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Effect of pH, buffer concentration and buffer composition on the absorption of theophylline from the small intestine of the rat

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Summary

The absorption of theophylline from the small intestine of the rat was investigated using buffer solutions of different pH (3.0–9.2), composition and concentration. The technique used, encloses luminal perfusion of an intestinal loop with collection of the blood draining the perfused loop, which enable calculation of absorption rates into the blood, disappearance rates from the lumen and mass balances. It was found, that in the presence of borate buffer, pH 9.2, the absorption rate of theophylline was decreased compared to the other perfusates. There was a significant difference between the absorption rate and the disappearance rate in the presence of phosphate and borate buffer. The low absorption rate in the presence of the borate buffer was attributed to a thickening effect of borate ions on intestinal mucus, thereby reducing the diffusion rate of theophylline. In another series of experiments, the absorption of caffeine was followed simultaneously and a Tris buffer was included to apply a pH of 9.2. In the presence of borate buffer, the absorption rate of both theophylline and caffeine was reduced compared to the other perfusates. In the presence of Tris buffers, there was a tendency to decreased absorption and disappearance rates for theophylline as wel as for caffeine. In the presence of phosphate and borate buffer, there was a difference between the absorption rate and the disappearance rate only for caffeine and not for theophylline. This was attributed to differences between the binding of caffeine and theophylline to intestinal mucus and/or tissue in the presence of phosphate and borate buffer.

It was concluded, that buffers were not able to influence the pH at the membrane (microclimate pH). Furthermore, buffer components influenced the absorption

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process by affecting intestinal mucus and/or tissue. Thus, buffers should not be used in absorption studies.

Introduction

Theophylline has already been used for a long time in the chronic treatment of asthma. Still the mechanism by which this drug is absorbed from the gastrointestinal tract is far from fully understood. In their work on rectal absorption of aminophylline, Crommelin et al. (1979) found no difference between the values of the disappearance rate of theophylline from phosphate buffer solutions of pH 5 and pH 10, respectively. At the same time they observed a considerable neutralizing capacity of the rectum. Nishihata et al. (1981) noted an enhanced absorption of theophylline from the rectum of the rat in the presence of salicylate. The mechanism of this enhancement is not known. On the other hand, theophylline is able to enhance the absorption of other drugs (Kakemi et al., 1969; Beubler and Lembeck, 1976; Nakamura et al., 1979; Sund and Hillestad, 1981) possibly by increasing the amount of cAMP at the absorption site and thus increasing the permeability of the tight junctions and increasing the bloodflow at the absorptive site. In the study of the absorption process, buffers are used to maintain a certain pH in the intestinal lumen. However, the buffer components used can influence the absorption process. According to Morishita et al. (1971) the disappearance rate of sulfonamides was reduced in the presence of a phosphate-citrate buffer compared to isotonic saline solution. Bechgaard (1973) found an increased absorption of salicylate from the human rectum in the presence of a glycine buffer compared to phosphate buffer.

In the study, presented here, the absorption of theophylline was studied in the presence of buffers with different pH and different ionic strength. Furthermore the validity of the pH-partition theory for the absorption of theophylline was investigated. The technique used in this study consists of a luminal perfusion with collection of the blood draining the intestinal segment under investigation. With this technique, it was possible to obtain independently values for the absorption rate constant and the disappearance rate constant from the intestinal lumen and thus the mass balance.

Materials and Methods

Animals

Male Wistar rats, 200–350 g, were fasted 16–20 h prior to the experiment. Water was available ad libitum.

Preparation

The preparation technique used, was based on the one described by Ochsenfahrt and Winne (1969). After anesthesia with urethane (1 ml/100 g body weight of a 10%

w/w solution), a midline abdominal incision was made, whereafter a suitable segment of jejunum was placed outside the abdomen on a heated platform (38°C). The rectal temperature of the animal was held between 37 and 38°C by a second heated platform. After rinsing with warm isotonic saline solution until the effluent was clear, glass cannulas were tied into the proximal and distal end of the segment. After injection of 500 IU of heparin into the jugular vein, the jejunal vein, collecting the blood draining the cannulated segment, was punctured.

Absorption experiments

The experimental set-up is shown in Fig. 1.

The blood was collected in a glass tube every 5 min. The blood flow was determined by weighing its contents and conversion into millimeters using a density of 1.059 g/ml (Ochsenfahrt and Winne, 1969). The blood pressure was measured via the carotid artery with a pressure transducer (Hottinger Baldwin Messtechnik,

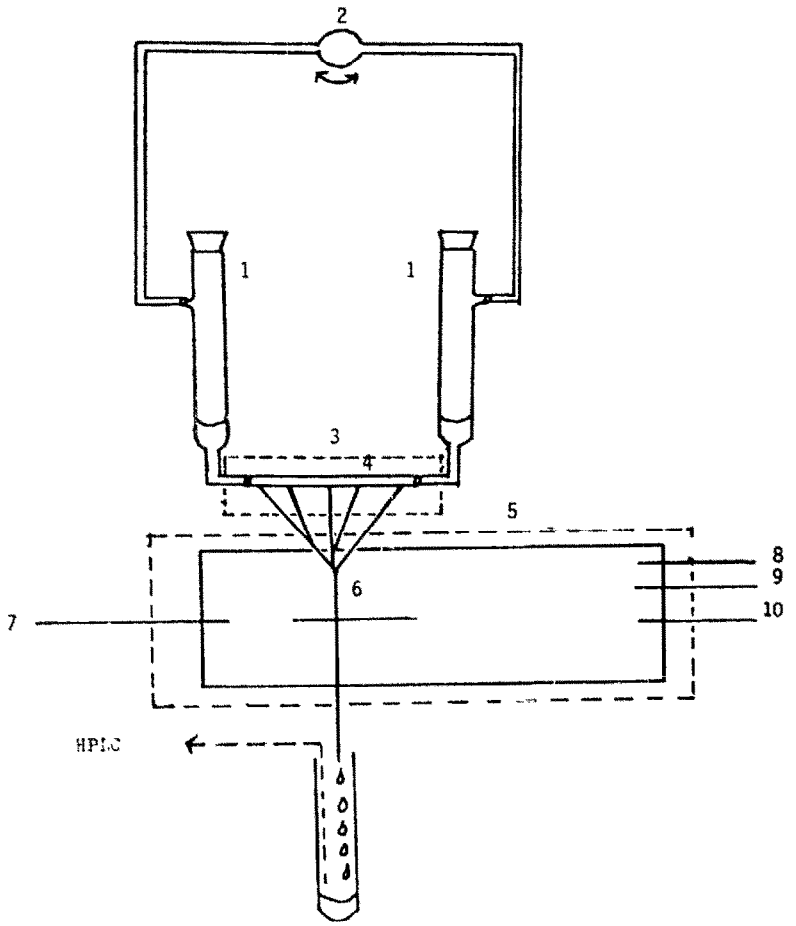

Fig. 1. Experimental set-up. 1 = cannula; 2 = pump; 3 = heated platform; 4 = jejunum; 5 = heated platform; 6 = jejunal vein; 7 = rectal temperature; 8 = blood pressure; 9 = respiration; 10 = donor blood into jugular vein.

Darmstadt, F.R.G.) The respiration was controlled by a respirator (V5kg Narco Biosystems, Houston, TX, U.S.A.). Donor blood, obtained immediately before the experiment from urethane-anaesthetized rats was infused into the jugular vein with an infusor (Perfusor ED.B. Braun, Melsungen, F.R.G.). The donor blood was diluted with a Ringer–lactate solution (Martindale, 1972) to a haematocrit of 0.40. At the end of the experiment, the jejunal segment was removed and opened, blotted and dried for 12 h at room temperature. Then the segment was dried for 2 h at 110°C and weighed. The absorption of theophylline from the small intestine of the rat is rather slow and no reliable absorption rate constants could be obtained with a recirculation technique. Therefore an oscillating technique, requiring a much smaller perfusate volume, was chosen. The perfusion was performed at a rate of 0.88 ml/min. This perfusion rate is in the same order of magnitude as the optimal perfusion rate (0.59 ml/min) determined with experiments with iopanic acid (Savina et al., 1981). For the oscillating perfusion a peristaltic pump (VRX-22, Verder, Dusseldorf, F.R.G.) provided with a custom made timer was used. Glass cannulas and the jejunal segment were filled with 2.0 ml of perfusion solution, leaving approximately 75% of the solution in the cannulas. The temperature of the solution in the cannulas was about 36°C throughout the experiment. Experiments with a final perfusate volume below 1.8 ml were discarded. A volume marker such as phenol red was avoided since its red color above a pH of about 7 prevents detection of an eventual luminal bleeding. A 10 μl perfusate sample was taken every 5 min up to 50 min. Then 10 μl samples were taken every 10 min until the end of the experiment. The following buffer solutions were prepared as described in the literature (Wissenschaftliche Tabellen, Ciba-Geigy, 1968): McIlvaine phosphate-citrate buffer pH = 3.0, Sorensen borate buffer pH 9.2, Tris buffer pH 7.4 (referred to as Tris-C), Tris buffer pH 9.2 (referred to as Tris-A) and the Sorensen phosphate buffer pH 7.4. In the Sorensen phosphate buffer the potassium was replaced by an equivalent amount of sodium because of the damaging effect large amounts of potassium have on the intestinal epithelium (Kojima and Miyake, 1976). The Tris buffer 0.175 M pH 9.2 (referred to as Tris B) is prepared by dissolving 5.85 g sodium chloride and 21.2 g Tris in 1.0 liter demineralized water. The components of the modified Krebs solution are listed in Table 1.

TABLE 1

COMPOSITION OF THE MODIFIED KREBS SOLUTION

Substance	g/l	mmol/l
NaCl	5.5	94.1
KCl	0.35	4.7
$MgSO_4 \cdot 7H_2O$	0.29	1.2
$CaCl_2 \cdot 2H_2O$	0.37	2.5
KH_2PO_4	0.16	1.2
$NaHCO_3$	2.1	25.0
Glucose	2.3	11.6

Perfusion solutions were prepared by dissolving 30 mg of theophylline monohydrate in 100 ml buffer (the concentration of theophylline was thus of the same order of magnitude as in the work of Crommelin et al., 1979). For the experiments with theophylline and caffeine, 15 mg of both substances were dissolved in 100 ml buffer. Ten-fold diluted buffers were prepared by diluting the original buffers with isotonic saline solution. Buffer solutions were adjusted to 310–320 mOsm/kg with sodium chloride. The calculated initial buffer capacities and the ionic strengths of the perfusion solutions are given in Table 2. The buffer capacity was calculated using the equation of Van Slyke (Martin et al., 1973).

High-performance liquid chromatography

The high-performance liquid chromatograph consisted of a model 710B Waters Intelligent Sample Processor and model 440 UV absorbance detector (Waters Associates, Milford, MA, U.S.A.). A 30 cm × 3.9 mm steel column filled with Lichrosorb 10 RP 18 (Merck, Darmstadt, F.R.G.) was used. The eluent, a water–methanol (90 : 10, w/w) mixture with 0.5% (v/v) glacial acetic acid, was pumped at a rate of 1.3 ml/min. The wavelength of detection was 280 nm. Chromatography was performed at 20°C.

Determination of theophylline

Perfusate samples were diluted with 500 μl of internal standard solution (containing 2.5 mg etophylline/100 ml demiwater) and analyzed without further treatment. To blood samples of 100 μl, 100 μl of internal standard solution and 500 μl acetonitrile were added. After vortexing and centrifugation at 2000 rpm for 10 min, the supernatant was collected and evaporated. The residue was dissolved in 500 μl demiwater and 50 μl were injected into the chromatograph. Calibration curves were constructed by analyzing perfusate and blood samples spiked with theophylline by

TABLE 2

CALCULATED INITIAL BUFFER CAPACITY (mol/l) AND IONIC STRENGTH (mol/l) OF THE BUFFERS (THE BUFFER CAPACITY IS CALCULATED USING THE EQUATION OF VAN SLYKE (MARTIN ET AL., 1973))

Buffer	pH	Buffer capacity (mol/l)	Ionic strength (mol/l)
Phosphate-citrate 1 st.	3.0	0.054	0.197
Phosphate-citrate 0.5 st.	3.0	0.027	0.176
Phosphate isotonic	7.4	0.068	0.177
Phosphate 1 st.	7.4	0.037	0.257
Phosphate 0.5 st.	7.4	0.019	0.206
Borate 1 st.	9.2	0.028	0.211
Borate 0.5 st.	9.2	0.014	0.183
Tris C	7.4	0.016	0.148
Tris B	9.2	0.026	0.104
Tris A	9.2	0 008	0.142

the same procedure and by plotting the peak height ratio (theophylline/internal standard) versus the concentration of theophylline.

Determination of caffeine and theophylline

When both theophylline and caffeine were present in perfusate and blood, the handling of the samples was as described above except for the internal standard and the eluent; the eluent was water–methanol (80 : 20 w/w) with 0.5% v/v glacial acetic acid and 8-Cl-theophylline was used as the internal standard.

Data analysis

After a short distribution phase, the absorption of theophylline in the steady-state situation follows a first-order absorption process. The time dependence of the luminal concentration, C, can thus be written as:

$$\ln (C_t/C_0) = -K_{abs} \times t \quad (1)$$

or

$$\ln (M_t/M_0) = -K_{abs} \times t \quad (2)$$

C_0, C_t and M_0 and M_t are luminal concentration (mg/ml) and mass (mg) of the model compound at time 0 and t, respectively and K_{abs} is the first-order absorption rate constant (1/min). Since $M_t = M_0 - M_{abs}(t)$, Eqn. 2 can be written as:

$$\ln((M_0 - M_{abs}(t))/M_0) = -K_{abs} \times t \quad (3)$$

The absorption rate constant, K_{abs} was calculated from plots of $\ln (M_0 - M_{abs}(t))$ vs t by linear regression.

All absorption rate constants were taken from the plots in the steady-state phase. The absorption rate constants obtained in different experiments cannot be compared as such. In the first place, K_{abs} must be standardized with respect to the "absorbing surface area" of the intestinal segment under investigation. Measuring the length of an intestinal segment was not accurate because stretching as well as shrinking occurred very easily by manipulating the intestine. Therefore, K_{abs} values were standardized to 100 mg dry tissue weight (dtw). A second variable was the blood flow. The mean blood flow in the different experiments was not equal. Therefore, the K_{abs} values were standardized with respect to a blood flow of 0.34 ml/min · 100 mg dtw.

Disappearance rates, K_{dis}, were calculated from semilogarithmic plots of the concentration of the model compound in the perfusate versus time by linear regression and expressed per 100 mg dtw and a blood flow of 0.34 ml/min · 100 mg dtw. To be comparable with the absorption rate constant, K_{dis} must be multiplied by the quotient of the volume of the perfusate left in the cannulas and the volume of the perfusate in a jejunal segment of 100 mg dtw. In experiments with isotonic saline solution this quotient was calculated to be 0.82 ± 0.13 (mean $\pm$S.D., n = 4), result-

ing in a corrected disappearance rate constant K'_{dis}. The mass balance is represented by ΔQ which is the non-recovered amount of drug (in μg) after summation of the cumulative amount of the drug in the blood and in the perfusate.

Results and Discussion

High-performance liquid chromatography

By the treatment with acetonitrile, 95% of the plasma proteins were precipitated (Blanchard, 1981). The recovery of theophylline from blood samples was found to be 95% ± 5% (mean ± S.D., n = 10) for the concentration range of 5–30 μg/ml theophylline. The absolute recovery of caffeine was 65% ± 5% (mean ± S.D., n = 10) for blood samples containing 5–30 μg/ml caffeine. The reproducibility of the determination was determined with blood samples spiked to different concentrations of theophylline. At 20 μg/ml and 50 μg/ml the coefficient of variation was 1.7% (n = 4) and 0.8% (n = 4), respectively. The minimal quantifiable concentration of theophylline and caffeine with this method was about 1 μg/ml.

Dependence of the flux of theophylline on the blood flow through the perfused loop

Experiments were performed to study the relationship between the blood flow through the segment under investigation and the absorption of theophylline, using recirculating perfusion with 25 ml of an isotonic saline solution containing 0.6 mg/ml of theophylline. A non-linear relation was found between the blood flow and the absorption rate of theophylline which could be described by Eqn. 4:

$$F = 0.02 + 0.13 \times Vb - 0.0011 \times Vb^2 \quad (4)$$

where F = flux of theophylline in $\mu g/min \cdot 100$ mg dtw; and Vb = blood flow in ml/min · 100 mg dtw.

The K_{abs} values per 100 mg dtw were multiplied by 21.8/F which was the quotient of the theophylline flux at a blood flow of 0.34 ml/min · 100 mg dtw and the theophylline flux at the actual blood flow measured.

Influence of buffer solutions on the structural integrity of the intestinal segment

The possible damaging effect of the buffer solutions on the intestinal segment was investigated by experiments using tritium-labeled inulin. Since this compound with a molecular weight of 5000 dalton, is absorbed to a negligible extent (Lauterbach, 1969), the damaging effect of a buffer on the intestine would result in a decreasing inulin concentration in the perfusate with time. Two ml of buffer solution containing 0.8 μCi of [^{3}H]inulin were oscillated through an intestinal segment at a flow rate of 0.88 ml/min during 60 min. Disappearance of [^{3}H]inulin from the lumen was measured by determining the decrease of the number of disintegrations per second (dps) between time zero and 60 min.

After a rapid initial loss, there was no further loss of inulin from the perfusate with time. A possible explanation for the initial loss of inulin is, that the inulin was

bound to intestinal mucus. Supporting evidence to this hypothesis was given by Schanker et al. (1958). They found that after perfusion of an intestinal segment with an isotonic solution containing inulin, some inulin was lost. By subsequent perfusion of the same segment with an isotonic saline solution, nearly all of the lost inulin was recovered (97%). It can be concluded that with the buffers used, the intestinal epithelium did not become leaky and that the structural integrity of the epithelium was maintained.

Absorption of theophylline from different buffer solutions

In Fig. 2 a representative plot is given of $\ln(M_0 - M_{abs}(t))$ vs time. From this figure it can be seen that, initially, the absorption of theophylline could be described by first-order absorption kinetics. In most of the experiments a second steady-state phase was observed that could be described by first-order kinetics too, but with a smaller absorption rate constant. This second phase, however, was not seen in all experiments. The biphasic absorption behaviour could be due to the existence of two compartments in the intestine. The absorption rate constants (K_{abs}) were calculated from the first steady-state phase by linear regression. In Table 3 the results for the different buffer solutions are given.

For the statistical evaluation of the results of the absorption experiments, the non-parametric test of Wilcoxon as well as the Student's *t*-test were used. Both tests gave the same results. For the statistical evaluation of the differences between absorption rate constant and disappearance rate constant in one group of experiments, the Student's *t*-test was used. Differences were considered significant when $P < 0.05$. Mutual comparison of the results for the different perfusates showed a

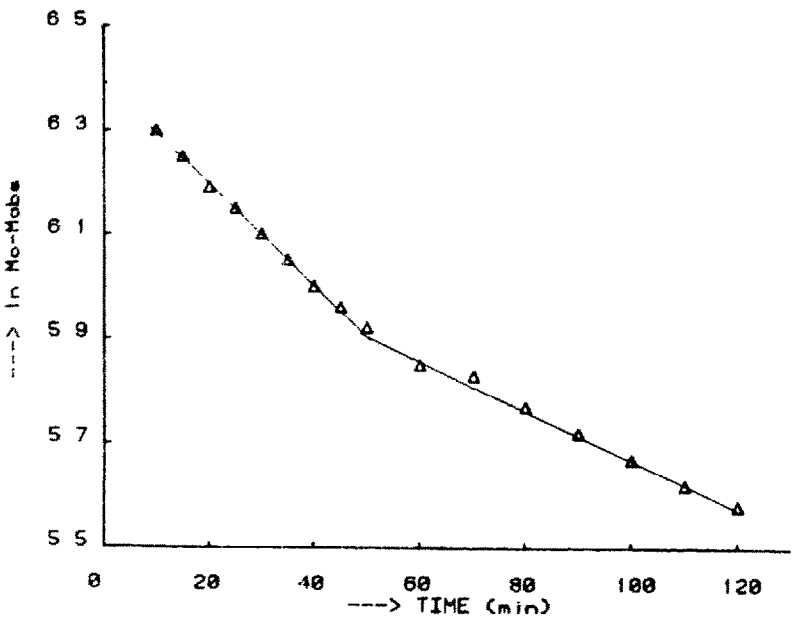

Fig. 2. Representative plot of $\ln(M_0 - M_{abs}(t))$ vs time for theophylline obtained with an undiluted phosphate buffer.

TABLE 3

ABSORPTION RATE CONSTANTS, DISAPPEARANCE RATE CONSTANTS (1/min) AND MASS BALANCES (%) FOR THEOPHYLLINE USING DIFFERENT PERFUSION SOLUTIONS CONTAINING 300 μg/ml THEOPHYLLINE

Perfusate	pH	$k_{abs} \cdot 10^3$	n	$k'_{dis} \cdot 10^3$	n	ΔQ(%)	n
Phosphate buffer	7.4	17.6 ± 1.7	5	22.4 ± 2.6	6	21 ± 4.8	4
Phosphate buffer 10 × dil.	7.4	17.5	2	17.6 ± 2.3	3	12 ± 3	3
Borate buffer	9.2	10.5 ± 3.8	9	13.9 ± 2.9	8	18 ± 3.8	8
Borate buffer 10 × dil.	9.2	16.0 ± 4.2	4	13.1	2	14	2
Phosphate citrate buffer	3.0	17.8 ± 3.3	5	18.0 ± 3.2	5	12 ± 7	5
Modified Krebs solution	7.4	20.1 ± 2.9	3	21.1 ± 1.7	3	11 ± 7.9	3
Isotonic saline solution	6.5	18.1 ± 2.9	4	18.1 ± 1.3	4	9 ± 2	4

ΔQ(%) = percentage of the initial amount of theophylline not recovered after summation of the cumulative amounts of drug in the blood and in the perfusate. Data are expressed as mean ± S.D.

significantly reduced absorption rate constant for theophylline with the borate buffer. The reduction was about 40%. Absorption rate constants with the other perfusates did not differ significantly. Looking at the K_{abs} and the K'_{dis} values in Table 3 there was a significant difference between K_{abs} and K'_{dis} for the phosphate and the borate buffer. The mass balances represented by ΔQ in Table 3 for these buffers were higher than for the other buffers. These differences point to a higher binding of theophylline to intestinal mucus or tissue under the influence of the buffer. It can also be seen from Table 3, that this effect was dependent on the concentration of the buffer components. The results with the phosphate-citrate buffer were the same as with the isotonic saline solution, so in contradiction to the results of Morishita et al. (1971) there seemed to be no influence of the phosphate-citrate buffer on the absorption of theophylline.

Reasons for the reduction in absorption rate with the borate buffer could be: (a) the borate buffer created a pH of about 9 at the membrane surface. This would cause an increase of the ionized fraction of theophylline and thus a reduced absorption rate (assuming that undissociated molecules are absorbed much faster than dissociated molecules); (b) an interaction between borate ions and intestinal mucus. This would lead to an increased viscosity of the mucus causing a reduction of the diffusion coefficient for theophylline (Davis and Deverell, 1977); and (c) the viscosity of the intestinal mucus increased by the high pH of the borate buffer leading again to a reduced diffusion coefficient for theophylline. Which of these factors was responsible for the decreased absorption rate constant with the borate buffer could not be deduced directly from the experiments with theophylline. Therefore experiments were performed with the addition of caffeine as a model compound and also additional buffers were used (Tris).

Absorption of theophylline and caffeine from different buffer solutions

Tris buffers of different pH values were used to clarify an eventual effect of Tris on the absorption of theophylline and caffeine. The absorption rate constant (K_{abs})

It might be, that the ionic strength, which is highest for the borate and the phosphate buffer (Table 2) is responsible for the observed effect, but this argument did not hold for caffeine, because diluting the phosphate buffer did not eliminate or decrease the effect. Taking into account the difficulties that can occur with the use of buffers in absorption studies (e.g. influence on intestinal mucus) it can be questioned whether the use of buffers can be recommended at all. A better approach would be to use a solution of the model compound in isotonic saline solution adjusted to a pH of 6.3, which is assumed to be the equilibrium pH in the jejunum of the rat.

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