MECHANISM OF DESENSITIZATION
TO B-AGONISTS IN AIRWAY SMOOTH MUSCLE
AND ITS MODULATION BY DRUGS - A STUDY

THESIS SUBMITTED FOR THE
DEGREE OF
DOCTOR OF PHILOSOPHY
IN PHARMACOLOGY
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A. STATEMENT REQUIRED UNDER ORDINANCE 0.770 .

I hereby state that the matter incorporated in to the thesis entitled "Mechanism of desensitization to B-agonists in airway smooth muscle and its modulation by drugs-a study" submitted for Ph.D., degree in pharmacology is my own contribution. I also state that I have not been awarded any degree or diploma or any other academic award of University of Bombay or any other University or body for this work.

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I state that the results obtained from the functional studies on the above subject for the thesis are my own findings and based on my own experiments and information derived from various biochemical studies. These findings contribute for further advancement of knowledge in the area of desensitization: an important mechanism of receptor regulation.

The literature concerning the present studies has been surveyed and necessary references have been cited. The laboratory studies have been carried out independently. Due acknowledgement has been made wherever facilities have been provided and availed.

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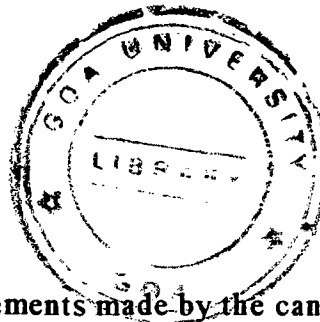
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Research Guide.

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CONTENTS

INTRODUCTION

Desensitization, tolerance, refractoriness and tachyphylaxis are all different terms used to denote a state of diminished responsiveness of a tissue or organ that follows its prior exposure to an agonist, a hormone or a drug (Lefkowitz et al., 1980). Desensitization is known to occur with beta adrenoceptor system. Over the last few years much work has been carried out to unravel the molecular mechanisms involved in beta adrenoceptor desensitization. From this work has come an appreciation of the diversity of mechanisms that may lead to desensitization. In some cases the desensitization is homologous and only responsiveness to beta adrenergic catecholamines is attenuated. In other cases the beta adrenergic refractoriness may be part of a more heterologous picture of desensitization in which hormonal responsiveness to a variety of receptor mediated responses is affected. Homologous desensitization may indicate a receptor specific lesion whereas heterologous desensitization may be mediated at post receptor levels (Stiles et al., 1984).

Beta adrenoceptor agonists are widely used as bronchodilators since the time epinephrine was introduced as bronchodilator in England by Mathew in 1909 and in Germany by Epheraim in 1910. Isoproterenol was synthesized by Konzett in 1940 and was the first adrenergic agonist without significant alpha adrenergic activity. However, with the discovery of the beta-2 selective agonists salbutamol (Brittain et al., 1968) and terbutaline (Bergman et al., 1969), the non-selective beta adrenergic agonist isoproterenol was replaced by these drugs in the treatment of bronchial asthma.

Another important advancement in the treatment of bronchial asthma was the development in the 1960s of pressurised aerosols which could deliver precisely metered doses of bronchodiilators suspended in microfine crystalline form in freon

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propellents. As these devices were very convenient they were very well accepted by both physicians and patients alike. This led to increased use of adrenergic aerosol preparations in the treatment of bronchial asthma.

In 1968, the attention of physicians and pharmacologists was drawn by a publication of Spiezer and his group that with the introduction of pressurised aerosols, the asthma mortality in England and Wales rose in the patients in the age group of 5 - 34 years, the highest mortality being in the age group of 10-14 years. It was also observed that 83% of these patients had used pressurised aerosols. Several patients were found dead with empty aerosol canister besides them.

This increased mortality in bronchial asthma patients led to a wide debate on the function of beta adrenoceptors in asthma and the role of drugs in modulating the function of the beta adrenoceptor. Some scientists like Szentivanyi (1968) argued that there is a primary defect in the beta receptors of asthmatics. He showed experimentally that when guinea-pigs were sensitized with B.pertussis vaccine there was apparent reduction in the beta adrenoceptor responses. Another group (Conolly & Greenacre 1977) argued that reduced responsiveness seen in asthmatics is not a primary defect as suggested by Szentivanyi (1968) but is the result of prolonged exposure to beta adrenergic bronchodilators. They studied the lymphocyte beta adrenoceptor function and found marked depression of cyclic AMP responses to isoproterenol in asthmatics using adrenergic drugs, but not in asthmatics of comparable severity who were exclusively treated with nonadrenergic drugs like cromolyn sodium or beclamethasone. Further, asthmatics weaned off their adrenergic drugs reverted back to their normal pattern of response. On the other hand obstetric patients, who were showing normal response previously showed

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marked attenuation of lymphocyte cyclic AMP response after 48 hours of beta agonist infusion to control premature labour. These observations were followed by many experimental and clinical investigation to elucidate the molecular mechanisms involved in the process of desensitization.

The current view is that the mechanism of desensitization may involve (1) loss of affinity of the beta adrenoceptor agonist to the receptor (Lin et al., 1977; Avner & Noland, 1978) (2) down regulation of receptors (Harden et al., 1980) and (3) variation of receptor turnover (Raaka & Samuels, 1981). According to Stiles et al (1984), desensitization in a number of systems is a two step process. The immediate reaction which occurs within minutes of exposure to low concentration of isoproterenol is an uncoupling of the receptor which is not associated with reduction in receptor number and is rapidly reversed as soon as the drug is removed. The second process occurs with more prolonged exposures or a brief exposure to a higher concentration of the drug. This is not readily reversible and is associated with loss of receptors (down regulation).

Clinical studies indicate that glucocorticoids restore airway responsiveness to beta agonists in some patients who are refractory to their bronchodilator effect. (Ellui-Micaleff & Fenech 1975 ; Tattersfield & Holgate 1976 ; Svedmyir 1990 and Qing et al ., 1992). In another experimental study in dogs Stephan et al (1980) showed that pretreatment of the animals with large doses of methyl prednisolone could prevent loss of responsiveness to isoproterenol. Glucocorticoids have also been shown to be effective in reversing the reduced beta adrenerigic responsiveness in some asthmatics (Parker & Smith 1973). It is possible that glucocorticoids facilitate the action of beta agonists in airway smooth muscle.

The involvement of prostaglandins in the desensitization of airway smooth muscle to beta agonists has been shown by Douglas et al(1977). It has also been demonstrated by Omini et al (1981) that PGE_2 is generated in isolated guinea-pig trachea relaxed by isoproterenol, an event which increased significantly after desensitization of beta adrenoceptors. This could be prevented by pretreatment of the preparation with indomethacin (Berti et al.. 1982).

Treatment of bronchial asthma with a combination of theophylline and a beta adrenoceptor agonist is a common procedure (Ogilvy .1978). The rational basis for the combination of theophylline with a beta adrenoceptor agonist is the synergism of their action on the metabolic pathway of cyclic AMP leading to an increase in intracelluar cyclic AMP which appears to regulate bronchodilation and mediator release in the bronchial smooth muscle (Campbell et al., 1977). Taylor (1987) has showed that aminophylline potentiates isoproterenol induced relaxation after desensitization with isoproterenol.

In the light of the above, this study was undertaken to elucidate the mechanisms involved in desensitization of beta adrenoceptor to its agonists. The study involves (1) induction of desensitization to various beta agonists that is isoproterenol, salbutamol, and terbutaline in beta adrenoceptors of guinea-pig trachea both in vivo and in vitro (2) measurement of affinities of the beta agonists for the beta receptors of the guinea-pig trachea and (3) studying the effect of pretreatment with hydrocortisone, indomethacin and theophylline on the process of desensitization as reflected by changes in affinity.

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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

characterstuc feature of asthma. However, Larsson et al(1976), Sims et al(1978). Wilson et al (1976) and Peel & Gibson (1980) failed to demonstrate the development of any tolerance.

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 bronehodilator 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

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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 prepartions. Other studies using the radiolabelled beta adrenoceptor agonist, $(\pm)$ (3_H) - hydroxybenzyl isoproterenol (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 (GDP) from the nucleotide regulatory protein(N) which is followed by

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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 moeity of the enzyme adenylase cyclase (C) to form N.GTP.C presumed to be the activee 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 erytyrocytes, 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 occurrs 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

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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 132lN1 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 32_p labelled frog erythrocytes with either dibutyryl cyclic AMP or isoproterenol promoted a stoichiometric three fold increase in the

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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 adreno receptor 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 B_1 and B_2 adrenoceptor agonists have shown that desensitization in humans is specific and beta sub type selective.

Venter et al (1980) proposed that autoantibodies to B_2 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 asthamatic with B_2 - 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

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al., 1979; Svedmyr 1990). 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 relase 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

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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 A_2 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 adrenoceptor 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 adrenocedptor 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 mechanicsin 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 where as 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 & Hedqust, 1972; Samuelsson & Wennmalm ,1971).

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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

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(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.

MATERIAL AND METHOD

1. Animals and Tissue

Male Haffkine strain guinea-pigs (Cavia porcellus) bred in the departmental animal house and weighing 350 - 500 grams were used in the study. The isolated tracheal tissue was used both in vitro as well as after pretreatment of the animals in vivo.

Equipment used.

i) Assembly for mounting the tissue.

A two-unit isolated bath assembly (INCO) consisting of a tissue bath of 10 ml capacity, mounted vertically inside a water bath was used.

ii. Recording Assembly.

The assembly for measuring the response of the muscle isometrically include the Encardio-rite Polygraph Model No.434 serial No.0043 Manufactured by M/s. Encardio-rite Electronics, Lucknow,

3. Composition of the Physiological Salt solution.

The physiological salt solution used in the study consisted of Kreb-Hensleitt solution of the following composition in gms.litre^{-1} NaCl: 6.9, Kcl: 0.35, CaCl$_2$: 0.28, Mg SO$_4$ 7 H$_2$O: 0.28,

NaHCO$_3$: 0.21, KH$_2$PO$_4$:0.16, Glucose: 1.5 Ascorbic Acid

0.2.

All chemicals used were of analytical reagent grade.

4. Drug solutions used in the study.

The following drugs were used in the study the drug solutions were freshly prepared on the day of study using double distilled water.

i) Carbamylcholine chloride (Carbachol) E.Merck.

An aqueous solution of Carbachol containing 1 mg.ml^{-1} was prepared and subsequently diluted as required.

ii) Isoproterenol Hydrochloride (Sterling Winthrop)

A 1 mg.ml^{-1} was prepared in distilled water containing 17 micrograms.ml^{-1}. of ascorbic acid and diluted as required.

iii) Salbutamol Sulphate (Cipla)

The drug was prepared freshly in 0.9% NaCl. and diluted as required.

iv) Terbutaline Sulphate (Astra IDL)

The drug was freshly prepared in 0.9% NaCl and diluted as required.

v) Hydrocortisone sodium Succinate (Allenbury/Lyka)

Vial containing Hydrocortisone 100 mg was diluted as required.

vi) Indomethacin (Sigma)

A 1 mg.ml^{-1} solution was prepared using sodium carbonate 0.5% solution and subsequently diluted.

vii) Theophylline (Sigma) was dissolved in 0.5 M NaOH and diluted in 0.9% NaCl.

viii) Phentolamine HCl (Ciba) Dissolved in alcohol and diluted in 0.9% NaCl.

ix) Reserpine (Sigma) Reserpine was dissolved using glacial acetic acid and diluted as required.

x) Pyrogallol (E. Merck) Dissolved in distilled water.

Isolation and mounting of Guinea-pig trachea:

Guinea-pigs were stunned and exsanguinated and trachea was dissected free. Tracheal spirals were prepared according to constantine (1965). From each guinea-pig two or three pieces of trachea were obtained. The response to different segments is not variable as indicated by the study of Hanna and Roth (1978). The tracheal strips were equilibrated for 90 minutes under initial tension of 8 gms in Krebs-Hensleitt solution at 37°C aerated with an air pump. The high initial tension

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was needed to ensure that resting tension at the end of equilibrium period was between 4 to 6 gms. Under these conditions the responses to agonists are reproducible since the muscle is near its maximal length (Stephen, 1970) One end of the tracheal spiral was tied to the tisse hook and the other end tied to a force displacement transducer FT 0.03 (Grass) and isometric response was obtained using an Encardiorite polygraph. It has been shown by Armour et al(1988) that isometric measurements may provide a more accurate representation of smooth muscle changes in response to agonists in vitro. The instrument was calibrated using 2 gm weight and at the end of the equilibration period a resting tension equivalent to 2 gms was provided to the tissue with the preclibrated instrument. The response was recorded with a sensitivity of 0.1 - 0.2mV.cm^{-1}

Standardisation of Technique:

The technique was standardised with each drug with respect to the dose and time required for the response to be recorded. A dose of carbachol producing submaximal contraction was chosen to increase the tone of the preparation and response was taken for 10 mintues.

Response with isoproterenol / salbutamol / terbutaline:

Relaxant responses with the above beta agonists were taken in tissues precontracted with carbachol. The drugs were added in a cumulative manner as per the method of Van-Rossum (1963). Two minutes was allowed for each dose to produce its response. Following the cumulative dose response to each beta agonist the tissue was washed repeatedly. Similar contractile response to carbachol was ensured before obtaining the next cumulative dose response. The above responses were obtained before and after incubation with various drugs for specified periods.

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Plan of study. The process of desensitization was studied by determining the change in affinity of the beta adrenoceptor to various beta-agonists and their change in potency in guinea-pig trachea. Deesensitization was produced both in vitro and in vivo.

A salient feature of the methodological approach was the study of auto-desensitization which involves desensitisation to and challenge with the same beta-agonist. Three beta agonists were studied, isoproterenol a non-selective catecholamine. salbutamol a beta - 2 selective saligenin derivative, and terbutaline a beta-2 selective resorcinol derivative, the last two beta-2 agonists being the most frequently used bronchodilators in asthma. The mechanism of desensitization and its modulation by pre-treatment with hydrocortisone. indomethacin and theophylline has been investigated.

Since it was not technically feasible to measure affinity of the agonist directly, the study utilised two indirect indices to reflect the same (1) relaxing potency(pD_2) of the beta-agonist: Cumulative dose response curves were obtained as per the method of Van-Rossum (1963) to beta-agonists using guinea-pig tracheal spirals either pretreated in vitro or obtained from guinea-pigs pretreated in vivo. The pD_2 values for the beta agonists were calculated in isolated guinea-pig trachea in (1) controls (2) desensitized tracheas and (3) treacheas treated with hydrocortisone, indomethacin or theophylline prior to desensitization. Similarly pD_2 values were calculated using isolated guinea-pig treacheas obtained from in vivo vehicle treated guinea-pigs, desensitized guinea- pigs and guinea pigs subjected to desensitization after pretreatment with hydrocortisone, indomethacin or theophylline. EC_{50} was calculated and relaxant potency PD_2 was calculated from the formula $pD_2 = -\log EC_{50}$

(ii) Apparent dissociation constant (K_B Value) was the second index employed. It represents the affinity of the beta receptor antagonist for the beta receptor (Furchgott 1955). The K_B value is computed by the equation

$$K_B = \frac{[A]\ [B]}{[A'] - [A]}$$

Where K_B=dissociation constant for the antagonist receptor complex, [B] is the concentration of the antagonist added to the bathing medium in the organ bath, [A] is the concentration of the agonist needed to produce a given magnitude of response in the absence of antagonist and [A'] is the concentration of the agonist needed to produce the same magnitude of response in the presence of the antagonist. The other details of the procedure were according to method of Lin et al (1977).

I. In vitro production of Desensitization.

Dose response curves for the relaxant beta agonists isoproterenol, salbutamol and terbutaline were plotted according to the cumulative method of Van-Rossum (1963) using guinea-pig trachea precontractted with (5×10^{-7}M) carbachol. Higher concentration of carbachol blocks the relaxant effect of beta agonists completely (Van Den Brink, 1973).

The EC_{50} to the beta agonist was then calculated. The trachea was then incubated for 30 minutes with a concentration of two hundred time the EC_{50} of the respective beta agonist to produce desensitization as per the method of Watanabe et al (1976). The tissue was washed at intervals of 10 minutes for 30 minutes the EC_{50} for the relaxant effect of the beta agonists was then calculated in the desensitized trachea as well in guinea-pig tracheas where desenstization was preceeded by

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treatment with hydrocortisone (4×10^{-5}M (30 minutes) / indomethacin 1.7×10^{-6} M (30 minutes) or theophylline 5.6×10^{-4} M (20 minutes). The relaxant potency pD_2 value of the agonist was then obtained. Similarly the apparent disassociation constant K_B value for proproanalol was calculated as per method of Lin et al (1977) for normal and desensitized guinea-pig tracheas pretreated with hydrocortisone / indomethacin/ theophylline prior to desensitization. The concentration of the beta antagonist used (propranalol) was 1×10^{-5}M.

Certain precautions were taken while preparing the isolated trachea for the in vitro experiments to impede processes which could influence the observed effect of beta receptor agonists as per the method of Buckner & Saini (1975) All tissues were taken from guinea pigs which had been pretreated with reserpine (5 mg.kg^{-1} I.P) 24 hours previously in order to minimise release of endogenous catecholamine during the experiment the alpha antagonist phentolamine was added in a concentration of 10^{-5}M 30 minutes prior to experiment and was present throughout. Pyrogallol, a catchol o-methyl transferase inhibitor was added 45 minutes prior to the experiment in a concentration of(3×10^{-5}M) and was present throughout the experiment as it has been shown that O-methylated metabolites of catecholamines are weak beta - adrenoceptor antagonists (Brine et al 1979; Goldie & Patterson, 1982) and may thus reduce tissue sensitivity to beta-agonists (Kenakin. 1980).

II) In vivo production of desensitization

i) Control group: The guinea-pigs in this group were pre-treated with 0.9% NaCl containing 20 microgram.ml^{-1} of ascorbic acid by intramuscular injection at intervals of 20 mintues for five hours. The animals were sacrificed at seven hours that is two hours after the last injection and trachea isolated and subjected to the study.

ii) Desensitization group. The animals in this group were desensitized as per the method used by Conolly et al (1971) using one of the beta agonists isoproterenol (4 microgram.kg^{-1},), Salbutamol (4 microgram.kg^{-1}) or terbutaline (20 microgram.kg^{-1}). The animal was injected intramuscularly any one of the above agonists at twenty minute intervals for five hours and sacrificed at the end of the seven hours. The tracheas were taken out and pD$_2$ and K$_B$ values were calculated as described earlier.

iii) Desensitization proceeded by treatment.

The animal was treated prior to desensitization with anyone of the following drugs; hydrocortisone 50 mg.Kg^{-1} I.M. eighteen hours prior to desensitization as per Brink et al (1977), indomethacin 20 mg.Kg^{-1} IP 4 hours prior to desesitization as per method of Chandra (1986), or theophylline 45 mg.kg^{-1} IP at the beginning of desensitization procedure as per the method of Madsen and Ribel (1981). Following treatment, desensitization in vivo was carried out as described above pD$_2$ and K$_B$ values were calculated.

Analysis of Results.

The results obtained in the studies were evaluated statistically using either paired or unpaired two tailed T tests.

OBSERVATIONS & RESULTS

I. Effect of beta agonists on auto-desensitization.

A. In Vitro

The results of in vitro experiments showed that desensitization was maximum with isoproterenol followed by salbutamol and terbutaline as reflected by pD_2 values. However, the decrease in potency was statistically significant only in the case of isoproterenol.(Vide Table-I.Figures 1,2,3.).

The decrease in affinity as reflected by K_B was highest with salbutamol followed by isoproterenol and terbutaline and the values were statistically significant.(Vide Table-II.Figures 4,5,6.).

B. In Vivo

In case of desenstization in vivo the decrease in potency was highest with salbutamol followed by isoproterenol and terbutaline all results were statistically significant.(Vide Table-III.Figures 7,8,9).

The decrease in affinity was hihest with salbutamol followed bi isoproterenol and terbutaline and statistically significant in case of all the three drugs. (Vide Table-IV,Figures 10.11,12).

II Effect of pretreatment with hydrocortisone indomethacin and theophylline on desensitization to isoproterenol.

A. In Vitro

Hydrocortisone completely prevented loss of potency due to isoproterenol desensitization while indomethacin and theophylline had a partial effect. (Vide Table-V, Figure 1).

Analysis of the K_B values showed that all the three drugs not only prevented loss of affinity resulting from desensitization but actually increased affinity beyond control level,the result being ststistically significant.(Vide Table - VI,Figure 4).

B. In Vivo

In vivo also hydrocortisone completely prevented desensitization to isoproterenol. Indomethacin not only failed to prevent desensitization but actually decreased pD_2 values further,theophylline also failed to protect aganist desensitization in vivo.(Vide Table - VII, Figure 7).

While hydrocortisone and theophylline produced increase in affinity beyond control,indomethacin failed to prevent loss of affinity.(Vide Table - VIII, Figure 10).

III. Effect of pretreatment with hydrocortisone,indomethacin and theophylline on desensitization to salbutamol.

A. In Vitro

Hydrocortisone and indomethacin completely prevented desensitization to salbutamol but theophylline failed to do so.(Vide Table - IX, Figure 2).

There was no change in affinity as compared with control in case of hydrocortisone and theophylline but indomethacin produced an increase in affinity beyond control level.(Vide Table - X, Figure 5).

B. In Vivo

In vivo also hydrocortisone and indomethacin completely prevented desensitization to salbutamol as reflected by pD_2 values.Theophylline however, had only a partial protective effect.(Vide Table - X, Figure 8).

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Analysis of K_B values however, gave an entirely different picture. All the three drugs did not prevent the loss of affinity caused by salbutamol.(Vide Table - XII, Figure 11).

IV. Effect of pretreatment with hydrocortisone, indomethacin and theophylline on desensitization to terbutaline.

A. In Vitro

All the three drugs prevented desensitization to terbutaline as reflected by pD_2 values. However, desensitization produced by terbutaline in vitro as reflected by pD_2 was statistically insignificant. (Vide Table - XIII. Figure 3).

Analysis of K_B values showed, howevr, that hydrocortisone could prevent the loss of affinity produced by terbutaline whereas indomethacin and theophylline failed to do so.(Vide Table - XIV, Figure 6).

B. In Vivo

In vivo all the three drugs could partially prevent loss of potency due to desensitization. Hydrocortisone was the most effffective followed by theophylline and indomethacin.(Vide Table - XV. Figure 9).

All the three drugs also prevented loss of affinity to terbutaline. (Vide Table - XVI, Figure 12).

Table - 1

pD_2 values in guinea-pig trachea desensitized with beta agonists.

Group	Control	Desensitized	P
Isoproterenol	14.117 ± 0.807 (9)	11.087 ± 1.009 (9)	0.042
Salbutamol	10.790 ± 2.710 (6)	8.850 ± 2.820 (6)	NS
Terbutaline	10.000 ± 2.800 (7)	9.360 ± 2.610 (7)	NS

The data are presented as the mean ± SE. The number in the parentheses represent number of experiments.

NS = Not significantly different from control.

Table - II

-Log K_B for propranalol in guinea-pig trachea desensitized with beta agonists.

Group	Control	Desensitized	P
Isoproterenol	9.3816 ±0.0687(13)	7.9085 ± 0.2734 (7)	0.0001
Salbutamol	9.2830 ±0.0898(12)	7.1580 ± 0.2000 (8)	0.0001
Terbutaline	9.2920 ± 0.1400 (6)	8.2870 ± 0.0990 (6)	0.0005

The data are presented as the mean ± SE. The number in paretheses represent the number of experiments.

Table - III

pD$_2$ values in tracheas from guinea-pigs desensitized with agonists.

Group	Control	Desensitized	P
Isoproterenol	14.25 ± 2.24 (17)	11.06 ± 1.50 (14)	0.001
Salbutamol	13.05 ± 1.10 (7)	7.28 ± 0.74 (7)	0.001
Terbutaline	11.29 ± 1.48 (8)	7.45 ± 0.85 (10)	0.001

The data are presented as the mean $\pm$ SE. The number in the parentheses represent number of experiments.

Table - IV

-Log K$_B$ for propranalol in tracheas from guinea-pigs desensitized with beta agonists.

Group	Control	Desensitized	P
Isoproterenol	9.065 ± 0.3999 (7)	7.525 ± 0.251 (7)	0.0068
Salbutamol	14.108 ± 0.8030 (6)	9.430 ± 0.242 (6)	0.0002
Terbutaline	8.330 ± 0.3550 (6)	6.931 ± 0.388 (6)	0.0237

The data are presented as the mean $\pm$ SE. The number in the parentheses represent number of experiments.

Table - V

pD$_2$ values in guinea-pig tracheas treated with different drugs before desensitization with isoproterenol.

Group	Control	Desensitized	P
Hydrocortisone	12.297 ± 1.456 (6)	12.837 ± 1.198 (6)	NS
Indomethacin	12.316 ± 2.306 (6)	11.508 ± 2.306 (6)	NS
Theophylline	16.447 ± 0.709 (7)	15.782 ± 0.734 (7)	NS

The data are presented as the mean ± SE. The number in the paretheses represent number of experiments.

NS = Not significantly different from control.

Table - VI

-Log K$_B$ for propranalol in guinea-pig tracheas treated with different drugs before desensitization with isoproterenol.

Group	Desensitized	Desensitized with pretreatment	P
Hydrocortisone	7.9085 ± 0.2734 (7)	15.5 ± 0.7637 (6)	0.0001
Indomethacin	7.9085 ± 0.2734 (7)	14.2832 ± 1.903 (6)	0.0042
Theophylline	7.9085 ± 0.2734 (7)	18.01 ± 0.4083 (6)	0.0001

The data are presented as the mean ± SE. The number in the parentheses represent number of experiments.

Table - VII

pD$_2$ values in the tracheas from guinea-pigs treated with different drugs before desensitization with isoproterenol.

Group	Control	Desensitized	P
Hydrocortisone	14.25 ± 2.24 (17)	13.65 ± 2.45 (7)	NS
Indomethacin	14.25 ± 2.24 (17)	8.18 ± 2.49 (8)	0.0001
Theophylline	14.25 ± 2.24 (17)	11.21 ± 2.0 (12)	0.01

The data are presented as the mean ± SE. The number in parentheses represent number of experiments.

NS = Not significantly different from control.

Table - VIII

-Log K$_B$ for propranalol in tracheas from guinea-pigs treated with different drugs before desensitization with isoproterenol.

Group	Desensitized	Desensitized with pretreatment	P
Hydrocortisone	7.525 ± 0.251 (7)	10.5767 ± 0.517 (7)	0.0002
Indomethacin	7.525 ± 0.251 (7)	8.4100 ± 0.431 (7)	NS
Theophylline	7.525 ± 0.251 (7)	13.0420 ± 0.502 (6)	0.0001

The data are presented as the mean ± SE. The number in the paretheses represent number of experiments.

NS = Not significantly different from the desensitized guinea-pigs.

Table - IX

pD$_2$ values in guinea-pig tracheas treated with different drugs before desensitization with salbutamol.

Group	Control	Desensitized	P
Hydrocortisone	9.91 ± 3.4 (6)	9.93 ± 3.59 (6)	NS
Indomethacin	14.50 ± 0.49 (5)	14.87 ± 0.49 (5)	NS
Theophylline	9.78 ± 2.39 (6)	7.925 ± 2.7 (6)	NS

The data are presented as the mean ± SE. The number in the parentheses represent number of experiments .

NS = Not significantly different from control.

Table - X

-Log K$_B$ for propranalol in guinea-pig tracheas treated with different drugs before desensitization with salbutamol.

Group	Desensitized	Desensitized with pretreatment	P
Hydrocortisone	7.158 ± 0.22 (8)	9.396 ± 0.434 (6)	0.0003
Indomethacin	7.158 ± 0.22 (8)	12.186 ± 0.393 (7)	0.0001
Theophylline	7.158 ± 0.22 (8)	9.076 ± 0.660 (6)	0.0092

The data are presented as the mean ± SE. The number in the parentheses represent number of experiments.

Table - XI

pD$_2$ values in tracheas from guinea-pigs treated with different drugs before desensitization with salbutamol.

Group	Control	Desensitized	P
Hydrocortisone	13.05 ± 1.1 (7)	13.56 ± 0.93 (5)	NS
Indomethacin	13.05 ± 1.1 (7)	13.97 ± 0.75 (5)	NS
Theophylline	13.05 ± 1.1 (7)	11.16 ± 1.14 (6)	0.05

The data are presented as the mean ± SE. The number in the parentheses represent number of experiments.

NS = Not significantly different from control.

Table - XII

-Log K$_B$ for propranalol in tracheas from guinea-pigs treated with different drugs before desensitization with salbutamol.

Group	Desensitized	Desensitized with pretreatment	P
Hydrocortisone	9.43 ± 0.242 (6)	9.76 ± 0.384 (6)	NS
Indomethacin	9.43 ± 0.242 (6)	12.33 ± 1.738 (6)	NS
Theophylline	9.43 ± 0.242 (6)	11.2294 ± 1.780 (6)	NS

The data are presented as the mean ± SE. The number in the parentheses represent number of experiments.

NS = Not significantly different from the desensitized guinea-pigs.

Table - XIII

pD$_2$ values in the guinea-pig tracheas treated with different drugs before desensitization with terbutaline.

Group	Control	Desensitized	P
Hydrocortisone	7.725 ± 0.66 (7)	7.45 ± 0.79 (7)	NS
Indomethacin	7.730 ± 2.15 (6)	6.83 ± 1.33 (6)	NS
Theophyllyine	7.620 ± 0.67 (7)	7.20 ± 1.046 (7)	NS

The data are presented as the mean ± SE. The number in parentheses represent number of experiments.

NS = Not significantly different from control.

Table - XIV

-Log K$_B$ for proparalol in guinea-pig tracheas treated with different drugs before desensitization with terbutaline.

Group	Desensitized	Desensitized with pretreatment	P
Hydrocortisone	8.287 ± 0.099 (6)	9.38 ± 0.3211 (6)	0.0089
Indomethacin	8.287 ± 0.009 (6)	8.762 ± 0.697 (6)	NS
Theophylline	8.287 ± 0.009 (6)	8.3527 ± 0.099 (6)	NS

The data are presented as the mean ± SE. The number in the parentheses represent number of experiments.

NS = Not significantly different from the desensitized guinea-pigs.

Table - XV

pD$_2$ values in the tracheas from guinea-pigs treated with different drugs before desensitization with terbutaline.

Group	Control	Desensitized	P
Hydrocortisone	11.29 ± 1.48 (8)	11.22 ± 1.56 (7)	NS
Indomethacin	11.29 ± 1.48 (8)	9.48 ± 2.63 (9)	NS
Theophylline	11.29 ± 1.48 (8)	10.11 ± 1.83 (8)	NS

The data are presented as the mean ± SE. The number in the parentheses represent number of experiments.

NS = Not significantly different from control.

Table -XVI

-Log K$_B$ for propranalol in tracheas from guinea-pigs treated with different drugs before desensitization with terbutaline.

Group	Desensitized	Desensitized with pretreatment	P
Hydrocortisone	6.931 ± 0.388 (6)	8.34 ± 0.168 (6)	0.0076
Indomethacin	6.931 ± 0.388 (6)	8.64 ± 0.299 (6)	0.0059
Theophylline	6.931 ± 0.388 (6)	8.68 ± 0.280 (6)	0.0045

The data are presented as the mean ± SE. The number in the parentheses represent number of experiments.
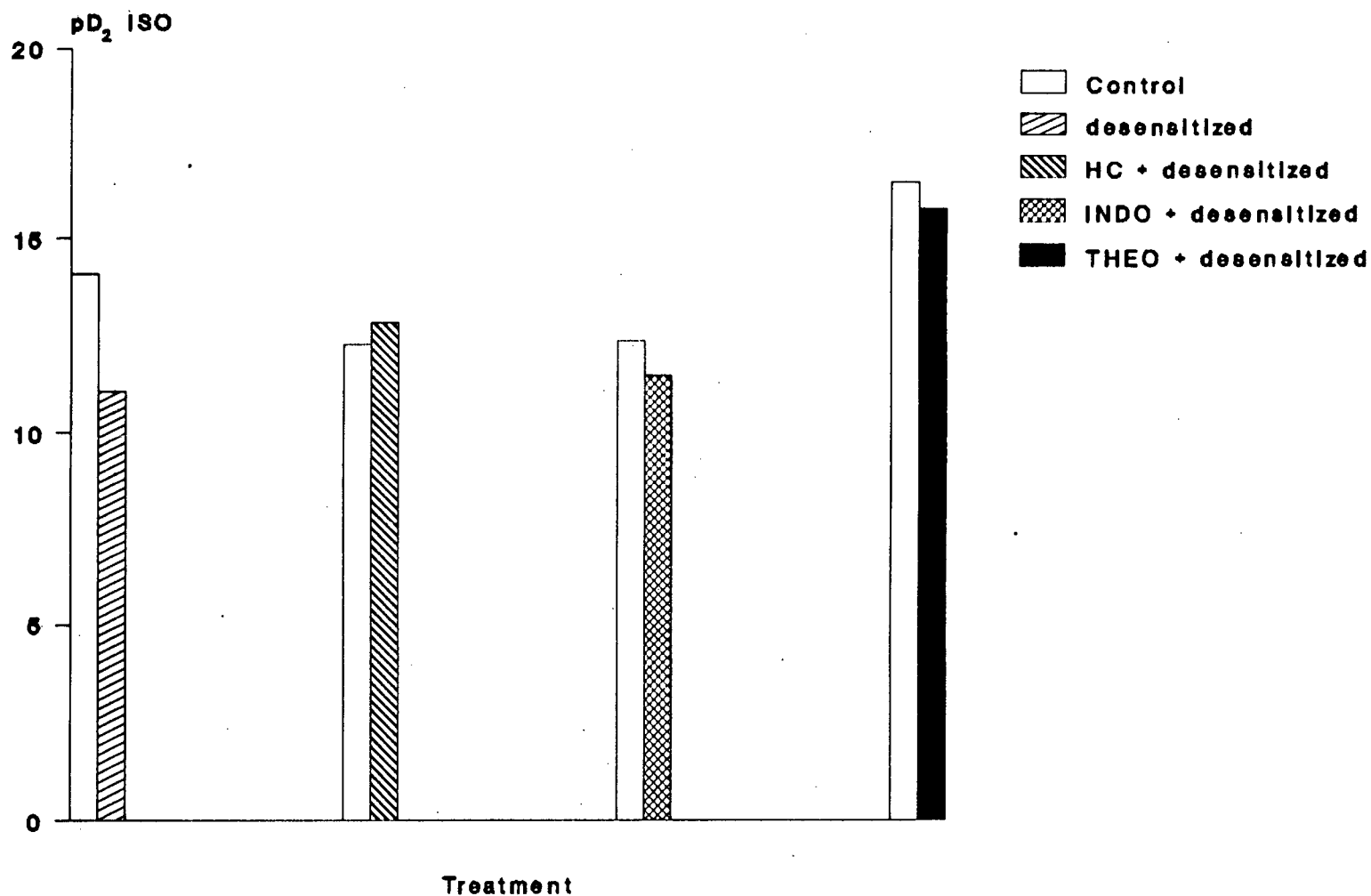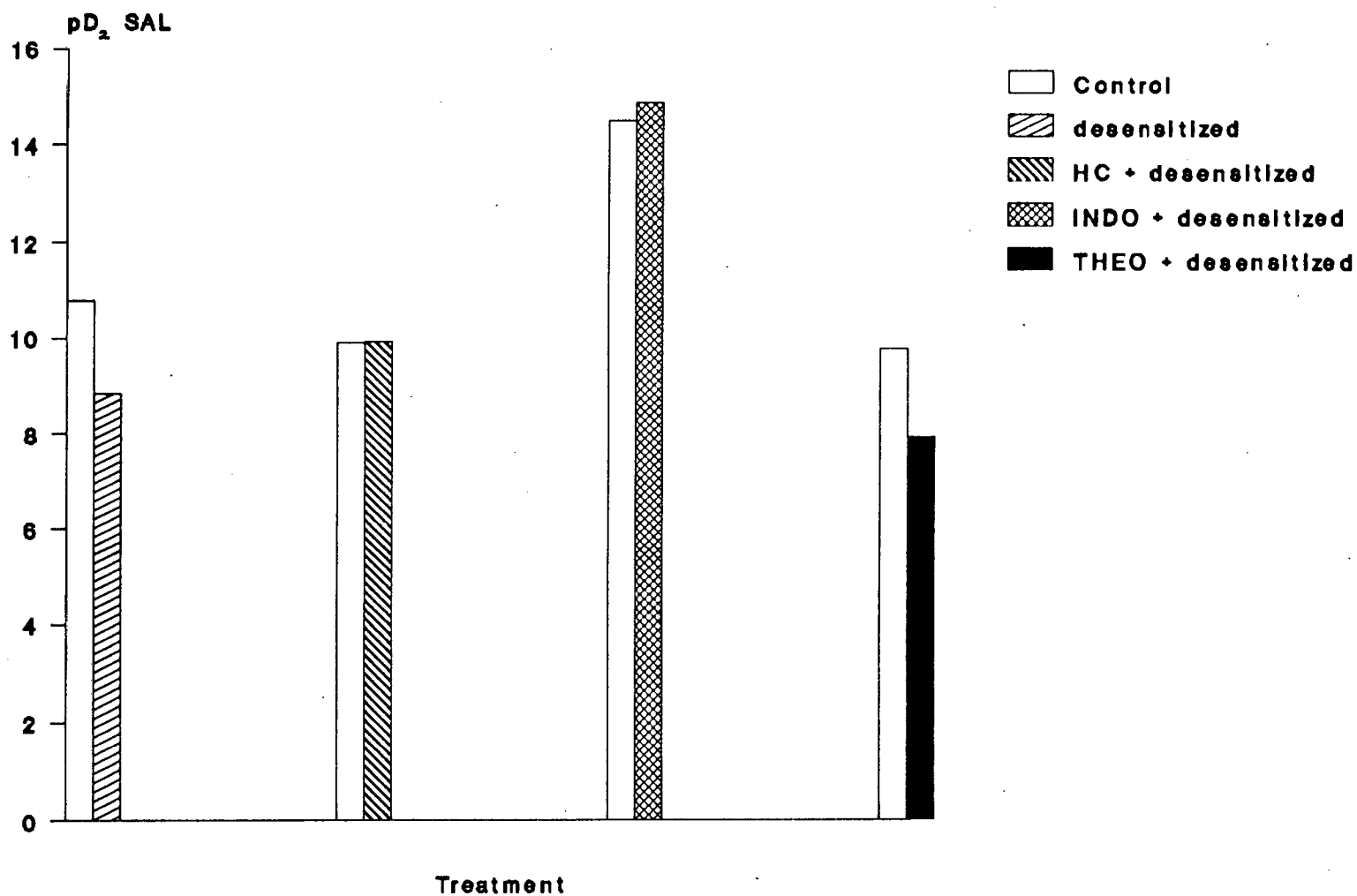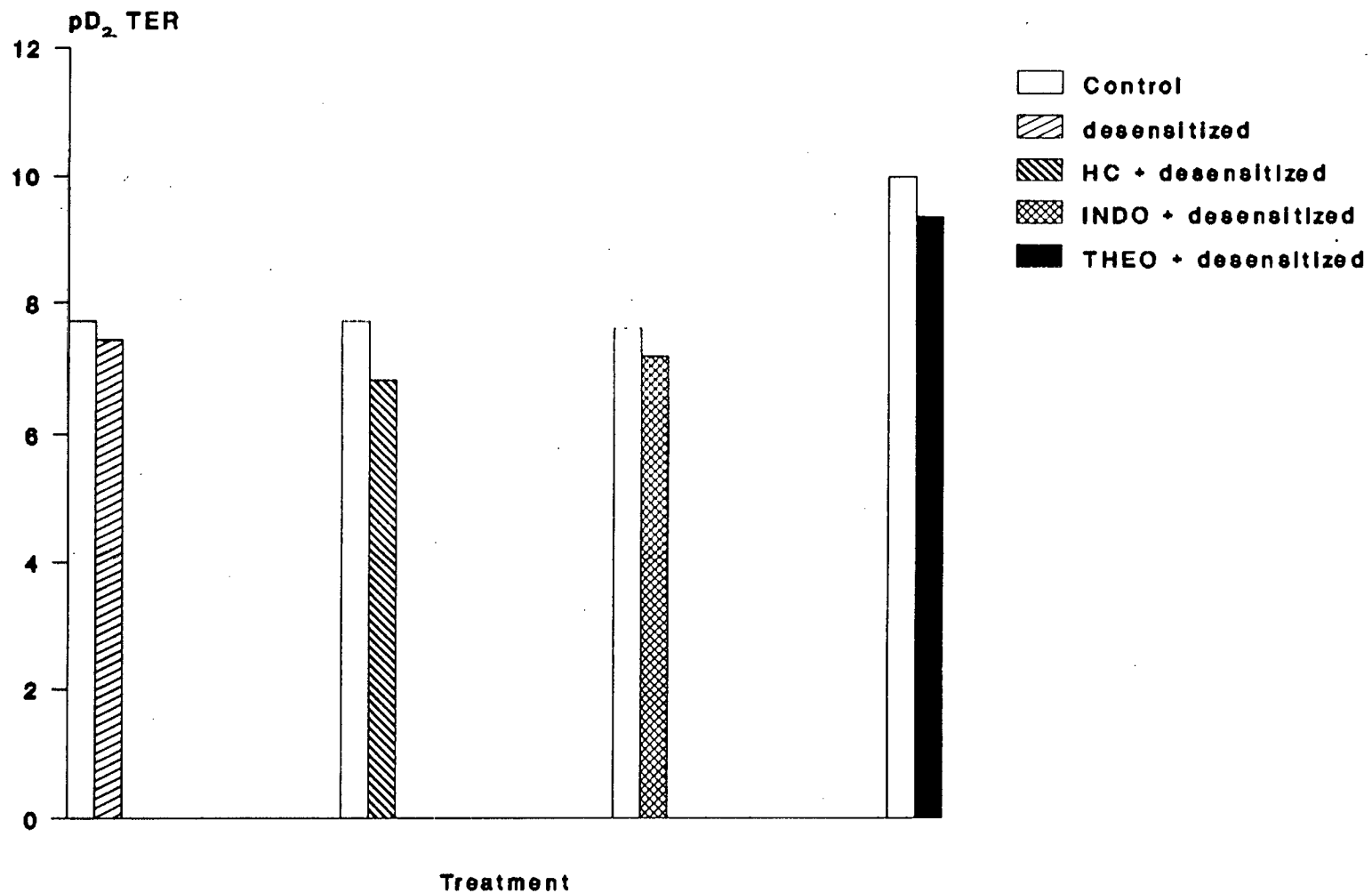
Fig. 1. Effect of In-vitro desensitization and desensitization after pretreatment with hydrocortisone (H.C) indomethacin (IND) and theophylline (THEO) on the potency pD_2 of isoproterenol (ISO) in isolated guinea-pig trachea.

Fig. 2. Effect of In-vitro desensitization and desensitization after
pretreatment with hydrocortisone (HC) indomethacin (INDO) and
theophylline (THEO) on the potency pD_2 of salbutamol (SAL) in
isolated guinea-pig trachea.

Fig.3 Effect of in-vitro desensitization and desensitization after pretreatment with hydrocortisone (HC) indomethacin (INDO) and theophylline (THEO) on the potency pD_2 of terbutaline (TER) in isolated guinea-pig trachea
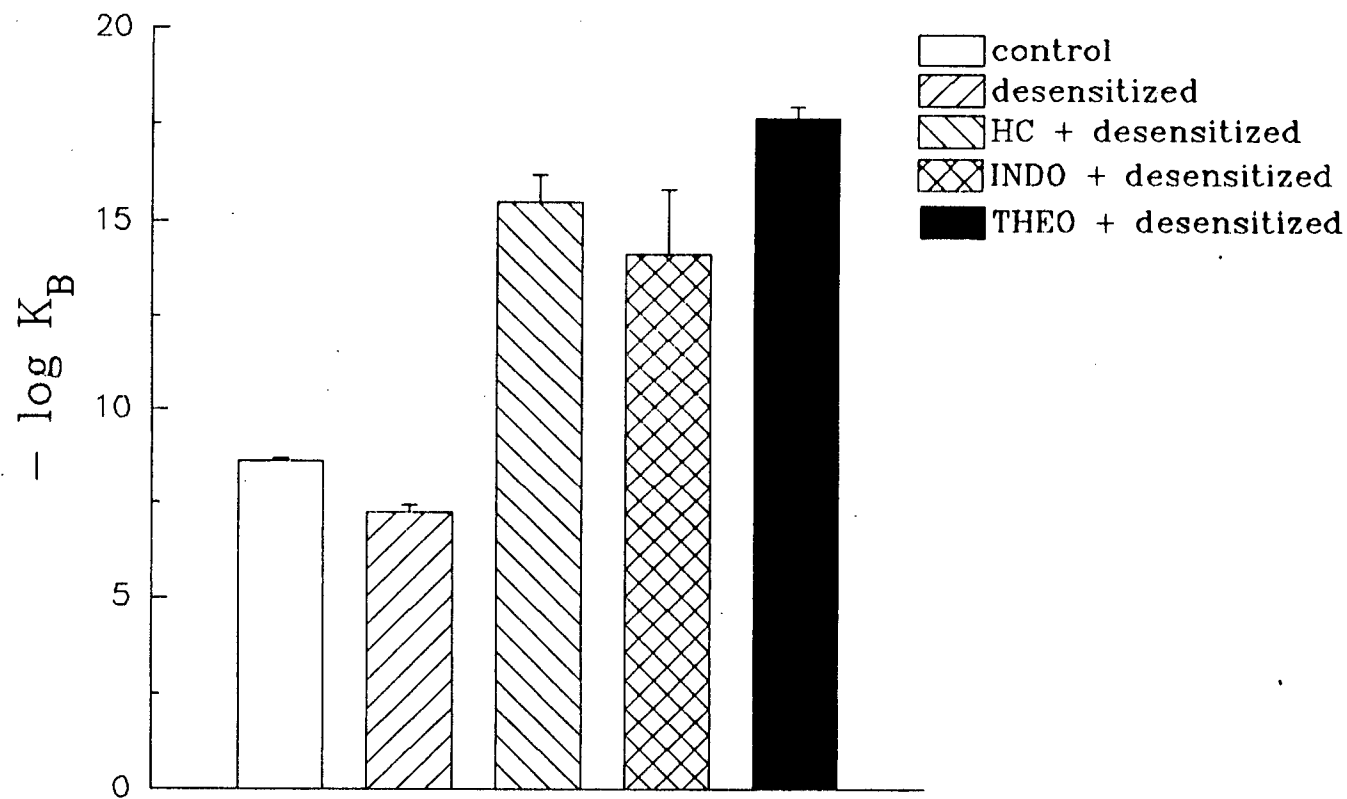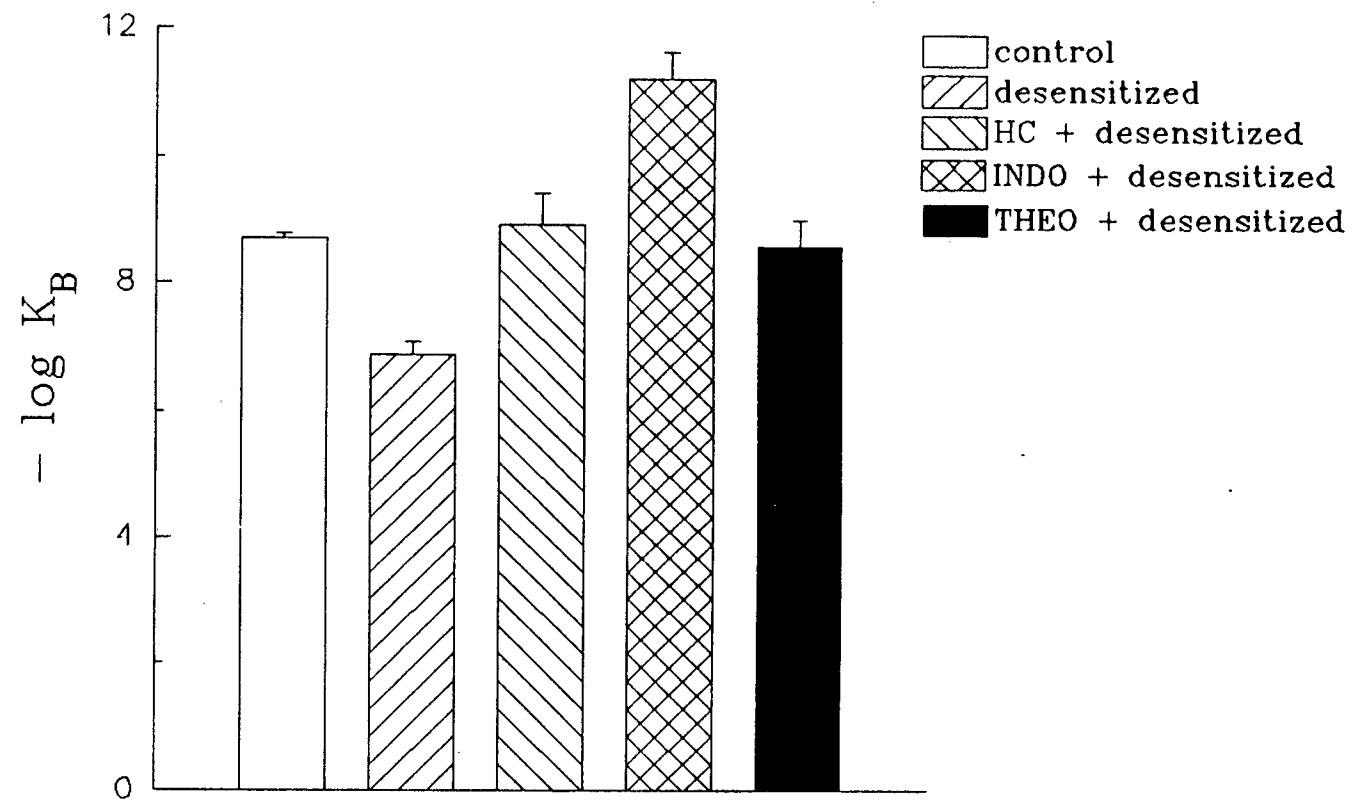
Fig.4 Effect of in-vitro desensitization after pretreatment with hydrocortisone (HC) indomethacin (INDO) and theophylline (THEO) on the apparent dissociation constant (K_B) for propranalol - beta receptor complex with isoprenaline (ISO) in isolated guinea-pig trachea
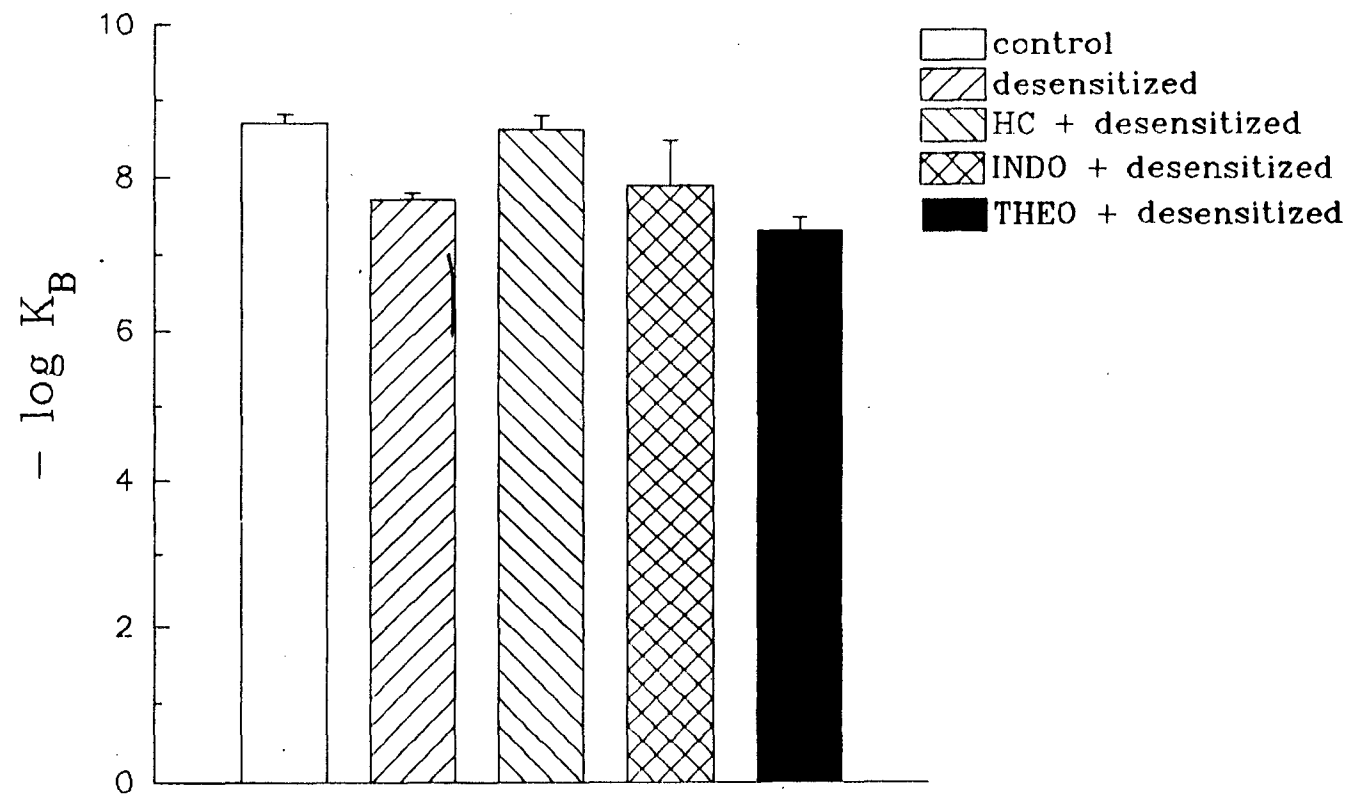
Fig.5 Effect of in-vitro desensitization and desensitization after pretreatment with hydrocortisone (HC) indomethacin (INDO) and theophylline (THEO) on the apparent dissociation constant (K_B) for propranalol - beta receptor complex with salbutamol (SAL) in isolated guinea-pig trachea

Fig.6 Effect of in-vitro desensitization and desensitization after pretreatment with hydrocortisone (HC) indomethacin (INDO) and theophylline (THEO) on the apparent dissociation constant (K_B) for propranalol - beta receptor complex with terbutalin (TER) in isolated guinea-pig trachea
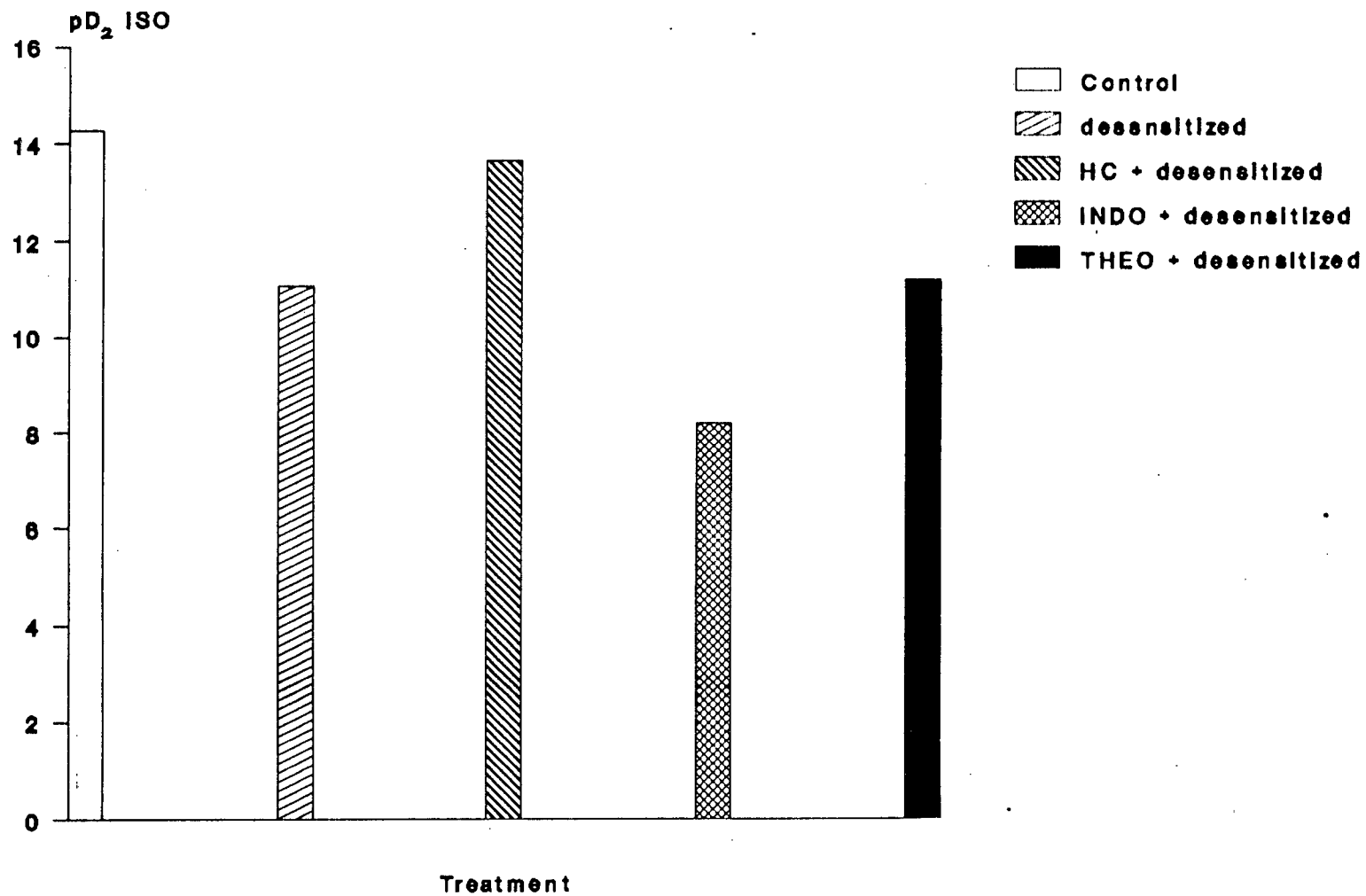
Fig.7 Effect of in-vivo desensitization and desensitization after pretreatment with hydrocortisone(HC) indomethacin (INDO) and theophylline (THEO) on the potency pD_2 of isoproterenol (ISO) in isolated guinea-pig trachea
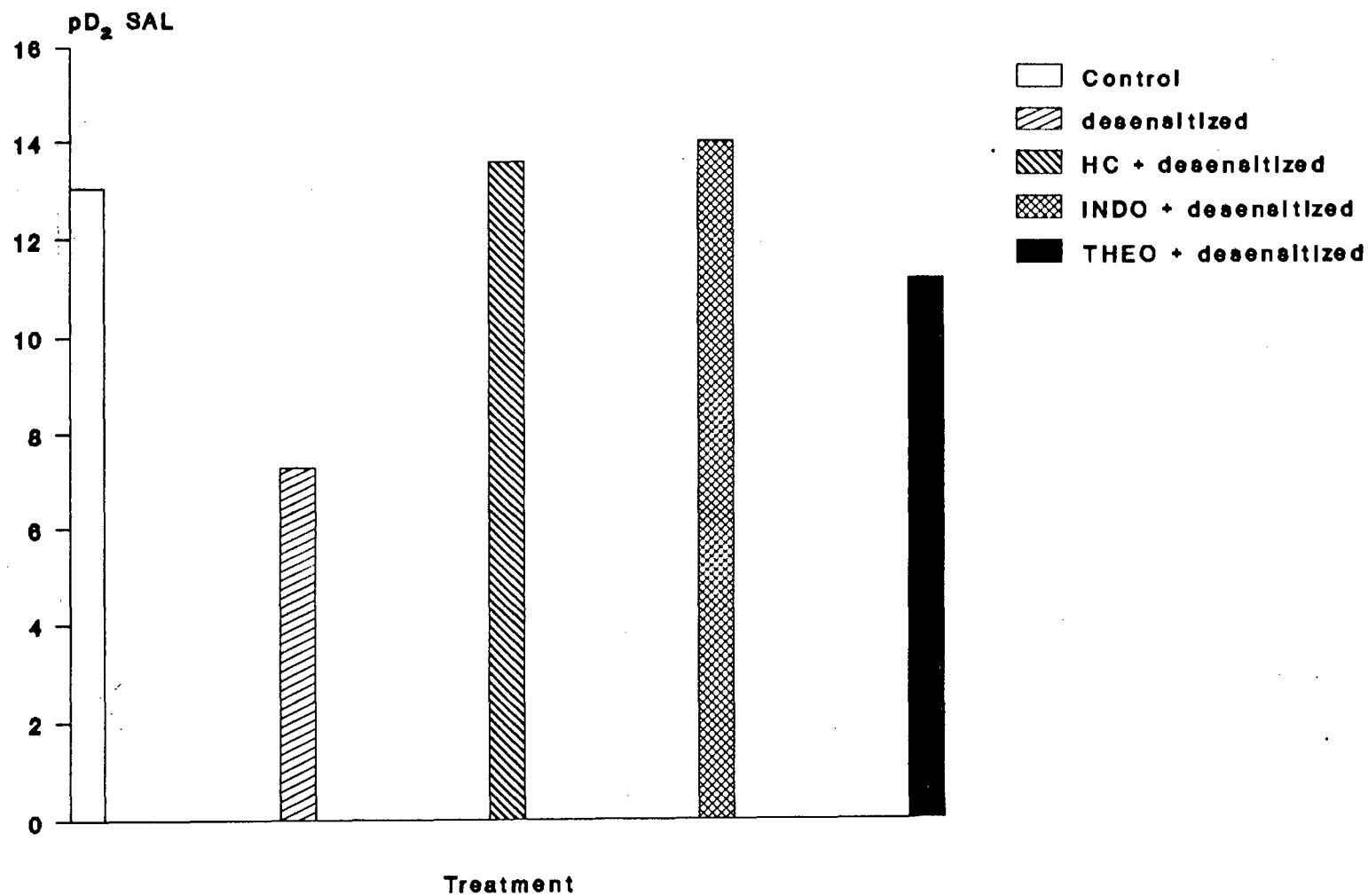
Fig.8 Effect of in-vivo desensitization and desensitization after pretreatment with hydrocortisone (HC) indomethacin (INDO) and theophylline (THEO) on the potency pD_2 of salbutamol (SAL) in isolated guinea-pig trachea

Fig.9 Effecto of in-vivo desensitization and desensitization after pretreatment with hydrocortisone (HC) indomethacin (INDO) and theophylline (THEO) on the potency pD_2 of terbutaline (TER) in isolated guinea-pig trachea
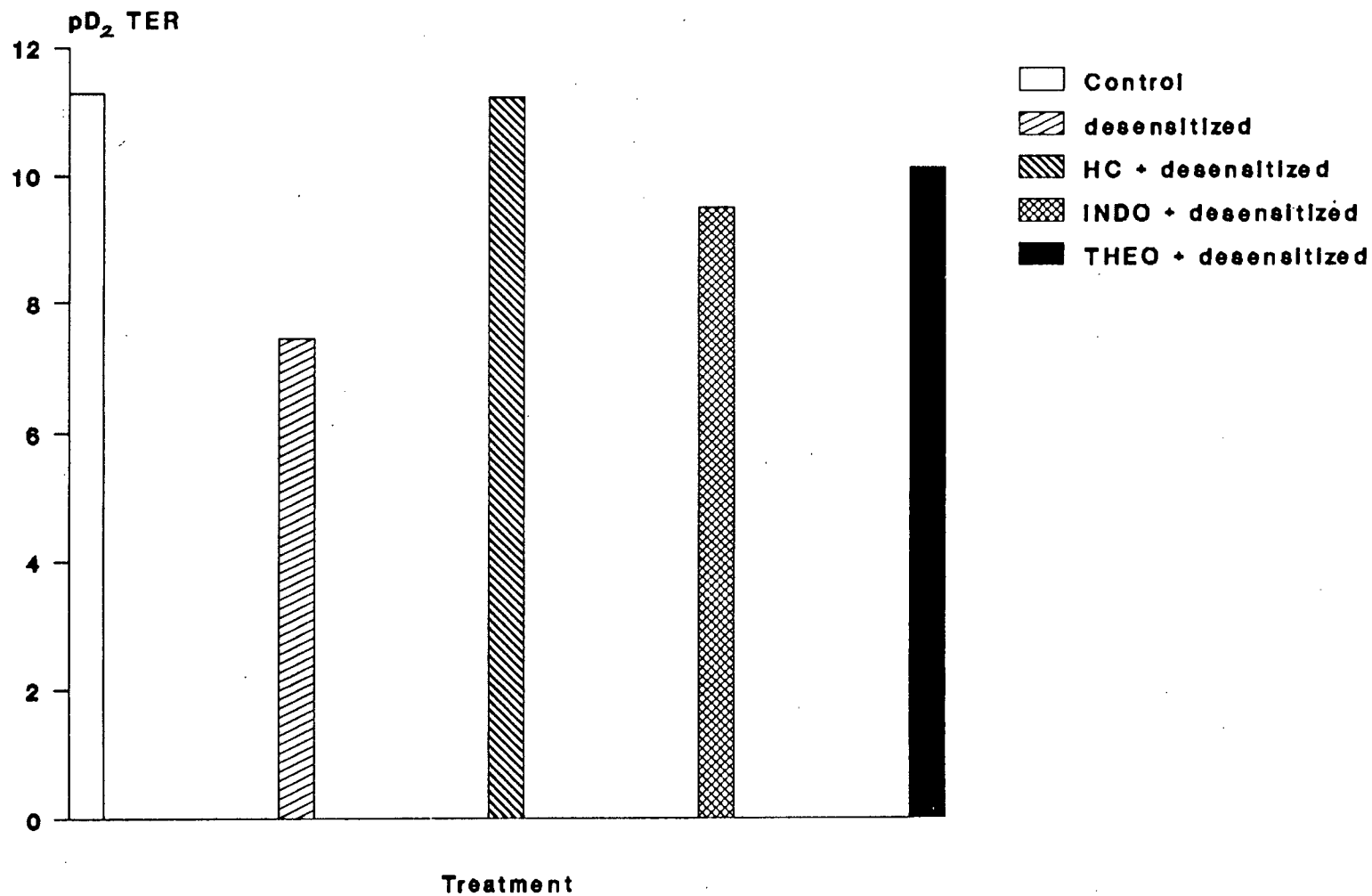
Fig.10 Effect of invivo desensitization and desensitization after pretreatment with hydrocortisone (HC) indomethacin (INDO) and theophylline (THEO) on the apparent dissociation constant (K_B) for propranalol - beta receptor complex with isoprenaline (ISO) in isolated guinea-pig trachea
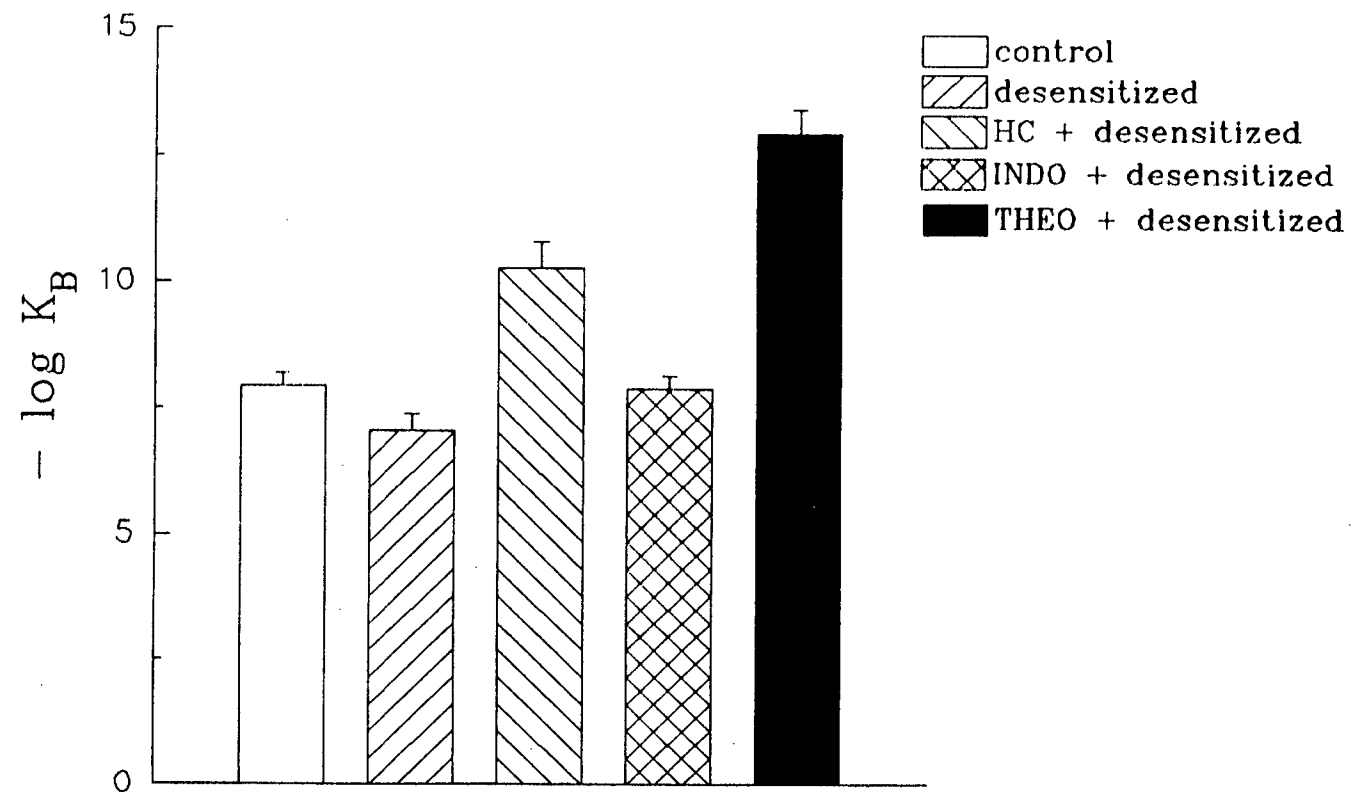
Fig.11 Effect of in-vivo desensitization and desensitization after pretreatment with hydrocortisone (HC) indomethacin (INDO) and theophylline (THEO) on the apparent dissociation constant (K_B) for propranalol - beta receptor complex with salbutamol (SAL) in isolated guinea-pig trachea
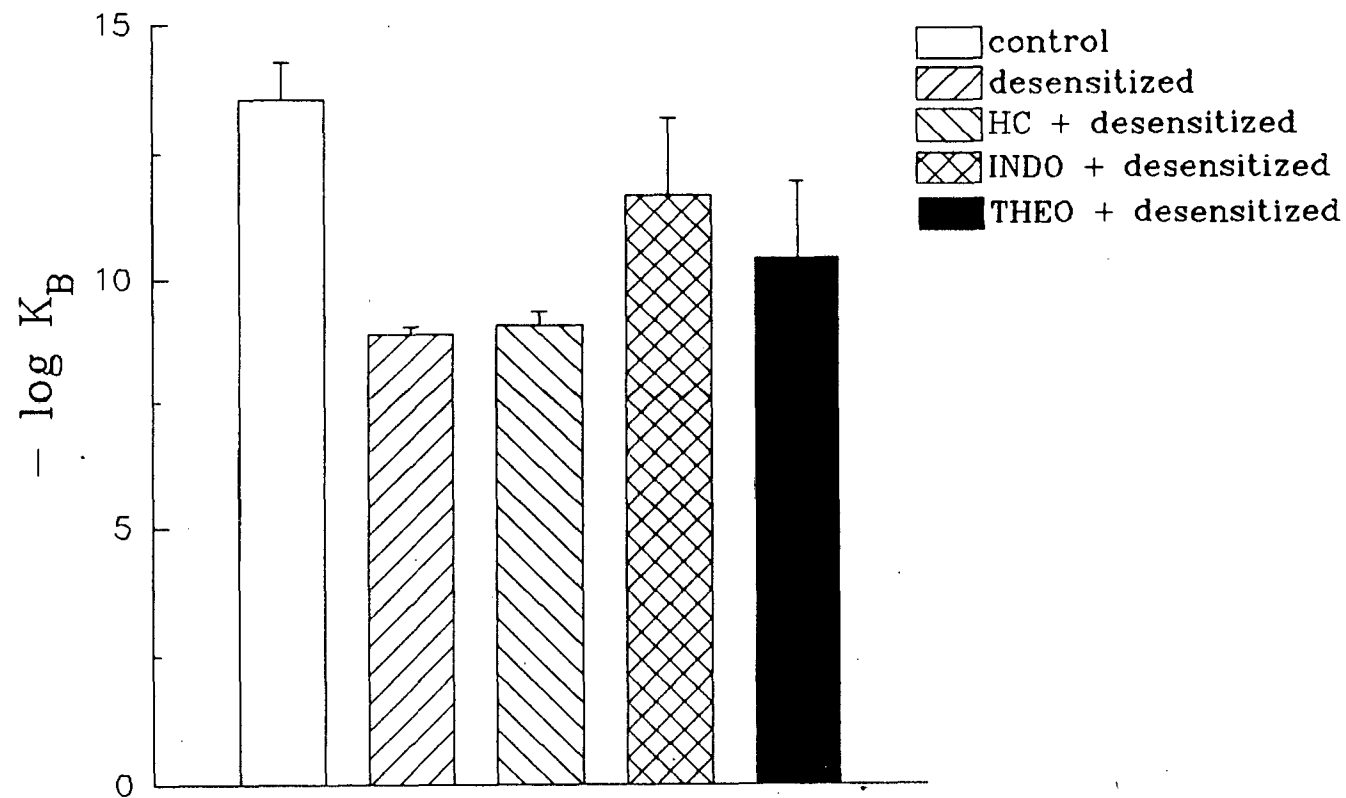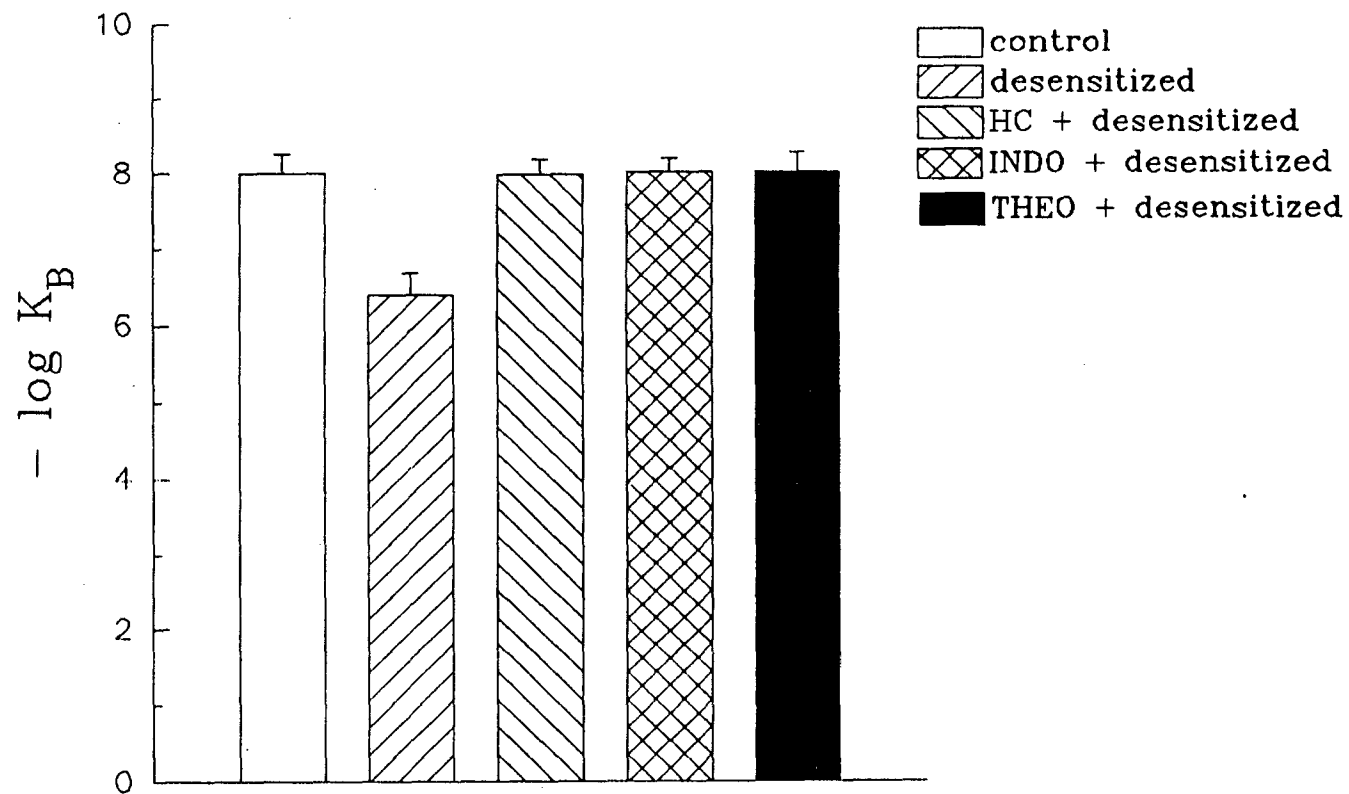
Fig.12 Effect of in-vivo desensitization and desensitization after pre-treatment with hydrocortisone (HC) indomethacin (INDO) and theophylline (THEO) on the apparent dissociation constant (K_B) for propranolol - beta receptor complex with terbutalin (TER) in isolated guinea-pig trachea

DISCUSSION

The results of this study indicates that desensitization of beta receptors occurs to catecholamines as well as non-catecholamine beta adrenoceptor agonists in the guinea-pig tracheal smooth muscle, both in vivo and vitro. This was reflected in two parameters tested (1) the pD_2 value as a measure of the relaxant potency of the beta agonist and (2) K_B value for the propranlol-beta adrenoceptor complex, a measure of the affinity of the beta adrenoceptor for the antagonist and indirectly a measure of beta agonistic affinity. The pD_2 value decreased with desensitization implying a decrease in potency of the three agonists while the K_B value increased suggesting loss of affinity.

Though desensitization to the catecholamine isoproterenol was reflected in both parameters (pD_2 and K_B) in vitro as well as in vivo, the same cannot be said of the two non-catecholamines used in this study salbutamol and terbutaline. In vitro, both of them showed a significant decrease in affinity in the desensitized tissue but the decrease in potency was not statistically significant. It has been postulated that the first event that occurs during desensitization is the uncoupling of receptors due to phosphorylation (Sibley et al.,1987),the subsequent steps being receptor sequestration (Chuang et al.,1980) and down-regulation (Lefkowitz & Williams,1977; Nishikawa et al.,1993). If this is so, it can be expected that the potency of a drug (a measure of uncoupling with or without receptor modulation) should be affected before its affinity (a measure of receptor related events). In vivo there was a decrease in potency as well as affinity to sabutamol and terbutaline probably because of the prolonged exposure to the drugs (7 hours) during which both uncoupling as well as receptor modulation can occur. In vitro, howevr, where the exposure to the drugs was shortlived (30 minutes) both salbutamol and terbutaline showed changes in affinity but not in potency. Our findings with the catecholamine isoproterenol leads

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us to suggest that the sequence of events in the process of desensitization may be different for non-catecholamines.

Another point which emerged from this study is that the ability of beta agonists to induce desensitization differs with in vivo and in vitro adminstration. With in vivo adminstration desenstization was markedly more in case of the non-catecholamine salbutamol as compared to the catecholamine isoproterenol. This may be because, unlike isoproterenol, salbutamol is not subject to metabolic degradation by COMT. Thus it acts on the beta receptors for a longer period, leading to greater desensitization. Further, the weak beta blocking effect of the metabolite of isoproterenol, 3 methoxy isoproterenol, may decrease the beta agonistic effect of isoproterenol when it is adminstered in vivo (Conolly et al., 1971; Morgan et al., 1969 and Buckner & Saini, 1975). On the other hand with in vitro adminstration, desensitization was more pronounced with the catecholamine isoproterenol as compared to the non-catecholamine, salbutamol and terbutaline. It should be noted that, by design, in the in vitro studies, the formation of 3 methoxy metabolite was eliminated using COMT inhibitor, pyrogallol. Thus, in the absence of the operation of these vitiating factors, desensitization may be more pronounced with isoproterenol.

Barnes(1992) while reviewing molecular pharmacology of beta receptors, pointed out that beta receptors are linked to G proteins. Further, beta-1 receptor is neuronal, beta-2 is horomonol, beta-3 metabolic and beta-4 is probably found in brown fat. It has been shown that both beta-1 as well as beta-2 receptors mediate the tracheal smooth muscle relaxation in the guinea-pig (Omini et al., 1979), unlike in the human trachea which has a homogeneous population of beta-2 receptors

(Carswell & Nahroski, 1983; Zaagsma et al., 1983). In the human lungs beta receptors are widely distributed, with fewer beta - 2 receptors in the airway smooth muscle and more at or beyond the terminal bronchiole. According to Barnes (1992) reduced effect seen with regular beta-2 agonist administration could be due to receptor modulation and uncoupling as well as the presence of inflammatory mediators. In the light of the above, once again, it is quite possible that the loss of potency seen in this study with desensitization, could be due to uncoupling whereas the loss of affinity may be due to receptor modulation.

Another important observation emerging from this study, is that, whether desensitization is produced in vitro or in vivo, of the 3 beta agonists tested, terbutaline produced the least - degree of desensitization as measured by both pD_2 as well as K_B values. On the other hand, salbutamol in general, produced the highest degree of desensitization. This has possible clinical relevance in guiding the selection of the beta agonist to be used in patients of bronchial asthma. It is difficult to explain the difference in the effect of salbutamol and terbutaline both of which are non-catecholamine beta-2 agonists. However, unlike salbutamol, terbutaline has a resorcinol ring in its structure which may be responsible for a different site of action on the beta adrenoceptor (Mena et al., 1978).

Pretreatment with hydrocortisone prevented the loss of potency as well as the loss of affinity to isoproterenol in desensitized tracheas, both in vitro and in vivo. It had, in general, a similar effect in case of salbutamol and terbutaline with the following two exceptions i.e., hydrocortisone failed to prevent the loss of affinity to salbutamol in vivo and the loss of potency to terbutaline in vitro. The protective effect of hydrocortisone has been attributed to several mechanisms. Among them,

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increase in both affinity and coupling to adenyl cyclase (Davies & Lefkowitz, 1981) increase in beta receptor number (Stephan et al., 1980, Sano et al., 1980) or a dampening of the ability of agoinsts to uncouple receptors without affecting receptor number (Samuelson & Davies, 1984). Our experimental observations are generally in conformity with the clinical observation of Ellul-Micallef & Fenech (1975) who showed that glucocorticoids can restore responsiveness to beta agonists in unresponsive asthmatic patients. However, the differential effects of the three beta agonists tested that is effect on both potency and affinity with isoproterenol, lack of effect on affinity in vivo with salbutamol and failure to affect potency in case of terbutaline in vitro, all point to the possibility that the process of desensitization is very complex and involves several mechanisms which hydrocortisone may affect in different ways.

Our results with both indomethacin and thophylline also confirm the above postulate. In vitro, indomethacin prevented the loss of potency as well as the loss of affinity to both isoproterenol and salbutamol in desensitized tissues. However, it did not affect desensitization to terbutaline. In vivo, the reslts were quite different. Indomethacin had no effect on desensitization to isoproterenol. In case of salbutamol it prevented the loss of potency, but did not prevent the loss of affinity while in case of terbutaline, it prevented both the loss of potency as well as loss of affinity.

Omini et al (1981) suggested that desensitization to beta agonists is a prostaglandin mediated phenomenon as they were able to prevent desensitization in guinea-pig tracheal tissue by pre-treatment with indomethacin. However, Fernandes et al (1988) & Matran et al (1989) reported negative results with indomethacin on

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isoproterenol induced desensitization. Brink (1981) demonstrated in his in vivo studies that indomethacin does not prevent beta adrenergic desensitization when this phenomenon is properly established. Omini et al (1985) were also unable to prevent desensitization in vitro with indomethacin when "stonger" desensitization procedure was used, involving higher concentration of isoproterenol for a longer period of time. They suggested that these results support the hypothesis that arachidonic acid metabolites might regulate the coupling between the receptor and the enzyme. Uncoupling of receptors from the adenylate cyclase moiety is an early event in receptor desensitization. If exposure to the agonist is continued, it results in the disappearance of receptors from the membrane surface (Chuang et al., 1980; Su et al., 1979). This, to a certain extent, may explain why in our experiments with isoproterenol and salbutamol. indomethacin had some protective effect in vitro (a shorter desensitization procedure were only uncoupling is involved) while it had no protective effect in vivo (a longer procedure involving down regulation of receptors). This explanation. however, does not apply to our results with terbutaline where indomethacin exerted a protective effect in vivo but failed to do so in vitro. This could possibly be because of a difference in the chemical structure of terbutaline as mentioned earlier.

Pretreatment with theophylline also yielded different results with different drugs, in vitro and in vivo. For example, though it had a protective effect in general against isoproterenol desensitization, it did not affect its potency in vivo. In case of salbutamol, theophylline prevented the loss of affinity in vitro and the loss of potency in vivo. In case of terbutaline, theophylline protected against desensitizatrion in vivo but not in vitro. This could be because of the high doses of theophylline used in vivo (45 mg.kg^{-1}.). This dose was used in view of the reports that when theophylline is

co-administered with terbutaline, only low concentrations of theophylline reach target organs as shown by HPLC (Madsen & Ribel, 1981).

Theophylline is an important bronchodilator drug used alone or in combination with beta agonists. Theophylline and beta agonists have been shown to be synergistic in relaxing isolated guinea pig trachea (Lefcoe et al., 1975) and human bronchial smooth muscle (Svedmyr, 1977). Both beta agonists and theophylline increase the intracellulor level of cyclic adenoisine 3',5' - monophosphate (cyclin AMP) through stimulation of adenylcyclase and inhibition of phosphodiesterase respectively. Taylor (1987) showed that in desensitized tissue pretreated with amino-phylline, the isoproterenol response was potentiated. He explained this observation by stating that phosphodiesterase exists in two forms: a high affinity form and low affinity form. When desensitization occurs, the high affinity form undergoes degradation as a result of exposure to lhigh concentration of cyclic AMP while the low affinity form persists for a longer time. Aminophylline affects this low affinity form of phosphodiesterase thereby potentiating the action of isoproterenol in the desensitized tissue. A similar mechanism may be involved in our studies in the protective effect of theophylline on isoproterenol desensitization.

In conclusion, the results of our study suggest that though desensitization can be produced to all the three beta agonists used, the sequence of events during desensitization with non-catecholamines may differ from that occuring with catecholamines. Further. the degree of desensitization differed with the beta agonist used, terbutaline producing the least desensitization. Hydrocortisone indomethacin and theophylline had different effects on desensitization produced by the three beta agonists leading us to suggest that the process of desensitization may differ with the beta agonist used.

SUMMARY & CONCLUSION

The results of the present study can be summarised as foiiows:

1. Desensitization to both catecholamine as well as non-catecholamine beta adrenoceptor agonists could be produced in the guinea-pig tracheal smooth muscle in vitro as well as in vivo.

2. In vitro, desensitization was maximum with catecholamine isoproterenol while in vivo, it was most with the non-catecholamine, salbutamol. Desensitization produced by terbutaline was the least, both in vitro and in vivo. This may have a clinical bearing in selection of drugs in the treatment of patients of bronchial asthma. .

3. The sequence of events during desensitization to the non-catecholamines salbutamol and terbutaline may differ from that occuring with the catecholamine isoproterenol.

4. Of the three drugs employed for pretreatment, hydrocortisone seemed to be generally, the most effective in preventing the development of desensitization as compared to indomethacin and theophylline.

5. Each of the three drugs used for pretreatment, that is hydrocortisone, indomethacin and theophylline had different effects on the desensitization produced by the three beta agonists studied.

It is suggested, that the process of desensitization may differ with different beta agonists used. This may be due to the difference in their chemical structure and site of action in the cascade of signal transduction at the beta receptor.

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