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Sympathomimetic Bronchodilators: Increased Selectivity with Lung-Specific Prodrugs

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Abstract: The development of selective bronchodilator β-adrenoceptor agonists is reviewed with emphasis on a *pharmacodynamic approach,* which is directed to drugs with high specificity for the β_2-adrenoceptor, and on a *pharmacokinetic approach* in which known β-adrenoceptor agonists are converted to prodrugs with selectivity for the lung. The pharmacodynamic approach has produced drugs that display high specificity for the β_2-adrenoceptor but still suffer from side-effects including tremor and palpitations. This is due to the fact that the β_2-adrenoceptors present in skeletal muscle and blood vessel are indistinguishable from those in the airways. On the other hand, the prodrug pharmacokinetic approach offers a promising way to obtain selectively acting bronchodilators with significantly fewer side-effects.

For more than ten years selective β_2-adrenoceptor (β_2-AR) agonists have been valuable drugs in the treatment of bronchial asthma. The development of these drugs is an example of the effective utilization of the principle of pharmacologic selectivity and receptor specific drug design. The history of this field began with the α-, β_1- and β_2-AR agonist epinephrine which was introduced at the turn of this century and proceeded via the β_1- and β_2-AR-agonist isoproterenol discovered in the fifties to the almost pure β_2-AR agonists we have today. This effort has resulted in the availability of safer drugs that provide improved and more convenient therapy. Increased safety of the newer β_2-AR agonists is mainly due to elimination of the undesirable β_1-AR cardiac stimulating activity found in many of the older drugs. The side-effects generally encountered with the β_2-AR agonists are tremor, palpitations and nervousness. Tremor usually is the dose-limiting factor with the newer drugs, whereas with the unselective β-AR agonists the more dangerous tachycardia is dose-limiting.

This review will focus on the search for more selectively acting β_2-AR bronchodilators, i. e. for compounds with a significantly increased therapeutic efficacy. The research in this field has progressed along the following two principal paths:

i) The pharmacodynamic approach in which the aim was to design agonists with increased selectivity for the β_2-AR.

ii) The pharmacokinetic approach in which known, therapeutically effective β_2-AR agonists are used as parent compounds in the design of lung-specific prodrugs.

This review will discuss the principles of drug design in this field and will use representative drugs to illustrate different approaches that attempt to optimize the therapeutic efficacy of candidate drugs through consideration of both pharmacodynamic and pharmacokinetic principles.

The β-Adrenoceptor

In 1948 Ahlquist (1) introduced a classification which described adrenergic receptors as α- and β-AR. A further refinement of this classification was made by Lands in 1967 (2). He proposed a subclassification of the β-AR into the β_1-AR and the β_2-AR with the suggestion that the β-AR of an organ could be classified as belonging to either of the subgroups. The variety of effects mediated through β-AR is given in Table I, with those effects relevant to the treatment of asthma printed in bold faced type. In 1972 Carlsson et al. (3) further refined Lands' theory by presenting evidence for the co-existence of both β_1- and β_2-AR in the same organ. Both receptors

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Table I. Effects mediated through β-adrenoceptors.

Tissue	Receptor	Effect
Airways	β_2	*Bronchodilation.* *Reduction in mediator release from mast cells.* *Increased ciliary activity* *Increase in pulmonary surfactant* *Antiedema effect*
Heart	β_1	Tachycardia Inotropic action
Blood vessels	β_2	Dilatation
Uterus	β_2	Relaxation
Skeletal Muscle	β_2	Tremor
Metabolic	β_2	Increase in glucose, insulin, lactate, FFA Decrease in potassium

appeared to mediate the same biological response. These authors also found that the relative concentrations of β_1- and β_2-AR differ from organ to organ. For instance, the heart contains mainly β_1-AR while bronchi and skeletal muscle contain mainly β_2-AR. These findings also seem to be valid in man.

In 1976 Ariens and Simons (4) put forward a view that β_1-AR might be physiologically "innervated" receptors, mediating responses to neuronally released norepinephrine, and that β_2-AR might be "hormonal" receptors mediating responses to circulating catecholamines, particularly epinephrine. Further support for this hypothesis has been presented by Bryan et al. 1981 (5).

It may be concluded that a substantial body of knowledge exists for both the distribution of and the effects mediated by the two β-AR subtypes. Moreover, the β-AR and their mechanisms of action have been thoroughly evaluated during recent years, mainly by Lefkowitz and his group. [For a recent review of this area, see ref. (6)].

Development of β_2-AR Agonists

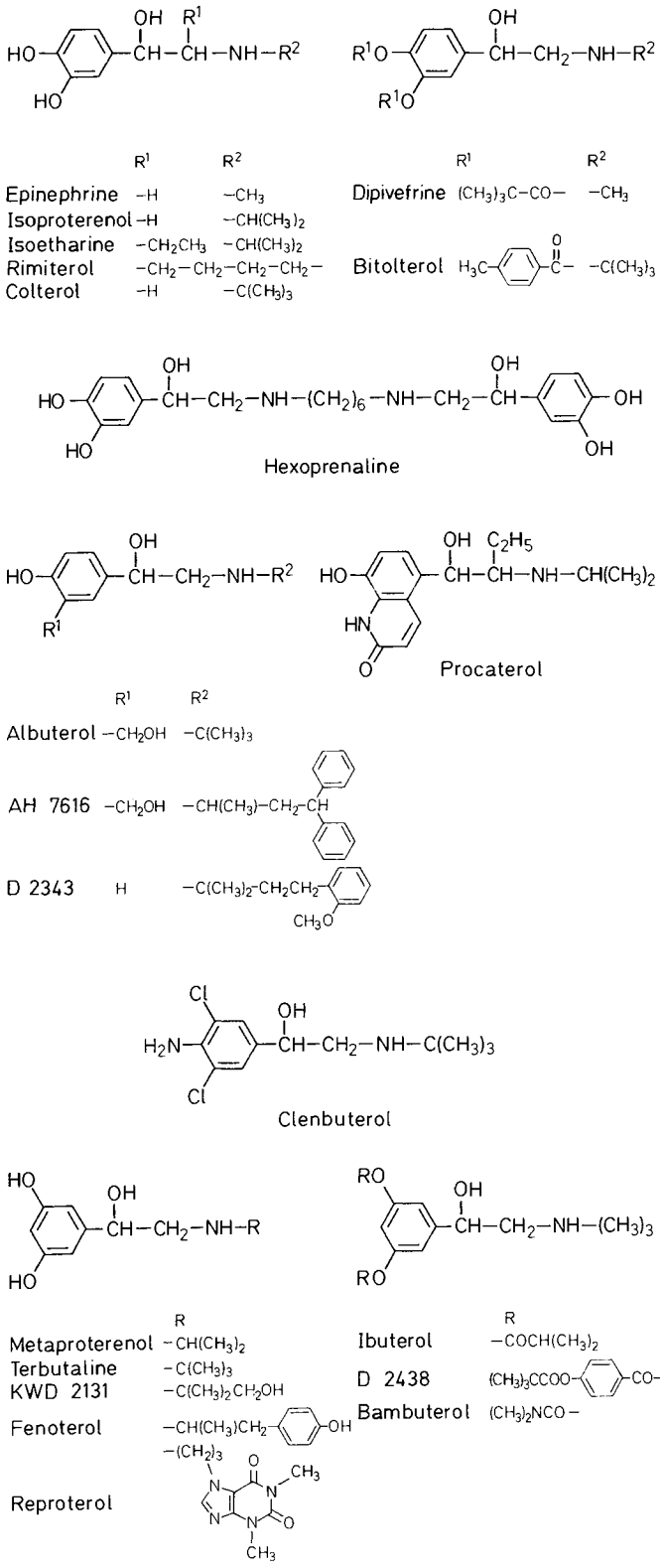
With the present knowledge of β-AR and the various effects which they mediate, it is difficult to visualize how the design of a β-AR-agonist with maximal selectivity for the β_2-AR should result in an increased effect/side effect ratio. Evidently many of the side effects result from interaction of the drug with β_2-AR in tissues outside the target organ, that is the lung.

Tremor is considered to be caused mainly by the direct effect of the β_2-AR on skeletal muscle receptors and represents an increase in physiological tremor (7). Another side effect, palpitations, is due to a reflex initiated response following the fall in blood pressure caused by stimulation of β_2-AR on blood vessels (8). However, in contrast to the bronchodilating effect, these side effects seem to decrease gradually as tachyphylaxis develops during chronic administration of β_2-AR agonists (9).

The situation, however, was different at the beginning of the 1960's when the search began for orally active and more selective bronchodilators than epinephrine and isoproterenol. In this context, epinephrine may be regarded as the prototype for all β-AR stimulating agents. In addition to its potent bronchodilating effect mediated via stimulation of β_2-AR, epinephrine also displays potent cardiovascular side effects mediated through stimulation of both α and β_1-AR. With the development of isoproterenol in 1940 the first step to more selectively acting β-AR agonists was undertaken. Even if isoproterenol now is being replaced by the more selective β_2-AR agonists, this drug was for a long time period the leading therapeutic bronchodilating agent. Isoproterenol is usually administered as an aerosol by inhalation, as it is ineffective after oral administration because of its extensive presystemic metabolism involving both sulfo-conjugation and metabolism by catecholamine *O*-methyl transferase (COMT). Unlike epinephrine, isoproterenol is not a good substrate for monoamine oxidase (MAO) (10). Moreover, isoproterenol, like other catecholamines, is inactivated by the uptake 2 mechanism which, in combination with metabolic inactivation, results in a short effect duration. Even though more selective than epinephrine, isoproterenol stimulates both β_1 and β_2-AR. Consequently a search for more selective drugs with less β_1-AR stimulating effect was begun to minimize potentially serious cardiovascular side effects. Some success was achieved by further development of the catecholamine β_2-AR agonists such as isoetharine (11), rimiterol (12) and hexoprenaline (13) (Scheme I). The latter compound surprisingly is active also after oral administration while the other two compounds, which are metabolized in the same fashion as isoproterenol, are used predominantly by the inhalation route.

The desired properties for a new selective β_2-AR agonist were identified as i) activity after oral administration and ii) prolonged duration of effect. The first compound with these properties to be introduced was metaproterenol in 1966 (14). Although this drug is only slightly more β_2-AR selective than isoproterenol, it is active after oral administration with a duration of action for a single dose of up to 5 hours. The minor change in the molecular structure when going from isoproterenol to metaproterenol – transformation of the 3,4-dihydroxy-catechol moiety to a 3,5-dihydroxy-resorcinol moiety – results in rather drastic pharmacokinetic changes. These changes are due to the fact that the resorcinol structure is neither a substrate for COMT nor for the uptake 2 mechanism. Metaproterenol is metabolized mainly by sulfo-conjugation and to a minor extent to a tetrahydroisoquinoline derivative (15). The introduction of metaproterenol established a new class of orally active bronchodilators. Selectivity for the β_2-AR, however, was only slightly improved when compared with the catecholamine derivatives, and duration of action was still considered to be too short to give adequate night-time protection for the patient. Therefore, in the early 1960's – even before Lands' classification was published – when it still was generally accepted that the bronchi and heart had the same type of β-AR, the search went on for more selective, orally active bronchodilators. In 1966 this effort led to the development of albuterol (16) and terbutaline (17). It is of interest that the patent applications for these two drugs were filed just a couple of weeks apart during the Fall of 1966 (18, 19).

Albuterol and terbutaline provided two pharmacological tools to further verify Lands' β_1- and β_2-AR concept. Two different approaches to overcome metabolic inactivation by COMT operate with albuterol and terbutaline. Terbutaline utilizes the resorcinol moiety, first introduced in metaproterenol, while albuterol – by insertion of a methylene group between the aromatic catechol nucleus and the *m*-hydroxyl group – is converted to a saligenin derivative, which no longer is a substrate for COMT. Both albuterol and terbutaline, however, still are substrates for conjugating enzymes. In man mainly the sulfo-conjugates are formed (20, 21). Surprisingly, in these two classic compounds with their rather different aromatic moieties, maximal β_2-AR agonist activity is found



when the substituent at nitrogen is a tertiary butyl group, while the corresponding catecholamine, colterol, is about 10 times less β_2-AR selective than terbutaline *in vitro* (22).

The bronchoselective effect of albuterol and terbutaline is well documented. Table II lists the quotients between the bronchospasmolytic effect (β_2-AR) and heart stimulation (β_1-AR) for these and related drugs in relationship to that of isoproterenol. In receptor binding studies the two agonists albuterol and terbutaline also show higher affinity for the β_2-AR than for the β_1-AR (23).

Table II. Selectivity quotient of some β-adrenoceptor agonists.

Compound	Selectivity quotient[1]	Selectivity quotient[2]
Isoproterenol	1.0	1.0
Terbutaline	31	42
Albuterol	17	25
Isoetharine	11	–
Metaproterenol	–	3.8
Fenoterol	–	35
Hexoprenaline	21	–

[1] Selectivity quotient obtained from EC50 for tracheal relaxation and EC50 for increase in heart rate in guinea-pigs *in vitro* (55).
[2] Selectivity quotient obtained from ED50 for bronchospasmolysis and ED50 for increase in heart rate in guinea-pigs *in vivo* (56).

Further Development of β_2-Agonists with different Characteristics

After the introduction of albuterol and terbutaline, a variety of new β_2-AR agonists have been prepared (see Scheme I). Even though at this time it was known that most of the side effects experienced with selective β_2-AR agonists were caused by stimulation of β_2-AR in extrapulmonary tissues, the search for even more selective β_2-AR agonists continued. Research mainly aimed at β_2-AR agonists having none, or significantly reduced tremorogenic activity. New compounds, in fact, were found which elicited significantly less tremor than albuterol and terbutaline in animals. One of these compounds, AH 7616 (24), seems not to have been examined in man, since animal studies revealed that the separation between tremor and bronchodilating effects most likely was caused by the compound's strong serotonin (5-HT) antagonist action. When acetylcholine, instead of 5-HT, was used as the spasmogen, separation of the bronchodilating and tremorogenic effects disappeared. Another example, D2343 (25), proved to be more effective in inhibiting histamine induced bronchospasm than in generating tremor. Since histamine is more likely to contribute to bronchospasms in asthma than 5-HT and since D2343 displays α_1-antagonist activity in addition to β_2-AR agonist activity, D2343 was brought to clinical studies in man. The α_1-AR blocking properties of D2343 may be of additional therapeutic value, since increased α_1-AR sensitivity usually is found among certain groups of asthmatics (26). However, studies in man showed that D2343, if anything, was slightly more tremorogenic than terbutaline (27). These disappointing results were partially explained by the efficient liver metabolism of D2343 to more potent but also less selective catechol derivatives (28).

KWD 2131 was another drug candidate with promising selective action. In addition to their bronchodilating action β_2-AR agonists also have an antiallergic effect. This effect is suggested to be caused by a β_2-AR mediated inhibitory action on histamine release from mast cells. KWD 2131 was an interesting compound in the sense that in animal experiments it displays a selective action in inhibiting the anaphylactic release

of histamine from sensitized guinea-pig lung tissue (29). In comparison with terbutaline, at equibronchospasmolytic doses, KWD 2131 is 10 to 25 times more effective in inhibiting histamine release. In this case, one could expect the combination of antiallergic and bronchodilating effects to give a beneficial effect/side effect ratio in the treatment of asthma. However, the results from clinical studies in man were again disappointing since KWD 2131 provided no clinical advantage in allergen provoked bronchospasms in extrinsic asthmatics (30).

These results together with similar findings with other currently used selective bronchodilators including the highly selective β_2-AR agonist procaterol (31), strongly suggest that it may prove impossible to separate the bronchodilating and tremor enhancing properties of β_2-AR agonists when administered orally.

All of the newer orally active β_2-AR agonists are also used in various inhalation formulations. In this way a small but highly effective dose is delivered to the lung. The small dose delivered, however, gives the desired therapeutic effect with a fast onset of action and a duration of action of up to 6 hours. After inhalation, the plasma concentration of active drug will be negligible, so the patient usually will not experience any side effects. Generally, however, only 10 % of the inhaled dose reaches the lung and the rest is swallowed (32). Improved selectivity in inhalation therapy has recently been achieved with new inhalation devices. With a newly developed pear-shaped spacer – Nebuhaler® – aerosolized drug is delivered more efficiently to the lung and less drug is swallowed or deposited in the mouth (33).

In summary, increased selectivity for the β_2-AR thus does not seem to give improved selective action with less side effects than experienced with bronchodilators with only moderate selectivity for the β_2-AR. Improvements, however, have been obtained with regard to the duration of action with drugs such as clenbuterol and procaterol. Consequently, dosing is possible b.i.d. instead of t.i.d. or even q.i.d. which generally is the case with other β_2-AR agonists. Prolonged duration of effect also may be achievable by pharmaceutical means, such as with slow release formulations.

Prodrug Development

A drug that displays good agonist properties but has limited therapeutic efficiency due to formulation problems or poor pharmacokinetic behavior is an ideal prodrug candidate for which suitable reversible derivatives should be sought (34, 35). The catecholamine type β-AR agonists all exhibit stability problems because of the ease of oxidation of the catechol moiety. In solution these drugs are rapidly destroyed unless antioxidants are present. The non-catechol β_2-AR agonists do not suffer this disadvantage because of the increased chemical stability of the various aromatic moieties present in these drugs.

Therapeutic limitations of a pharmacokinetic nature also are apparent with the catecholamine type drugs, since they generally are inactive after oral administration. Even after administration in aerosol form locally to the lung, these compounds are rather short acting. As mentioned earlier, the reason for this behavior is that in general catecholamines are extensively metabolized both presystemically by conjugation and systemically by COMT and the uptake 2 mechanism. The latter mechanism also inactivates such drugs in lung tissue.

Most of the non-catechol β_2-AR agonists also undergo presystemic metabolism to some degree, usually by conjugation, but since they generally are resistant to both COMT and uptake mechanisms, they are orally active with durations of action ranging from 4 to 12 hours. In some cases the hydrophilic nature of the β_2-AR agonist molecule leads to slow and/or incomplete absorption which may result in inter- and intraindividual variations in plasma levels of the active drug. Among the catecholamine and many of the non-catecholamine β_2-AR agonists the vulnerable phenolic hydroxyl groups are the structural elements mainly responsible for the pharmaceutic and pharmacokinetic based disadvantages. Therefore, modification of the hydroxyl groups in these compounds with a suitable and chemically inert protecting group should lead to improved chemical stability, decreased rate of metabolic inactivation and, with increased lipophilic character, enhanced adsorption. Chemical derivatization generally results in the conversion of agonists to pharmacologically inactive molecules. Therefore, it is essential to design protecting groups so that they are cleared as soon as the specific task of the prodrug has been achieved. A widely used prodrug linkage is that of an ester. In the case of β_2-AR agonists, the hydroxyl groups are converted to the corresponding esters with a suitable carboxylic acid reagent.

The ester is an appropriate prodrug derivative since esterases, which are widely distributed within the human body, will catalyze their hydrolysis to produce the active drug. In addition, presystemic conjugation reactions at the phenolic hydroxyl groups cannot take place as long as the protective ester group is intact. The ester prodrug technique thus seems to offer possibilities to address both stability and pharmacokinetic problems of catechol and related β_2-AR agonists. A few examples of such prodrugs will be given below.

The main issue is now whether selectivity can be improved in this way so that undesirable side effects like palpitations and tremor are avoided at therapeutically effective doses. Results indicating increased selectivity were first obtained with the prodrugs ibuterol (36) and bitolterol (37) in the asthma field and with dipivefrine (38) in glaucoma. Bitolterol and dipivefrine are catecholamine derived prodrugs, while bitolterol is the bis-*p*-toluate of colterol and dipivefrine is the bis-pivalate of epinephrine. Ibuterol is the bis-isobutyrate of terbutaline. As expected, conversion of epinephrine to dipivefrine results in improved stability and increased lipid solubility. Additionally this prodrug was found to be about 100 times more effective than epinephrine in the management of glaucoma and about 100 to 400 times weaker than epinephrine in affecting the cardiovascular system (39). Dipivefrine, however, seems not to have found any use in the treatment of asthma.

Animal studies with bitolterol (40) revealed some interesting properties of this prodrug. Data from these studies indicate that bitolterol is absorbed following oral administration and subsequently is retained as the intact ester in the lung. After intravenous administration of ^{3}H-bitolterol to rats and dogs radioactivity was preferentially retained in the lung compared to the blood and the heart. Lung radioactivity after intraduodenal administration was 2 to 4 times lower but significiantly higher than blood radioactivity. When the radioactivity remaining in the lung was analyzed with respect to intact bitolterol, the prodrug was found to be present in lung tissue after either route of administration. Esterases in human lung and plasma hydrolyze bitolterol somewhat faster than the corresponding esterases in the dog. The slower rate of hydrolysis of bitolterol in the dog is reflected in the mean elimination

of radioactivity from plasma where the value for the dog is 15 hours. The corresponding value in man is approximately 3 hours. After oral administration in man, intact bitolterol in plasma accounts for only 1 % of the total radioactivity (41).

Good bronchodilating activity with a prolonged duration of action was obtained with bitolterol when compared with colterol and isoproterenol after intraduodenal administration to dogs (40). In addition, bitolterol showed significantly less chronotropic effect than albuterol at equibronchospasmolytic doses, both after intraduodenal and aerosol administration in dogs. The relevance of such effect comparisons, however, in single dose studies is doubtful since the distribution of the drugs may be quite different at steady state reached after repeated doses. It also was found that the cardiovascular effects of bitolterol and isoproterenol were similar in asthmatic patients (42). When oral administrations of albuterol and bitolterol were compared in patients over a period of four weeks, bitolterol caused more adverse drug reactions and was not found to be more effective than albuterol (43).

Bitolterol aerosol gives a relatively long acting bronchodilating effect when compared with isoproterenol and the parent compound colterol. Application of the prodrug technique on colterol thus has resulted in an improved duration of activity. The long lasting effect of bitolterol is believed to be due to the high affinity of the prodrug for lung tissue. Consequently, bitolterol is retained in lung where it subsequently is hydrolyzed by esterases to colterol which then exerts its bronchospasmolytic effect by stimulation of β_2-AR. A prolonged duration of activity after oral administration of bitolterol, however, seems not to have been realized (37).

One may speculate that the low cardiovascular effects observed in the bitolterol single dose studies in dogs may have been due to either a distribution effect or by a β_1-AR-antagonistic effect exerted by bitolterol itself. Similar findings in our laboratory were obtained with various ester prodrugs of terbutaline. The bis-3,5-dibenzoate of terbutaline and ester prodrugs of similar hydrolytic stability were found to block the effect of epinephrine when given after the test compound in an isolated tracheal preparation (44). Similar results were also obtained with ibuterol which, in one study, was found to produce a non-competitive antagonism of chronotropic responses to isoproterenol in guinea-pig atria (45). Like bitolterol, ibuterol was also found to be retained in lungs from rat and guinea pig (46).

In clinical studies intravenously infused ibuterol – in doses producing bronchodilation equal to that of terbutaline – showed significantly less effects on heart rate, blood pressure and tremor (47). Similarly, in a study with orally administered ibuterol, the prodrug was found to be less tremorogenic than terbutaline (36). Although these studies deal with single doses of ibuterol, the data obtained in the intravenous infusion study may be explained in that under these circumstances intact ibuterol possessing β_1-AR-antagonistic activity may be present at low concentrations to modulate the cardiovascular effects. Hydrolysis of ibuterol to terbutaline seems not to take place in skeletal muscle (48) but occurs in lung where ibuterol accumulates. In this way the lower incidence of tremor after intravenous ibuterol may be explained. After oral administration ibuterol, however, is quantitatively hydrolyzed to terbutaline during the first-pass through the gut wall and liver. Therefore, no intact ibuterol is found in the general circulation. This behavior makes it difficult to find any explanation for the lower incidence of tremor observed after oral administration of ibuterol.

The improvements achieved with oral ibuterol included a faster onset of action and a better reproducibility of the bronchodilator effect because of the more favorable absorption of the prodrug. Its short duration of action following oral administration, however, was a drawback. The reason for this was that the ester groups in ibuterol were not sufficiently stable to resist presystemic hydrolysis and thereby protect against presystemic conjugation. In fact, a higher degree of presystemic conjugation than with terbutaline was found after oral administration of the prodrug (36).

It now became obvious to us that if the lung retention of a β_2-AR agonist prodrug was to be exploited, esters with high presystemic stability were required. If such a prodrug could resist presystemic metabolism to a substantial degree, the intact prodrug could distribute into the general circulation and be retained in lung tissue where the active β_2-AR agonist would be released by hydrolysis.

With this hypothesis in mind two new terbutaline ester prodrugs, D2438 and bambuterol, were designed with the goal that they would possess high presystemic stability (49). The cascade ester D2438 is derived from *p*-pivaloyloxybenzoic acid and was designed to undergo first pass hydrolysis and conjugation at the *p*-pivaloyloxybenzoic acid moiety, distal from the active resorcinol moiety in terbutaline. D2438 itself is active in the isolated guinea pig trachea and displays prolonged duration of action both after inhalation in guinea pigs and after oral administration in dogs. When D2438 was tested in man, who generally seems to have a higher esterase activity than the dog, this prodrug did not show the high presystemic hydrolytic stability observed in the dog.

On the other hand, bambuterol, the bis-*N,N*-dimethylcarbamate of terbutaline, displays presystemic hydrolytic stability also in man where bambuterol produces prolonged plasma concentration profiles of terbutaline. The improved hydrolytic stability of bambuterol mainly depends on the dimethylcarbamate groups which make the prodrug behave like an esterase inhibitor. In this way, bambuterol slows down its own rate of hydrolysis by reversibly inhibiting the esterase participating in its hydrolysis, such as pseudocholinesterase. Bambuterol displays high affinity and selectivity for pseudocholinesterase without affecting acetylcholinesterase (50). In addition to producing a significantly prolonged duration of action in asthmatic patients, bambuterol, at steady-state, was found to produce bronchodilating effects at lower terbutaline plasma levels than generally are required when terbutaline itself is given (51). Since the side effects at steady-state are proportional to the plasma concentration of terbutaline, this behavior results in a significantly lower incidence of side effect such as tremor when equibronchodilating doses of bambuterol and terbutaline are compared in asthmatic patients. These results are consistent with the actions of a site specific prodrug.

As demonstrated for bitolterol and ibuterol, bambuterol also displays a certain affinity for lung tissue (52). In fact, this behavior seems to be a common feature among basic drugs with the phenylethylamine structure (53). Moreover, the carbamoyl methyl groups of bambuterol are oxidized in the liver in a cytochrome P-450 catalyzed reaction to yield metabolites that still are lipophilic carbamate prodrugs of terbutaline (54). This pathway seems to be a more efficient way to generate terbutaline from bambuterol than direct hydrolysis of bambuterol via the monocarbamate. Studies on isolated perfused guinea pig lungs have demonstrated that bambuterol and some of its metabolites are retained in lung tissue. This opens the possibility that terbutaline may be generated *in situ* in the

lung (52). In this way the data obtained with bambuterol in patients could be explained in that therapeutic terbutaline levels may be obtained locally in lung tissue, even at low plasma concentrations of terbutaline. Another explanation is that bambuterol and its metabolites are active bronchodilators themselves. In animal studies, however, both bambuterol and some of its metabolites lack β_2-AR stimulating effects.

Clinical studies in asthmatic patients with oral bambuterol thus indicate that, in addition to a significantly prolonged duration of action, this prodrug also displays an increased ratio of bronchodilating effect to side effects. The apparent selectivity obtained when terbutaline is converted to bambuterol appears to be the result of altered tissue distribution and pharmacokinetics rather than increased β_2-AR specificity. Consistent with this view, terbutaline, when given as such, behaves differently both with respect to duration of action and incidence of side effects.

Conclusion

The pharmacological approach to the development of bronchodilators with increased selectivity for the β_2-AR has not led to greatly enhanced selectivity, since many of the side effects of β-agonists are mediated through stimulation of β_2-AR in tissues outside the lung. On the other hand the pharmacokinetic approach in which therapeutically effective β_2-AR agonists are converted to lipophilic prodrugs with affinity for lung tissue, seems to have been more successful in achieving enhanced selectivity. In addition, such prodrugs also seem to have a long lasting bronchodilating effect. The increased duration of action realized in these new agents provides additional advantages over traditional β-agonists in the treatment of the asthmatic condition.

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RESEARCH ARTICLES

Absorption of Etoposide (VP-16-213) from the Small Intestine of the Rat. The Potential Role of Mucus as an Absorption Rate Limiting Barrier

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Abstract: The absorption of etoposide (VP-16-213) was investigated in a perfused intestinal loop. The absorbed amount of drug was determined by collecting the blood draining the loop via cannulation of the efferent jejunal vein. The absorption rate of VP-16-213 strongly depended on the composition of the perfusion medium. The addition of taurocholate to an aqueous etoposide solution enhanced the absorption rate. When *N*-acetylcysteine was added to an aqueous solution, the absorption rate dropped significantly.

The absorption of etoposide in man after oral administration is erratic (1). In rats relatively high concentrations of etoposide were found in the intestine. This finding may indicate binding of etoposide to intestinal mucus. In the literature, mucus binding has been described for various drugs (e. g. 2–7). In most cases, the transport rate of the drug from the intestinal lumen to the blood was decreased if a drug-mucus interaction occurred. Only 10–20 % of benzomethamine, a monoquaternary compound, was taken up over 3–4 hours; absorption mainly occurred during the first 45 minutes. It was shown, that binding to mucus was responsible for the slow absorption rate (8, 9). Meli et al. (10) observed that ethynylestradiol cyclopentyl ether disappeared from the intestinal lumen by 85 % within 7.5 minutes. Almost the entire amount that disappeared was bound to the intestinal tissue from where it was released very slowly. The absorption of some water soluble dyes was linearly related to the binding of these substances to the intestinal mucus (11–14). The binding to intestinal mucus was essential for the absorption of these dyes, because digestion of the mucus glycoproteins, and even the glycocalyx, caused a severe decrease in the absorption of these dyes.

If the intestinal mucus is a significant barrier to the absorption of a drug, attempts can be made to promote absorption by reducing the influence of the mucus. A possible way to increase the diffusion rate through the mucus might be to decrease the apparent viscosity of the gel. Organic compounds, containing thiol groups such as dithiothreitol, 2-mercaptoethane-sulfonate, glutathione and cysteine derivatives can reduce the disulfide bridges in the core of the glycoprotein molecule, thereby, breaking up the native structure of the glycoproteins (15–17). This results in a decreased elasticity and an increased ciliary transport rate (18–20). The addition of 2 % *N*-acetylcysteine (N-ac) causes a 40 % reduction in consistency of the mucus after a contact time of 60 minutes (17). The optimal pH for the action of *N*-acetylcysteine (N-ac) lies around 7 (21, 22). *N*-acetylcysteine also enhances the binding of some antibiotics to mucus (2).

The resistance of the mucus layer in the absorption process can also be changed by bile salts. It was shown that bile salts affect mucus viscosity and elasticity (23); therefore, these compounds might influence the absorption kinetics of mucus interacting drugs. The benificial effect of bile salts on the absorption process of a number of drugs has been reported (24–26). In general, the mechanism behind the observed phenomena is not clear. An increase in the "apparent" solubility, or a change in membrane or mucus characteristics might play a role.

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