# PHARMACODYNAMIC STUDY OF A NEW $B_2$-AGONIST "TULOBUTEROL"

T H E S I S

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By

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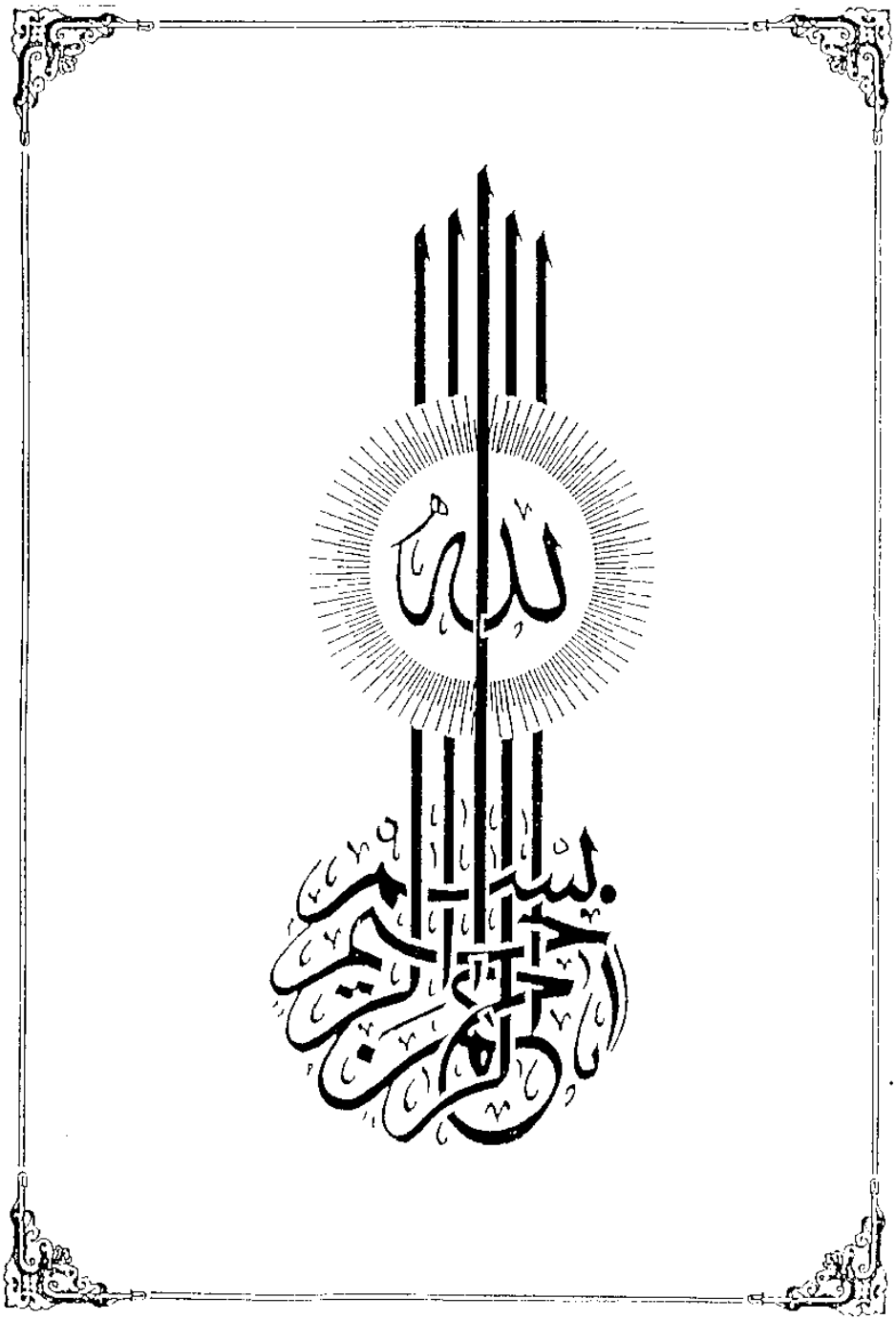

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# C O N T E N T S

# INTRODUCTION

# I N T R O D U C T I O N

Bronchial asthma is a disease characterized by an increased responsiveness of the trachea and bronchi to various stimuli and manifested by a widespread narrowing of the airways that changes in severity either spontaneously or as a result of therapy ( O'Connor et al., 1986; Daniele, 1988 ).

This conceptual definition was proposed by the American Thoracic Society in 1962 ( O'Connor et al., 1986 ) . One of the major classes of drugs that are used for treatment of bronchial asthma is the bronchodilator drugs . Some bronchodilator drugs in addition to antibronchoconstrictor effect have other properties that may be important in relieving airway obstruction e.g. effects on small blood vessels, mast cells, ciliary activity and mucus secretion ( Paterson et al., 1979; Barnes, $1986_a$) . A brief consideration of a number of aspects of lung pharmacology is necessary for a scientific understanding of bronchodilator therapy .

Airway calibre is influenced by many hormones, neurotransmitters, drugs and mediators which produce their effects by binding to specific recognition sites or receptors on the external surface of various target cells in the airways ( Barnes, $1986_a$) . Smooth muscle extends from the trachea to the alveolar ducts and is under the control of autonomic

nervous system ( Fernandez & Cherniack, 1986 ) .

Autonomic innervation of the airways is complex ( Nadel & Barnes, 1984; Barnes, $1986_b$ ) . In addition to classical cholinergic pathways and adrenergic mechanisms, there is more recently recognised component of autonomic control which is neither cholinergic nor adrenergic (NCNA) ( Barnes, 1984 ) . Parasympathetic innervation of the bronchi occurs via the vagus nerve as far down as the terminal bronchioles, but evidence for direct innervation by the sympathetic nervous system is incomplete ( Staub, 1975 ) . However there is excellent evidence for the presence of adrenoceptors in bronchial smooth muscle ( Paterson et al., 1979 ) .

Originally beta receptors of airway smooth muscle were classified as beta-2-receptors, but using direct receptor binding techniques and selective beta antagonists confirmed the coexistence of beta-1- and beta-2-receptors in animal and human lung ( Rugg et al., 1979; Engel, 1981 ) .

Functional studies of tracheal smooth muscle from the dog showed that relaxation to exogenous beta agonists is mediated by beta-2-receptors, but relaxation to sympathetic nerve stimulation is mediated by beta-1-receptors ( Barnes et al., $1983_a$ ) . In cat trachea which has a dense sympathe-

tic nerve supply, relaxation to beta agonists is mediated predominantly by beta-1-receptors, whereas in lung strips which contain bronchioles devoid of sympathetic nerves, response is mediated by beta-2-receptors ( Lulish el al., 1976 ) . Similarly in guinea pig trachea which has a sparse sympathetic innervation, binding studies showed that approximately 15 % of beta receptors are of the beta-1-subtype ( Carswell & Nahorski 1983 ) .

In human airway smooth muscle, recent autoradiographic studies of beta receptor subtype have confirmed that the beta receptors of human airways smooth muscle from bronchi down to terminal bronchioles are entirely of the beta-2-subtype ( Carstairs et al., 1985 ) . These findings are consistent with the hypothesis that beta-1-receptors are regulated by sympathetic nerves (neuronal beta receptors), whereas beta-2-receptors are regulated by circulating adrenaline (hormonal beta receptors) ( Ariens & Simonis, 1983 ) .

Alpha receptors which mediate contraction of airway smooth muscle have been found in many species including man ( Kneussl & Richardson, 1978 ) . With development of selective alpha antagonists, two subtypes of alpha receptors have been recognised : $\alpha_1$ receptors are the classical alpha receptors which mediate contractile effects and are selectively

blocked by prazosin; whereas those presynaptic alpha receptors mediating negative feedback of noradrenaline release which are selectively blocked by yohimbine are termed $\alpha_2$ receptors ( Hoffman & Lefkowitz, 1980 ). Such $\alpha_2$ receptors have recently been found postsynaptically ( Barnes, $1986_a$ ). There is no alpha adrenergic response in normal canine or human airway smooth muscle in vitro even after beta blockade, but with disease human airways or following pretreatment of normal canine airways with histamine or serotonin, a marked alpha adrenergic contractile response is found ( Kneussl & Richardson, 1978; Barnes et al., $1983_b$ ) . This response suggesting that alpha adrenergic responses are activated by mediators or disease, and also the enhanced responsiveness does not involve any change in airway alpha receptors density or affinity ( Barnes et al., $1983_b$ ) .

There is a small resting bronchomotor tone due to vagal activity with release of acetylcholine, and this resting tone can be demonstrated by blockade of the cholinoceptors with atropine ( Vincent et al., 1970 ) . Autoradiographic study revealed a high density of muscarinic receptors on smooth muscle of large airways and the density decreases as airways become smaller, so that terminal bronchioles are almost devoid of muscarinic receptors ( Barnes et al., $1983_a$ ).

The muscarinic receptors may be subclassified into at least two subtypes : in the gut, $M_1$ receptors are localised to nerve terminals, ganglia and secretory cells, whereas $M_2$ receptors are localised to smooth muscle ( Hammer et al., 1980 ), However there is evidence for prejunctional $M_1$ receptors on vagal nerve endings in guinea pig airways ( Fryer & Mac lagan, 1984 ) . The inhibitory effects of sympathomimetic drugs on cholinergically mediated contractions of guinea pig isolated tracheal muscle was studied and showed that cholinergic neurotransmission in airway smooth muscles can be inhibited by postjunctional $B_2$ adrenoceptors or by prejunctional $\alpha_2$ adrenoceptors ( Kamikawa & Shimo, 1986 ) . The anticholinergic drugs appear to be more effective in chronic bronchitis, probably because they block vagal tone, which has a greater effect if the airways are already narrowed for geometric reasons ( Gross & Skorodin, 1984 ) .

The existence of a nervous system in the gastrointestinal tract which is neither adrenergic nor cholinergic (NANC) has been established for many years, and because the airways develop embryologically from the foregut, it is not surprising to find that NANC nerves are also present in the lung ( Barnes, 1984 ) . A non-adrenergic inhibitory nervous pathway has been found in airways in several species, and in human airways it is the only inhibitory nervous mecha-

nism in smooth muscle from the trachea to the smallest bronchi ( Richardson, 1979; Palmer et al., 1985 ) . A non-cholinergic excitatory nerves have also been described ( Andersson & Grundström, 1983 ) . Originally it was proposed that the neurotransmitter might be a purine nucleotide such as ATP or adenosine, since in the gut these purines are released on nerve stimulation and both exogenous ATP and adenosine mimic some of the effects of NANC nerve stimulation, and therefore were termed purinergic ( Burnstock, 1972 ) . However, although ATP relaxes isolated guinea pig airway smooth muscle ( Kamikawa & Shimo, 1976 ), its antagonist does not block NANC relaxation either in vitro or in vivo ( Kamikawa & Shimo, 1976; Irvin et al., 1982 ) .

Several regulatory peptides have been identified by radioimmunoassay in lung tissue of several species including man; they include vasoactive intestinal peptide (VIP), substance P, bombesin, cholecystokinin and somatostatin ( Hakanson et al., 1983 ) . Immunocytochemical studies have demonstrated that some of these peptides are localised to nerves within the airway ( Barnes, 1984 ) .

VIP is a potent relaxant of isolated human bronchi, mimicking the effect of non adrenergic nerve stimulation ( Palmer et al., 1985 ) . Electrical field stimulation of guinea pig and bovine trachea releases VIP into the bathing medium and the release is blocked by the nerve toxin tetro-

dotoxin, indicating that VIP is derived from nerve stimulation ( Matsuzaki et al., 1980 ) .

A closely related peptide; peptide histidine isoleucine (PHI) and, in humans peptide histidine methionine (PHM) coexists with VIP in airway nerves, and is equipotent with VIP in dilating human airways ( Palmer et al., 1986 ). VIP and PHM activate a specific receptor and activation of receptor stimulates adenylate cyclase to increase intracellular cyclic AMP ( Frandsen et al., 1978 ) . VIP and PHM appear to coexist with acetylcholine in airway vagal nerves and these peptides may therefore interact functionally with cholinergic mechanisms ( Barnes, 1984; Palmer et al., 1986 ) .

Substance P is localised to afferent nerves in the airways of several species including man, and may be released by antidromic conduction ( Pernow, 1983 ) . Electrical stimulation of guinea pig bronchi in vitro produces a component of bronchoconstriction which is not inhibited by atropine but is blocked by substance P antagonist ( Lundberg et al., 1983 ) . It has been possible to differentiate at least two types of substance P receptor, in airway smooth muscle the receptors appear to be of the E subtypes ( Barnes, $1986_a$ ), whereas the receptor mediating mast cell mediators release in skin is probably of the P subtype ( Foreman et al., 1983 ) .

Bronchial edema has been known for many years as one of the causes of airway narrowing in asthma ( Paterson et al., 1979 ) . Histamine induces contraction of endothelial cells in the venular wall and thus increasing microvascular permeability. This effect is mediated through $H_1$-receptor ( White & Eiser, 1983 ) . Histamine also increases lung epithelial permeability and therefore perhaps airway mucosal permeability via $H_2$-receptors ( Braude et al., 1984 ) . Prior treatment with beta agonistswill reduce histamine-induced microvascular leakiness in airway of guinea pigs, suggesting that beta receptors also regulate bronchial microvascular permeability ( Persson et al., 1982). It has also been shown in laboratory animals that the anti-inflammatory property of catecholamines is probably due to a beta agonist effect ( Green, 1972 ) .

Respiratory secretions consist of mucus produced by submucosal glands in the bronchi and goblet cells, and tissue fluid ( Wanner, 1986 ) . Human bronchial mucus glands appear to be innervated by both cholinergic and adrenergic fibres ( Pack & Richardson, 1984 ); they also respond to both muscarinic and adrenoceptor agonists ( Sturgess & Reid, 1972 ) . Immunocytochemical studies have demonstrated that VIP increases the cyclic AMP content in submucosal glands indicating the presence of VIP receptors on these cells ( Lazarus et al., 1986 ) .

Beta agonists stimulate secretion of mucus, resulting in a more viscous secretion by selective stimulation of the mucus rather than the serous cells in submucosal glands ( Phipps et al., 1982 ) . Autoradiographic studies confirm a high density of beta receptors in airway glands, with greater labelling of mucus than serous cells ( Barnes, $1986_a$ ) . In addition beta agonists stimulate ion transport across human airway epithelium in vitro ( Knowles et al., 1984 ) . Thus, beta agonists may increase mucus and watery secretion into airways, which result in increased mucociliary clearance ( Wanner, 1977 ) .

Stimulation of the vagus nerve and cholinergic agonists both potently stimulate mucus secretion in animals and in man ( Baker et al., 1985 ), and cholinoceptor agonists have also been shown to stimulate mucociliary transport in both lower and higher animals ( Kensler & Battista, 1966 ) . It might therefore be expected that antimuscarinics such as atropine would impair mucociliary transport . Atropine inhibits production of tracheobronchial secretions and volume of sputum ( Lopez-Vidriero et al., 1975 ), and thus might impair mucociliary transport ( Annis et al., 1976 ) . VIP also stimulates mucus secretion ( Peatfield, 1983 ) and fluid transport across airway epithelium ( Nathanson et al., 1983 ) . Thus, VIP nerves may regulate secretion of both mucus and water, and so influence mucociliary transport in the airways ( Barnes, 1984 ) .