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Review

β-Adrenoceptor agonists and asthma—100 years of development

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Abstract

Inhaled β_2-adrenoceptor agonists are by far the most effective and safe bronchodilators currently available. They have not been surpassed by any other bronchodilating principle. The way to this position has been long and started with the first successful treatment of acute, severe asthma with s.c. injections of adrenaline 100 years ago. Over the years, synthetic congeners of adrenaline have been produced and tested for their pharmacological properties. During the first decades, little attention was given airway smooth muscle. The discovery of isoprenaline in 1940 was the first major step towards selective bronchodilation. This compound became a key tool for the classification of adrenoceptors into α and β. Salbutamol and terbutaline were the first to show a significant attenuation of the cardiostimulant effect and confirmed the subdivision of β-adrenoceptors into β_1 and β_2. Much effort was made to eliminate the next dose-limiting side effect, skeletal muscle tremor but in vain. Prolonged duration of action was achieved in three ways: with bambuterol, an orally active carbamate ester prodrug of terbutaline, salmeterol, an inhaled β_2-adrenoceptor agonist emerging from a purposeful research project, and formoterol which was found, accidentally, to have a long duration of action when inhaled. Throughout the 20th century, β-adrenoceptor agonists have been developed and marketed as racemates. The pharmacological activity usually resides in the (*R*)-enantiomer. Despite claims for the opposite, there is so far no compelling evidence that the presence of the less active (*S*)-enantiomer is of any harm to the patient. One hundred years of experience of structural modifications of adrenaline has shown that the possibilities to modify the properties of this endogenous prototype appear to be unlimited.

Keywords: β-Adrenoceptor agonist; Adrenaline; Enantiomer; Asthma; Bronchodilation

1. Inhaled β_2-adrenoceptor agonists, the mainstay in asthma treatment

"Inhaled β_2-agonists are by far the most effective bronchodilators currently available" (Barnes, 1997). This is the way many articles in this field begin today. However, the confidence in sympathomimetics has not always been that obvious. Fifty years ago, remedies for asthma offered by various pharmaceutical companies included formulations containing ephedrine, supplemented with theophylline, caffeine, sedatives and analgetics. Moreover, the increased death rates in asthma in the mid-1960s was associated with the increased use of isoprenaline in pressurized, metered dose inhalers (Inman and Adelstein, 1969). Since then, waves of concern regarding adverse effects of β-adrenoceptor agonists have emerged followed by pleas for their safety (Sears et al., 1990; Giuntini and Paggiaro, 1995; Kips and Pauwels, 2001). The experience today is that, in patients taking inhaled glucocorticosteroids, regular use of the long-acting β_2-adrenoceptor agonists formoterol and salmeterol increase asthma control compared with monotherapy with the steroid (Pauwels et al., 1997; Woolcock et al., 1996). Over the years, the β_2-adrenoceptor agonists have not been surpassed in efficacy and safety by any other bronchodilating principle.

This review will take us back to the very beginning, the first successful use of adrenaline in asthma. From there we will follow, step by step, the development of synthetic analogues of adrenaline with improved pharmacodynamic and pharmacokinetic properties up to the long-acting and selective β_2-adrenoceptor agonists available today. Although β-adrenoceptor agonists have a number of effects on various airway effector cells, emphasis will be on bronchodilation, the principal anti-asthmatic effect of this class of compounds (Barnes, 1997). There is an unfortunate confusion of generic names in this field, starting with the prototype adrenaline which got the name epinephrine in American usage (Max, 1988). This confusion extends to the name of these drugs as a class where β-adrenoceptor agonists and β-adrenergic ago-

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Table 1
Generic names of some β-adrenoceptor agonists, their synonyms or laboratory codes

Generic name	Alternative generic name or laboratory code
Adrenaline	Epinephrine
Isoprenaline	Isoproterenol
Orciprenaline	Metaproterenol
Salbutamol	Albuterol
Fenoterol	Th 1165a
Procaterol	OPC 2009
Formoterol	Eformoterol, BD 40A

nists are used synonymously, or simply the short form β-agonists. To assist literature search a list of synonyms and important laboratory codes are given in Table 1.

2. Adrenaline, ephedrine and the sympathomimetic amines

In the beginning of the 20th century, Bullowa and Kaplan (1903) reported on an extremely successful treatment of severe acute asthma with subcutaneous injections of adrenaline. The patients were dramatically relieved within a few minutes. In those days, the dyspnoe in asthma was thought to be due to turgidity of the bronchial mucosa, the so-called angioparetic theory. The rationale to try adrenaline was its pronounced vasoconstrictor effect, which at that time was a new discovery. The authors concluded "In conformity with the angioparetic theory of an attack, the dose must be such as will cause prompt general vasoconstriction". It was only years later that the relaxing capacity of adrenaline on airway smooth muscle was recognized (Kahn, 1907), let be that this author suggested an indirect inhibitory action via ganglia in the tracheal wall rather than a direct relaxing effect on the muscle fibres. This misconception and confusion on the mode of action may have delayed the development of bronchodilating analogues of adrenaline significantly. Though highly efficient, adrenaline was not the ideal drug. Besides the desired therapeutic effect, bronchodilation, it caused hypertension, tachycardia and tremor. Moreover, due to metabolic instability, it had a short duration of action and had to be given parenterally.

During the first decade after the chemical structure of adrenaline had been established, a large number of derivatives were synthetized and tested for their pharmacological properties. Barger and Dale (1910), in their classical paper, coined the term "sympathomimetic" for the action of this class of compounds. Although they described motor as well as inhibitory effects of their test compounds, focus was on vasoconstriction and pressor effects. Little attention appears to have been given airway smooth muscle and, as expressed in a coeval review of the field, during the following years nothing of particular interest developed (Hartung, 1931). So the next step in the symptomatic treatment of asthma was not a synthetic derivative of adrenaline but the introduction

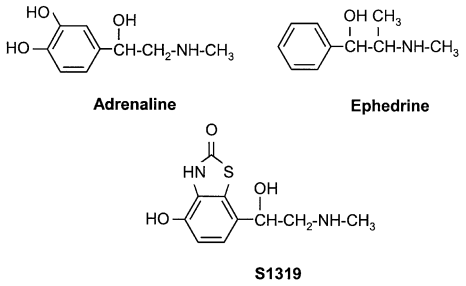


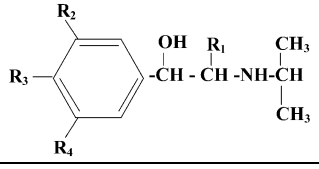
Fig. 1. Three naturally occurring β-adrenoceptor agonists. Adrenaline is unselective, ephedrine is largely indirectly acting whereas S1319 is highly selective for β_2-adrenoceptors.

in western medicine of the natural product, ephedrine by Chen and Schmidt (1924). Ephedrine, the active principle of the old Chinese anti-asthma drug Ma Huang, is structurally related to adrenaline (Fig. 1) but it is a poor β-adrenoceptor agonist (Trendelenburg et al., 1963; Waldeck and Widmark, 1985) and has largely an indirect mode of action (Aviado, 1970). It is still unknown to what extent ephedrine acts by direct bronchodilation or by mucosal decongestion in asthma. Ephedrine had an advantage, however, because it is metabolically more stable than adrenaline and can thus be taken orally. Moreover, it has a longer duration of action.

3. Isoprenaline, the first step towards selective action on bronchial smooth muscle

While attempts had been made to find analogues of adrenaline with an improved bronchodilatory profile (Tainter et al., 1934; Cameron and Tainter, 1936), only Konzett (1940a) could demonstrate a significant step forward with isoprenaline (Table 2), regarded as the first sympathomimetic amine completely devoid of pressor effects. As a matter of fact, isoprenaline causes a pronounced depression of blood pressure (Konzett, 1940b). However, this does not appear to be the full truth. In a comprehensive investigation of hydroxylated derivatives of ephedrine, Schaumann

Table 2
Chemical structures of β-adrenoceptor agonists with an isopropyl group attached to the nitrogen atom



Compound	R_1	R_2	R_3	R_4
Isoprenaline	H	HO	HO	H
Isoetharine	C_2H_5	HO	HO	H
Orciprenaline	H	HO	H	HO
Soterenol	H	CH_3-SO_2-NH	HO	H
Dichloroisoprenaline	H	Cl	Cl	H

(1931) showed that α-ethyladrenaline (termed β-Äthyl-Suprarenin in his study), being equipotent with adrenaline in stimulating the heart, had no pressor effect but indeed lowered the blood pressure dose-dependently in the anaesthetized dog. Unfortunately, airway smooth muscle was not included in the study but the available data suggest, retrospectively, that this compound might have been a good bronchodilator.

Konzett denoted the isopropyl group attached to the nitrogen atom as "broncholysophore", i.e. carrying the bronchospasmolytic effect. The unique properties of isoprenaline contributed decisively to Ahlquist's classification of adrenoceptors into α and β 8 years later (Ahlquist, 1948) and from now on we can talk about β-adrenoceptor agonists as a class. Isoprenaline is an early example of cooperation between university and industry since Konzett, at that time pharmacologist at the Vienna University, got the compound for evaluation from chemists at Boehringer-Ingelheim in Germany (Konzett, 1981). Isoprenaline for inhalation soon became a popular remedy for asthma, but there were still problems with considerable cardiac stimulation, even with a fatal outcome (Inman and Adelstein, 1969). Moreover, being a catecholamine like adrenaline, isoprenaline is rapidly metabolized. This limits the duration of effect and the compound has a poor oral availability.

The concept of a "broncholysophore" group inspired to modifications. Thus a series of phenylisopropyl-derivatives of isoprenaline were synthetized and tested for their bronchospasmolytic effect (Biel et al., 1954). Among these compounds, protokylol gained some clinical use as an orally active and longer acting analogue of isoprenaline (Weiner, 1980). The molecular skeleton of protokylol with its phenyl-isopropyl-group attached to the nitrogen has been adopted in numerous of the β-adrenoceptor agonists to follow (Fig. 2). It is therefore interesting to note that Biel's intention with this side chain was to incorporate a "pressor amine" in the molecule, apparently to potentiate the bronchodilator properties and balance the marked depressor effect displayed by isoprenaline thus creating a clinically more satisfactory antiasthmatic agent. This expectation was not fulfilled, rather an α_1-adrenoceptor antagonistic action was observed among members of this group of potent β-adrenoceptor agonists (Decker et al., 1982). Soterenol, another derivative of isoprenaline (Table 2) should later be shown to possess an appreciable α-adrenoceptor stimulant component in addition to its β-adrenoceptor agonism (Dungan et al., 1968).

More familiar as a metabolically stable and longer acting analogue of isoprenaline is its resorcinol-derivative, orciprenaline (Engelhardt et al., 1961). This compound, apparently the first synthetic resorcinol β-adrenoceptor agonist, resistant against catechol-*O*-methyl transferase, is still in use but its selectivity was not regarded as sufficient and the cardiac problems had to be solved. Among the numerous analogues of isoprenaline, synthetized and evaluated for their pharmacological action, dichloroisoprenaline (Table 2) deserves a special mention. This low efficacy β-adrenoceptor agonist turned out to antagonize competetively the relaxing effect of adrenaline and isoprenaline on airway smooth muscle (Powell and Slater, 1958). This discovery made dichloroisoprenaline the prototype for all β-adrenoceptor antagonists to follow.

4. Salbutamol and terbutaline, the first β_2-selective adrenoceptor agonists in general clinical use

Inspired by the work of Lands and his group (Lands et al., 1950; Lands and Tainter, 1953; Lands and Brown, 1964), even before the definitive subclassification of β-adrenoceptors into β_1 and β_2 (Lands et al., 1967a,b), independent investigators considered the possibility of developing a bronchodilating β-adrenoceptor agonist without adverse effects on the heart. A key observation was that noradrenaline, being equipotent with adrenaline in stimulating the heart, is a poor bronchodilator. This difference in physiology was taken as a possibility of finding a drug with reversed properties, efficient bronchodilation with minimal cardiac stimulation. As a matter of fact, with isoetharine (the α-ethyl derivative of isoprenaline, Table 2) a step was taken in this direction (Lands et al., 1950).

The real break-through came with salbutamol (Brittain et al., 1968; Cullum et al., 1969) and terbutaline (Bergman et al., 1969; Persson and Olsson, 1970), compounds invented and developed independently, and obviously during the

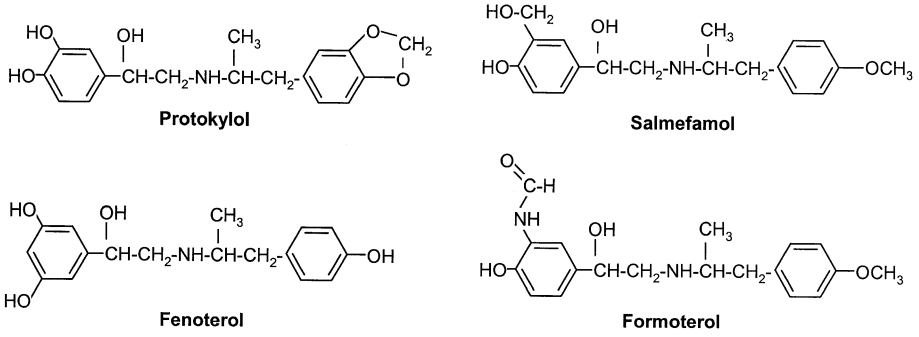


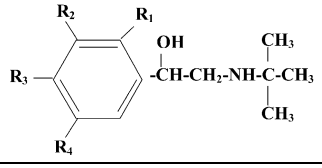
Fig. 2. β-Adrenoceptor agonists of the phenylethanolamine type with a substituted phenyl-isopropyl group attached to the nitrogen atom.

same period of time, by a British and a Swedish research team, respectively. Behind the work that led to terbutaline, there was also the observation that some β-adrenoceptor antagonists blocked the effects of isoprenaline on airway smooth muscle better than the effects on the heart (Persson, 1995). While salbutamol is a saligenin derivative and terbutaline a resorcinol, both compounds have a *tert*-butyl group attached to the nitrogen, a substitution which became very useful in later developments (Table 3 and Fig. 3). Salbutamol and terbutaline have very similar pharmacological properties. Since both compounds are non-catechol derivatives of adrenaline they are, like orciprenaline, resistant against catechol-*O*-methyl transferase. This promotes oral availability and a prolonged duration of action (Nyberg, 1997).

Salbutamol and terbutaline became the first truly β_2-selective adrenoceptor agonists in general clinical use. They show a strong relaxing capacity on airway smooth muscle in concentrations where effect on cardiac muscle is low. In fact, the two compounds were the tools by which Lands' β_2-adrenoceptor concept became established. It should be kept in mind, however, that the development of salbutamol and terbutaline and the subdivision of β-adrenoceptors into β_1 and β_2 were based solely on observation of functional effects in vivo and in vitro. Subsequent research has shown that the high selectivity in action of terbutaline and salbutamol is due to differences in efficacy rather than the subtle differences in affinity for the two receptor types (Rugg et al., 1978; Waldeck et al., 1986; Johansson et al., 1990). From this, it follows that with a screening procedure based on ligand binding to the receptor, a technique which was not available at that time, these valuable drugs may have been dropped.

Ligand binding studies, on the other hand, has contributed to the current understanding that the β_2-adrenoceptor is a surface-membrane bound, G-protein coupled 7TM-receptor, operating with c-AMP as a second messenger (Liggett and Green, 1997). In this way, β_2-adrenoceptor agonists act as functional antagonists, i.e. they relax airway smooth muscle whatever the contractile stimulus is. This is of great importance in asthma where a multitude of mediators, such as histamine, acetylcholine, leukotrienes and tachykinines, contribute to the bronchoconstriction. More recent research has revealed that there are genetic variations of the receptor molecule, which relate to differences in the response to β_2-adrenoceptor agonists. Thus it was found that in an asthmatic cohort, five different β_2-adrenoceptor haplotypes could be identified (Drysdale et al., 2000). Significant differences in the bronchodilating effect of salbutamol between different haplotype groups were observed. If it can be shown that the order of relative potency or efficacy of structurally different β_2-adrenoceptor agonist differ among haplotypes, then medication may be individualized and new drugs developed. There is a risk, however, that the enormous growth of the gene technology hampers the integration of the new with the old knowledge. The following statement, quoted from a recent news article will illustrate the confusion: "Salbutamol, a drug that is aimed at spurring the muscle relaxing gene into greater action to treat asthmatic wheezing worked very well." (Gottlieb, 2000).

Table 3
Chemical structures of some β_2-adrenoceptor agonists with a *tert*-butyl group attached to the nitrogen atom



Compound	R_1	R_2	R_3	R_4
Colterol	H	HO	HO	H
Terbutaline	H	HO	H	HO
Salbutamol	H	$HO-CH_2$	HO	H
Carbuterol	H	$H_2N-CO-NH$	HO	H
Sulfonterol	H	$CH_3-SO_2-CH_2$	HO	H
Clenbuterol	H	Cl	H_2N	Cl
Tulobuterol	CP	H	H	H

5. "Me too" products in the wake of salbutamol and terbutaline

After the first break-through with a clinically significant selectivity for β_2-adrenoceptors in airway smooth muscle, a large number of congeners emerged from various pharmaceutical companies. The first in this series appears to have been fenoterol (Th 1165a), a congener of orciprenaline, extended with a *p*-hydroxyphenyl group on the isopropyl moiety (Fig. 2). Perhaps it may be unfair to classify fenoterol as a "me too" product. With reference to unpublished material (Schuster and Baum, 1969), this compound appears to have emerged from the laboratory as a bronchodilating β-adrenoceptor agonist with improved selectivity already in 1964 and was evaluated in asthmatic patients with good results a few years later (Mattila et al., 1967). However, solid evidence for the β_2-adrenoceptor selectivity of fenoterol was presented only after the publication of the first results on salbutamol and terbutaline (O'Donnell, 1970).

Also hexoprenaline (Stormann and Turnheim, 1973) and clenbuterol (Engelhardt, 1972, 1976) appear to have been identified as bronchodilating β-adrenoceptor agonists with reduced cardiovascular effects before the pharmacology of salbutamol and terbutaline became public knowledge. Hexoprenaline, two molecules of noradrenaline with the amino groups linked together with a hexamethylene chain (Fig. 4), has a remarkable chemical structure where one side of this symmetric molecule may interact with the active site of the β-adrenoceptor while the counterpart might govern the β_2-selectivity. As we will find later on, the hexamethylene bridge is critical as a spacer arm for extended substitutions

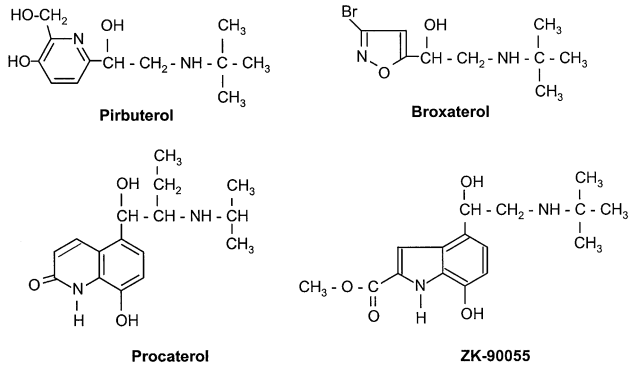


Fig. 3. Four β_2-adrenoceptor agonists with a heterocyclic ring or ring system substituted for the phenyl ring of the prototype, adrenaline.

of the phenylethanolamine molecule. Clenbuterol, besides its bronchodilating effect, appears to have a pronounced anabolic effect in animals with increases in body mass and muscle protein (Emery et al., 1984). This property has led to an illicit use of clenbuterol in animal breeding and cases of intoxication after ingestion of bovine liver from cattle treated in this way have been described (Martínez-Navarro, 1990). The subjects affected suffered from muscle tremor and tachycardia for up to 40 h. Nevertheless, clenbuterol has got a legal use in veterinary medicine for the treatment of horses with chronic obstructive pulmonary disease (COPD) (Erichsen et al., 1994).

Other β_2-selective adrenoceptor agonists which emerged from the first wave are tulobuterol (Kubo et al., 1975), formoterol (Ida, 1976a,b), carbuterol (Colella et al., 1977), and procaterol (Yabuuchi, 1977; Yabuuchi et al., 1977). Two compounds, pirbuterol (Moore et al., 1978) and broxaterol (Chiarino et al., 1986) have a heterocyclic ring substituted for the phenyl ring (Fig. 3). The rich freedom in subtitution of β-adrenoceptor agonists may be illustrated also by AA-497 in which the side chain of phenylethanolamine has been incorporated into an aminotetraline structure (Inatomi et al., 1980). All these compounds differ in potency and efficacy and in the degree of β_2-selectivity as well. Procaterol proved to be highly potent and extremely selective for β_2-adrenoceptors and is a very useful tool for exploring subpopulations of β-adrenoceptors in tissues (Johansson and Persson, 1983), but in clinical practice procaterol offered no substantial advantage compared to salbutamol (Crowe et al., 1985). This is because the limit for functional selectivity is set by the physiology: the presence of β_2-adrenoceptors mediating chronotropic and inotropic effects in the human heart (Ask et al., 1985; Levine and Leenen, 1989). With the exception of formoterol, none of the β_2-selective adrenoceptor agonists which followed early after salbutamol and terbutaline offered a clinically significant advantage over the prototypes and their place on the market remained limited.

Ironically enough, 30 years after the development of synthetic β_2-adrenoceptor agonists, an extremely potent, naturally occurring and highly β_2-adrenoceptor selective agonist was found in a marine sponge by a Japanese research team (Suzuki et al., 1999a). This compound, given the laboratory code S1319, is a benzothiazol-2-one analogue of adrenaline (Fig. 1). It shows in vitro a relaxing capacity on airway smooth muscle, in potency exceeding that of salbutamol a thousand-fold and with a higher efficacy (Suzuki et al., 1999b). This means that it is orders of magnitude more potent than adrenaline. Like other β_2-adrenoceptor agonists, it also shows a mast cell stabilizing effect (Suzuki et al., 2000). It would be most interesting to know what physiological function this peculiar compound may have in a marine organism.

6. Trouble with tremor

It seems to have been forgotten that the next objective in the development of bronchodilating β-adrenoceptor agonists was not to increase their duration of action but to eliminate the skeletal muscle tremor which was considered a dose-limiting side effect, particularly after oral administration. The sympathomimetic-induced muscle tremor has been shown to be mediated via peripheral β-adrenoceptors (Marsden et al., 1967). Several pharmaceutical companies worked hard for almost 10 years to find a new β-adrenoceptor agonist without this disturbing side effect. The experimental rationale was the observation by Bowman and Zaimis (1958) that adrenaline causes a decrease in fusion and tension of subtetanic contractions of slow contracting skeletal muscle. This effect, which is open to elaborate studies in vivo (Bowman and Nott, 1970) as well as in vitro (Waldeck, 1976; Holmberg and Waldeck, 1980), is thought to result in amplification of the physiological tremor occurring in the range 8–12 Hz (Bowman, 1980).

For most of the compounds investigated, the bronchodilating effect was accompanied by effects on the skeletal muscle (Bowman and Rodger, 1972; Malta and Raper, 1976; Apperley et al., 1976; Olsson et al., 1979). However, a number of compounds emerged which in some animal experiments showed a certain degree of selectivity between airway smooth muscle and skeletal muscle such as sulfonterol (Kaiser et al., 1975), AH7616 (Apperley et al., 1976), QH25 (Larsen and Hermansen, 1977) and D2343 (Andersson et al., 1982). Among these compounds, D2343 and QH25 were selected for evaluation in asthmatic patients. D2343 was compared to terbutaline after i.v. injection and QH25 was compared to salbutamol after oral administration (Löfdahl et al., 1982). Both compounds failed to separate tremor from bronchodilation. Apparently, the β-adrenoceptors in the skeletal muscle are too similar to those in airway smooth muscle to permit a pharmacological differentiation. However, a significant attenuation of the systemic side effects is achieved with the inhaled route of administration as demonstrated for terbutaline (Thiringer and Svedmyr, 1976) and previously suggested for adrenaline (Graeser, 1939).

7. Nocturnal asthma, pro-drugs and soft-drugs

Facing the fact that elimination of tremor was not feasible, new lines of development were considered. One was to find compounds with extended duration of action to offer protection during a whole night's sleep. The bronchodilating effect of salbutamol and terbutaline, albeit considerably more durable than that of isoprenaline, waned within 6 h. Since D2343 had already been approved for clinical studies and indicated a prolonged effect in animal experiments (Olsson and Ekdahl, unpublished), the next step was to compare its duration of action with that of terbutaline after inhalation. In one study on patients with asthma, D2343 showed a bronchodilating action with a slightly longer duration of action than that of terbutaline (Löfdahl and Svedmyr, 1984). Similar results appear to have been obtained with salmefamol compared to salbutamol (Sillett et al., 1976). However, the improvements achieved with these compounds were not sufficient to be clinically significant and the search for new compounds continued.

A project, based on the pro-drug principle, led to the development of bambuterol, the *bis*-dimethylcarbamate of terbutaline (Olsson and Svensson, 1984). Bambuterol has no affinity for the β_2-adrenoceptor per se, but it is slowly converted to terbutaline via a complex oxidative and hydrolytic metabolism (Lindberg et al., 1989). The result is a very smooth plasma concentration of terbutaline for up to 24 h, enabling once daily oral administation (D'Alonzo et al., 1995). Bambuterol is not metabolized to a major extent locally in the lung and is thus not active after inhalation. On the other hand, activity after inhalation was achieved with another pro-drug, bitolterol, the *bis*-*p*-toluate of colterol (Minatoya, 1978). But colterol is a catecholamine (Table 3) and subject to a more rapid metabolism than terbutaline. The duration of action of a pro-drug is determined by a subtle balance between the rate of formation of active metabolite and the rate of elimination of the latter. Thus it is questionable whether bitolterol offers a clear advantage to salbutamol.

Contrasting a pro-drug, a soft-drug is active per se when given locally but is rapidly inactivated when it reaches the general circulation. Thus, a long duration of action at a minimum of systemic side effects may be achieved after inhalation of a β_2-agonist designed in this way (Albrecht and Loge, 1985). One such example is ZK-90055 (Fig. 3), an indol derivative with a hydrolysable ester bond on the ring system (Albrecht et al., 1985, 1987). Unfortunately, the development of ZK-90055 was stopped before clinical trials were commenced and we do not know if the concept holds. Another β-adrenoceptor soft drug candidate, a phenylethanolamine with the ester bond in the *N*-substituent, has been suggested for topical treatment of psoriasis (Gill et al., 1994). After hydrolysis, a free carboxyl group in the side chain is formed and the agonistic activity of the compound at β-adrenoceptors is eliminated. Gill et al. (1994) also suggested development of combined pro- and soft-drugs to achieve both long effect duration and a minimum of systemic effects. This approach, however, requires a critical balance between activating and inactivating metabolism, which may be very difficult to achieve.

8. Formoterol and salmeterol, accidental and purposeful discoveries

New drugs may emerge in two different ways, as a result of target-directed research based on a specific hypothesis or through accidental discoveries. The latest achievements in the field, the inhaled, long-acting β_2-adrenoceptor agonists, offer examples of both tracks. Salmeterol was the result of a search for a compound with an extremely long duration of action in preclinical experiments (Ball et al., 1991; Johnson, 1995). It was thought that, by extension of the substituent at the amino group of a phenylethanolamine, the molecule would be able to "anchor" at an "exosite" outside the active site of the β_2-adrenoceptor. This philosophy was outlined in a review article (Brittain et al., 1976), apparently before the project leading to salmeterol was initiated (Johnson, 1995). Like hexoprenaline, salmeterol has a hexamethylene-bridge linking the two parts of the molecule together (Fig. 4). The first clinical trial with salmeterol in patients with asthma was carried out in the mid-1980s by Ullman and Svedmyr (1988) and it confirmed the preclinical evidence of a considerable extension of the duration of the bronchodilating effect compared to salbutamol.

At about the same time, Svedmyr and his team were asked to evaluate formoterol. The pharmacology of this highly potent and β_2-adrenoceptor selective agonist had been described about 10 years earlier by Ida (1976a,b), in vitro as well as in vivo. There was no convincing evidence of a prolonged duration of action compared to salbutamol. Moreover, an oral formulation of formoterol had just been introduced and it did not appear to offer a clear advantage to salbutamol (Tasaka, 1986). Svedmyr suggested the inhaled mode of administration and now, together with Löfdahl, he could show in patients with asthma that formoterol had a duration of action of at least 8 h, significantly longer than that exerted by salbutamol (Löfdahl and Svedmyr, 1989). Given orally, formoterol was not longer acting than salbutamol.

Subsequent clinical studies directly comparing formoterol and salmeterol have shown that both compounds have a duration of action of 12 h or more (Rabe et al., 1993; van Noord et al., 1996; Palmqvist et al., 1997). This contrasts with certain results obtained in animal experiments where formoterol appears to be shorter acting than salmeterol on airway smooth muscle in vitro as well as in vivo (Nials et al., 1994). Using different experimental conditions, however, it has been possible to demonstrate a long duration of formoterol in guinea-pig airways in vivo (Erjefält and Persson, 1991) and in vitro (Jeppsson et al., 1994). Obvi-

ously the predictive value of preclinical experiments in the evaluation of the effect duration of bronchodilator drugs for local administration is limited, suffering from both false positive and false negative results (Jeppsson et al., 1989). A crucial point may be that the duration of action of formoterol, but not salmeterol, appears to be concentration-dependent (Anderson et al., 1996). In any way, the exosite hypothesis, suggested but disputed for salmeterol (Nials et al., 1993; Green et al., 1996; Bergendal et al., 1996; Teschemacher and Lemoine, 1999), does not seem to be applicable to formoterol and a more general hypothesis, "the plasmalemma diffusion microkinetic model", based on differences in physico-chemical properties has been offered (Anderson et al., 1994).

Differences in physico-chemical properties may also explain the slow onset of action of salmeterol compared to formoterol, a difference first observed on isolated airway smooth muscle (Jeppsson et al., 1989) and repeatedly demonstrated in clinical investigations (van Noord et al., 1996; Palmqvist et al., 1997). The fast onset of action of formoterol enables this drug to be used both as reliever medication and for maintenance treatment. Another difference between the two drugs is that salmeterol has a relatively low efficay whereas the efficacy of formoterol is high (Lindén et al., 1993). This efficacy difference has been demonstrated in patients with stable asthma, in whom the broncho-protective effect of formoterol against metacholine provocation was dose-related and reached a higher maximum than that of salmeterol (Palmqvist et al., 1999), but at the expence of a higher tremor score at the highest doses. This means that salmeterol behaves as a partial agonist at human β_2-adrenoceptors. Consequently, salmeterol acts as a competitive antagonist at β_2-adrenoceptors against agonists with higher efficacy as demonstrated in isolated airway smooth muscle preparations for isoprenaline (Dougall et al., 1991), salbutamol, terbutaline and formoterol (Källström et al., 1994; Molimard et al., 1998). However, in patients with mild asthma, the combined bronchodilating effect of salmeterol and salbutamol appears to be largely additive (Smyth et al., 1993). Whether the difference in efficacy and potential for antagonism in vivo with salmeterol is of clinical significance, particularly during periods of acute severe asthma, is still an open question. Another open question is whether there is a difference in tolerance development to the high efficacy formoterol compared to the low efficacy salmeterol. Only well controlled clinical studies will settle this point.

Formoterol and salmeterol were followed by a number of other long-acting β_2-adrenoceptor agonists from various companies (Waldeck, 1996). Of these compounds TA-2005 (Kikkawa et al., 1991) is of particular interest because preliminary clinical trials indicated a duration of effect exceeding 24 h after inhalation of only 3 μg (Voss, 1994). TA-2005, possessing structural elements from both formoterol and procaterol, binds very firmly to the β_2-adrenoceptor (Voss et al., 1992), a property shared by some other agonists which like TA-2005 are based on a carbostyril skeleton (Standifer et al., 1989). However, unlike the condition for the first generation of β_2-adrenoceptor agonists, long-acting candidates which followed after formoterol and salmeterol have not reached the market. Not even TA-2005, which from the beginning was developed as a pure enantiomer, appears to have been further promoted. This will take us to the enantiomer issue.

9. The enantiomer issue

The biosynthesis of adrenaline involves the stereoselective introduction of a hydroxyl-group in the side chain by dopamine β-hydroxylase. Thus, endogenous adrenaline is levorotatory and has the (*R*)-configuration with modern denomination (Patil et al., 1970). Synthesis of adrenaline and its analogues using classical chemical methods results in a racemic mixture of the (*R*)- and the (*S*)-enantiomers. It was early found that the pharmacological activity of racemic adrenaline resides predominantly in the (−)-(*R*)-enantiomer (Cushny, 1926). In spite of this old knowledge, synthetic congeners of adrenaline were developed and used as racemates throughout during the 20th century. For technical and economic reasons, full-scale production of pure enantiomers was not judged feasible. Whenever tested in laboratory scale it was found, with few exceptions, that the bronchodilating property of new, synthetic β-adrenoceptor agonists stayed with the (−)-(*R*)-enantiomer, as shown for salbutamol (Hartley and Middlemiss, 1971) and terbutaline (Wetterlin, 1972), and that the presence of the (+)-(*S*)-enantiomer did not appear to interfere (Brittain et al., 1973; Jeppsson et al., 1984). To that comes that the toxicological documentation did not give a hint on effects unrelated to stimulation of β-adrenoceptors.

A more complex situation arises when a drug has two or three chiral centres enabling four and eight stereo-isomers, respectively. In those cases, a diastereomer pair has usually been selected for development since diastereomers are more easily separated than are enantiomers. Thus formoterol is a racemic mixture of the highly potent (*R*,*R*)-enantiomer and a virtually inactive (*S*,*S*)-enantiomer as demonstrated in vitro (Trofast et al., 1991) as well as in vivo (Fozard and Buescher, 2001). Isoetharine and procaterol comprise racemic mixtures of an active (1*R*,2*S*)-enantiomer and a less active (1*S*,2*R*)-enantiomer (Mardle et al., 1974; Yoshizaki et al., 1977) The native compound, ephedrine with its two chiral carbon atoms has the (1*R*,2*S*)-configuration (Patil et al., 1970).

In recent years, a suspicion has been raised that the (*S*)-enantiomer of racemic β_2-adrenoceptor agonists may cause airway hyper-reactivity and even contribute to increase in asthma death (Handley et al., 1998). This suspicion was originally based on experiments on guinea-pigs showing increased reactivity to the asthma-mediator

histamine after treatment with (*S*)-enantiomers of isoprenaline, salbutamol or terbutaline (Sanjar and Morley, 1988; Mazzoni et al., 1994). These and some other preclinical observations, which have been reviewed in depth elsewhere (Waldeck, 1999), led to the development of the pure (*R*)-enantiomer of salbutamol (levalbuterol) which was introduced to the US market recently. The weighed clinical evidence obtained with levalbuterol so far does not indicate a significant advantage of (*R*)-salbutamol to the racemate (Waldeck, 1999; Lötvall et al., 2001; Ahrens and Weinberger, 2001). If, however, a new β_2-adrenoceptor agonist with improved antiasthmatic properties should appear, the pure active enantiomer ought to be developed.

While there may be reasons to continue using a well established β_2-adrenoceptor agonist in racemic form for the treatment of asthma, there is no excuse to use racemates in experimental pharmacology when pure enantiomers are available. As a matter of fact, enantiomer pairs are valuable experimental tools in receptor pharmacology. Furthermore, there is a great variability in metabolism between enantiomers of β_2-adrenoceptor agonists (Nyberg, 1997) and this may cause problems in the interpretation of pharmacokinetic data (Ariëns, 1984). To use racemates of β-adrenoceptor agonists without mention of their chiral state and the implication thereof is both ignorant and misleading (Waldeck, 1993).

10. Hybride drugs

While the main-stream development of β-adrenoceptor agonists has been to increase their selectivity towards a single receptor, some effort has been made to find molecules with multiple effects other than those exerted by adrenaline. One such attempt was reproterol, which is a phenylethanolamine linked to theophylline via a propyl chain (Habersang et al., 1977). In clinical practice, reproterol proved to be nothing else than another β_2-selective adrenoceptor agonist and has not been able to compete with the pioneer compounds, salbutamol and terbutaline. Another approach to incorporate a second pharmacologic property is the dual dopamine D_2-receptor and β_2-adrenoceptor agonist AR-C68397AA (Fig. 4), also known as Viozan® (Bonnert et al., 1998). The rationale is the combination of the bronchodilating activity of a β_2-adrenoceptor agonist with the sensory afferent modulating effect of a dopamine D_2-receptor agonist in one and the same molecule. This combination is a new approach to the management of COPD (Newbold et al., 2001). However, the role of dopamine in the lung remains to be established. It is worth noting that the benzothizol structure of the synthetic compound AR-C68397AA was subsequently found to occur in the natural β_2-adrenoceptor agonist, S1319 (Suzuki et al., 1999a). A retrospective finding is that the β_2-adrenoceptor agonist, formoterol has an inherent anticholinergic component

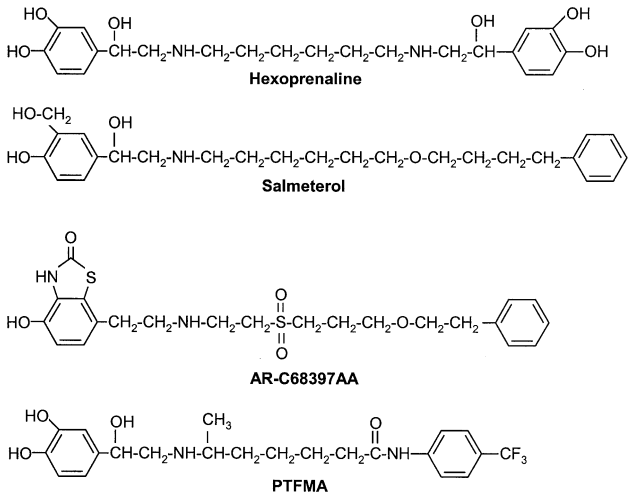


Fig. 4. β-Adrenoceptor agonists with a six-carbon atom (or the equivalent) "spacer arm" connecting the basic β-agonist structure (to the left) with a "carrier molecule" (to the right).

(Teschemacher et al., 1998), but only in very high concentrations.

11. Carrier molecules and spacer arms

In the foregoing, we have met some phenylethanolamine derivatives such as hexoprenaline and salmeterol which have a substituted aryl ring linked to the amino group via a linear carbon chain (Fig. 4). AR-C68397AA is designed in a similar way (Bonnert et al., 1998). This molecular construction has been explored more systematically by Jacobson et al. (1983). They synthetised a number of derivatives of isoprenaline, characterized by an extension of the *N*-isopropyl group by a linear alkyl chain of varying length, terminated by a substituted amide function, for example *p*-trifluoromethyl anilide (Fig. 4). It was found that the length of the alkyl chain, or "spacer arm", was critical for high agonistic activity, six carbon atoms usually being optimal. With this prerequisite a wide variation in the amide substituent or "carrier molecule" was possible and highly active β-adrenoceptor agonists were found with both aryl rings (Rosenkranz et al., 1983a) and oligopeptides (Rosenkranz et al., 1983b). This basic design of a β-adrenoceptor agonist opens for unlimited possibilities to modify pharmacodynamic and pharmacokinetic properties of the product. With multiple stereoisomers the variability is further increased (Eimerl et al., 1987).

12. Future development and the medical need

We have made a journey starting with adrenaline and ending with new, unexplored opportunities. One hundred years of experience of structural modifications of the adrenaline molecule has shown that the possibilities to modify

pharmacodynamic and pharmacokinetic properties of this endogenous prototype appear to be unlimited. Thus the duration of the bronchodilatory action may be further increased, the systemic side effects reduced using the "soft drug principle", and additional pharmacophores may be built into the molecule. The only limits set for the development of an anti-asthma β-adrenoceptor agonist with a new product profile are the medical need and marketing opportunities.

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