REVIEW OF LITERATURE
The phenomenon of desensitization in biological regulation, also referred to as tachyphylaxis, tolerance or refractoriness, is well documented in literature. It is most commonly observed as a loss of cellular responsiveness to a hormone or drug after repeated or prolonged exposure to that agent. The phenomenon of desensitization is frequently observed both in the laboratory as well as clinically. (Goldstein et al., 1974; Britton, 1993). Desensitization has been reported for a number of pharmacological agents like cholinomimetics (Rang & Ritter 1970) insulin (Gavin et al., 1974) prostaglandins (Remold-O'Donnel, 1974), Opiates (Collier, 1965) and catecholamines (Fleisch & Titus, 1972; Bouhuys et al., 1972).

Desensitization in case of beta agonist has been reported to occur in a number of tissues and cell types including rat aorta and trachea (Fleisch & Titus, 1972), guinea-pig trachea (Bouhuys et al., 1972), frog erythrocytes (Mukerjee et al., 1975) and lymphocytes (Makman. 1971). Avner & Jenne (1981) have demonstrated in the isolated human bronchial muscle that cross desensitization occurs between beta agonistic drugs isoproterenol, terbutaline and isoetharine but does not occur with the methylxanthine aminophylline. Aminophylline was as effective in normal as in desensitized tissues.

Though there is uniformity in vitro studies, clinical studies have produced conflicting results. Drug tolerance of a modest degree has been shown to occur in man with oral salbutamol (Nelson et al., 1977) and oral terbutaline (Jenne 1977). The studies of Miller (1978), Chervinsky (1978), Weber et al(1982) & Repsher et al(1984) have also shown a degree of bronchial tolerance with prolonged use of adrenergic bronchodilators. Daffonchio et al (1990) have suggested that desensitization may be related to the genesis or bronchial hyper-reactivity, a

Beta-agonists have been widely used as bronchodilators since the discovery of isoproterenol in 1940 by Konzett. This was followed by the introduction of the selective beta-2 agonists salbutamol in 1968, terbutaline in 1969 and several others thereafter. The importance of desensitization in airway smooth muscle was highlighted by Conolly et al (1971) who proposed that a possible reason for the sudden rise in asthma deaths in England and Wales during the sixties, could be due to an acquired resistance to beta adrenoceptor agonists. His observation was followed by many experimental as well as clinical investigations to elucidate the molecular mechanisms involved in the process of desensitization.

The guinea-pig isolated tracheal smooth muscle is known to have a predominance of beta-2 adrenoceptors (Zaagsma et al., 1979; O'Donnell & Wanstall, 1979; Carswell & Nahorski, 1983) and therefore is a useful preparation for studying the relaxant responses to beta agonists which are known to cause relaxation of the airway smooth muscles via the beta-2 receptors.

Van Den Brink (1973) used the term functional antagonism to describe the protective effect of isoproterenol on the constriction produced by methacholine or histamine in guinea-pig trachea. This phenomenon is of potential importance in evaluating bronchodilator response. It has been shown by Torphy (1983) in isolated canine tracheal smooth muscle that if the dosage of cholinergic constrictant is high there is no relaxant response to beta agonist. Further work on such functional
antagonism has been carried out by Buckner & Saini (1975) and Jones et al (1974) in the guinea-pig trachea.

According to Lin et al (1977) and Avner & Noland (1978) one demonstrable cellular change that occurs in the rat tracheal smooth muscle during desensitization, is a pronounced reduction in the affinity of isoproterenol for the beta receptor. However, biochemical and radioreceptor binding studies have shown a reduction in adenylate cyclase, cyclic AMP levels followed by decreased number of beta adrenoceptors without change in affinity (Lefkowitz & Williams 1977, Nishikawa et al., 1993). Further Liao et al (1993) have shown that isoprterenol induced internalization and down regulation of beta receptors was time and concentration dependent in lung preparations. Other studies using the radiolabelled beta adrenoceptor agonist, \((\pm)(3H)-(\text{HBI})\) (Galant et al., 1978 & Wessels et al., 1979), have shown that desensitization involves a selective loss of high affinity binding sites, the remaining sites binding the agonists with much lower affinity. Wolfe & Harden (1981) using radio labelled antagonists have shown that guanine nucleotides caused a 2 to 4.5 fold increase in apparent affinity of these antagonists in addition to reduction of apparent binding affinity of the agonist isoproterenol for the beta receptor of the L6 myoblast membranes.

Lefkowitz (1982) has proposed a ternary complex model for activation of adenylate cyclase by beta adrenergic agonists and guanine nucleotides. According to this, when a hormone agonist \((H)\) binds to a receptor \((R)\) it somehow induces the formation of the ternary complex \((HRN)\) with nucleotide binding protein \((N)\). Formation of this complex is associated with loss of tightly bound guanosine diphosphnate \((\text{GDP})\) from the nucleotide regulatory protein \((N)\) which is followed by
binding of stimulatory guanosine triphosphate (GTP) to this protein. Interaction of this GTP with ternary complex (HRN) seems to destabilise it so that the receptor reverts to its free low affinity form and the nucleotide regulatory protein now occupied by GTP is also released. NGTP presumably interacts with the catalytic moiety of the enzyme adenylase cyclase (C) to form N.GTP.C presumed to be the active form of the enzyme. This form is short lived because the GTP ase activity associated with the regulatory protein cleaves the GTP to GDP. This deactivates the enzyme and presumably N and C dissociate at this point. N is now occupied by GDP which was formed by the last cycle of GTPase activity. At this point if the hormone agonist is still present the cycle repeats again and enzyme remains activated. Many physiological circumstances modulate the formation of HRN complex and such modulation represents an important mechanism for the control of tissue sensitivity to catecholamine action. Some circumstances which dampen the ability of beta agonists to stimulate adenylate cyclase are 1) desensitization due to prolonged exposure to agonist (Kent et al., 1980) and 2) hypothyroidism (Malbon et al., 1980).

Stiles et al (1984) have shown that desensitization in a number of systems (frog erythrocytes, human leukocytes) is a two step process. The first, most immediate reaction, occurring within minutes of exposure to low concentrations of isoproterenol is an uncoupling of the receptor which is not associated with receptor number. This uncoupling stops as soon as the drug is removed. The second process occurs with more prolonged exposure to low concentrations of the drug or even with a brief exposure to higher concentrations. It is not readily reversible and is associated with a loss of receptors (down regulation). This process is apparently due to the internalisation of the receptors into vesicles which remove them from the cell surface, thus reducing the number of receptors available for binding studies. Once
internalised, receptors remain intact for a while as shown by the ingenious fusion experiment of Strulovici et al (1983) but will slowly recycle back to the cell membrane if agonists are removed. If agonists are not removed they are destroyed by lysosomal proteases and must be resynthesized.

According to Raaka & Samuels (1981) one of the mechanisms by which drug or hormone induced desensitization occurs is by variations of receptor turnover. Perkins et al (1984) using 1321N1 human astrocytoma cells exposed to catecholamines showed that the rate of turnover of beta adrenoceptors was not appreciable in the absence of catecholamines. However, in the presence of catecholamines, there was an increased turnover of beta adrenoceptors, initiating a number of reactions that decrease cellular responsiveness to catecholamines. These eventually result in loss of functional beta adrenergic receptors from the cell.

Su et al (1976) classified desensitization to catecholamines into two broad categories namely “homologous” and “heterologous”. Homologous desensitization refers to a situation wherein, after prolonged exposure to a beta adrenoceptor agonist, the cell becomes refractory to further doses of the same agonist. Heterologous desensitization on the other hand, refers to a situation where the cell, after prolonged exposure to beta receptor agonist, becomes refractory not only to that agonist but also to other drugs and hormones.

Sibley et al (1987) have shown that both heterologous and homologous forms of adenylate cyclase desensitization involve phosphorylation of beta adrenergic receptors. Incubating 32P-labelled frog erythrocytes with either dibutyryl cyclic AMP or isoproterenol promoted a stoichiometric three fold increase in the
phosphorylation of the beta adrenergic receptor which occurs predominantly on the serine residues. However, if the cells are incubated with both dibutyryl cyclic AMP and isoproterenol then the adenylate cyclase desensitization as well as the phosphorylation of the beta adrenergic receptors are greater than those observed with either agent alone. These results indicate that heterologous and homologous desensitization of adenylate cyclase-coupled beta adrenergic receptors is mediated by different biochemical pathways involving phosphorylation of the receptor proteins at different sites. Lefkowitz & Caron (1990) have reviewed the role of two protein kinases involved in phosphorylation: the cAMP-independent kinase called the beta adrenergic receptor kinase (BARK) a specific kinase which can phosphorylate only the agonist occupied beta adrenoceptor leading to homologous desensitization. The other is a cyclic AMP dependent protein kinase A which can also phosphorylate other receptors causing heterologous desensitization.

Brodde et al (1990) using specific B₁ and B₂ adrenoceptor agonists have shown that desensitization in humans is specific and beta subtype selective.

Venter et al (1980) proposed that autoantibodies to B₂ adrenoceptors may play a role in desensitization. They speculated that such antibodies may constitute the primary mechanism for beta adrenergic “resistance” at the receptor level.

Elfellah & Turnbull (1978) suggested that tolerance to sympathomimetic bronchodilators may be due to a reduction in the functioning of receptors and/or the catalytic subunits, reflecting an adaptation of receptors/catalytic subunits to chronic exposure to the agonist.
Several experimental as well as clinical studies have shown that agonist induced subsensitivity of beta adrenoceptors can be reversed by glucocorticoids (Samuelson & Davies, 1984; Hui et al., 1982; Sauder et al., 1993). Corticosteroids have been reported to hasten recovery from tachyphylaxis induced in vitro in human bronchial muscle (Davis & Conolly, 1980) canine bronchus (Stephan et al., 1980) and bovine tracheal strips (Mackenzie, 1982). In a study on healthy volunteers the oral administration of prednisolone has been shown to accelerate the recovery of terbutaline induced beta adrenoceptor subsensitivity within eight to ten hours of glucocorticoid administration (Brodde et al., 1985). It has also been shown that in a group of asthmatic with B2-adrenoceptor subsensitivity a single intravenous dose of prednisolone restored responsiveness to inhaled isoproterenol (Ellul-Micallef & Fenech, 1975).

Potentiation of the response to catecholamines by glucocorticoids has been attributed to blockade of extraneuronal uptake (Geddes et al., 1974) and increased catecholamine receptor affinity (Besse & Bass, 1966). However, Rinard et al. (1983) questioned the hypothesis of Geddes et al. (1974) that the potentiating action of glucocorticoids is due to inhibition of reuptake of isoproterenol as they found that potentiation occurred even at saturating isoproterenol concentrations. Glucocorticoids have been reported to bring about an increase in receptor density, enhanced coupling and increased adenylate cyclase activity (Davies & Lefkowitz, 1984). Fraser & Venter (1980) have shown that glucocorticoids induce new receptors in cultured human lung cells within 12 hours of exposure. This is preceded by an increase in mRNA (Collins et al., 1988). Other studies have also provided evidence that corticosteroids have permissive effect on the function of pulmonary beta-2 adrenoceptors by preventing and reversing their down regulation (Mano et
The human beta-2 adrenergic receptor gene is now known to contain specific sequences of DNA known as glucocorticoid responsive elements (GRE) which interact directly with activated corticosteroid-glucocorticoid receptor complex to increase the rate of transcription of the receptor-protein. (Chung et al., 1987; Emorine et al., 1987; Kobika et al., 1987). Preliminary in vivo evidence using positron emission tomography to visualise pulmonary beta-2 receptors is supportive of the in vitro data and suggests that corticosteroid administration may both prevent and reverse down-regulation or uncoupling of these receptors in the asthmatic airway (Qing et al., 1992).

The first evidence that prostanoids might have a physiological role in airway smooth muscle responses was by Orhek et al. (1973, 1975) who showed that a number of structurally unrelated nonsteroidal antiinflammatory drugs reduce the basal tone of the isolated guinea-pig trachea. This led to the conclusion that locally generated prostaglandins are responsible for basal tone. The same group of workers also showed by bioassay that histamine induced the formation of PGE$_2$ mainly by interacting with histamine H$_1$ receptor and this release could be inhibited by antihistamines (Orehek et al., 1975). They also observed that the prostaglandins have modulating action on contractility and inhibition of PGE$_2$ synthesis leading to enhanced contractility.

Omini et al. (1981, 1985) have shown that isoproterenol and salbutamol cause relaxation of guinea-pig trachea and selectively induce release of PGE$_2$ in guinea-pig trachea in vitro. This release of PGE$_2$ was shown to be specific for beta adrenoceptor agonists. Further, Lew et al (1992) have shown that PGE$_2$ synthesis elicited by
adrenergic stimuli in guinea-pig trachea is mediated primarily via activation of beta 2 adrenergic receptors. These investigators suggest that arachidonic acid might regulate the coupling of beta adrenergic receptors to adnylate cyclase system. It has also been demonstrated that phospholipase A2 activity which catalyses the rate limiting step of the arachidonic acid cascade, is significantly increased in a model of experimental asthma, parallel to reduced beta adrenergic responsiveness (Taki et al., 1986).

Tachyphylaxis resulting from chronic exposure of tissue to catecholamines has also been associated with increased prostanoid synthesis. Douglas et al., (1977) first suggested that induction of tachyphylaxis to catecholamines in guinea-pig traches is more difficult in tissues pretreated with nonsteroidal anti-inflammatory drugs like indomethacin. Subsequently Brink (1981) showed that in vivo induction of tachyphylaxis to catecholamines could, under certain circumstances, be prevented if the catecholamines were coadministered with indomethacin. These observations were later confirmed by Omini et al (1981, 1985), Berti et al (1982) and Lew et al (1992). Daffonchio et al(1990) have demonstrated the role of eicosanoids in beta adrenocedptor desensitization induced by antigen challenge in guinea-pig trachea. Pretreatment of tissues with either hydrocortisone or indomethacin prevented the development of desensitization.

Prostaglandins play an important role in desensitization of beta adrenoceptor in the lung (Omini, 1985). However, prostaglandins do not participate directly in the relaxing action of beta agonists, as it has been shown that indomethacin does not antagonize the relaxant action of isoproterenol and salbutamol (Omini et al., 1981). Evidence available in literature indicates that prostaglandins of the E series can cause
refractoriness of adenylate cyclase in various cell types as well as a marked reduction of beta receptor mediated responses in myometrium (Strulovici et al., 1981; Tongui et al., 1980). Omini et al (1985) have suggested that prostaglandins modulate to some extent the early molecular event leading to desensitization of beta adrenoceptor. However, if desensitization is already established indomethacin does not effect it. Brink (1981) has also obtained similar results in guinea-pigs in vivo. Dihydroalprenolol (DHA) binding studies by Abbrachio et al (1983) in lung membranes have shown that prostanoids act on the adenylate cyclase system and not directly on the beta adrenoceptor as indomethacin does not prevent loss of DHA binding sites after the desensitizing procedure.

Indomethacin does not alter baseline pulmonary function in healthy volunteers (Ogilvy et al., 1981) in asthmatic patients (Smith, 1975) and in patients with allergic rhinitis (Fish et al., 1981). However, indomethacin alters pulmonary mechanics in certain animal species as it has been shown to reduce airway resistance in spontaneously breathing unanaesthetized guinea-pigs (Brink et al., 1978). Pretreatment with indomethacin did not alter the response to bronchial provocation tests either with histamine or methacholine (Ogilvy et al., 1981; Smith, 1975) Brink (1987) has suggested that the effect of PGE$_2$ is basically dependent on the basal tone. Where the basal tone is low, PGE$_2$ is contractile whereas if the basal tone is high, PGE$_2$ is relaxant. On the other hand PGE$_1$ is always relaxant. According to Tatterfield (1987) when asthmatic patients inhale PGE$_2$, the predominant effect is bronchodilatation which is not increased either by salbutamol or PGI$_2$. Prostaglandins may also be involved in modulating release of neurotransmitters from nerve endings (Von Euler & Hedquist, 1972; Samuelsson & Wennmalm, 1971).
Taylor (1987) has shown in guinea-pig trachea that the action of isoproterenol is potentiated by theophylline to a greater extent after desensitization than in control. He postulated the existence of two forms of phosphodiesterase, one with high affinity and the other with low affinity. In case of desensitization the low affinity phosphodiesterase concentration may increase causing the observed potentiation of aminophylline action. A similar mechanism may be operative in severely ill patients in whom theophylline has been reported to produce a greater potentiation of beta adrenoceptor agonist action (Svedmyr, 1977). However, in vitro studies by Bergendal et al (1992) failed to demonstrate any effect of theophylline on isoproterenol tachyphylaxis.

Salonen et al (1985) have shown in guinea-pigs that the magnitude of the bronchodilator action of theophylline when combined with either terbutaline or ipratropium depends on the severity of cholinergic airway obstruction. They suggested that theophylline has a more peripheral site of action than either terbutaline or ipratropium. The study also showed that the dosage of beta agonist has to be large enough to produce an enhanced bronchodilator effect with theophylline or larger airways. Combined treatment with beta adrenoceptor agonist and theophylline is used in order to achieve proper bronchodilatation with minimal side effects (Svedmyr, 1981; Shenfield, 1982). This combination induces in vitro, a synergistic relaxation of guinea-pig and human tracheo-bronchial smooth muscle, (Lefcoe et al., 1975) guinea-pig peripheral airway (Mitchell et al., 1979), and bronchial smooth muscle of asthmatic patients (Svedmyr, 1977). Theophylline and beta agonists have a synergistic action on the inhibition of mediator release from human leukocytes (Lithchenstein & Margolis 1968). Theophylline may act as prostaglandin antagonist.
(Horrobin et al., 1977). It may also affect intracellular calcium (Brisson et al., 1972) and increase binding of cyclic AMP to cAMP binding protein (Miech et al., 1979).

In view of the above, it is clear that the process of desensitization is an intriguing and multifaceted one and mechanisms involved are complex. What is clear however is that the process of desensitization whether, in vitro or in vivo, whether in the laboratory or in the clinic can be modulated favourably with corticosteroids, theophylline and NSAIDS. The present investigation is an attempt to elucidate further the mechanisms involved in the process of desensitization to beta adrenergic agonists in a laboratory setting.