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Research Article

Bambuterol: Uptake and Metabolism in Guinea Pig Isolated Lungs

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The lung uptake and biotransformation of ^{3}H-bambuterol, a prodrug to terbutaline, were studied using isolated perfused and ventilated guinea pig lungs. ^{14}C-Sucrose was used as an extracellular marker. The lung uptake of bambuterol was significantly ($0.05 \geq P \geq 0.001$) higher than that found for sucrose in single-pass perfusion experiments. High-performance liquid chromatographic (HPLC) analysis showed that 95.6 ± 3.6% of the effluent ^{3}H radioactivity was attributable to bambuterol. In recirculating experiments (120 min) the lung biotransformation of ^{3}H-bambuterol (8.5 pmol/ml) was studied. Both oxidative and hydrolytic metabolism took place. The dominating metabolites were hydroxylated bambuterol and the monocarbamate derivative which is a product of hydrolysis of bambuterol. Traces of terbutaline were also formed. The results show that bambuterol has a certain affinity to lung tissue and that the drug is, to some extent, biotransformed in the guinea pig lung.

KEY WORDS: bambuterol; uptake; metabolism, guinea pig; lung.

INTRODUCTION

Bambuterol, the bis-*N,N*-dimethylcarbamate of terbutaline, is a prodrug of the β_2-adrenoceptor stimulating agent terbutaline (1,2). This prodrug is slowly metabolized via oxidative and hydrolytic pathways to, e.g., terbutaline. The biotransformation of bambuterol as revealed by *in vitro* studies in liver microsomes and plasma is shown in Fig. 1 (3).

Clinical studies with bambuterol given orally to asthmatic patients have shown that its duration of action is so sustained that once-a-day treatment is possible (4). These clinical studies have also shown that bambuterol, compared with terbutaline, gave effective bronchodilation at lower terbutaline plasma levels and hence gave a lower incidence of systemic side effects. Since bambuterol as well as those metabolites, having at least one of the phenolic hydroxyl groups blocked, lacks bronchodilating activity, the effect compartment for bambuterol-generated terbutaline seems to be more oriented toward the lung, whereas that of terbutaline itself seems to be in plasma (2,4). The terbutaline part of bambuterol is effectively protected from extensive first-pass metabolism, which offers a possibility for bambuterol to be taken up by the lung and be biotransformed locally to active drug.

In order to get information about the uptake, biotransformation, and retention of bambuterol in the lung, experiments were performed using isolated perfused and ventilated lungs. Lungs from guinea pigs were used since a large part of the pharmacological studies has been performed with this species. ^{3}H-Bambuterol was given via the pulmonary circulation and either single-pass or recirculating perfusion was used. In the uptake and retention studies, ^{14}C-sucrose was used as a marker of the extracellular space.

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MATERIALS AND METHODS

Compounds

^{3}H-Bambuterol (TRQ-2750-1B; sp act, 0.97 μCi/nmol) and ^{14}C-sucrose (Code CFB.4 batch 68; sp act, 10 μCi/mmol) were obtained from the Radiochemical Centre, Amersham, U.K. The ^{3}H-bambuterol was purified by the authors, and the radiochemical purity was 98–99%, when analyzed on the high-performance liquid chromatographic (HPLC) system described below. The radiochemical purity of ^{14}C-sucrose was 98% according to the Radiochemical Center.

Lung Perfusion

Male Dunkin–Hartley guinea pigs, weighing 250–300 g, were anesthetized with pentobarbital (~ 60 mg/kg i.p.). The surgical procedure and the perfusion system used were that described by Ryrfeldt and Nilsson (5). The lungs were perfused with Krebs–Ringer biocarbonate buffer (pH 7.4) containing 4.5% bovine serum albumin (fraction V, Sigma) and 0.1% glucose for 10–15 min at 37°C to establish constant perfusion flow and ventilation. The lungs were perfused in either a single-pass or a recirculating system.

The lungs were ventilated by creating an alternating negative pressure inside the thoracic chamber relative to the ambient atmosphere. This was created by a respirator (Model 665, Harvard Apparatus) and a vacuum source con-

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nected to the thoracic chamber giving a pressure variance of −1 to −10 cm H_2O. The frequency of respiration was 85–90 breaths/min.

The thoracic pressure, tracheal airflow, and tidal volume were measured and recorded continuously on a Grass Model 7D polygraph. These parameters were used for simultaneous computer calculations of lung resistance (R_L) and dynamic compliance (C_{DYN}) according to Amdur and Mead (6). Spirometric values before the infusion of the compounds to the lungs in the single-pass experiments were as follows: tidal volume (V_T), 1.39 ± 0.09 ml; lung resistance (R_L), 0.76 ± 0.06 cm H_2O/ml/sec; and dynamic compliance (C_{DYN}), 0.09 ± 0.01 ml/cm H_2O (N = 8). In the recirculating system the parameters were as follows: V_T 1.68 ± 0.41 ml; R_L, 0.28 ± 0.07 cm H_2O/ml/sec; and C_{DYN}, 0.13 ± 0.04 ml/cm H_2O (N = 3). The perfusion flow in the single-pass experiments was kept at 16 ml/min and in the recirculating experiments the perfusion flow was 13 ml/min. The perfusate pH was measured and recorded continuously (Grass Model 7D polygraph) and kept within 7.4–7.6 (7).

Drug Administration

When the lungs were perfused using the single-pass mode, two buffer reservoirs were connected to the pulmonary circulating via a three-way valve. One reservoir contained control buffer, and the other buffer containing ^{3}H-bambuterol (0.01 nmol/ml) and ^{14}C-sucrose (1.0 nmol/ml). The infusion time of the drug was 15 min; after this time the lungs were perfused with control buffer for 20 min.

In the recirculating system the lungs were initially perfused for 2 hr with 50 ml perfusion medium containing 0.01 nmol/ml ^{3}H-bambuterol. When starting the perfusion with the bambuterol-containing medium, this medium was diluted with 9 ml bambuterol-free medium remaining in the tubings from the equilibration perfusion. The concentration of ^{3}H-bambuterol in the recirculating system was thus 8.5 pmol/ml.

Sample Collection

Samples (~1 ml) were taken from the lung effluent as well as from the reservoirs at various times during the single-pass experiments. Two milliliters was taken from the recirculating perfusion medium 0, 5, 30, 60, 90, and 120 min after initiation.

All biological samples were stored in a frozen state (−20°C) until time to measure the total radioactivity and, in some cases, to determine the metabolite profile.

Measurement of Total Radioactivity

The total radioactivity of ^{3}H-bambuterol and ^{14}C-sucrose in the perfusate in both the single-pass and the recirculating system was counted by dispersing 0.2 ml of sample in 10 ml of scintillation cocktail (Optifluor Packard). The radioactivity of ^{3}H-bambuterol and ^{14}C-sucrose in the samples was determined simultaneously with Tri-Carb liquid scintillation spectrometers (Packard). Preset condition for the isotope pair $^{14}C/^3H$ was used and quench correction was performed by the external standard procedure.

The lungs were homogenized in 2 vol of distilled water with a Polytron homogenizer (CH 6005 Luzern, Switzerland). The ^{3}H and ^{14}C activities in the lung homogenates were determined as follows: a sample of each lung homogenate (250–300 mg) was combusted in a Packard Tri-Carb Model 306 sample oxidizer to determine the total ^{3}H (as ^3H_2O) and ^{14}C (as $^{14}CO_2$) content. The recovery of ^{3}H and ^{14}C in the sample oxidizer was 98.2 and 95.4%, respectively.

Sample Preparation for HPLC Analysis

Single-Pass Perfusion Technique

Perfusate (400 μl) from the lung perfusion experiments was precipitated with 400 μl 5% perchloric acid (PCA). Fifty microliters of a standard solution (containing unlabeled bambuterol, the monocarbamate, and terbutaline) was mixed with 200 μl of supernatant from the perfusate, and 200 μl was injected into the HPLC system. Lung homogenate (400 μl) was precipitated with 400 μl 5% PCA. Standard solution (50 μl) and 200 μl supernatant from the lung were mixed, and 200 μl of the solution was injected onto the HPLC system.

Recirculation Technique

Perfusate samples (1600 μl) from the lung perfusion experiments were precipitated with 400 μl 35% PCA. Three hundred microliters of the standard solution was mixed with 1500 μl supernatant from the perfusate, and 800 μl was injected onto the HPLC system. Lung homogenate (1600 μl) was precipitated with 400 μl 35% PCA. Standard solution (200 μl) and 1000 μl supernatant from the lung were mixed, and 800 μl of the solution was injected onto the HPLC system.

HPLC System

The HPLC system consisted of two Waters M-45 pumps, a Waters M 440 UV detector (254 nm) connected to a Kipp and Zonen BD 41 recorder, a Waters 710 B Intelligent Sample Processor, and a Waters Model 680 automated gradient controller. The outlet of the detector was connected to a LKB 2112 Redirac fraction collector, and 0.5-min fractions were collected. Optifluor (10 ml) was added to each fraction.

The column was Nucleosil 10SA (150 × 5-mm i.d.). Mobile phase A consisted of 19.3 g of ammonium acetate and 14.4 ml of acetic acid diluted with 1000 ml of water. Mobile phase B was 19.3 g ammonium acetate and 14.4 ml of acetic acid, diluted with 1000 ml of 50% acetonitrile in water (v/v). The flow rate was 1.0 ml min. The gradient program was as follows: initial conditions, 90% A/10% B; linear change to 10% A/90% B during 0–20 min; and linear change to 90% A/10% B during 23–26 min. At least 7 min of equilibration time at initial condition was used between the runs.

Identification of Metabolites

Bambuterol, the monocarbamate, and terbutaline were identified by retention-time resemblance to the synthetic standards present in each HPLC run. The double peak called "metabolite II" was recently, in a rat liver microsomal system, identified as hydroxylated bambuterol (Fig. 1) and demethylated bambuterol by means of mass spectrometry (manuscript in preparation). In the present study,

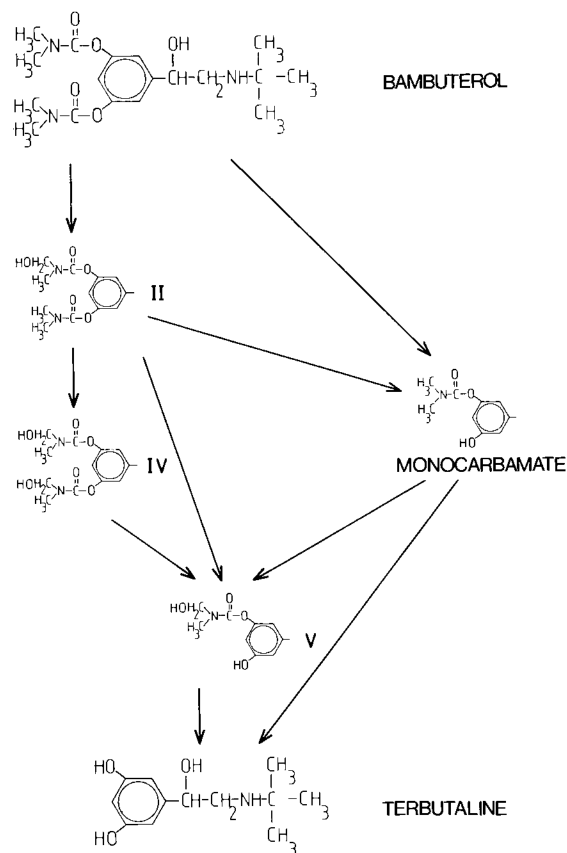

Fig. 1. Bambuterol and metabolic pathways.

metabolite II was tentatively identified by retention time relative to the standards.

Kinetic and Statistical Calculations

Lung extraction (E) = $(C_{in} - C_{out})/C_{in}$, where C_{in} and C_{out} are the concentrations of drug in the inflowing and the outflowing medium.

The efflux constants K (min^{-1}) were obtained by computer-performed linear regression analysis using the method of least squares. Results are given as mean values ± SE (N = 8). Significance analyses were performed using Student's t test.

RESULTS

Single-Pass Experiments

The lung extraction ratios (E) of ^{3}H-bambuterol and ^{14}C-sucrose (both total radioactivity) are given as a function of perfusion time in Fig. 2. A statistically significant ($0.05 \geq P \geq 0.001$) higher lung extraction was noted for bambuterol than for sucrose from 30 sec of perfusion and to the end of the perfusion period (900 sec). At 30 sec the lung extraction ratios (E) for bambuterol and sucrose were 0.121 ± 0.009 and 0.068 ± 0.017, respectively. The corresponding values at 900 sec were 0.016 ± 0.003 and 0.001 ± 0.003.

The concentrations of radioactivity expressed as unchanged compounds in the effluent during and after termination of infusion of ^{3}H-bambuterol and ^{14}C-sucrose are shown in Fig. 3. After the termination of infusion, at least two

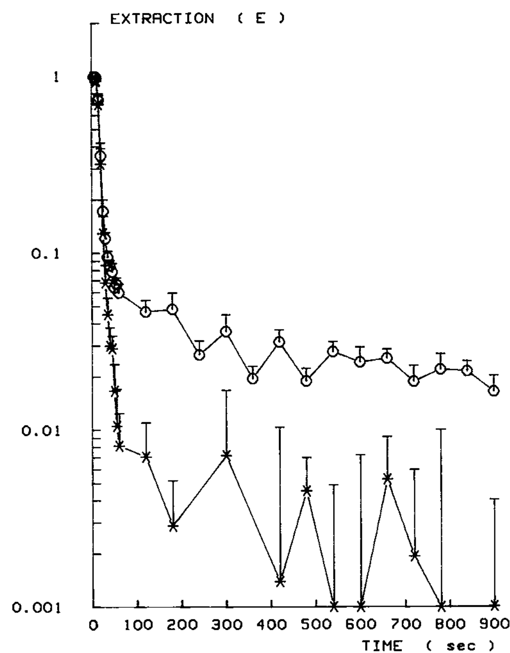

Fig. 2. Lung extraction (E) of total radioactivity during infusion of ^{3}H-bambuterol (O——O) and ^{14}C-sucrose (*——*) with time using isolated perfused and ventilated guinea pig lungs. Inflowing concentrations of bambuterol and sucrose were 0.01 and 1.0 nmol/ml, respectively. Values are means ± SE (N = 8).

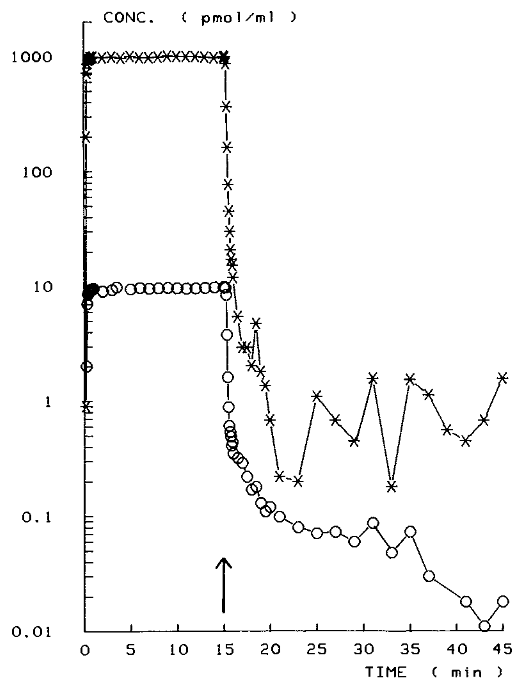

Fig. 3. Effluent concentrations of total radioactivity after infusion of ^{3}H-bambuterol (O——O) and ^{14}C-sucrose (*——*) to an isolated guinea lung preparation, perfused using the single-pass mode. Inflowing concentrations of bambuterol and sucrose were 0.01 and 1.0 nmol/ml, respectively. Arrow shows termination of infusion of bambuterol and sucrose.

phases, one rapid (α) and one slow (β), were discernible for bambuterol and at least one rapid for sucrose. Efflux constants, K (min^{-1}), are given in Table I. Due to unreliable measurements of the very low amounts of radioactivity attributable to ^{14}C-sucrose, it was not possible to calculate K values for a slow phase of sucrose.

The total amount of bambuterol (^{3}H activity) remaining in the lung, after a 30-min efflux period, amounted to 30.5 ± 4.8 pmol/lung, corresponding to 1.31% of the total dose of bambuterol given to the lungs. The corresponding values for sucrose (^{14}C activity) were 280 ± 62 pmol/lung and 0.12%, respectively. HPLC analysis of perfusate samples showed that 95.6 ± 3.6% of the total perfusate ^{3}H activity was attributable to unchanged bambuterol.

Recirculatory Experiments

In these experiments the lung uptake of bambuterol was 96 ± 22 pmol/lung (N = 3), corresponding to almost 19% of the amount of drug initially present in the recirculating system. About 15% of the radioactivity present in the lung, after 2 hr of perfusion, represented metabolized drug. In the perfusate 93–98% of the radioactivity was unchanged bambuterol after 2 hr of perfusion. These results are summarized in Table II.

HPLC chromatograms from perfusate and lung tissue samples are shown in Fig. 4. Metabolites with retention times identical to those of metabolite II and the monocarbamate derivative, a hydrolysis product of bambuterol, were found. Traces of radioactivity with a retention time identical to that of terbutaline were also detected in the lung samples.

DISCUSSION

Various phenylethanolamine derivatives, such as β_2-adrenoceptor agonists, seem to have some affinity to lung tissue (8–10). However, large variations exist. Lipophilicity may be one important factor to determine the lung affinity. For drugs, such as β_2-adrenoceptor agonists, used in the treatment of respiratory disorders, knowledge of the fate of these compounds in the lung is of importance to understand their mode of action, e.g., effect duration. Also, increased affinity to the lung tissue may be of importance to increase target organ specificity and possibly minimize adverse effects on other organ systems such as the cardiovascular system (palpitations), the skeletal muscle (tremor), and the central nervous system (CNS; nervousness).

In our studies with bambuterol, using a clinically relevant concentration, lung uptake was noted. In the recirculating experiments, primarily aimed to study the metabolism of bambuterol, a decrease in the perfusate concentration of the radioactivity of about 20% was noted during the first 5–10 min of perfusion. This was in excellent agreement with the amount of unchanged drug and metabolites present in the lung tissue after the 2-hr perfusion period, 96 ± 22 pmol (N = 3) corresponding to 19% of the total amount of bambuterol initially present in the recirculating system. The weight of the lungs was about 1.9 g, which means that the concentration of bambuterol and metabolites in the lung tissue was 52.4 ± 14.2 pmol/g wet weight at steady state. Their concentration in the perfusate, at the same time, was 6.7 pmol/ml. Bambuterol and its metabolites were thus concentrated by at least a factor of 7 in the lung tissue.

Table I. Values for Efflux Constants K (min^{-1}) for Total Radioactivity from Isolated Guinea Pig Lungs After Discontinuation of ^{3}H-Bambuterol and ^{14}C-Sucrose Infusion (15 min)[a]

Compound	Conc. (nmol/ml)	K (min^{-1})	
		α	β
^{3}H-Bambuterol	0.01	2.415 ± 0.402	0.052 ± 0.007
^{14}C-Sucrose	1.0	3.257 ± 0.271	—

[a] Values are means ± SE (N = 8).

In the single-pass studies a significantly (0.05 ⩾ P ⩾ 0.001) high lung uptake (E) was found for bambuterol than for the extracellular marker sucrose. The lung uptake of the parent compound terbutaline (E = 0.013–0.021) in the isolated lung preparation (rat and guinea pig) has been reported (8). Although the experiments with bambuterol and terbutaline were not performed at the same time, the data indicate that bambuterol has a higher lung uptake than terbutaline. This is what can be assumed since the amphophilic bambuterol (log k_D = 3.5, where log k_D is the partition coefficient between chloroform and water) is more lipophilic than terbutaline (log k_D = −3.9, chloroform and water).

From the efflux studies with bambuterol at least two phases, one rapid (α) and one slow (β), could be discerned. The rapid phase very likely reflects washout of compound from the vascular compartment and other compartments rapidly in equilibrium with the vascular compartment. This assumption is supported by the fact that the K_e value for the α phase of bambuterol (2.415 ± 0.402 min^{-1}) was similar to the corresponding value for sucrose (3.257 ± 0.271 min^{-1}), which is used as an extracellular marker. For bambuterol a slow (β) phase could also be discerned, probably representing washout of drug and metabolites from tissue compartments, thus indicating a certain tissue uptake. No such β phase could be seen with sucrose. This seems plausible, since sucrose, being an extracellular marker, has a very low tissue uptake.

Experiments were also performed to investigate whether any lung metabolism of bambuterol occurred. This is of special importance since bambuterol per se lacks smooth muscle relaxing properties and only the end product terbutaline is the active bronchodilator. In the guinea pig trachael preparation bambuterol is inactive but does not influence the effect of terbutaline (2). The drug seems also to lack acute bronchospasmolytic effects when given in nebulized form locally to the lungs in anesthetized guinea pigs challenged with intravenously administered histamine (2). Whether this is due to slow absorption of bambuterol or too short observation time or other things is not known. Bambuterol, however, demonstrates bronchospasmolytic properties when given orally to conscious guinea pigs (2). These data suggest that bambuterol partly is metabolized during the passage from the gastrointestinal tract to the systemic circulation. Experiments with isolated perfused guinea pig livers have shown that bambuterol is extensively metabolized at the carbamate part of the prodrug molecule and that, e.g., terbutaline is formed (3). Our experiments with perfused guinea pig lungs showed that this organ also biotransforms bambuterol. Oxidative as well as hydrolytic metabo-

Table II. Metabolism of ^{3}H-Bambuterol in Isolated Perfused Guinea Pig Lungs During Recirculation[a]

Lung No.	Time (min)	Bambuterol	OH-bambuterol	Monocarbamate	Terbutaline
I					
P	0	98.9	0.4	0.3	0.3
P	120	97.6	0.5	1.9	0
L	120	84.2	9.6	5.6	0.6
II					
P	0	99.6	0.2	0.2	0.1
P	120	93.2	1.2	4.3	1.4
L	120	87.6	4.0	7.7	0.7
III					
P	0	99.6	0.2	0.2	0.1
P	120	95.4	1.0	3.5	0.1
L	120	86.7	5.1	7.6	0.7

[a] P, perfusate; L, lung tissue. The numbers indicate the percentage of the total radioactivity found in perfusate or lung tissue, with correction for impurities in the ^{3}H-bambuterol. The results were calculated as illustrated in Fig. 4.

lism occurred since hydroxylated bambuterol as well as the monocarbamate derivative and traces of terbutaline were detected after a 2-hr perfusion period (Fig. 4). The metabolites formed seemed to be retained in the lung tissue since only traces of them were found in the perfusate. Whether the amounts of metabolites formed in the lung, in particular terbutaline, can contribute to the bronchospasmolytic action noted after oral administration is not known. It can also be critical regarding in which type of lung cells uptake and metabolism to active metabolites occur and the site(s) of action for the active metabolites. Even small amounts of active metabolites, formed close to their site of action, may contribute to the antiasthmatic properties. In this respect the prodrug technique may offer a possibility to achieve better localization of the active drug since the more lipophilic prodrug can penetrate into tissues to which the more hydrophilic parent drug does not penetrate (12).

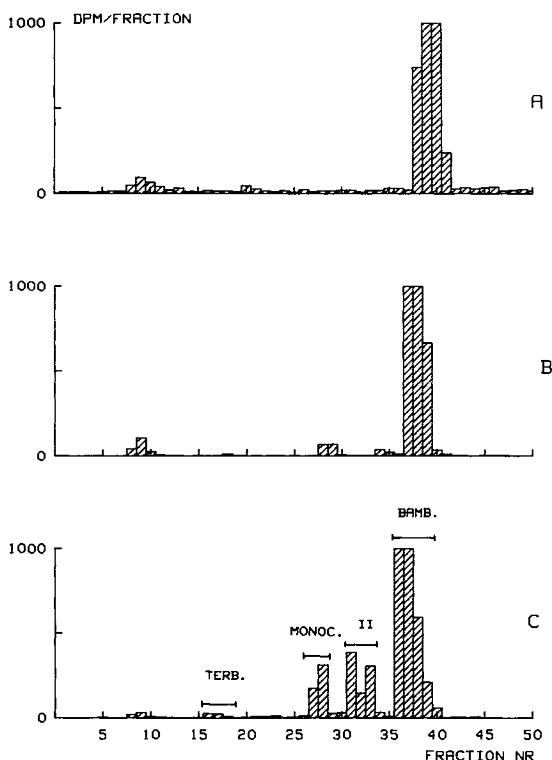

Fig. 4. Metabolism of ^{3}H-bambuterol during recirculating conditions. HPLC elution profiles of a blank perfusate (A); a perfusate 120 min after the start of drug administration (B), and a sample obtained from a lung perfused with ^{3}H-bambuterol for 120 min (C). BAMB., bambuterol; II, hydroxylated bambuterol; MONOC., monocarbamate, TERB., terbutaline (see Fig. 1).

In conclusion, a certain lung uptake of bambuterol occurs. Oxidative as well as hydrolytic metabolism takes place in the guinea pig lung, suggesting that terbutaline formation from bambuterol does occur in the lung. Whether the amount of terbutaline formed is high enough and formed at critical sites to exert antiasthmatic properties cannot be settled from the experiments performed in this study. However, these findings may offer one explanation to the results obtained in clinical studies with bambuterol. Thus, the finding that bambuterol, in comparison with terbutaline, was found to be an effective bronchodilator at lower terbutaline plasma levels and, hence, gave a lower incidence of systemic side effects may be explained by an enhanced delivery to the target organ of terbutaline via uptake and metabolism in the lung of bambuterol and some of its metabolites.

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