Effects of Chronic Terbutaline and Abana Pretreatments on Beta-Adrenoceptor Mediated Relaxant Responses of Guinea-pig Isolated Trachea

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SUMMARY

Effects of chronic pretreatment with Abana (a herbomineral formulation) on beta-adrenoceptor mediated guinea-pig isolated tracheal relaxation was studied. Chronic terbutaline (TER) pretreatment in the guinea pig significantly reduced the contractile response of the isolated tracheal chain preparation to pilocarpine (PILO; 3.68 x 10^6 M) while the response to KCl (1.78 x 10^2 M) was not modified. The maximal relaxant responses to isoprenaline (ISO), TER and aminophylline (AMINO) were significantly depressed in PILO-contracted isolated tracheal chain preparations but were not modified in KCl-contracted preparations. Chronic Abana pretreatment significantly depressed the contractile response to PILO and significantly enhanced that to KCl. The maximum relaxation to the three relaxant drugs were not modified in PILO-contracted preparations while in KCl contracted preparations an increased sensitivity was observed. Chronic pretreatment with Abana plus TER caused marked depression of the contractile responses of the preparation to PILO; while KCl induced contractions were not modified. The maximal relaxations to the three relaxant drugs were markedly depressed in PILO-contracted preparations which were not significantly different from those in the chronic TER-pretreated groups. In KCl-contracted preparations maximal relaxations by ISO, TER and AMINO were not modified. It is concluded that chronic Abana pretreatment in the guinea-pig does not affect either beta-adrenoceptor mediated tracheal relaxation or the decreased responsiveness of the tracheal smooth muscle induced by chronic TER pretreatment.

Key words: Abana; beta-adrenoceptor; tracheal relaxation

The adrenoceptors of the human respiratory tract are predominantly of the β subtype and following chronic administration of terbutaline (TER), a selective adrenoceptor agonist, desensitization/down regulation of these receptors occurs resulting in decreased beta-adrenoceptor mediated relaxation of the respiratory tract smooth muscle.

Abana is a proprietary herbomineral formulation reputed to have beneficial effects chiefly in cardiovascular diseases. Following chronic pretreatment of rabbits with Abana beta-adrenoceptor mediated responses of the isolated atria and intestine to isoprenaline (ISO) are reduced. It is likely that chronic Abana pretreatment could also effect β2 adrenoceptor-mediated relaxation of the respiratory tract smooth muscle.
The present study therefore attempts to investigate the comparative effects of chronic pre-treatment of the guinea-pig with Abana or TER on beta-adrenoceptor mediated relaxation of isolated trachea contracted with two different spasmogens, viz. pilocarpine (PILO) and KCl.

**MATERIALS AND METHODS**

Healthy, adult guinea pigs of either sex or weighing 400-600 g were divided into four groups. The first group (control, n=5) received subcutaneous injections of normal saline (vehicle) 0.1-ml/kg body weight every 12 h for 14 days. The second group (n=5) received subcutaneous injections of TER 10 µg/kg every 12 h for 14 days. The third group (n=5) was fed Abana powder (3 g/kg/d) for 21 days. The daily oral dose of Abana was given at 9.30 A.M. regularly after mixing with a small amount (approximately 10 g) of the standard food (grated Bengal gram and banana pulp mix). The rest of the food was given daily only after ensuring total consumption of the Abana mixed food. The fourth group (n=4) received the same dose of Abana in an identical manner for 21 days, but in addition, also received 12 hourly subcutaneous injections of TER (10 µg/kg) for the last 14 days.

At the end of the treatment period each guinea pig was sacrificed and a part of the trachea between the larynx and carina was promptly removed and placed in a Petri dish containing carbogenated, warm Krebs-Henseleit physiological salt solution of the following composition: (mM): NaCl-117.6; KCl-5.2; CaCl$_2$-1.9; MgSO$_4$, 7H$_2$O-0.56; NaH$_2$PO$_4$-0.8; NaHCO$_3$-25.0 and glucose 11.1. After thorough cleansing a tracheal chain was prepared from it by the method of Akcasu$^6$.

The preparation was suspended in a 30 ml organ bath containing Krebs-Henseleit solution (pH 7.4) at 37.0 ± 0.5°C temperature and continuously bubbled with carbogen. The preparation was stabilized over 90 min with regular washes every 15 min. Isotonic frontal writing lever with 10-fold magnification and 500 mg tension was used to record the drug responses.

Steady spasm of the preparation was induced by 15 min contact with a single, submaximal concentration of either PILO (3.68 x 10$^{-6}$M) or KCl (1.78 x 10$^{-2}$M) added to the bath each time before eliciting relaxant responses to the cumulative concentrations of ISO (sulphate salt), TER (sulphate salt) and aminophylline (AMINO). For each spasmogen separate groups of treated animals were used. Graded concentrations of ISO and TER were added every 2 minutes and those of AMINO were added every 5 min to the bath until maximum relaxation of the preparation was produced by each drug. Following this the basal tone of the preparation was recovered over 60-90 min with regular washes every 10 minutes.

For each spasmogen the mean maximum height of spasm in the corresponding control group was taken as 100 per cent for comparison with those in the other groups. For constructing the dose response curves of ISO, TER and AMINO relaxation equal to the corresponding control mean maximum height of spasm was considered as 100 per cent response. The level of statistical significance of difference was obtained by Student’s unpaired ‘t’ test. P value less than 0.05 was considered statistically significant.
RESULTS

Contractile responses: PILO ($3.68 \times 10^{-7} - 1.11 \times 10^{-4}$M) and KCl ($4.42 \times 10^{-3} - 1.43 \times 10^{-1}$M) produced concentration related steady contractions of the preparations. The submaximal contractions produced by PILO ($3.68 \times 10^{6}$M) were very significantly reduced in chronic TER-pretreated, chronic Abana-pretreated and in chronic Abana plus TER-pretreated groups (Figure 1a).

The submaximal contractions produced by KCl ($1.78 \times 10^{2}$M) were not significantly modified by either chronic TER or Abana plus TER-pretreatments. However, in the chronic Abana pretreated group the contractions were significantly increased (Figure 1B).

Relaxant responses: ISO ($3.23 \times 10^{-9} - 6.62 \times 10^{8}$M), TER ($1.46 \times 10^{-1} - 1.49 \times 10^{5}$M) and AMINO ($1.75 \times 10^{-5} - 1.12 \times 10^{-3}$M) produced concentration-dependent relaxation of the preparations contracted by either PILO or KCl in all the four groups;

(i) Responses to ISO: In PILO-contracted preparations ISO-induced maximal relaxation was significantly depressed in the chronic TER-pretreated group. Chronic Abana pretreatment did not significantly change the concentration-response curve of ISO. In chronic Abana plus TER-pretreated group, the responses to lower concentrations of ISO were significantly
(p<0.05) increased with marked depression of the maximal relaxation (Figure 2a).

In KCl-contracted preparations chronic TER pretreatment neither produced any shift of the concentration response curve, nor significantly affected the maximal response to ISO. Abana pretreatment significantly (p<0.01) shifted the curve to the left, but the maximal relaxation was not significantly increased from control. Chronic Abana plus TER pretreatment also significantly (p<0.01) shifted the curve to the left at lower concentrations of ISO; however, the maximal relaxation produced was not significantly different from that in the control group (Figure 2b).

(ii) Responses to TER: PILO-contracted preparations from chronic TER- or Abana plus TER pretreated groups showed significant depression of maximal relaxant responses to TER. However, chronic Abana pretreatment showed no effect on relaxant responses to TER (Figure 3a).

In the chronic Abana pretreated group the concentration response curve of TER was significantly (p<0.01) shifted to the left in KCl contracted preparations, but the maximal relaxation was not significantly altered. In chronic TER or Abana plus TER pretreated groups there was no significant alteration of either sensitivity or maximal relaxation (Figure 3b).
(iii) Responses to AMINO: The maximal relaxation and responses to AMINO in PILO-contracted preparations were significantly depressed in the chronic TER- or Abana plus TER pretreated groups, but not in the preparations from chronic Abana-pretreated group (Figure 4a).

(iv) KCl-contracted preparations chronic Abana-pretreatment caused significant ($p<0.01$) leftward shift of the concentration response curve of AMINO with significant increase in the maximal relaxation. Chronic TER or Abana plus TER-pretreatment did not significantly affect the maximal relaxation (Figure 4b).

**DISCUSSION**

The contractile effects of PILO and KCl and the relaxant effects of ISO, TER and AMINO on the tracheal chain were differently modified by the chronic pretreatment of guinea pigs with TER, Abana or Abana plus TER.

PILO-induced contraction of the preparation is mediated by stimulation of the muscarinic cholinooceptors of the tracheal smooth muscle\(^7\) and unlike KCl this does not involve influx of the extracellular calcium ion\(^8\). The markedly reduced PILO-induced contractions observed in the chronic TER, Abana or Abana plus TER-pretreated groups indicated decreased responsiveness of the muscarinic cholinooceptors of this tissue. On the contrary, significantly increased KCl-induced contractions observed in the chronic Abana pretreated group could be due to Abana-induced enhancement of smooth muscle cell membrane permeability to Ca\(^{2+}\) during K\(^+\)-induced membrane depolarization. Similar observations were made by Pasnani *et al.*\(^5\) for rabbit tissues.

In the guinea-pig chronic TER-pretreatment is known to produce decreased beta-adrenoceptor mediated relaxation of the tracheal smooth muscle due to impaired generation
of cyclic AMP (CAMP) by the beta-adrenoceptor agonists like ISO\(^9\). The same mechanism could be responsible for the significant depression of the maximal relaxations by ISO and TER in PILO-contracted preparations obtained from the chronic TER and Abana plus TER-pretreated groups. The maximal relaxations in these two groups were not significantly different from each other. The relaxation induced by ISO and TER was not significantly reduced in the chronic Abana-pretreated group, implying that chronic Abana-pre-treatment neither significantly reduces the beta-adrenoceptor mediated relaxation, nor does it affect the decreased beta-adrenoceptor mediated relaxation of the trachea induced by chronic TER-pretreatment.

*In vitro* preparations of airway smooth muscle are relaxed by relatively high concentrations of theophylline or its salts (e.g. AMINO) which act by inhibiting the cyclic nucleotide phosphodiesterase enzyme, thereby allowing accumulation of CAMP and consequent smooth muscle relaxation\(^10\). AMINO-induced maximal relaxations were similar in magnitude to those produced by ISO and TER in the PILO-contracted preparations obtained from chronic TER- and Abana plus TER-pretreated groups. This suggested that prevention of hydrolysis of CAMP by AMINO was not responsible for maximum achievable relaxation because generation of CAMP is impaired by chronic pre-treatment with TER or other beta-adrenoceptor agonists. These observations are consistent with those made by Benoy *et al.*\(^11\) on guinea-pig isolated trachea.

Although Pasnani *et al.*\(^5\) had reported decreased beta adrenoceptor mediated responses of isolated atria and intestine following chronic Abana-pretreatment of the rabbit, such an effect has not been observed in the present study with guinea-pig trachea. However, agents like thyroid hormones have been shown to induce differential regulation of beta-adrenoceptors in different tissues from different species\(^12,13\). It is possible that a similar differential modification of beta-adrenoceptor function by Abana in different tissues and species may be responsible for this variation.

One of the major mechanisms for relaxation of airway smooth muscle by ISO TER and AMINO involves CAMP-dependent protein kinase-induced enhancement in the membrane Na\(^+\)-K\(^+\)-ATPase activity which increases K\(^+\) influx\(^14\) and associated Ca\(^{2+}\) efflux resulting in hyperpolarization of smooth muscle cell membrane\(^15\). Chronic Abana-pretreatment is likely to induce increased membrane permeability to Ca\(^{2+}\) and thereby considerably elevate the intracellular level of Ca\(^{2+}\) during KCl-induced contraction\(^5\). During relaxation of such contracted preparations by ISO, TER and AMINO, more intracellular Ca\(^{2+}\) can be extruded and accompanying K\(^+\) influx can also be augmented if the K\(^+\) concentration of extracellular fluid is elevated. This could explain the increased sensitivity to the three relaxant drugs observed only in the KCl-contracted preparations and not in the PILO-contracted preparations obtained from the corresponding chronic Abana pretreated group. Chronic TER- or Abana plus TER-pretreatment do not appear to affect Ca\(^{2+}\) permeability and therefore possibly do not alter the sensitivity of the KCl-contracted preparations to the three relaxant drugs.
It is concluded that in the guinea-pig chronic TER-pretreatment induces decreased relaxations with ISO, TER and AMINO in PILO-contracted tracheal preparations. Chronic Abana-pretreatment does not significantly affect this probably due to the lack of its effects on the beta-adrenoceptor functions of the guinea-pig airway. Chronic Abana-pretreatment probably also induces enhanced membrane permeability to Ca^{2+} which increases KCl-induced contractions and increased sensitivity to the relaxant effects of ISO, TER and AMINO following such contractions.

REFERENCES


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