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Influence of Bloodflow on the Absorption of Theophylline from the Jejunum of the Rat

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Abstract: The influence of the jejunal bloodflow on the absorption of theophylline was investigated. The bloodflow through a segment under investigation was varied by changing the systemic blood pressure by means of a donor blood infusion into the jugular vein or by an infusion of isoprenaline or levarterenol into a femoral vein, and was measured by collecting the venous outflow from the intestinal segment. Above a bloodflow of approximately 0.40 μl/min/cm the flux/flow ratio is reduced, and it is proposed that above this flow the intestinal epithelium provides the rate limiting step in the absorption of theophylline. When the bloodflow was held low for a prolonged time, the flux of theophylline decreased. The absorptive site bloodflow was calculated to be 18 % of the total bloodflow through the segment under investigation.

Introduction

The intestinal absorption of many nutrients is limited by the intestinal bloodflow. Only for slowly absorbed substances the rate limiting step consists of the resistances of the epithelium and the mucosal unstirred layer (1, 2). There are few studies describing the influence of bloodflow on the absorption of drugs. A few drugs (salicylic acid, antipyrine and amidopyrine) are mentioned by Ochsenfahrt and Winne (3). Crouthamel (4) investigated the influence of the bloodflow on the absorption of sulfaethidol. To investigate the influence of bloodflow on the absorption, there are several ways to change the intestinal bloodflow: (a) increasing or decreasing the systemic bloodpressure by means of an intravenous infusion of donor blood (1, 3), (b) decreasing the total small intestinal bloodflow by constricting the superior mesenteric artery (4), (c) decreasing the venous outflow from a segement under investigation by compressing the collecting vein or raising the level of the venous outflow (5); the increased venous pressure results in a constriction of arterioles (6). Furthermore, the bloodflow can be varied by hormones and neurotransmitters, such as levarterenol (1, 5, 7) or drugs, such as isoprenaline (5, 8). Finally, a decrease in bloodflow can be achieved by hypothermia (9). The bloodflow can be measured by collecting the venous outflow of the intestinal segment under investigation (3, 10, 11). This method provides no information on the distribution of the bloodflow to the different intestinal tissue layers. Other methods to measure the bloodflow are the microsphere technique (11, 13) and the isotope-fractionation technique (11, 14). These methods provide information on the distribution of the bloodflow to the different intestinal tissue layers but are not suitable for simultaneous determination of the bloodflow and the absorption. The absorptive site bloodflow, the fraction of the total bloodflow that passes immediately underneath the absorbing surface has been measured by determining the blood to lumen flux of drugs, such as barbital (9) and the lumen to blood flux of tritiated water (9, 11). A method that provides a very good control of the bloodflow is the vascularly perfused intestine technique (15). This type of experiment consists of simultaneous vascular and luminal perfusion of an isolated intestinal segment. In the rat this technique is difficult.

The purpose of the present study was to investigate the influence of the bloodflow on the absorption of theophylline. The bloodflow was varied by different methods:

1. Variation of the donor blood infusion rate.
2. Infusion of isoprenaline.
3. Infusion of levarterenol.

The technique we used was described in detail by Ochsenfahrt and Winne (3), that is simultaneous luminal perfusion and collection of all the blood draining the segment under investigation.

Theory

In order to calculate the fraction of the venous outflow that passes immediately under the absorbing epithelium ("absorptive site bloodflow"), Ochsenfahrt and Winne (3) derived, on the basis of a three compartment model, a relationship between the bloodflow, the perfusate flow through the intestinal lumen and the flux of a model compound. With the same equation, a permeability coefficient for the model compound under investigation can be calculated. The working equation is equation *(1)*:

(1) $Q = Cb \cdot Vb = Cd \cdot Vd\,[1 - \exp\,[-\,A1/[Vd\,(1 + A2/Vb)]]]$

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with A1 = permeability coefficient = k1 · Fd; A2 = k2 · Fd/αa1; Q = flux of the model compound [mg/min · g tissue weight (tw)]; Cb = concentration in the outflowing jejunal blood (mg/ml); Vb = bloodflow (ml/min · g tw); Cd = concentration in the perfusate (mg/ml); Vd = perfusion rate through the intestinal lumen (ml/min · g tw); k1 = permeability coefficient lumen-interstitium (ml/min · cm^2); k2 = permeability coefficient interstitium-lumen (ml/min · cm^2); α = fraction of the total venous outflow that passes immediately under the absorbing surface (absorptive site bloodflow); a1 = Cb/Cpl = quotient of blood concentration and free concentration of the model compound in the plasma; Fd = absorbing surface area (cm^2/g tw).

For model compounds that are not sensitive to different pH values at both sides of the intestinal membrane, k1 and k2 are equal. Inserting k1 = k2 into equation *(1)* and rearranging gives:

(2) $-1/[Vd \cdot \ln [-(Q/Cd \cdot Vd-1)]] = 1/a1Vb \cdot 1/\alpha + 1/A1$

When $-1/[Vd \cdot \ln [-(Q/Cd \cdot Vd-1)]]$ is plotted against 1/a1Vb, the slope of the curve gives $1/\alpha$, that is the reciprocal of the absorptive site bloodflow, and the intercept on the ordinate is 1/A1, that is the reciprocal of the permeability coefficient of the model compound.

Materials and Methods

Animal Procedures

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

After anesthesia with urethane (1 g/kg body weight), a midline abdominal incision was made, and a suitable segment of jejunum was placed outside the abdomen on a heating platform (38° C) (see Fig. 1). The rectal temperature of the animal was maintained by a second heating platform. After rinsing the segment with warm isotonic saline solution until the effluent was clear, glass cannulas were tied into the proximal and the distal end of the segment. After injection of 500 IU heparin into the jugular wein, the jejunal vein, which collects all the blood draining the cannulated segment, was punctured. The outflowing blood was collected in glass tubes during periods of 5 or 3 min. The bloodflow was determined by weighing the contents of the glass tubes. Respiration was controlled by a respirator (V 5 kg Narco Biosystems Inc., Houston, Tx.). During the experiment, donor blood was infused into the jugular vein with an infusor (Perfusor ED, B. Braun, Melsungen, W. Germany). The donor blood was obtained immediately before the experiment from urethane-anesthetized rats and diluted with Ringer-lactate solution (15) to a hematocrit of 0.40. To obtain a high bloodflow, the initial donor blood infusion rate was 50 ml/h. For intermediate bloodflow the infusion rate was 30 ml/h, and for low bloodflow the infusion rate was 15 ml/h. Infusions of isoprenaline or levarterenol were given into a femoral vein. The blood-pressure was measured in the femoral artery of the same leg. Simultaneously, the bloodflow in the superior mesenteric artery was measured using a perivascular electromagnetic flowprobe (Skalar-Medical, Delft, Holland, diameter: 0.75 mm).

Perfusion Experiments

The experimental set-up is shown in Fig. 1. 25.0 ml isotonic saline solution, containing 65 mg theophylline monohydrate

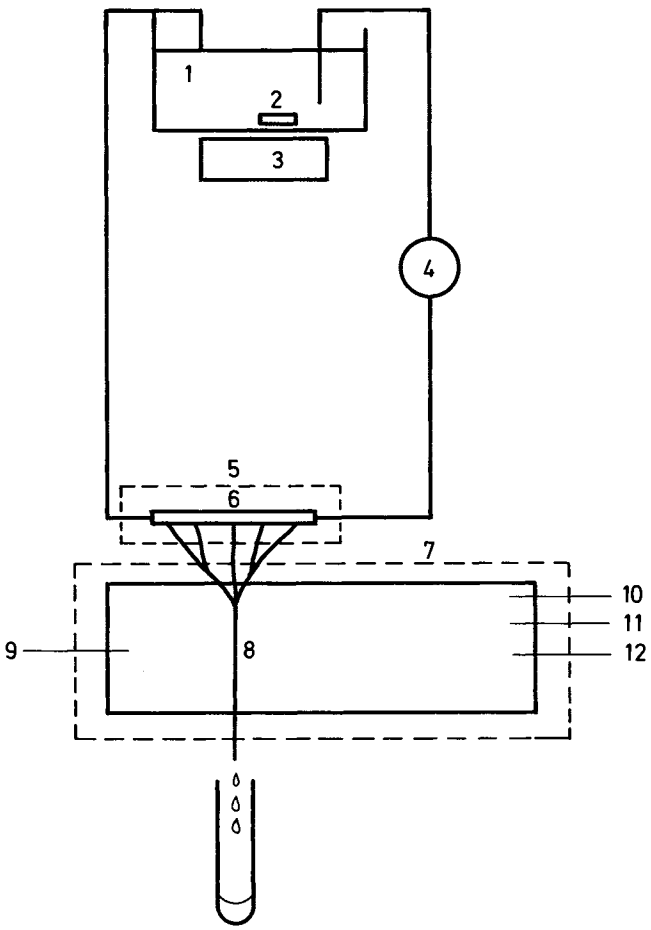

Fig. 1 Experimental set-up.
1 = perfusate (38° C), 2 = stirring bar, 3 = stirrer, 4 = pump, 5 = heating platform, 6 = jejunum, 7 = heating platfrom, 8 = jejunal vein, 9 = rectal temperature, 10 = blood pressure, 11 = donor blood into jugular vein, 12 = respiration.

per 100 ml (37° C) was recirculated through the intestinal segment with a flow rate of 0.88 ml/min. For the perfusion a peristaltic pump (VRX-22, Verder, Düsseldorf, W. Germany) was used, which was equipped with polyethylene tubing. The volume of the perfusate at the end of the experiment is measured by transferring it to a calibrated glass tube. Experiments with a final volume below 22 ml were discarded.

At the end of an experiment the intestinal segment is removed and the length is measured after stretching it for 60 sec with a weight of 10 g.

Analytical Procedure

To blood samples of 100 μl, 100 μl of internal standard solution (2.5 mg etophylline in 100.0 ml demineralized water) and 500 μl acetonitril were added. After mixing and centrifugation for 10 min at 2000 rpm the supernatant was collected and evaporated. The residue was dissolved in 500 μl water and 50 μl were injected onto a Lichrosorb 10-RP-18 column (Eluent : water : methanol 10 : 90 w/w + acetic acid 0.5 % g/v). The concentration of theophylline was determined with the aid of spiked blood samples. The recovery of theophylline with this method was 95 ± 5 % (n = 10).

Data Analysis

The theophylline flux was calculated by multiplication of the concentration (μl/ml) and the flow (ml/min), divided by the

sampling interval (min), and is expressed in μg per min per cm. The flux at time (t) is multiplied by the quotient of the initial amount of theophylline present in the solution and the difference between the initial amount of theophylline present in the solution and the cumulative amount of theophylline present in the blood at time (t −1) to correct for the decreasing concentration gradient between intestinal lumen and blood. The measured bloodflow is expressed in μl per min per cm. The bloodflow, measured in grams, is converted to milliliters by the factor 1.054 g/ml (3).

Results and Discussion

From the bloodflow through an intestinal segment during the first 10 minutes after puncture of the jejunal vein, a value for the resting bloodflow could be obtained since in this period the jejunal outflow was not influenced by the infusion rate into the jugular vein. For the resting bloodflow in the proximal jejunum 80 ± 20 μl/min · cm was found (n = 7), and in the distal jejunum 60 ± 20 μl/min · cm was found (n = 11). The preparation time had no influence on this bloodflow. Although urethane anesthesia and laparotomy have a negligible influence on the intestinal bloodflow and the distribution of the bloodflow (18, 19), it is possible that these values are too low; first, because manipulating the intestine might reduce bloodflow (20), and second, because prior to puncture of the jejunal vein, the vein is tied off for a few seconds which leads to increased venous pressure and eventually to a slight constriction of arterioles as predicted by the "myogenic control" theory of pressure-flow autoregulation (6). It is not clear to what extend these factors influence the bloodflow in our preparation, but the obtained results are in agreement with values obtained from the literature. Takacs and Vajda (21) found 60 μl/min · cm. Steiner and Mueller (22) found 80 μl/min · cm for the jejunum, and Csaky and Varga (9) found 80 μl/min · cm for the ileum.

In separate experiments, we confirmed that when the bloodflow is constant the flux is also constant during the experiment, so there is no change in the absorption of theophylline over time because of changes in absorptive properties of the intestinal epithelium.

Variation of the Donor Blood Infusion Rate

In these experiments, the bloodflow is changed from high to low values. The relationship among theophylline flux and blood flow is shown in Figure 2. For clarity, the results are further presented as shown in Figure 3. Curve A in Figure 3 (triangles) is obtained starting at high (100 to 120 μl/min · cm) and intermediate bloodflow levels (60 to 70 μl/min · cm). Above a flow of 40 μl/min · cm there is only little further increase of the theophylline flux. Curve B in Figure 3 is obtained starting at low bloodflow levels (30 to 40 μl/min · cm); in this case there is no further increase of the theophylline flux above a bloodflow of 20 μl/min · cm. The value of the flux at a flow of 40 μl/min · cm is significantly smaller in curve B compared with curve A. (The curves in Figure 3 are extrapolated to zero, since when there is no bloodflow, hardly any theophylline will appear in the blood. This extrapolation is only justified when blood data are used and not when dissappearence data from the lumen are used). One possible explanation of the observed difference in plateau value is that after being held at a low bloodflow for some time, there is a redistribution of the bloodflow through the epithelium and the muscularis favoring the bloodflow through the muscularis,

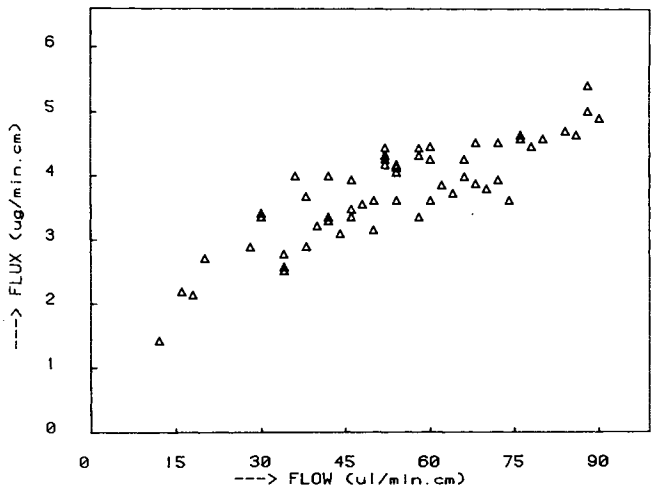

Fig. 2 Example of a curve obtained in bloodflow experiments. The bloodflow is changed by variation of the donor blood infusion rate.

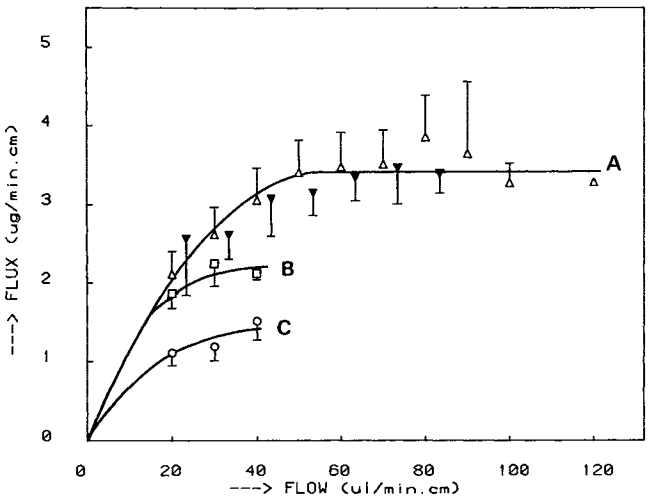

Fig. 3 Relation between the flux of theophylline and the bloodflow through the segment under investigation. Curve A (triangles): bloodflow changed by variation of the donor blood infusion rate starting at high initial levels (n = 5). Curve A (reversed triangles): bloodflow changed by isoprenaline infusion (n = 7). For clarity of the figure, the reversed triangles are shifted to the right. Curve B: bloodflow changed by variation of the donor blood infusion rate starting at low initial levels (n = 4). Curve C: bloodflow change by levarterenol infusion (n = 3).

which results in a new steady state situation. This explanation, however, would be contradictory to the "metabolic control" theory of pressure-flow autoregulation. According to this theory, the bloodflow at low levels should be shunted through the mucosa, because the demand for oxygen in this tissue is higher than in the muscularis due to higher metabolic activity. Another explanation might be that at low bloodflow a degeneration of mucosal cells begins. This could lead to a decreased flux of theophylline, although it is not known whether a flow of 40 μl/min · cm is already critical for tissue degeneration. A comparable effect was described by Winne (1) for antipyrine, salicylic acid and phenylalanine. When the jejunal bloodflow was changed from high to low values (10 μl/min · cm) and then to high values again, the level of the initial flux of the model compound was not reached, but remained significantly decreased.

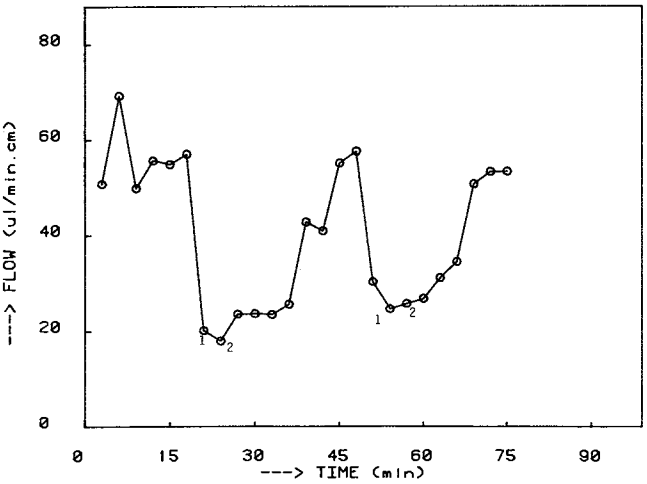

Fig. 4 Variation of intestinal bloodflow with time caused by isoprenaline infusion. Data points 1 and 2 are referred to in the text. (Arrows indicate start of the isoprenaline infusion).

Variation of the Bloodflow Using Isoprenaline

In low doses, isoprenaline gives an increased bloodflow through the intestinal bed (1, 7) (see below). In higher doses, however, isoprenaline gives a pronounced reduction in bloodpressure and a concomitant reduction in flow through the intestine. Isoprenaline was infused at a high dose (57 nM/min · kg) to achieve rapid changes in bloodflow. An example of a flow versus time curve is given in Figure 4. The results of these experiments are shown in Figure 3, curve A, reversed triangles. The results with infusion of isoprenaline are identical to the results obtained with high initial donor blood infusion rates. An interesting observation is that immediately after the start of the isoprenaline infusion, there is a considerable decrease in flow accompanied by a considerable decrease in flux during the first sampling period (Table I). In the second sampling period, however, the flux increases while the flow remains low. These periods are indicated in Figure 4 as 1 and 2.

The flow and flux data for the different experiments in period 1 and period 2 are given in Table I. In the second period of low flow, the decrease in flux is approximately 44 % less than in the first period. It can be concluded that after a fast decrease in bloodflow, the bloodflow through the epithelium is increased resulting in an increased theophylline flux immediately after the decrease in bloodflow. This observation is in good agreement iwth the prediction of the "metabolic control" theory. A fast decrease in bloodflow is tolerated well by the tissue, whereas a slow decrease over longer periods results in a new steady state situation with decreased maximal theophylline flux. Therefore, when the intestinal bloodflow is decreased for some time, such as after prolonged fasting of the animals, the absorption rate constant for theophylline, and probably for other compunds too, will be decreased (23) below that prevailing under normal physiological conditions.

Table I. Variation of the Theophylline-Flux with the Bloodflow.

Experiment	Period	ΔFlow (%)	ΔFlux (%)
1	1	−61	−43
	2	−64	−26
2	1	−59	−39
	2	−61	−22
3A	1	−53	−43
	2	−61	−21
3B	1	−51	−45
	2	−59	−16
4A	1	−65	−58
	2	−69	−39
4B	1	−48	−32
	2	−58	−20

A and B are periods taken from the same experiment.

In separate experiments we used lower concentrations of isoprenaline (0.15–0.30 nM/min · kg) to achieve an increase of bloodflow through the intestine. It was found that there was only a 10 to 14 % increase in conductance of the intestinal vascular bed resulting in an increase of about 10 % of the bloodflow. No increase in the flux of theophylline was found.

Variation of the Bloodflow Using Levarterenol

Sympathic stimulation gives a reduction in absorptive site bloodflow because of constriction of the precapillary sphincters (24). To achieve low bloodflow levels, experiments were done using a levarterenol-infusion (70 nM/min · kg). The results are shown in Figure 3, curve C. The flux of theophylline is hardly influenced by changes in the bloodflow. Compared with curve B the flux of theophylline is significantly lower. It can be concluded that under the influence of levarterenol, there is a constriction especially of the arterioles underneath the epithelium and that the bloodflow is shunted through the vessels of the submucosa or the muscularis.

From our experiments it can be concluded that the flux of theophylline does not increase above a bloodflow of approximately 40 μl/min · cm. This can be ascribed to a limiting diffusion rate of theophylline through the epithelium.

As mentioned under "Theory", the factor α is an estimate of the absorptive site bloodflow. Assuming a binding percentage of theophylline to serum albumine of 50 % (25), the factor a1 becomes 2. Inserting a1 = 2 into equation (2) and plotting $-1/Vd \cdot \ln (Q/Cd \cdot Vd - 1)$ against 1/2Vb gives the curve shown in Figure 5. The assumption that α is constant is only valid between values of 3.2 and 2.0 for 1/2Vb. This corresponds to a bloodflow of 12 to 20 μl/min · cm. At higher bloodflow rates the curve levels off which means that equation (2) is no longer valid. From the slope of the curve it can be calculated that α = 0.18, i.e., 18 % of the total bloodflow from the segment under investigation passes immediately underneath the absorbing epithelium. Extrapolation to 1/2Vb = 0 gives a1 = 0.086. The value of α is comparable with the values found by Winne (3) for antipyrine (0.20) and amidopyrine (0.14). An absorptive site bloodflow of 18 % is much higher than the absorptive site bloodflow given by Granger (5), namely 5 to 7 % of the bloodflow through the intestine. It is, however, more comparable with values given by Mailman (7) namely 150–300 μl/min · g wet tissue weight. This is approximately 16 to 30 % of the total bloodflow through the intestine. The permeability coefficient for theophylline (0.086) is in the same order of magnitude as the values found by Winne (3) for antipyrine (0.191) and amidopyrine (0.176). The difference in permeability coefficient for theophylline, antipyrine and amidopyrine can be only qualitatively explained by the log P values for the water/octanol system for theophylline (−0.02), antipyrine (0.23) and amidopyrine (0.80) (26). It might well be that another barrier is more important to intestinal transport, e.g. the intestinal

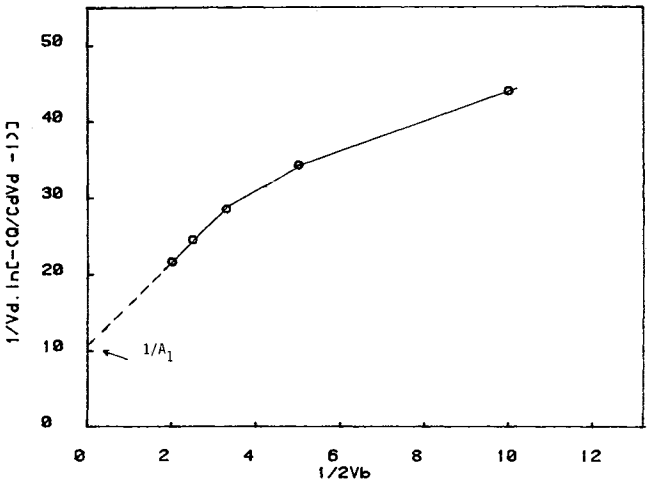

Fig. 5 Plot for the determination of α (i. e. the fraction of the total venous outflow that passes immediately under the absorbing surface = "absorptive site bloodflow") and the permeability coefficient for theophylline. The reciprocal of α is calculated from the initial slope of the curve and the reciprocal of the permeability coefficient (1/A1) is given by the intercept on the vertical axis.

mucus. Unfortunately, diffusion coefficients for these compounds through intestinal mucus are not yet available.

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