RESEARCH ARTICLES

New Lipophilic Terbutaline Ester Prodrugs with Long Effect Duration

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Received: March 10, 1983; accepted: June 2, 1983

Abstract: Two new lipophilic terbutaline ester prodrugs – the biscarbamate bambuterol (pINN) and the cascade ester D 2438 – have been designed with the goal to achieve enhanced absorption and high hydrolytic stability during first-pass in order to prolong the effect duration of the parent compound. Bambuterol, the bis-N,N-dimethylcarbamate of terbutaline, displays improved hydrolytic stability, partly by inhibition of its own hydrolysis, and has been shown to survive first-pass hydrolysis in the dog to a high degree. Bambuterol per se is inactive; however, after oral administration to guinea-pigs, the ED50 value for protection from histamine-induced bronchospasm is similar to that of terbutaline. Moreover, the terbutaline plasma level-time profile after oral doses of bambuterol in dogs is significantly prolonged. The cascade ester of terbutaline (D 2438), derived from p-pivaloyloxybenzoic acid, was designed to undergo first-pass hydrolysis and conjugation at the p-pivaloyloxybenzoic acid moiety; i. e. distal from the active resorcinol moiety in terbutaline. The prodrug itself is active in the isolated guinea-pig trachea and displays prolonged effect duration both after inhalation in guinea-pigs and after oral administration in dogs. The cascade ester prodrug (D 2438) has a somewhat shorter effect duration than bambuterol in these species.

Introduction

The bronchodilator terbutaline is a rather polar molecule, which suffers from slow and incomplete absorption in the GI tract and undergoes first-pass conjugation in the gut wall and liver to a high extent (1, 2). The resorcinol moiety of terbutaline contributes to its high hydrophilicity, and the phenolic groups are targets for the first-pass conjugation reactions with sulfuric and glucuronic acid.

A single oral dose of terbutaline has a duration of action of 5–7 hours; the dose regimen is generally 5 mg t.i.d. A prolonged effect duration is desirable, however, as patients commonly present their worst asthmatic symptoms at night and during the early morning hours. Consequently, the last dose taken at bedtime may not be adequate to maintain effective plasma levels of terbutaline throughout the whole night.

As it would probably be more difficult to find a "new terbutaline" with high bioavailability and long duration of action, the prodrug concept offered an interesting approach to improve the characteristics of the parent drug. We therefore hypothesized that a lipophilic terbutaline ester with sufficient presystemic hydrolytic stability would not only be well absorbed but also might display a prolonged effect duration due to distribution of the prodrug to different tissues where it would slowly hydrolyze to terbutaline.

We had earlier observed that common fatty acid esters of terbutaline gave improved absorption (3). In clinical studies on one of these esters, ibuterol (Fig. 1), it was found, however, that improved absorption was accompanied by *shorter* effect duration and a slightly *increased* extent of first-pass metabolism (4). Since the hydrolytic stability of the esters in this series is mainly a function of steric hindrance near the acyl carbonyl group (5), it became evident that exploitation of such factors alone would not lead to esters of terbutaline with adequate stability to resist first-pass hydrolysis.

In this paper we wish to present two new compounds, the biscarbamate bambuterol and the cascade ester D 2438 (Fig. 1), which represent two different approaches to the design of first-pass surviving ester prodrugs of terbutaline (6). Bambuterol is the N-isostere of ibuterol. We expected this compound to display improved hydrolytic stability for the following reasons:

1. Introduction of the nitrogen atom reduces the reactivity of the acyl function due to resonance effects from the electron-donating nitrogen atom.
2. Dimethylcarbamates are well-known cholinesterase inhibitors, so that bambuterol with its two carbamoyl groups may well function in the same way. We could thus expect the hydrolysis of bambuterol to be partially inhibited because of reversible inhibition of the esterases responsible for its own hydrolysis.

The cascade ester D 2438 was designed to undergo first-pass hydrolysis and conjugation at a site in the prodrug molecule distal from the active resorcinol moiety in terbutaline. We thus expected this cascade ester, constructed from p-hydroxybenzoic acid and pivalic acid, to undergo first-pass hydrolysis mainly at the p-pivaloyloxy bond followed by conjugation at the resulting p-*hydroxy*-benzoyl moiety. In this way the active resorcinol moiety in terbutaline would be protected during first-pass, and eventually free terbutaline may be generated from hydrolysis of the conjugated or free p-hydroxybenzoates, during and after the distribution phase. The reason for choosing the actual combination of pivalic and p-hydroxybenzoic acids is based on the hydrolytic stability of the corresponding esters of terbutaline. The half-life of terbutaline dipivalate in human plasma is less than five minutes (5) but that of the di-p-hydroxybenzoate is approximately one hour; therefore, it is primarily the pivalate bond in the cascade ester that should undergo hydrolysis during first-pass.

It seems to be a common feature among basic drugs with the phenylethylamine structure to display a certain affinity for lung tissue (7). The degree of affinity generally seems to increase with increasing lipophilicity. In the isolated perfused and ventilated rat and guinea-pig lung preparations the lung extraction ratio for terbutaline ranged from 0.013 to 0.021,

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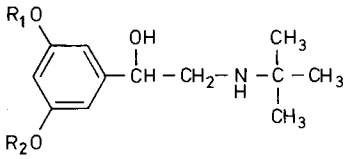


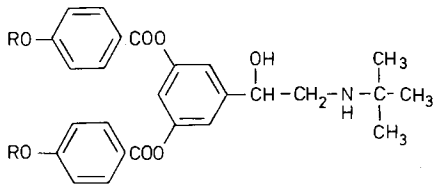
$R_{1,2}$ = H Terbutaline

$R_{1,2}$ = $(CH_3)_2CHCO$ Ibuterol

$R_{1,2}$ = $(CH_3)_2NCO$ Bambuterol (PINN)

R_1 = H, R_2 = $(CH_3)_2NCO$ D 2439

Fig. 1a



R = H D 2435

R = $(CH_3)_3CCO$ D 2438

Fig. 1b

Fig. 1 Chemical structures of terbutaline, its ester prodrugs ibuterol and bambuterol, the cascade ester D 2438 and several of their hydrolysis products.

while the more lipophilic ibuterol gave values from 0.32 to 0.41 (8). We could therefore expect bambuterol and the cascade ester to have a rather high affinity for lung tissue and thus function as site-directed prodrugs as they partly will distribute in the organism as the intact lipophilic ester.

The biochemical and pharmacological properties of bambuterol and the cascade ester D 2438 have been examined *in vitro* and *in vivo* and the results are summarized below.

Experimental

Tracheal and heart preparations

Male guinea-pigs, DH, 400–500 g, were used. The spirally cut trachea and the spontaneously beating heart auricles were prepared for isometric recording in organ baths with carbogenated Krebs solution at 37 °C (9). The preparations were pretreated with 10^{-6} M eserine in order to avoid esterase catalyzed hydrolysis of the esters in the tissues.

Inhalation experiments in anesthetized guinea-pigs

Guinea-pigs, DH, 400–700 g, were narcotized (barbital or chloralose) and ventilated by a constant volume respirator. The increase in intratracheal pressure (ITP) after *I. V.* histamine or *I. V.* acetylcholine, or after electrical stimulation of n. vagus was recorded. The degree of inhibition of the increase in ITP after inhalation from a Bird inline nebulizer of an aqueous solution of the test compounds was determined.

Effect studies in conscious guinea-pigs

In male guinea-pigs, DH, 200–300 g, a state of respiratory distress was induced by exposing the animals to nebulized histamine generated from 0.02 % histamine and 3 % glycerol in a Bird inline nebulizer. The test compounds were given by a stomach tube before the histamine challenge and the dose dependent inhibition of respiratory distress was observed (10).

Locomotor activity

Male mice, NMRI, 20–25 g, were given water (controls) or drug by stomach tubes. The animals were placed in a Motron activity meter 30 or 60 minutes after dosing and activity was recorded during 25 minutes.

Peroral administration to trained dogs

Trained, male Beagle dogs (13–18 kg) were starved the night before the experiment (water *ad lib.*). The drug was given perorally as a water solution or suspension. Blood was collected from the cephalic veins in the forelegs. Heart-rate was determined as a mean of three determinations (stethoscope and/or ECG) during a five minute period before administration of drug and before each blood sampling. Because of the use of trained dogs, heart-rate measurements could be performed with good accuracy and the individual values never differd more than max 10 beats/min. All blood samples were drawn into tubes preloaded with either DFP (diisopropyl fluorophosphate) or eserine in order to stop esterase catalyzed hydrolysis of the esters.

Bioanalytical methods

An assay using gas chromatography chemical ionization mass spectrometry (GCCIMS) with ammonia as the reagent gas was used to measure plasma levels of terbutaline and D 2439. Deuterated internal standards were used and the compounds were determined as their TMS-derivates (11). Bambuterol itself was assayed in a similar manner after methylene chloride extraction from plasma (Lindberg, C., et al., to be published). The same GCCIMS assay was used for the determination of half-lives of terbutaline formation from the ester prodrugs (0.3–3 μM) in human plasma.

Results and Discussion

The stability in human plasma of bambuterol, the cascade ester D 2438 and some of their hydrolysis products, namely the di-p-hydroxybenzoate D 2435 and the monocarbamate D 2439 (Fig. 1), has been studied, and the half-lives for formation of terbutaline have been calculated (Fig. 2). Both bambuterol and the cascade ester have half-lives in the order of 10 hours, and D 2435 and D 2439 are also fairly stable compared to ibuterol (5). The half-lives for bambuterol and the monocarbamate D 2439 are difficult to determine as both compounds, as expected, effectively inhibit their own hydrolysis. Moreover, the hydrolysis of the cascade ester in dog plasma was inhibited in the presence of an equimolar concentration of bambuterol.

In the guinea-pig tracheal and heart preparations, bambuterol per se is inactive and does not influence the effect of terbutaline. The cascade ester per se is similarly without effect in the guinea-pig heart preparation, but in the pilocarpine-contracted tracheal preparation, the ester itself has an EC50 = 4.8 ± 3.5 x 10^{-5}M (terbutaline EC50 = 3.1 ± 2.2 x 10^{-7}M). This effect is not blocked in the presence of 3 x 10^{-6}M propranolol (Fig. 3). Moreover, low, ineffective doses of the cascade ester D 2438 (10^{-6}M) potentiate the effect of terbutaline in the trachea (Fig. 4). Thus, the cascade ester itself seems to have an additional bronchorelaxing effect

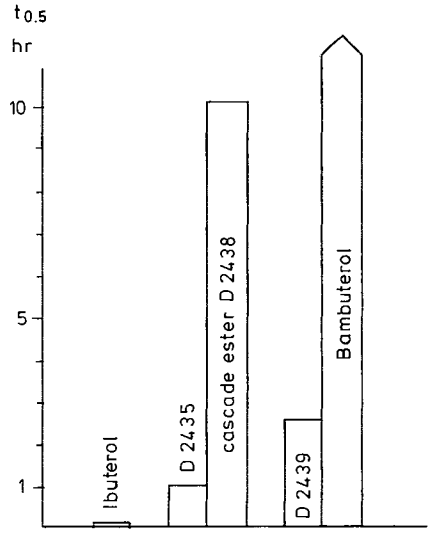


Fig. 2 Half-lives in human plasma for formation of terbutaline from some ester prodrugs of terbutaline.

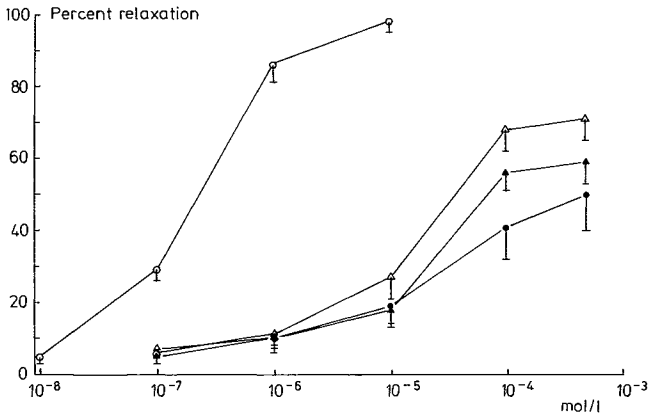


Fig. 3 Cumulative dose-response curves for relaxation of pilocarpine-contracted guinea-pig tracheas. The ordinate gives % relaxation and the abscissa the concentration of drug in the organ bath.
— ○ — terbutaline
— △ — D 2438
— ● — terbutaline + 3 x 10^{-6}M propranolol
— ▲ — D 2438 + 3 x 10^{-6}M propranolol

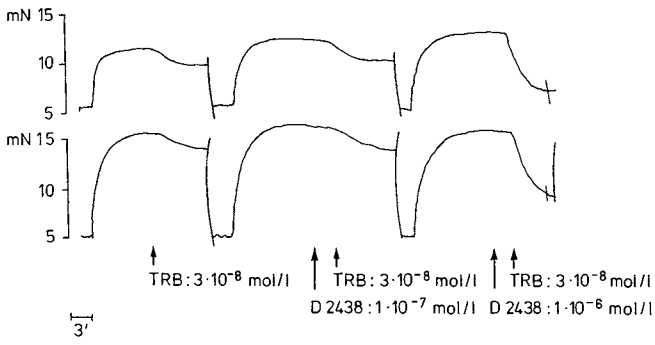


Fig. 4 Representative, simultaneous recordings from two guinea-pig tracheal strips. (A total of 8 strips from 5 animals were used). The tension is induced by pilocarpine (4 μmol/l) in the presence of physostigmine (0.5 μmol/l). D 2438 in the concentrations of 0.1 and 1 μmol/l shows no intrinsic effect but the last mentioned level increases the relaxing activity of 0.03 μmol/l of terbutaline.

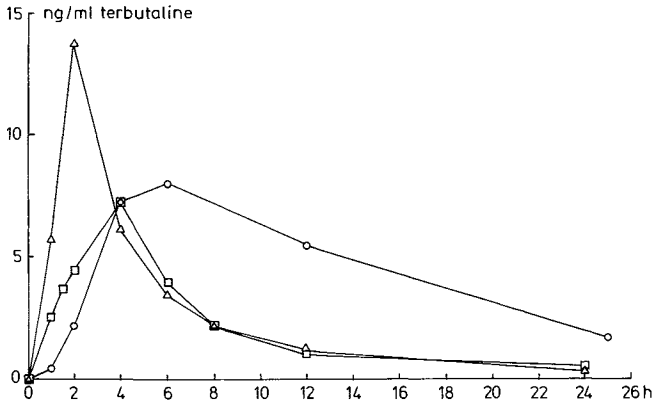


Fig. 5 Representative terbutaline plasma concentration profiles obtained after oral administration of single oral doses of terbutaline and bambuterol to trained dogs, and for comparison after a single oral dose of terbutaline to man.
— △ — Terbutaline 0.44 μmol/kg, dog n = 1
— □ — Terbutaline 0.40 μmol/kg, man n = 3
— ○ — Bambuterol 1.5 μmol/kg, dog n = 1

mediated via a mechanism different from that of the β_2-receptor. The duration of the effect of bambuterol and the cascade ester is somewhat prolonged when given orally to conscious guinea-pigs, and both compounds give ED50 values similar to terbutaline (ED50 = 2.5 ± 0.9 μmol/kg) for the protection against histamine-induced bronchospasm (bambuterol ED50 = 6.7 ± 2.2 μmol/kg; cascade ester D 2438 ED 50 = 1.6 ± 0.7 μmol/kg).

Bambuterol is inactive when given in nebulized form locally to the lungs in anesthetized guinea-pigs. The cascade ester, D 2438, however, is effective, and has an ED50 value of 4.5 ± 2.9 x 10^{-4}M, which is about twice that of terbutaline. Moreover, the effect duration of the cascade ester is 3–5 times longer than that of the parent compound.

Since bambuterol and the cascade ester D 2438 are considerably more lipophilic compounds than terbutaline, their ability to penetrate into the CNS could also be increased. We therefore have examined their influence on the spontaneous motor activity in mice. The cascade ester was found to be without effect in oral doses up to 2 x 10^{-4} mol/kg. Motor activity was slightly reduced when bambuterol 2.5 x 10^{-4} mol/kg was administered.

Both esters have been given orally to trained dogs and the plasma levels of terbutaline followed for up to 48 hours.

Man and dog treat terbutaline pharmacokinetically in a rather similar way; both species metabolize terbutaline preferentially to the sulfate conjugate, and a "therapeutic" plasma level of terbutaline (2–7 ng/ml) is maintained for 5–7 hours after a single oral dose of 0.26–0.4 μmol/kg terbutaline (2). After oral administration of different doses of bambuterol to dogs, 1.5 μmol/kg was found to maintain "therapeutic" terbutaline levels for as long as 20 hours (Fig. 5).

Similarly, 0.9 μmol/kg of the cascade ester D 2438 produced terbutaline plasma concentrations between 2–6 ng/ml during a period of 12 hours (Fig. 6). The analytical procedure (GCCIMS) allowed us to simultaneously analyze bambuterol, its monocarbamate metabolite D 2439, and terbutaline in plasma samples. We were therefore able to show that bambuterol, as expected, survives first-pass metabolism to a substantial degree and that terbutaline is formed via hydrolysis of bambuterol and the monocarbamate D 2439 (Fig. 7). For both bambuterol and the cascade ester it was also observed that the

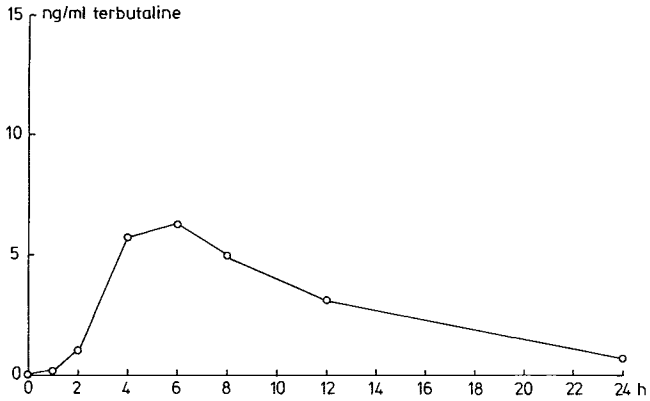


Fig. 6 Representative terbutaline plasma concentration profile after peroral administration of a single dose of the cascade ester D 2438 (0.9 μmol/kg) to a trained dog.

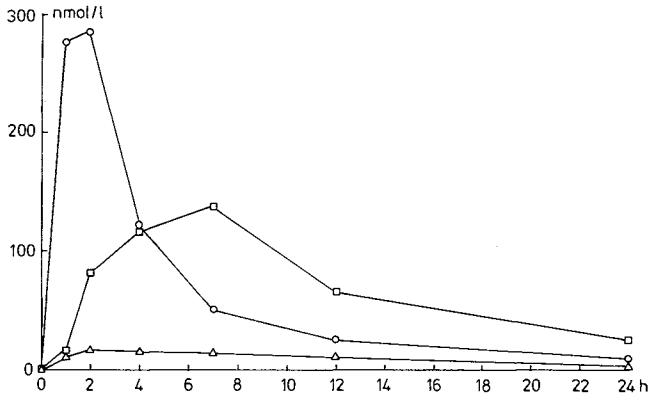


Fig. 7 Plasma concentration profiles of bambuterol (○), the monocarbamate D 2439 (△), and terbutaline (□) obtained after administration of a single oral dose of bambuterol, (2.5 μmol/kg) to a trained dog.

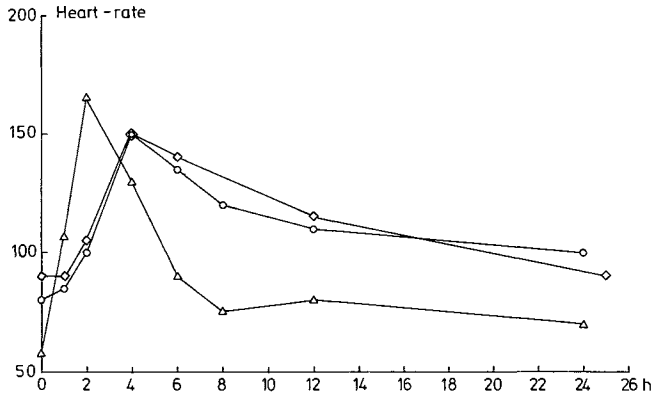


Fig. 8 Comparative heart-rate effect profiles of terbutaline (△), bambuterol (◇), and D 2438 ○) after peroral administration to dogs. (Same experiment as illustrated in Figs. 5 and 6).

plasma peak concentration of generated terbutaline was delayed compared to administration of terbutaline itself. The peak concentration appeared approximately 5 hours after dosing (Figs. 5 and 6).

The analytical assay was not sensitive enough to examine if the cascade ester to some extent survives first-pass, either as the intact ester, or as free or conjugated terbutaline p-hydroxybenzoates. On the other hand, we have found that both the dibenzoate and the di-p-hydroxybenzoate of terbutaline fail to give prolonged terbutaline plasma concentration profiles after oral administration to dogs. This observation shows that the benzoyl ester function alone is not responsible for the prolonged terbutaline plasma profiles obtained with the cascade ester D 2438, and suggests that the cascade ester prodrug may function as postulated; i. e. to protect the active terbutaline moiety from first-pass conjugation.

The dog heart is sensitive to sympathomimetic agents, and terbutaline has been found to induce a dose related tachycardia after peroral administration to dogs (12). The prolonged terbutaline plasma concentration profiles obtained after peroral administration of bambuterol and the cascade ester D 2438 are reflected in the heart-rate effect profiles as illustrated in Fig. 8.

Bambuterol seems to be a rather selective pseudocholinesterase inhibitor, as not even the high doses given to rats (1100 μmol/kg) and dogs (160 μmol/kg) in the toxicological studies over one month show any clinical or pathological effects that are due to inhibition of acetylcholinesterase.

A further indication that inhibition of acetylcholinesterase by bambuterol does not occur in lung tissue was obtained in chloralose-narcotized, propranolol-treated guinea-pigs. The animals were ventilated with a constant volume respiration pump, and the increase in intratracheal pressure (ITP) after vagal stimulation was measured. Thus, an i. v. injection of 0.25 μmol/kg of the unselective cholinesterase inhibitor eserine produced a 50–100 % increase in the ITP, i. e. severe bronchoconstriction, while bambuterol given intraduodenally in doses of 2.5–250 μmol/kg or inhaled in nebulized form failed to influence this parameter.

In conclusion, the animal studies presented here show that the terbutaline ester prodrugs bambuterol and the cascade ester D 2438 function as effective bronchodilators with prolonged effect duration compared with the parent compound. They are presently undergoing early phase I and II clinical evaluation in man.

Acknowledgement

The authors wish to thank Mrs. K. Tegnér for performing the hydrolysis expleriments in dog and human plasma, Dr. C. Lindberg and Mr. S. Jönsson for performing the GCCIMS analysis, and Mrs. A. Ekdahl and Mrs. B. L. Ahlquist for their skilful technical assistance.

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

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Received: May 5, 1983; accepted: June 28, 1983

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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