Review Article

Physiological perspectives of therapy in bronchial hyper-reactivity

Lexley M. Pinto Pereira MD, Fitzroy A. Orrett MD,
Merle Balbirsingh MD

Purpose: This paper reviews the literature on the aetiology and therapy of bronchial hyperreactivity to describe the underlying pathophysiology, identify patients at risk and update knowledge on new and existing therapies.

Source: Information was obtained from monographs on *New Drugs for Asthma*, *Respiratory Medicine: recent advances*, *Agents and Actions Supplements*, *Pulmonary Pharmacology*, *Anesth Analg*, the *European Journal of Respiration* and a Medline literature search.

Principal findings: Reduced airway calibre, increased bronchial contractility, altered permeability of the bronchial mucosa, humoral and cellular mediators, and dysfunctional neural regulation are critical factors for bronchial hyperreactivity, a characteristic feature of hyperreactive airways which results in bronchoconstriction after exposure to varied stimuli. Preoperative anaesthetic considerations in these patients include FEV₁ and PEF_R testing to assess the severity and for optimal control of the condition. Bronchospasm causing hypoxaemia is the major intraoperative problem anticipated in these patients. Current therapeutic management of bronchoconstriction focusses on the β_2 agonists, theophylline and steroids. Besides relaxing the airway smooth muscle these agents are all capable of altering bronchial inflammatory

responses. Future developments of therapy are directed towards the inflammatory components of the disease.

Conclusion: This review has presented background information on physiological mechanisms of smooth muscle contractility, pathophysiological alterations of bronchial contractility and the pharmacological basis of therapy in bronchoconstrictive disease. Information is presented to enable the prompt arrest and reversal of airway constriction, and to maintain prophylactic treatment during the perioperative period. Intraoperative bronchospasm is managed by adequate oxygenation and reversal of bronchoconstriction.

Objectif: Cet article parcourt la littérature portant sur l'étiologie et le traitement de l'hyperréactivité bronchique dans le but d'en exposer la physiopathologie, d'identifier les patients à risque et d'actualiser notre connaissance des thérapeutiques nouvelles et actuelles.

Sources: L'information a été recueillie des monographies *New Drugs for Asthma*, *Respiratory Medicine*, des progrès récents, des publications *Agents and Actions Supplements*, *Pulmonary Pharmacology*, *Anesth analg*, *European Journal of Respiration* et une recherche dans Medline.

Constatations principales: Les voies aériennes hyperréactives sont caractérisées par la diminution du calibre des voies aériennes, l'augmentation de la contractilité bronchique, l'altération de la perméabilité de la muqueuse bronchique, la libération de médiateurs humoraux et cellulaires et par le dysfonctionnement de la régulation neurale. Ces changements provoquent une bronchoconstriction à la suite d'une exposition à des stimuli variés. En préopératoire, il faut tenir compte des épreuves respiratoires du VEMS et du DEP pour évaluer la gravité de la maladie et optimiser son contrôle. L'hypoxémie provoquée par le bronchospasme constitue le problème périopératoire le plus important à anticiper chez ces patients. La gestion thérapeutique actuelle de la bronchoconstriction fait principalement appel aux agonistes β_2 , à la théophylline et aux stéroïdes. En plus de relâcher les muscles lisses des voies aériennes, ces agents sont tous capables d'altérer la réponse inflammatoire bronchique. Les progrès

Key words

ANAESTHESIA: emergency;
AIRWAY: reversible obstruction;
LUNG: bronchial hyperreactivity;
PHARMACOLOGY: β_2 agonists, theophylline,
glucocorticoids.

From the Department of Paraclinical Sciences, Faculty of Medical Sciences, University of the West Indies, St. Augustine, Eric Williams Medical Sciences Complex, Champs Fleurs, Trinidad, Trinidad and Tobago.

Address correspondence to: Dr. L.M. Pinto Pereira.

Phone: (1-809) 645-2640. Fax: (1-809) 663-9836.

Accepted for publication 22nd February, 1996.

futurs du traitement sont liés à la gestion des composantes inflammatoires de l'asthme.

Conclusion: *Ce survol a permis d'offrir des renseignements fondamentaux sur les mécanismes physiologiques de la contractilité du muscle lisse, sur les altérations physiopathologiques de la contractilité bronchique et la base pharmacologique du traitement de la maladie bronchoconstrictive. L'information pertinente à l'arrêt rapide et à la neutralisation de la constriction des voies aériennes ainsi qu'à l'entretien du traitement préventif à la période périopératoire a été présentée. Pendant l'intervention, le traitement du bronchospasme consiste à oxygéner et à faire lever la bronchoconstriction.*

Despite active research in the last two decades, asthma still affects 10% of the paediatric population and 5% of the adult population in developed countries. Efficacy of the currently available drugs is limited by adverse reactions and frequent dosing with short intervals, which often impede patient compliance. Inhalation is often the preferred route since a lower dose is needed and systemic side-effects may be reduced.¹ Improved drug delivery systems and approaches for high drug bioavailability in the lower airway remain therapeutic targets.

The goal of therapy in bronchospasm is to return the calibre of the airway to the resting state by increasing the radius of the airways. If basal resting tone of the bronchial smooth muscle increases, the calibre of the airway declines provoking excessive breathing responses. Bronchial hyperresponsiveness a characteristic feature of asthma, results in bronchoconstriction after a variety of stimuli. Contraction of the smooth muscle of the airway was previously regarded as the singular pathogenic feature of asthma, but has now been replaced by bronchial hyperreactivity secondary to inflammatory mucosal oedema.² Sporadic attacks of bronchial constriction may manifest with other allergic and chronic obstructive respiratory diseases such as emphysema, bronchitis and allergic rhinitis. These pathologies predispose the airways to bronchospasm from a multitude of factors; and the end result is a state of bronchial hyperreactivity secondary to inflammatory mucosal oedema.²

Impedance to the flow of atmospheric air into the pulmonary gas-exchanging units increases the work of breathing and is the predominant manifestation of bronchoconstrictive disease. For spontaneous breathing in patients with respiratory failure, the work of breathing must be maximally efficient, as any alteration in the normal pressure/volume relationship of the lung can alter the work of breathing. The work done in breathing is proportional to the product of pressure change and

volume change. Pressure change is the change in transpulmonary pressure required to overcome the elastic work of breathing and the resistive work of breathing. The former overcomes the recoil of the chest wall, the pulmonary parenchyma and the surface tension of the alveoli. The resistive work overcomes tissue resistance and to a major extent airway resistance, seen in bronchoconstriction. The first second forced expiratory volume (FEV₁) on the curve of forced vital capacity (FVC) is most sensitive to changes in expiratory airway resistance, and these indices of expiratory flow are markedly reduced during an asthmatic attack. Lung compliance is illustrated by the relationship between transpulmonary pressure and lung volume. High compliance is demonstrated by a steep slope on the pressure volume curve so that small changes in distending pressure cause large changes in volume. Compliance may become so inadequate that ventilation even at high inflation pressures becomes almost impossible, and the associated high inspiratory pressures when transmitted to the thorax, may impair venous return and depress cardiac output.

Perioperative management of the patient with reactive airways

Prevention

Perioperative bronchospasm in patients with reactive airway disease presents a major risk to the anaesthetist during surgery and in the immediate postoperative period due to postoperative pulmonary complications and cardiac arrest. Bronchospasm may have a diurnal variation occurring usually at night or in the early morning, and it may be prudent to defer elective surgery in these patients to later in the day. Factors which may initiate bronchospasm in the perioperative period include surgical stimulation, foreign bodies in the airway e.g., (a suction catheter or endotracheal tube) and movement of the patient's head. Acetylcholinesterase inhibitors which increase tissue concentrations of acetylcholine (ACh) and drugs such as morphine or the barbiturates (which release histamine), and propranolol may cause bronchospasm. Anaesthetists must also be aware that skeletal muscle relaxants such as tubocurarine³ and to a lesser extent atracurium, mivacurium and metocurine also liberate histamine,⁴ putting patients at risk of developing dysrhythmias and myocardial infarction from the hypoxaemia resulting from bouts of bronchospasm. Control of preoperative and intraoperative airway secretions helps prevent bronchospasm. Intraoperatively continuous auscultation of breath sounds and warmed humidified inhaled gases will permit removal of respiratory secretions.

Preoperative management

Kingston and Hirshmann⁵ have classified asthmatic patients into three preoperative groups. The first group comprises asymptomatic patients without recent attacks who are not receiving bronchodilators, and who do not require any workup. Group two patients have recurrent attacks, are without active wheezing, and are being treated with bronchodilators; their management consists of tests of pulmonary function, blood gas and theophylline levels. Some clinicians recommend performing lung function tests in these patients and delaying elective surgery if the values are <80% of the predicted. Patients in the third group are actively wheezing with signs of laboured breathing at examination and complain of worsening respiratory distress. Elective surgery is delayed in them until theophylline therapy is optimal. Corticosteroids may be considered and examination undertaken to determine the causes of refractoriness and the presence of complications of asthma.

Preoperative evaluation of these three groups of patients involves eliciting a careful history to determine severity of the disease. Important points to note are the age of onset, known triggers and allergies, case details of the last attack, average number of hospitalisations in a year, presence of cough and sputum (recording colour, consistency, mucus plugs), medications currently taken and a previous anaesthetic history.⁶

Assessment of pulmonary function is obtained by the Peak Expiratory Flow Rate (PEFR) and the FEV₁. The asthmatic patient in need of urgent care will have a PEFR and FEV₁ of approximately 35% of the predicted values. In 55% of patients values are <40% and in a fifth of patients they range between 40 to 60% of normal, and the blood gases indicate mild hypoxaemia (PaO₂ 8.8–9.2 kPa mmHg) except in severe obstruction when metabolic acidosis is present. Bronchodilator therapy is advised if FEV₁ is <70–80% or improves by >15% after a bronchodilator.⁷ Postural drainage is indicated in patients with severe asthma to assist in removal of secretions, chest expansion and to improve the respiratory reserve during the postoperative period. A single preoperative examination may not indicate the extent and the appearance of new findings, so communication with the primary physician may be helpful.

Intraoperative considerations

Two major intraoperative problems can be anticipated of which bronchospasm (especially in older asthmatics) is a likelihood. The airway should be cleared prior to induction of anaesthesia and known triggers (noted from history) should be avoided. Severe hypoxaemia which can be identified from blood gas analysis, may occur in the presence of respiratory depressants and sedatives.

Abnormal ventilation-perfusion ratios may increase the alveolar-arterial oxygen gradients requiring increased concentrations of inspired oxygen and necessitating the expeditious completion of surgery. Additional intraoperative monitoring such as pulse oximetry and arterial catheterisation for blood sampling may be considered.

For premedication to control anxiety, respiratory depressant drugs should be avoided. The benzodiazepines (e.g., diazepam) which do not increase airway resistance in asthmatics may be a better substitute.⁶ Atropine may be detrimental because in drying secretions it increases their viscosity and impairs elimination.

Intravenous thiopentone, midazolam and ketamine may be used to induce anaesthesia in asthmatics. Ketamine offers rapid induction and decreases airway resistance by releasing endogenous catecholamines; therefore it should be avoided or given with diazepam in patients receiving aminophylline. Intraoperative bronchospasm can occur in any patient and should be anticipated in every asthmatic. Signs of wheezing, prolonged filling of the reservoir bag, increased inflation pressures and over expansion of the chest with minimal excursion during ventilation are early features of bronchospasm. Immediate recognition and treatment are essential in the face of life-threatening tension pneumothorax and endotracheal tube obstruction. Manual rather than mechanical ventilation must be maintained and arterial blood gas analysis should guide the adjustments of FiO₂ and minute ventilation. Bronchoconstriction may be suppressed or reversed by increasing the concentration of the volatile anaesthetic (halothane, enflurane or isoflurane) or by administering lidocaine or ketamine *iv*. Halothane reduces airway resistance through a direct effect on smooth muscle and by inhibiting vagal reflex activity. Although the increased concentrations of volatile anaesthetics may improve bronchospasm, the depressant effect on cardiovascular function, via vagal activity should not be overlooked. Aerosolised bronchodilators with predominant β_2 receptor selectivity are effective for intraoperative bronchospasm and can be administered via the endotracheal tubing using adaptors placed in the anaesthetic circuit, for the metered dose inhalers. At least four to six puffs are recommended. Intravenous aminophylline may be considered in patients not receiving inhaled anaesthesia or if they are not already being treated with it. Patients whose lungs cannot be adequately ventilated may need intravenous isoproterenol (0.1–0.3 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), with electrocardiogram monitoring for β_1 -cardiac effects. If bronchospasm is severe or the patient has not received preoperative corticosteroid therapy, methylprednisolone 1 $\text{mg} \cdot \text{kg}^{-1}$ *iv* may be administered but the effects are usually seen after three to four hours. Observing the elec-

trocardiogram for β_1 effects and monitoring blood pressure, breath sounds and peak airway pressure for β_2 effects will indicate the therapeutic and any adverse drug effects. Postoperatively, extubation is favoured while airway responses are still depressed by anaesthesia.

Physiological mechanisms, pathophysiological alterations and therapeutic considerations

Airway smooth muscle tone

CALCIUM CHANNELS

Contraction of the airway smooth muscle has been recognised as the central component of the asthmatic response.⁸ Excitation-contraction of airway smooth muscle cells is dependent on the level of free calcium ions in the myoplasm of the cell.⁹ Though a concentration gradient for calcium ions exists from the extracellular to intracellular compartment, homeostatic mechanisms limit the entry of free calcium into the cell inhibiting induction of contraction. High intracellular calcium concentrations (Ca^{++}) are toxic to cell metabolism. Regulation of Ca^{++} occurs by two extrusion processes involving sodium-calcium ions exchange¹⁰ and an efflux of calcium ions,¹¹ and a third process of intracellular sequestration of calcium ions within the sarcoplasmic reticulum.¹² An excitatory or contractile stimulus (e.g., drug receptor activation) renders the cell membrane permeable to extracellular calcium which flows down the electro-chemical gradient through specific calcium channels.

Two distinctive calcium channels in the plasmalemma, the voltage operated (VOC) and receptor operated channels (ROC), have been proposed for cellular entry of calcium.⁶ There is little evidence supporting the involvement of VOCs in smooth muscle contraction by physiological agonists.^{13,14} Drugs like verapamil, diltiazem and nifedipine which block the VOCs are not effective in managing bronchoconstriction induced by allergens, methacholine or exercise, indicating that these channels are not important in contraction of smooth muscle bronchial cells. Calcium entry through the ROCs is more important for contraction of airway smooth muscle, and drugs acting on these channels are currently under development.¹⁵

INTRACELLULAR CALCIUM

Agonists acting on extra-cellular receptors signal the sarcoplasmic reticulum to release calcium through the metabolites of inositol phospholipids which act as second messengers. Hydrolysis of membrane phosphoinositides (PI) is crucial for the release of intracellular

FIGURE 1 Phosphoinositide hydrolysis as a signal event for secretion and contraction in the airway.

calcium following activation of surface receptors and triggers signal transduction in excitable cells.¹⁶ On interacting with bronchial cell surface receptors agonists catalyse the enzymatic cleavage of phosphatidylinositol 4,5-bisphosphate (PIP_2) by phosphoinositidase C through an intermediary regulatory guanine specific G protein. Two intracellular second messengers, myo-inositol 1,4,5-triphosphate (IP_3), and 1,2-diacylglycerol (DAG) are formed (Figure 1) of which IP_3 is proposed as the intra-cellular mediator of calcium release from sarcoplasmic reticulum.^{17,18} Prompt phosphorylation of IP_3 by an IP_3 kinase to 1,3,4,5-tetrakisphosphate (IP_4) seems essential to release calcium from the IP_3 -sensitive calcium pool in the sarcoplasmic reticulum.¹⁹ Muscarinic agonists and leukotrienes generate cellular accumulation of the metabolites of IP_3 in airway smooth muscle, and the turnover of phosphoinositides is correlated to the receptor occupancy.²⁰

The intracellular messenger IP_3 , reacts with specific receptors on the external sarcoplasmic reticular membrane to cause Ca^{++} efflux from the sarcoplasmic stores.^{17,18} Contraction of smooth muscle has been

demonstrated after IP_3 induced mobilisation of Ca^{++} from sarcoplasmic reticular stores.²¹ Asthmatics are known to be hyperreactive to inhaled spasmogens, and their airway smooth muscle is more sensitive to contractile stimuli (such as histamine or muscarinic agonists) which raise intracellular free calcium.²² High intracellular Ca^{++} activates 5-phosphatase to degrade IP_3 ; its metabolite IP_4 acts as an intracellular modulator of a plasmalemmal calcium channel²³ and permits Ca^{++} entry to replenish the sarcoplasmic reticular stores. Drugs which inhibit effects or turnover of phosphoinositide are potentially beneficial in asthma. Heparin is a potent competitive inhibitor of IP_3 binding in airway smooth muscle,²⁴ but does not penetrate cells and has no therapeutic utility. Analogues of IP_3 are among future drug development.

Hydrolysis of PI, also yields DAG which regulates maintenance of smooth muscle tension through membrane Protein Kinase C (PKC).²⁵ The free cytosolic Ca^{++} concentration is critical for activation of PKC. Physiological functions of PKC extend from cell repair processes²⁶ to negative feedback control on IP_3 generation.²⁷ Physiological effects include phosphorylation of the catalytic subunit of adenylate cyclase,²⁸ and changes in neuronal excitability and smooth muscle contractility,²⁹ which may modulate β adrenoceptor responses. Antagonists of PKC such as staurosporine have been described³⁰ but they lack specificity, and recognition of the several isoenzymes of PKC may contribute to future development of selective blockers for certain cell functions.³¹

CONTRACTILE MUSCLE PROTEINS

The sequence of biochemical events underlying contraction of airway smooth muscle is illustrated in Figure 2. Contractile agonists interact with specific bronchial cell receptors to activate phosphoinositidase C and cleave PIP_2 , generating IP_3 and DAG. Interaction of IP_3 with specific receptors on the external sarcoplasmic reticular membrane releases free Ca^{++} .^{16,17,21} When Ca^{++} increases, it binds with calmodulin, (the calcium binding protein), to activate Myosin Light Chain Kinase (MLCK) and phosphorylate the 20-kDa light chain of myosin. The increase in tension is determined by the rate of cross-bridge cycling, which in turn is determined by the magnitude of MLCK phosphorylation. Release of Ca^{++} from the sarcoplasmic reticulum is continuously achieved by IP_3 ; the latter provides an initial burst of Ca^{++} to induce rapid cross-bridge cycling and the development of tension.¹¹ Homeostatic mechanisms to remove cytosolic Ca^{++} (discussed earlier), cause it to dissociate from calmodulin, switching off MLCK activity and that of myosin ATPase too. The tension devel-

FIGURE 2 Sequential summation of events in the excitation-contraction coupling in airway smooth muscle. Hydrolysis of PI releases IP_3 and IP_4 which activates Ca^{++} release from sarcoplasmic reticulum to complex with calmodulin and turn on the calmodulin-MLCK pathway.

oped is sustained by an altered type of attachment of myosin and actin in the form of slowly-cycling cross bridges, termed "latch bridges"³² which maintain tone. Calmodulin's ubiquitous distribution limits its pharmacological alteration for therapy. Blocking the IP_3 -induced calcium release may prevent rapid cross-bridge cycling, but the inter-dependence of the rapid and the latch bridge cross-cycling remains to be defined.

Neural control

Neural regulation of airway tone, extends beyond sympathetic and parasympathetic activity, to non-adrenergic, non-cholinergic (NANC) neuropeptide co-transmitters.^{33,34} An imbalance in the neural control of the airways can provoke airway hyperresponsiveness and current therapeutic approaches may modulate effects of the autonomic neuro-transmitters on bronchial tone.

The airways are supplied by both parasympathetic and sympathetic nerves. The dominant control for airway smooth muscle and submucosal glands is from the parasympathetic nerves which contain agranular vesicles with acetylcholine and dense-cored vesicles con-

taining neuropeptides.³⁵ Cholinergic M₃ receptors are predominant in larger airways, and density of β_2 adrenergic receptors is higher in peripheral lung tissue. Baseline bronchomotor tone is attributed to be a function of parasympathetic activity.³⁶

MUSCARINIC SUBTYPES

On activation of muscarinic receptors in airway smooth muscle a specific G protein is stimulated, activating inositol phosphate metabolism, mobilising internal calcium³⁷ and decreasing cAMP by inhibiting adenylate cyclase.³⁸ Cholinergic tone is mediated via muscarinic receptor subtypes of which five distinct receptor proteins have been cloned,³⁶ and three pharmacologically recognised.³⁹ Receptors with a high affinity for pirenzepine, designated M₁,⁴⁰ found in the cerebral cortex and ganglia, facilitate neurotransmission via nicotinic receptors, and probably close K⁺ channels causing slow depolarisation of the ganglion cell.⁴¹ Prejunctional M₂ receptors situated on the short post ganglionic parasympathetic nerves in the airways, provide inhibitory feedback to reduce ACh release.⁴² They limit the degree of cholinergic bronchoconstriction and are postulated to be deficient in asthmatic patients.⁴³ Postjunctional M₃ receptors localised to airway smooth muscle and glands, mediate bronchoconstriction and mucus secretion.³⁶ Selective M₃ receptor antagonists will spare the M₂ receptors but are difficult to develop because the binding site for ACh is very similar for each muscarinic receptor sub-type.⁴⁴

Although anticholinergics are less effective bronchodilators than the β_2 agonists, studies suggest they may be as effective in acute exacerbations of bronchospasm illustrating the major role of cholinergic bronchoconstriction in airway narrowing.⁴⁵

Several mechanisms exaggerate cholinergic tone in hyperresponsive airway disease. Inflammatory mediators such as prostaglandins and histamine, released from airway cells increase cholinergic tone either by augmenting afferent receptor stimulation or enhancing ACh release from nerve terminals,⁴³ implying cholinergic mechanisms may underlie bronchial hyperresponsiveness.

Ipratropium bromide,^{46,47} a quaternary ammonium anti-cholinergic agent available as an aerosol, is almost free of systemic side-effects as its actions are confined essentially to the mouth and the airways after inhalation.^{48,49} Bronchodilatation with ipratropium is less intense and has a slower onset (20–30 min) than that produced by the β agonists (4–6 min), but is more prolonged. Therapeutic response is usually better in the older age group. Like atropine, ipratropium is non-selective and blocks pre-junctional M₂ and postjunctional M₃

receptors. Inhibition of the M₂ autoreceptors may explain the paradoxical bronchoconstriction which is associated with the use of ipratropium.

Glycopyrrrolate, another quaternary ammonium compound produces considerable bronchodilatation when administered either *iv* (1.0 mg) or as an aerosol.⁵⁰ Anticholinergic drugs have a slower onset of action than the β agonists, but their separate mode of action combined with their much longer duration of response (greater than eight hours), allows them to enter into successful combination with β agonists providing enhanced efficacy, without unwanted side-effects.⁵¹ Concerns on the alteration in production, clearance and viscosity of secretions, with resultant inspissation have not been observed even after high doses of anticholinergic drugs in patients with airway disease.⁵⁰ Their slow onset of action makes them suitable for prophylaxis, when administered intravenously about half an hour before induction of anaesthesia.

ADRENERGIC CONTROL

Sympathetic innervation is sparse in human airways, and the sympathetic influence on bronchial tone is mediated via circulating catecholamines which balance cholinergic tone.

Beta₂ adrenoceptor agonists are potent relaxants of airway smooth muscle from the trachea to the terminal bronchi. Stimulation of G regulatory proteins catalyse adenylate cyclase conversion of ATP to cAMP, phosphorylating protein kinases and inactivating MLCK causing smooth muscle relaxation. Adrenergic receptors are postulated to be tonically activated in asthmatics and their therapeutic exploitation stems both from their ability to relax airway smooth muscle⁵² and the inhibition of mediator release from mast cells.⁵³ They are widely distributed in the epithelium, blood vessels and alveolae of the lung where their other pharmacological effects on reversal of oedema, increase in surfactant release and increased mucociliary function are operative.⁵⁴ The β_2 -adrenoceptor agonists also reduce leakage from the microvasculature and inhibit eosinophil infiltration and activation.⁵⁵ Though sympathetic innervation has little direct effect on airway smooth muscle, it modulates parasympathetic control at the ganglion, synapses of parasympathetic nerves.⁵⁶

Salbutamol introduced in 1969⁵⁷ was the forerunner of selective β_2 agonists and, despite development of new chemical entities with differing pharmacokinetic profiles (Table), remains a drug of first choice. Beta agonists may be administered by almost all routes but aerosolised therapy delivers drug directly into the lung minimising systemic side-effects. Nebulised agonists have the same efficacy as subcutaneous or intravenous

TABLE Kinetics of aerosolised β -agonists

Drug	β_2 Specific	Onset of action (min)	Peak effect (min)	Duration (hr)
Isoproterenol	No	5	15	1
Isoetharine	Yes	10	30	2-3
Metaproterenol	Yes	10	45	3-6
Terbutaline	Yes	10	60	4-6
Albuterol	Yes	10	60	4-6
Pirbuterol	Yes	10	60	4-6
Bitolterol	Yes	10	60	3-8
Fenoterol	Yes	10	60	4-8
Salmeterol	Yes	10	60	8-10
Formoterol	Yes	10	60	8-10

doses, with fewer extra-pulmonary effects.⁵⁸ Inhalational therapy is limited by the short duration of action, which has been addressed in attempts to improve drug dosing and delivery.

Higher doses of the β_2 agonists do not offer added benefit, as dose increases provide the same improvement in daily peak air flow and number of symptom free days, as does their regular inhalation.⁵⁹ The apparent lack of efficacy encountered with β_2 agonist aerosols results from inadequate pulmonary drug delivery, usually from inco-ordination of the apparatus with inspiration; advances in drug delivery systems, such as breath actuated dry powder systems and the turbuhaler have addressed this problem. The new β_2 selective agonists salmeterol and formoterol have durations of action lasting >12 hr,^{15,59} indicating their potential to relieve nocturnal symptoms.

Salmeterol, an exciting new concept in drug delivery, is a substituted N-aryloxyalkyl analogue of salbutamol,⁶⁰ with an exquisitely simple but unique action. The substituted portion of the molecule binds with high affinity to an exo-site adjacent to the β receptor on the cell membrane. This attachment has no pharmacological effect, but permits the active head of the molecule to interact freely and continuously with the receptor. When the receptors are fully occupied the arm of the molecule moves away with leverage at the carrier molecule attached at the exo-site. Consequently the action of salmeterol is maintained, but its binding to the receptors can be readily reversed by a β receptor antagonist like propranolol. When the antagonist is removed the relaxation effects of salmeterol are "reasserted" and the prolonged duration of drug action remains without association to either tolerance or tachyphylaxis.⁶¹

Salmeterol produces long lasting suppression of mediator release from human mast cells *in vitro*. Acting on β_2 receptors on the endothelium and the macrophage, it suppresses plasma exudate following histamine chal-

lenge and alveolar macrophage function.⁶² In long term studies formoterol inhibited histamine release, gave higher peak flow values and required less rescue medication than salbutamol.⁶³ These long acting inhaled β agonists may be combined with inhaled steroids to provide bronchodilator and anti-inflammatory control through the day and night.

Adrenergic agents are the treatment of choice for acute bronchoconstriction. When administered with spacer devices, rapid initial particle velocity is eliminated, irritant properties are reduced, systemic bioavailability is decreased and the bronchodilatation achieved is as effective as after *iv* administration.⁶⁴ The inhaled route is preferred to oral administration except in children and geriatric patients, who have co-ordination problems or need adjunctive therapy for persistent nocturnal symptoms. Tremor is a side-effect which limits their oral administration.

To achieve bronchodilatation with decreased side-effect potential, recent approaches include development of pro-drugs and controlled release drug delivery. Bambuterol a pro-drug of terbutaline with high metabolic stability, has a single dose profile compared with twice-a-day dosing with terbutaline.⁶⁵ Constant drug levels of salbutamol over a prolonged period are achieved by incorporating it in an orally active, osmotically-controlled delivery system. The drug is released at a constant rate over nine hours, independent of food and pH.⁶⁶ The currently available slow release delivery systems for the beta-adrenoceptor agonists allow longer duration of therapeutic action.⁶⁷ Regular and chronic use of the β_2 agonists has recently been questioned.⁶⁸ Their continuous use is unlikely to influence further development of severe airway inflammation and they should be administered in association with an anti-inflammatory agent.⁶⁹ As regular medication they may mask the underlying inflammatory process and the need for anti-inflammatory therapy. In contrast when administered on

an "as required" basis, an increase in use is an indication for more anti-inflammatory therapy.⁷⁰

The importance of β adrenoceptor agonist therapy in bronchoconstriction, gives concerns regarding the use of β blocking agents to patients with reactive airway disease. Cardioselective beta-blockers reduce but do not eliminate the risk of blocking pulmonary β_2 receptors and caution is advised even in administering cardio-selective β_1 blocking agents such as atenolol or metoprolol, which in larger doses are not cardio-selective and do affect pulmonary function adversely.⁷¹

Beta agonists do not affect either the chronic inflammatory process or the late allergic response, nor do they reduce bronchial hyperreactivity (a reflection of airway inflammation).⁷² When β agonists are stopped there may be an associated "rebound" in bronchial hyperreactivity.⁷³

Tremors, palpitations and tachycardia are the most frequent side-effects which diminish with continued administration. At higher doses hypokalaemia from stimulation of β_2 receptors in skeletal muscle may manifest as a metabolic disturbance (from increased potassium uptake).

Anti-inflammatory interventions

GLUCOCORTICOIDS

Glucocorticosteroids (GCS), are the most effective anti-inflammatory drugs currently available to alleviate symptoms of bronchoconstriction. Alternate-day dosing schedules, followed by inhalational therapy with beclomethasone dipropionate (BDP), (originally a topical skin therapy), opened new vistas of cellular action. Doses as small as 400 μ g of inhaled BDP differentiated anti-asthmatic efficacy from systemic depression of plasma cortisol. At application sites in the lung, BDP and its first hydrolysis product, beclomethasone monopropionate act as potent GCS. After absorption they both undergo hepatic biotransformation into beclomethasone which has very low GCS activity. Unlike hydrocortisone, BDP has a very high affinity for pulmonary GCS receptors, permitting topical action at very low doses.⁷⁴ Betamethasone 17-valerate and BDP were the first GCS⁷⁵ to differentiate between local anti-asthmatic and systemic effects and initiated screening of steroid compounds, with high intrinsic activity at the application site and low systemic activity.

Budesonide, approximately twice as potent as BDP has high local metabolism implying higher inhaled doses may be administered without risking serious systemic toxicity. The drug also undergoes extensive hepatic metabolism and what is not metabolised locally is inactivated in the liver.⁷⁶ The improved ratio that budes-

onide offers between topical anti-inflammatory and systemic side-effects has positive implications for prolonged inhalational steroid therapy, especially since it inhibits allergen and exercise-induced asthma, attenuates airway hyperreactivity and improves baseline lung function.⁷⁷ Topical budesonide and flunisolide, have high pulmonary bioavailability, with low oral bioavailabilities, 11% and 20% respectively.⁷⁴ Its rapid metabolism after absorption may explain why it has more tissue selectivity in the lung as compared to beclomethasone⁷⁸; this feature combined with a high first pass effect (approx 90%) is consistent with a high therapeutic index and good systemic tolerance.⁷⁹

Glucocorticosteroid efficacy results from a combination of biochemical-cellular effects. The formation-release of PAF, LTD₄, LTB₄, histamine and PGE₂,⁷⁹ and the production of cytokines e.g., IL-1 β , IL-2, IL-6, TNF and GM-CSF is inhibited at the level of gene transcription and translation; tissue responsiveness is also reduced.⁸⁰ Besides GM-CSF, steroids reduce production of IL-3, and probably also IL-5 from lymphocytes or macrophages, thereby preventing their longer survival times, and the priming effects induced by these cytokines on the eosinophils.^{81,82} At the receptor molecule GCS exert a "hit and run" effect which explains their rather long latency of onset and duration of action. The GCS-receptor complex undergoes a conformational change, binds to DNA, triggers mRNA transcription, and induces synthesis of many proteins such as the beta-receptor, and lipocortin⁷⁹ and vasocortin⁸³ which are responsible for anti-inflammatory activity. The mRNA and proteins formed, have a longer half-life than the GCS, so the duration of action persists long after the receptor has been hit by the steroid moiety; the same mechanism explains the delayed onset of bronchodilatation. Topical GCS should be used 10–15 min after adrenoceptor β agonist induced vasodilatation and muscle relaxation, to permit the steroid particles to penetrate deep enough. The synthesis and density of β receptors, receptor affinity, and coupling to adenylyl cyclase are increased by GCS. Catecholamine uptake is decreased and the raised levels of endogenous catecholamines potentiates the action of sympathomimetics.⁸⁴ Aerosolised steroids such as beclomethasone and budesonide, with a high degree of topical activity and minimal adrenal suppression are considered the major development in asthma therapy. Oropharyngeal candidiasis, the commonest associated problem is readily treated with nystatin.

Steroids only suppress the inflammatory response and are not curative. Parenteral steroids are important preoperative medication in the patient with reactive airway disease, and also for intraoperative bronchospasm.

Hydrocortisone 1–2 mg·kg⁻¹ (or equivalent doses) is recommended one to two hours before induction in symptom-free patients. Patients with a history of severe asthmatic episodes may require three to four such doses in the 24 hr preceeding surgery. Corticosteroids enhance and prolong the duration of effect of β adrenergic agonists,⁸⁴ and significant improvement in airway function can be observed within two hours after dosing. Recently introduced recombinant human lipocortin presents problems in the delivery and degradation at inflammatory sites.¹⁵

Methylxanthines in bronchoconstriction

Xanthines are unique bronchodilator agents. They relax tracheobronchial smooth muscle independent of the contractile stimulus and without tachyphylaxis. Acknowledged as useful in the control of obstructive airway disease, their mode of action remains under speculation.

Adenosine antagonism has been demonstrated with theophylline,⁸⁵ but not with enprofylline,⁸⁶ a more potent compound with negligible CNS side-effects and minimal inhibition on phosphodiesterase.

The efficacy of the xanthines in chronic asthma and COPD suggests a strong anti-inflammatory rather than bronchodilator activity. Phosphodiesterase inhibitors selectively decrease eosinophil, mast cell, platelet and macrophage activity.⁸⁷ Theophylline and enprofylline prophylactically reduce inflammatory and stimulus-induced plasma-exudate;⁸⁸ the anti-migratory effects on inflammatory cells and mediators are synergistically enhanced by isoprenaline. Theophylline increases the number and activity of suppressor T cells, decreased in asthmatics.⁸⁹ Bronchodilatation has been ascribed to inhibition of phosphodiesterase; but levels of theophylline which produce increased cAMP concentrations are much higher than those for bronchodilatation and clinically toxic. Theophylline's efficacy is now believed to be the result of strong mucociliary clearance, reduced mediator and molecular leakage, enhanced diaphragmatic contractility, and decreased respiratory muscle fatigue.

Clearance of theophylline is decreased in infancy and geriatric patients, but children over one year clear it rapidly. Its metabolism is decreased by liver disease and increased cigarette smoking. The concomitant administration of erythromycin, cimetidine and oral contraceptives causes toxic levels, and upward dosage adjustment is necessary with phenobarbital, phenytoin, and furosemide. Theophylline can interact with inhalational agents like halothane which sensitise the myocardium to the effects of catecholamines. Theophylline has a narrow therapeutic window (10–20 $\mu\text{g} \cdot \text{L}^{-1}$) and therapeutic

drug monitoring assays have improved patient management. Side-effects correlate well with its plasma concentration and can be avoided by a gradual dosage increase when initiating therapy. The commonest side-effects are nausea and headache, but arrhythmias and seizures may occur at high concentrations. Despite its narrow therapeutic range, aminophylline, the water-soluble ethylene diamine salt of theophylline (approximately 80% theophylline) is considered standard maintenance therapy in patients with bronchoconstrictive disease. The potential of aminophylline to cause arrhythmias⁹⁰ makes its role in the prevention or treatment of bronchospasm controversial and β adrenergic agents or deepening anaesthesia are suggested as the more appropriate therapy.⁹¹

Potassium channels

Potassium channels exist in cardiac, skeletal and smooth muscle, in neurons and in secretory cells.⁹² Plasma membranes of airway smooth muscle cells are poorly excitable, do not generate action potentials and have little resting tone. On stimulation the membrane gives rise to a graded depolarisation which is related to the level of tone.⁹² The plasma membrane of airway smooth muscle has an inherent affinity to limit depolarisation and therefore contraction. Opening of voltage dependent potassium channels in the plasma membrane, prevents the development of action potentials in these cells.⁹³ As the membrane starts to depolarise the channels open, and K⁺ ions leave the cell down a concentration gradient and repolarisation commences. Besides these voltage dependent channels, potassium also leaves the cell by channels which open only when cytosolic calcium levels rise.⁹⁴

Blocking potassium channels increases excitability, and conversely their activation repolarises or hyperpolarises the cell. Potassium channel activators like cromakalim have been effective in relaxing airway smooth muscle in animal models,⁹⁴ healthy volunteers⁹⁵ and in asthma patients.⁹⁶

Cromakalim inhibits contraction of bronchial smooth muscle after histamine, carbachol, and neurokinin A, subsequent to hydrolysis of PI and intracellular Ca⁺⁺ release, and not depolarisation. Therefore, its effects probably extend beyond hyperpolarisation of the cell and involve lowering intracellular Ca⁺⁺, by pumping calcium back to intracellular stores. Bronchial relaxation by potassium channel blockers is not altered by blockade of the β_2 , H₁, or muscarinic receptors. The efficacy of these drugs approaches that of the β agonists and Barnes⁹⁷ suggests the latter may act by activation of potassium channels. Cromakalim has a half-life of 24 hr and peak plasma concentrations reach the early hours of

the morning after a single dose taken at night,⁹⁸ inhibiting the "morning dip" of FEV₁⁹² and suggesting the application of this class of drugs in patients with nocturnal asthma. Side-effects such as headache, flushing, and postural hypotension from vasodilatation have limited its clinical application. Inhalational potassium channel activators with selectivity for bronchial rather than vascular smooth muscle are considered future developments.

Airway blood flow

MICROVASCULAR LEAKAGE

Although the bronchial circulation receives only 1–2% of the total cardiac output, studies on the anatomical distribution of the bronchial microcirculation in casts of human airways reveal the presence of abundant capillary-venular plexuses in the submucosal layer,⁹⁹ indicating the importance of the vascular network in the airways. Plasma exudate or transudate from postcapillary venules and intercellular gaps, (from contraction of endothelial cells), is responsible for the physical alterations of bronchial asthma. Plasma derived mediators affecting microvascular leakage, include histamine, serotonin, leukotrienes, platelet activating factor (PAF), thromboxane, bradykinin, and substance P, all significant in the pathogenesis of bronchoconstriction.^{100–4} Extravasated plasma (an invariable sign of active inflammation), contains factors which inhibit mucociliary clearance predisposing to mucus plugs, besides inflammatory precursor proteins such as bradykinin and complement which are formed in the bronchial lumen. Microvascular leaks cause oedema of the airways and exaggerate contractile responses to spasmogens. Pharmacological intervention with α -adrenergic agonists like epinephrine, or the corticosteroids directly affects post capillary venular endothelial cells; the direct effect of inhaled β agonists on endothelial cells makes it the preferred route to intravenous application since the latter may actually increase bronchial blood flow. Xanthines have a primary "anti-leakage" effect on human nasal mucosa.¹⁰⁵

Hyperaemia consequent to mediator release, increases airway thickness and reduces airway calibre. The latter is not a sole function of intrinsic smooth muscle tone, but is also influenced by mucosal edema, secretions and inflammation of the airway.

Conclusion

The precise cause of bronchial hyperresponsiveness at the cellular level is not defined, but important aetiological factors include reduced basal calibre of the airways, increased contractility of the bronchial smooth muscle,

altered permeability of the mucosa, presence of circulating and humoral mediators, and disorders of neural regulation. At any time in the perioperative period the anaesthetist may be confronted with bouts of bronchoconstriction, which can potentially produce cardiac arrhythmias or infarction, and thus need to be vigorously and immediately addressed. Beta₂ agonists are the most effective bronchodilators in small and large airways. They prevent mediator release, and microvascular leakage and modulate cholinergic neurotransmission. Higher selectivity is limited by side-effects like tremors and hypokalaemia which are mediated by the β_2 receptor. An important recent advance is the introduction of the long acting β_2 agonists such as salmeterol, without significant side-effects. Theophylline retains a critical role in the management of severe bronchospasm. Besides being a bronchodilator it has anti-inflammatory and immunomodulating activity. Selective antagonists of the M₃ receptor which overcome blockade of the autoregulatory M₂ receptors are yet to be developed. The potassium channel activators are an important area of future development. High potency inhaled steroids with significant topical activity without systemic bioavailability have high therapeutic indices and the combination of steroids with β_2 agonists is effective and well tolerated. The contemporary management of airway hyperreactivity requires a knowledge of bronchial contractility, pathophysiology of the process and therapeutic interventions, which this review has sought to provide, to enable prompt reversal and prophylaxis of the condition.

Acknowledgement

The authors acknowledge Dr. H.L.S. Moseley, Head, Department of Anaesthesia and Intensive Care Unit, The University of the West Indies, Faculty of Medical Sciences, Queen Elizabeth Hospital, Bridgetown, Barbados, for the encouragement in preparing this manuscript.

References

- 1 Newhouse MT, Dolovich MB. Control of asthma by aerosols. *N Engl J Med* 1986; 315: 870–4.
- 2 Chung KF. Inflammatory mechanisms in asthma. *In*: Barnes PJ (Ed.). *Respiratory Medicine: Recent Advances*. Oxford: Butterworth-Heinemann Ltd, 1993: 32–56.
- 3 Miller RD. Skeletal muscle relaxants. *In*: Katzung BG, (Ed.). *Basic and Clinical Pharmacology*, 6th. ed. Connecticut: Appleton and Lange, 1995: 404–18.
- 4 Fung D, Hanowell L, Smith NT. Anaesthetic considerations in asthmatic patients. *In*: Gershwin ME, Halpern GM (Eds.). *Bronchial Asthma, Principles of Diagnosis and*

- Treatment, 3rd ed. New Jersey: Humana Press, 1994: 639–60.
- 5 Kingston HG, Hirshman CA. Perioperative management of the patient with asthma. *Anesth Analg* 1984; 63: 844–55.
 - 6 Allen SJ. Management of the patient with reactive airway disease. In: Stanley TH, Sperry RJ (Eds.). *Anaesthesia and the Lung*. The Netherlands: Kluwer Academic Publishers, 1992: 141–55.
 - 7 McFadden ER Jr, Hejel R. Asthma. *Lancet* 1995; 345: 1215–20.
 - 8 Knight DA, Phillips MJ, Stewart GA, Thompson PJ. The interaction of acetylcholine and histamine on human bronchial smooth muscle contraction. *Eur Respir J* 1991; 4: 985–91.
 - 9 Bolton TB. Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol Rev* 1979; 59: 606–718.
 - 10 Chideckel EW, Frost JL, Mike P, Fedan JS. The effect of ouabain on tension in isolated respiratory tract smooth muscle of humans and other species. *Br J Pharmacol* 1987; 92: 609–14.
 - 11 Torphy TJ. Action of mediators on airway smooth muscle: functional antagonism as a mechanism for bronchodilator drugs. In: O'Donnell SR, Persson CGA (Eds.). *Directions for New Anti-asthma Drugs, Agents and Actions Supplements*, Vol 23. Basel: Birkhauser Verlag, 1987: 37–53.
 - 12 Rodger IW. Calcium ion mechanisms in airway smooth muscle: potential targets for novel symptomatic drugs for asthma. In: O'Donnell SR, Persson CGA (Eds.). *Directions for New Anti-asthma Drugs, Agents and Actions Supplements*, Vol 23. Basel: Birkhauser Verlag, 1987: 69–87.
 - 13 Rodger IW. Drugs affecting calcium ions in airway smooth muscle. In: Barnes PJ (Ed.). *New Drugs for Asthma*. London: IBC Technical Services Ltd., 1989: 45–56.
 - 14 Rodger IW. Calcium channels in airway smooth muscle. *Amer Rev Resp Dis* 1987; 136: S15–6.
 - 15 Barnes PJ. Asthma therapy in the future. In: Barnes PJ (Ed.). *Respiratory Medicine: Recent Advances*. Oxford: Butterworth-Heinemann Ltd., 1993: 72–87.
 - 16 Berridge MJ. Inositol triphosphate and diacylglycerol as second messengers. *Biochem J* 1984; 220: 345–60.
 - 17 Hoffman BB. Adrenoceptor-activating and other sympathomimetic drugs. In: Katzung BG, (Ed.). *Basic and Clinical Pharmacology*, 6th. ed. Connecticut: Appleton and Lange, 1995: 115–31.
 - 18 Berridge MJ, Irvine RF. Inositol triphosphate, a novel second messenger in cellular signal transduction. *Nature* 1984; 312: 315–21.
 - 19 Morris AP, Gallacher DV, Irvine RF, Petersen OH. Synergism of inositol trisphosphate and tetrakisphosphate in activating Ca^{++} -dependent K^{+} channels. *Nature* 1987; 330: 653–5.
 - 20 Grandordy BM, Barnes PJ. Airway smooth muscle and disease workshops: phosphoinositide turnover. *Amer Rev Resp Dis* 1987; 136: S17–20.
 - 21 Hashimoto T, Hirata M, Ito Y. A role for inositol 1,4,5-triphosphate in the initiation of agonist-induced contractions of dog tracheal smooth muscle. *Br J Pharmacol* 1985; 86: 191–9.
 - 22 Garland LG. Is there a biochemical lesion in intrinsic asthma? In: Schmitz-Schumann M, Menz G, Costabel U, Page CP (Eds.). *Intrinsic Asthma, Agents and Actions Supplements*, Vol 28. Basel: Birkhauser Verlag, 1989: 135–45.
 - 23 Irvine RF, Moor RM. Micro-injection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on external Ca^{+} . *Biochem J* 1986; 240: 917–20.
 - 24 Chilvers ER, Challiss RA, Willcocks AL, Potter BU, Barnes PJ, Nahorski SR. Characterisation of stereospecific binding sites for inositol 1,4,5-triphosphate in airway smooth muscle. *Br J Pharmacol* 1990; 99: 297–302.
 - 25 Rasmussen H, Barrett PQ. Calcium messenger system: an integrated view. *Physiol Rev* 1984; 64: 938–84.
 - 26 Nishizuka Y. The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature* 1984; 308: 693–8.
 - 27 Kotlikoff MI, Murray RK, Reynolds EE. Histamine-induced calcium release and phorbol antagonism in cultured airway smooth muscle cells. *Am J Physiol* 1987; 253: C561–6.
 - 28 Yoshimasa T, Sibley DR, Bouvier M, Lefkowitz RJ, Caron MG. Cross-talk between cellular signalling pathways suggested by phorbol-ester-induced adenylate cyclase phosphorylation. *Nature* 1987; 327: 67–70.
 - 29 Rang HP, Dale MM. How drugs act: molecular aspects. In: *Pharmacology*, 2nd ed. London: Churchill Livingstone Inc., 1993: 24–48.
 - 30 Tamaoki T, Nomoto H, Takahashi I, Kato Y, Morimoto M, Tomita F. Staurosporine, a potent inhibitor of phospholipid/ Ca^{2+} -dependent protein kinase. *Biochem Biophys Res Commun* 1986; 135: 397–402.
 - 31 Nishizuka Y. The molecular heterogeneity of protein kinase C and its implications for cellular regulation. *Nature* 1988; 334: 661–5.
 - 32 Guyton AC. Contraction and Excitation of Smooth Muscle. *Textbook of Medical Physiology*, 8th ed. Philadelphia: W.B. Saunders Company, 1991: 87–95.
 - 33 Ellis JL, Undem BJ. Pharmacology of non-adrenergic, non-cholinergic nerves in airway smooth muscle. *Pulm Pharmacol* 1994; 7: 205–23.
 - 34 Ramnarine SI, Rogers DF. Non-adrenergic, non-cholinergic

- gic neural control of mucus secretion in the airways. *Pulm Pharmacol* 1994; 7: 19–33.
- 35 Widdicombe JG. The neuronal component of asthma. In: Schmitz-Schumann M, Menz G, Costabel U, Page CP (Eds.). *Intrinsic Asthma, Agents and Actions Supplements*, Vol 28. Basel: Birkhauser Verlag, 1989; 213–24.
 - 36 Smart SJ, Casale TB. Pathogenesis of asthma. In: Gershwin ME, Halpern GM (Eds.). *Bronchial Asthma, Principles of Diagnosis and Treatment*, 3rd ed. New Jersey: Humana Press, 1994: 23–55.
 - 37 Tattersfield AE. Prospects for bronchodilating drugs in the 1990s. In: Anderson GP, Chapman ID, Morley J (Eds.). *New Drugs for Asthma Therapy, Agents and Actions Supplements*, Vol 34. Basel: Birkhauser Verlag, 1990: 157–76.
 - 38 Jones CA, Madison JM, Tom-Moy M, Brown JK. Muscarinic cholinergic inhibition of adenylate cyclase in airway smooth muscle. *Am J. Physiol* 1987; 253: C97–104.
 - 39 Bonner TI, Buckley NJ, Young AC, Brann MR. Identification of a family of muscarinic acetylcholine receptor genes. *Science* 1987; 237: 527–32.
 - 40 Hammer R, Berrie CP, Birdsall NJ, Burgen AS, Hulme EC. Pirenzepine distinguishes between different subclasses of muscarinic receptors. *Nature* 1980; 283: 90–2.
 - 41 Barnes PJ. Muscarinic receptor subtypes in airways (Editorial). *Eur Respir J* 1993; 6: 328–31.
 - 42 Faulkner D, Fryer AD, MacLagan J. Postganglionic muscarinic inhibitory receptors in pulmonary parasympathetic nerves in the guinea-pig. *Br J Pharmacol* 1986; 88: 181–7.
 - 43 Barnes PJ. New developments in anticholinergics and cholinergic pharmacology. In: Barnes PJ (Ed.). *Drugs for Asthma*. London: IBC Technical Services Ltd., 1989: 33–44.
 - 44 Kurtenbach E, Curtis CA, Pedder EK, Aiken A, Harris AC, Hulme EC. Muscarinic acetylcholine receptors. Peptide sequencing identifies residues involved in antagonist binding and disulfide bond formation. *J Biol Chem* 1990; 265: 13702–8.
 - 45 Barnes PJ. Muscarinic receptor subtypes: implications for therapy. In: Hansel TT, Morley J (Eds.). *New Drugs for Allergy and Asthma, Agents and Actions Supplements*, Vol 43. Basel: Birkhauser Verlag, 1993: 243–52.
 - 46 Schlueter DP. Ipratropium bromide in asthma. A review of the literature. *Am J Med* 1986; 81: 55–60.
 - 47 Cugell DW. Clinical pharmacology and toxicology of ipratropium bromide. *Am J Med*. 1986; 81: 18–22.
 - 48 Gross NJ. Ipratropium Bromide. *N Engl J Med* 1988; 319: 486–94.
 - 49 Connolly CK. Adverse reaction to ipratropium bromide *BMJ* 1982; 285: 934–5.
 - 50 Gal TJ. Bronchial hyperresponsiveness and anaesthesia: physiological and therapeutic perspectives. *Anaesth Analg* 1994; 78: 559–73.
 - 51 Rebuck AS, Chapman KR, Abboud R. *et al.* Nebulised anticholinergic and sympathomimetic treatment of asthma and chronic obstructive airways disease in the emergency room. *Am J Med* 1987; 82: 59–64.
 - 52 Lefkowitz RJ, Hoffman BB, Taylor P. Neurohumoral transmission: the autonomic and somatic motor nervous system. In: Gilman AG, Rall TW, Nies AS, Taylor P (Eds.). *Goodman's and Gilman's The Pharmacological Basis of Therapeutics*, 8th ed. USA: Pergamon Press, 1990, 84–121.
 - 53 Church MK, Hiroi J. Inhibition of IgE-dependent histamine release from human dispersed lung mast cells by anti-allergic drugs and salbutamol. *Br J Pharmacol* 1987; 90: 421–9.
 - 54 Paterson JW, Lulich KM, Goldie RG. An overview of the current status of the drug therapy of asthma. In: O'Donnell SR, Persson CGA (Eds.). *Directions for New Anti-asthma Drugs, Agents and Actions Supplements*, Vol 23. Berlin: Birkhauser Verlag, 1988; 15–33.
 - 55 Anderson GP. Pharmacology of formoterol: an innovative bronchodilator. In: Anderson GP, Chapman ID, Morley J (Eds.). *New Drugs for Asthma Therapy, Agents and Actions Supplements*, Vol 34. Basel: Birkhauser Verlag, 1990: 97–115.
 - 56 Barnes PJ. Beta-adrenoceptors on smooth muscle, nerves and inflammatory cells. *Life Sci* 1993; 52: 2101–9.
 - 57 Warrell DA, Robertson DG, Howes JN, *et al.* Comparison of cardiorespiratory effects of isoprenaline and salbutamol in patients with bronchial asthma. *BMJ* 1970; 1: 65–70.
 - 58 Bloomfield P, Carmichael J, Petrie GR, Jewell NP, Crompton GK. Comparison of salbutamol given intravenously and by intermittent positive pressure breathing in life threatening asthma. *BMJ* 1979; 1: 848–50.
 - 59 Anderson GP. Formoterol: pharmacology, molecular basis of agonism, and mechanism of long duration of a highly potent and selective β_2 -adrenoceptor agonist bronchodilator. *Life Sci* 1993; 52: 2145–60.
 - 60 Johnson M, Butchers PR, Coleman RA, *et al.* The pharmacology of salmeterol. *Life Sci* 1993; 52: 2131–43.
 - 61 Johnson M. Salmeterol: a novel drug for the treatment of asthma. In: Anderson GP, Chapman ID, Morley J (Eds.). *New Drugs for Asthma Therapy, Agents and Actions Supplements*, Vol 34. Basel: Birkhauser Verlag, 1990; 79–95.
 - 62 Anderson GP. Long acting inhaled beta-adrenoceptor agonists the comparative pharmacology of formoterol and salmeterol. In: Hansley TT, Morley J (Eds.). *New Drugs in Allergy and Asthma*. Basel: Birkhauser Verlag, 1993: 252–69.
 - 63 Lofdahl CG, Chung KF. Long-acting β_2 -adrenoceptor

- agonists: a new perspective in the treatment of asthma. *Eur Respir J* 1991; 4: 218–26.
- 64 *International Report on Asthma*. International Consensus Report on Diagnosis and Treatment of Asthma. *Eur Respir J* 1992; 5: 601–41.
 - 65 *Svensson L-Å*. Bambuterol, a bronchodilator prodrug with sustained action. In: O'Donnell SR, Persson CGA (Eds.). *Directions for New Anti-asthma Drugs, Agents and Actions Supplements*, Vol 23. Basel: Birkhauser Verlag, 1987: 271–6.
 - 66 *Palmer JBD*. β -Agonists in asthma-current status and future developments. In: Barnes PJ (Ed.). *New Drugs for Asthma*. London: IBC Technical Services Ltd., 1989: 12–20.
 - 67 *Palma-Carlos AG*. Basic aspects of therapy in intrinsic asthma. In: Schmitz-Schumann M, Menz G, Costabel U, Page CP (Eds.). *Intrinsic Asthma, Agents and Actions Supplements*, Vol 28. Basel: Birkhauser Verlag, 1989: 263–77.
 - 68 *Sears MR, Taylor DR, Pruitt C, et al*. Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet* 1990; 336: 1391–6.
 - 69 *Pauwels R*. The pharmacological treatment of asthma in adults. In: Gershwin ME, Halpern GM (Eds.). *Bronchial Asthma*, 3rd ed. New Jersey: Humana Press, 299–303.
 - 70 *Barnes PJ*. Overview of current therapy. In: Barnes PJ (Ed.). *New Drugs for Asthma*. London: IBC Technical Services Ltd. 1989: 1–11.
 - 71 *Hoffman BB and Lefkowitz RJ*. Adrenergic receptor antagonists. In: Gilman AG, Rall TW, Nies AS, Taylor P (Eds.). *Goodman's and Gilman's, The Pharmacological Basis of Therapeutics*, 8th ed. USA: Pergamon Press, 1990: 221–43.
 - 72 *Cockcroft DW, Murdock KY*. Comparative effects of inhaled salbutamol, sodium cromoglycate and beclomethasone dipropionate on allergen-induced early asthmatic responses, late asthmatic responses, and increased bronchial responsiveness to histamine. *J Allergy Clin Immunol* 1987; 79: 734–40.
 - 73 *Vathenen AS, Knox AJ, Higgins BG, Britton JR, Tattersfield AE*. Rebound increase in bronchial responsiveness after treatment with inhaled terbutaline. *Lancet* 1988; 1: 554–8.
 - 74 *Dompeling E, van Schayck CP, Molema J, Folgering H, van Grunsven PM, van Weel C*. Inhaled beclomethasone improves the course of asthma and COPD. *Eur Respir J* 1992; 5: 945–52.
 - 75 *McAllen MK, Kochanowski SJ, Shaw KM*. Steroid aerosols in asthma: an assessment of betamethasone valerate and a 12-month study of patients on maintenance treatment. *BMJ* 1974; 1: 171–5.
 - 76 *Barnes PJ*. Pulmonary Disorders. In: Melmon KL, Morrelli HF, Hoffman BB, Nierenberg DW (Eds.). *Melmon and Morrelli's Clinical Pharmacology*, 3rd ed. USA: McGraw-Hill Inc., 1992: 186–218.
 - 77 *Andersson PT, Persson CGA*. Developments in anti-asthma glucocorticoids. In: O'Donnell SR, Persson CGA (Eds.). *Directions for New Anti-asthma Drugs, Agents and Actions Supplements*, Vol 23. Basel: Birkhauser Verlag, 1988: 239–60.
 - 78 *Persson CG*. Antiinflammatory therapy with glucocorticoids in intrinsic asthma. In: Schmitz-Schumann M, Menz G, Costabel U, Page CP (Eds.). *Intrinsic Asthma*, Basel: Birkhauser Verlag, 1989: 279–91.
 - 79 *Brattsand R*. Glucocorticosteroids for inhalation. In: Barnes PJ (Ed.). *New Drugs for Asthma*, London: IBC Technical Services Ltd., 1989: 117–30.
 - 80 *Kern JA, Lamb RJ, Reed JC, Daniele RP, Nowell PC*. Dexamethasone inhibition of interleukin 1 beta production by human monocytes. Posttranscriptional mechanisms. *J Clin Invest* 1988; 81: 237–44.
 - 81 *Gillis S, Crabtree GR, Smith KA*. Glucocorticoid-induced inhibition of T cell growth factor production. I. The effect on mitogen-induced lymphocyte proliferation. *J Immunol* 1979; 123: 1624–31.
 - 82 *Raghavachar A, Fleischer S, Frickhofen N, Heimpel H, Fleischer B*. T lymphocyte control of human eosinophilic granulopoiesis. Clonal analysis in an idiopathic hypereosinophilic syndrome. *J Immunol* 1987; 139: 3753–8.
 - 83 *Carnuccio R, DiRosa M, Guerrasio B, et al*. Vasocortin: a novel glucocorticoid-induced anti-inflammatory protein. *Br J Pharmacol* 1987; 90: 443–5.
 - 84 *Morris HG*. Mechanisms of action and therapeutic role of corticosteroids in asthma. *J Allergy Clin Immunol* 1985; 75: 1–13.
 - 85 *Boushey HA*. Bronchodilators and other agents used in asthma. In: Katzung BG (Ed.). *Basic and Clinical Pharmacology*, 6th ed. Connecticut: Appleton & Lange, 1995: 305–21.
 - 86 *Persson CG*. Development of safer xanthine drugs for treatment of obstructive airway disease. *J Allergy Clin Immunol* 1986; 78: 817–24.
 - 87 *Barnes PJ*. New drugs for asthma. *Eur Respir J* 1992; 5: 1126–36.
 - 88 *Persson CG*. Role of plasma exudation in asthmatic airways. *Lancet* 1986; 2: 1126–9.
 - 89 *Barlinski J, Lockhart A, Frossard N*. Modulation by theophylline and enprophylline of the excitatory non-cholinergic transmission in guinea-pig bronchi. *Eur Respir J* 1992; 5: 1201–5.
 - 90 *Stirt JA, Sullivan SF*. Aminophylline. *Anesth Analg* 1981; 60: 587–602.
 - 91 *Tobias JD, Kubos KL, Hirshman CA*. Aminophylline does not attenuate histamine-induced airway constriction during halothane anaesthesia. *Anesthesiology* 1989; 71: 723–9.
 - 92 *Black JL, Johnson PR, McKay KO, Carey D, Armour CL*.

- Levcromakalim- and isoprenaline-induced relaxation of human isolated airways-role of the epithelium and of K⁺ channel activation. *Pulm Pharmacol* 1994; 7: 195–203.
- 93 *McCann JD, Welsh MJ.* Calcium-activated potassium channels in canine airway smooth muscle. *J Physiol (Lond)* 1986; 372: 113–27.
- 94 *Arch JR, Buckle DR, Bumstead J, Clarke GD, Taylor JF, Taylor SG.* Evaluation of the potassium channel activator cromakalim (BRL34915) as a bronchodilator in the guinea-pig: comparison with nifedipine. *Br J Pharmacol* 1988; 95: 763–70.
- 95 *Buckle DR.* Prospects for potassium channel activators in the treatment of airways obstruction. *Pulm Pharmacol* 1993; 6: 161–3.
- 96 *Williams AJ, Lee TH, Cochrane GM, et al.* Attenuation of nocturnal asthma by cromakalim. *Lancet* 1990; 336: 334–6.
- 97 *Barnes PJ.* Control of airway Caliber. *In: Fishman A (Ed.). Update: Pulmonary Diseases and Disorders.* New York: McGraw-Hill Inc., 1992: 53–66.
- 98 *Williams AJ.* Potassium Channel Activators. *In: Barnes PJ (Ed.). New Drugs for Asthma.* London: IBC Technical Services, 1989: 57–65.
- 99 *McCormack DG, Crawley DE, Evans TW.* New perspectives in the pulmonary circulation and hypoxic pulmonary vasoconstriction. *Pulm Pharmacol* 1993; 6: 97–108.
- 100 *Persson CG.* Role of plasma exudation into asthmatic airways. *Lancet* 1986; 2: 1126–9.
- 101 *Kroegel C.* Does airway smooth muscle care about platelet-activating factor? (Editorial). *Eur Respir J* 1992; 5: 915–8.
- 102 *Shindo K, Matsumoto Y, Sumitomo M, Matsumura M, Okubo T.* Measurement of leukotriene C4 and D4 in arterial blood of asthmatic patients during remission. *Ann Allergy* 1991; 66: 405–10.
- 103 *Barnes NC, Piper PJ, Costello JF.* Comparative effects of inhaled leukotrienes C4, leukotriene D4, and histamine in normal human subjects. *Thorax* 1984; 39: 500–4.
- 104 *Persson CG, Draco AB.* Xanthines as airway anti-inflammatory drugs (Letter). *J Allergy Clin Immunol* 1988; 81: 615–7.