significant differences between any of the groups, i.e. all P values were > 0.05. These results agree with the findings of Hurwitz and Sheehan (1971) who found that salts of magnesium other than the hydroxide had no effect on the in vitro intestinal absorption of pentobarbital in the rat.

The lack of effect of hyperosmolar solutions on the transfer of theophylline across the everted gut was interesting since Mayersohn and Gibaldi (1971) found a statistically significant increase in the transfer of riboflavin across an everted rat intestinal preparation when isotonic mucosal and serosal solutions were replaced by hypertonic (500 mOsm) bathing solutions. While they also observed that the transfer of salicylate and sulfanilamide was increased using hypertonic bathing solutions, the changes were not statistically significant. These data, together with our results for theophylline indicate that tonicity changes may affect the transfer of some compounds, but not others.

Intestinal transfer of some xanthine homologs

The intestinal clearance of theophylline and 6 other xanthine derivatives was compared in order to determine the relationship, if any, between the degree of N-alkyl substitution of the xanthine nucleus and the clearance across the everted rat jejunum. The compounds chosen for investigation differed only in their alkyl substitution at the N-1, N-3, and N-7 positions except for 8-chlorotheophylline which, in addition to the N-methylation at N-1 and N-3, has a chlorine atom at C-8. In all but one compound, 7-(2-chloroethyl) theophylline, the substituents were methyl groups.

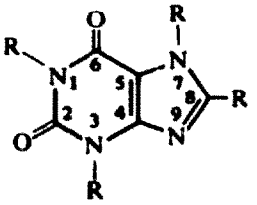
Except for xanthine itself, all transfer experiments were conducted under identical conditions of pH 7.4, 37°C, and a mucosal drug concentration of 50 μg/ml. In order to examine the transfer of xanthine across the intestinal membrane, the enzyme xanthine oxidase, which exists in high concentrations in the columnar epithelial cells of the rat intestine, had to be inhibited (Berlin and Hawkins, 1968a) since it catalyzes the oxidation of xanthine to uric acid. It was confirmed that unless xanthine oxidase activity was blocked, accurate measurements of xanthine transfer across the intestinal membrane were impossible. Inhibition of this enzyme was achieved by pretreating rats with an oral dose of 25 mg/kg of allopurinol at least 3 h prior to sacrifice, and by adding allopurinol (1 mg/ml) to the mucosal bathing solution. Neither allopurinol nor uric acid interfered with the HPLC assay used.

The theoretical degree of ionization of the 7 xanthine derivatives at pH 7.4 was calculated from their pK_a values using the Henderson-Hasselbalch equation. The lipid solubilities of these compounds at pH 7.4 were estimated from their apparent partition coefficients. Table 1 summarizes these data along with the clearances and pK_a values of this series of xanthine derivatives.

It can be seen that increasing the N-substitution of the xanthine moiety results in a large increase in the relative lipid solubility. Methylation at positions N-1, N-2,

TABLE 1

PHYSICAL PROPERTIES OF SOME XANTHINES AND THEIR CLEARANCE ACROSS THE EVERTED RAT INTESTINE AT PH 7.4



Compound	R_1	R_3	R_7	R_8	pK_a	Fraction unionized at pH 7.4	APC [a]	Clearance (Cl) (ml/min)×10^2	
								0–30 min	30–60 min
Xanthine (1) [b]	H	H	H	H	7.53 [c]	0.5743	0.001	1.421	1.985
3-Me-xanthine (2)	H	CH_3	H	H	8.5 [c]	0.9264	0.013	0.518	1.385
8-Cl-theophylline (3)	CH_3	CH_3	H	Cl	5.44	0.0108	0.039	0.623	1.255
Theophylline (4)	CH_3	CH_3	H	H	8.5 [c]	0.9264	0.325	1.721	2.236
Theobromine (5)	H	CH_3	CH_3	H	10.05 [c]	0.9978	0.601	0.952	1.647
Caffeine (6)	CH_3	CH_3	CH_3	H	13.9 [c]	1.000	20.10	2.883	3.143
7-(2-chloroethyl) theophylline (7)	CH_3	CH_3	C_2H_4Cl	H	14.0 [d]	1.000	53.90	3.018	3.256

[a] The APC was determined in a chloroform/pH 7.4 phosphate buffer system.
[b] Numbers in parentheses correspond to those in Fig. 2.
[c] The sources for the listed pK_a values, by compound number, are as follows: (1) Murgia et al., 1973; (2) and (4) Bergmann et al., 1972; (5) Anon, 1976; (6) Rowland and Tozer, 1980.
[d] The pK_a value was estimated to be approximately equal to that of caffeine.

and N-3 (as in caffeine) results in an APC about 20,000 times greater than that of xanthine and 60 times greater than theophylline. Replacement of the N-7 methyl group of caffeine with a 2-chloroethyl group increases the lipid solubility to about 3 times that of caffeine. In contrast, substituting a chlorine atom at the 8-position of theophylline reduces the APC to about 1/8 that of theophylline. This is a result of the strong electron-withdrawing effect of the chlorine atom which increases the acidity of the hydrogen at the N-7 position.

A relationship recently used (Sanvordeker et al., 1977; Plá-Defina and Moreno, 1981) for correlating the clearance of compounds across everted intestinal sacs with their partition coefficients was utilized in an attempt to determine whether a similar relationship existed for the xanthine homologs used here. The equation expressing this relationship is:

$$\log Cl = M \log APC + \log k \qquad (2)$$

where Cl equals the clearance of a compound, k is the clearance constant, APC is the apparent partition coefficient, and M is an exponential term referring to the chloroform/pH 7.4 buffer system. Plotting log Cl versus log APC should result in a straight line with slope M and intercept log k.

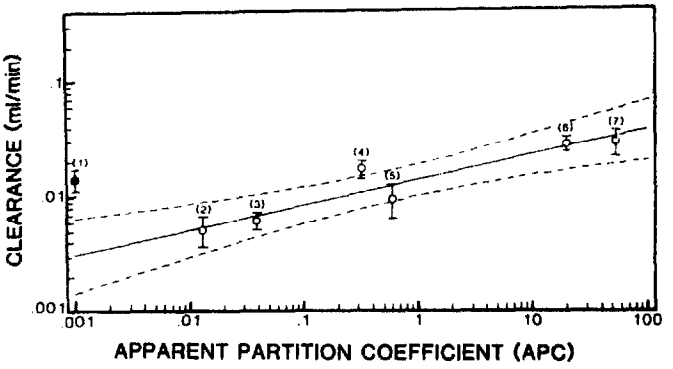


Fig. 2. Relationship between the 0–30-min clearance (Cl) and the apparent partition coefficient (APC) of several xanthines. The identification numbers above each compound correspond to those listed in Table 1. Xanthine (solid circle) was not included in the regression. Each data point represents the mean Cl ± 1 S.D. from 6 intestinal segments. Dashed lines represent the 95% confidence interval.

Fig. 2 illustrates this plot for the xanthines tested, except for xanthine which appeared to deviate from this relationship. Regression analysis of the observed data yielded an excellent linear correlation ($r = 0.9458$; $P < 0.005$). The equation which best describes this relationship for the xanthine derivatives tested is:

$$\log Cl = 0.2175(\pm 0.0374) \log APC - 1.8544(\pm 0.0495) \quad (3)$$

$$n = 6,\ s = 0.1203$$

where n is the number of data points fitted, s is the standard error of the estimate, and the values in parentheses are the standard deviations of the constants.

The unusually large clearance of xanthine, which excludes it from the clearance/APC relationship, cannot be explained by examining the physical or chemical data on this compound. Even though its pK_a indicates that at pH 7.4 it is 57.4% unionized, its APC is so low that its intestinal transfer should be the slowest of all the xanthine derivatives. In fact, its clearance is not significantly different from that of theobromine and theophylline, both of which are considerably more lipid-soluble than xanthine. The result for xanthine is consistent with some previous observations by Kakemi et al. (1969). These authors found that the absorption of xanthine, theobromine and caffeine from the rectum of rats was in perfect rank-order agreement with their chloroform/phosphate buffer partition coefficients. However, when the absorption of these 3 compounds from the small intestine was measured they found that xanthine, which had the smallest partition coefficient, the fastest rate of absorption. Unfortunately, these authors did not inhibit the xanthine oxidase known to be present in the rat small intestine (Westerfeld and Richert, 1949) and known to be capable of rapidly converting xanthine to uric acid (Khan et al., 1975). Thus their results for the intestinal transfer of xanthine are questionable, especially in view of the fact that they utilized a spectrophotometric assay which would not likely differentiate xanthine from uric acid.

Schanker and Tocco (1960) concluded that thymine and uracil cross the intestinal membrane by both active and passive processes, which accounts for their large

intestinal absorption despite their low lipid solubility. It was suggested (Berlin and Hawkins, 1968a and b) that a similar transport mechanism might exist for the oxypurines: xanthine, hypoxanthine and uric acid. However, Khan et al. (1975) could not find evidence of an active transport mechanism for these 3 oxypurines in rat or hamster jejunum. Although no evidence for the active transport of theophylline was observed in this study no attempts were made to examine this possibility for xanthine.

An obvious change in clearance at about 30 min was also observed for all of the xanthine homologs tested, except caffeine and 7-(2-chloroethyl) theophylline. This change in clearance corresponds to the change in slope observed in Fig. 1 for theophylline. The two compounds with the smallest change in slopes were caffeine and 7-(2-chloroethyl) theophylline (see Table 1), both of which are very lipophilic and essentially unionized at pH 7.4. The largest change in slope was observed for 3-methylxanthine and 8-chlorotheophylline. The 3-methylxanthine is transferred slowly due to its low lipophilicity while the low clearance of 8-chlorotheophylline is presumably due to its high degree of ionization at pH 7.4.

This observation supports the conclusion presented by Benet et al. (1971) and Gibaldi and Grundhofer (1972) who postulated that this rate of change of cumulative transfer is due to some time-dependent alteration in the in vitro intestinal membrane that affects only those compounds that are poorly lipid-soluble.

Conclusions

The metabolic inhibitor 2,4-dinitrophenol and the Na^+, K^+-ATPase inhibitor, strophanthin-K, had no demonstrable effect on the intestinal clearance of theophylline, indicating that its transfer across the everted rat jejunum is a passive process.

The intestinal transfer of theophylline from a 2 : 1 theophylline–phenobarbital complex was shown to be essentially identical to that of theophylline alone.

Neither magnesium ion (120 mM) nor hypertonicity (600 mOsm) affect the in vitro intestinal transfer of theophylline at pH 5.5.

The clearance of all the xanthine homologs, except for xanthine, were related to their chloroform/pH 7.4 phosphate buffer partition coefficients.

Acknowledgement

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