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 propranolol-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 salbutamol 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, however, 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
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 horomonal, 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
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 $\text{pD}_2$ 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 aswell as the loss of affinity to isoproterenol in desensitized tracheae. 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,
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 agonists 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 theophylline 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 results 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
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 "strong" 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 desensitization 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 intracellular level of cyclic adenosine 3',5' - monophosphate (cyclic 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 high 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 occurring 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.