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TOLERANCE OF GUINEA-PIG AIRWAY MUSCLE PREPARATIONS TO RELAXANT AGONISTS INDUCED BY CHRONIC EXPOSURE TO ISOPRENALINE *in vivo*

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1 The histamine sensitivities of complete tracheal spiral preparations from guinea-pigs were similar to those of paired half tracheal tissues.

2 Airway muscle preparations from animals chronically treated with isoprenaline showed a significant increase in resting tone and a significant decreased responsiveness to histamine.

3 The paired half tracheal preparations exhibited no significant difference when either their isoprenaline or theophylline sensitivities were compared.

4 Paired half tracheal muscle preparations from chronically treated animals (0.4 μmol, 4.0 μmol, and 40.0 μmol isoprenaline, 3 times daily for 21 days, s.c.) showed a significantly reduced sensitivity to isoprenaline when compared to appropriate controls.

5 Theophylline concentration-effect curves for the paired half tracheal preparations from chronically treated guinea-pigs (4.0 μmol and 40.0 μmol isoprenaline) were significantly reduced compared with appropriate controls.

6 Indomethacin treatment reversed the isoprenaline desensitization induced by chronic treatment (0.4 μmol isoprenaline) but was ineffective in animals that received the higher doses of isoprenaline *in vivo*.

Introduction

A variety of tissues when exposed to high concentrations of agonists *in vitro* become refractory (Fleisch & Titus, 1972; Watanabe, Olivo & Kasuya, 1976; Spilker & Tyll, 1976; Lin, Hurwitz, Jenne & Avner, 1977; Douglas, Lewis, Ridgway, Brink & Bouhuys, 1977). The induction of tolerance to sympathomimetic agents in respiratory tissues has been considered to be responsible for the increase in asthma deaths in the early 1960's (Conolly, Davies, Dollery & George, 1971). Attempts to elucidate the mechanism of action of desensitization in animal tissues have involved both *in vivo* and *in vitro* models. Recently it has been shown that tolerance can be induced by chronic exposure *in vivo* to sympathomimetic agents (Benoy, El-Fellah, Schneider & Wade, 1975; Anderson & Lees, 1976; Avner & Noland, 1978). Previous work dealing with the process of desensitization used whole lung preparations rather than isolated airway muscle (Benoy, *et al.*, 1975), while others have used tracheal tissue from the rat, a species known to be refractory to isoprenaline (Avner & Noland, 1978). In addition, Anderson & Lees (1976) observed β-adrenoceptor desensitization in uncontracted tracheal muscle preparations where the effects of isoprenaline were monitored on the basal tone of the preparations.

Since there was little information on the effects of chronic treatment on guinea-pig airway muscle *in vivo*, the present paper describes the effects of pretreatment of guinea-pigs with different doses of isoprenaline for a fixed duration. Tracheal muscle preparations from these animals were then examined for their sensitivity to histamine, isoprenaline and theophylline.

Methods

Female guinea-pigs (175 to 350 g) were used. The animals were from an outbred population, that is, the results of random crossing between English short hair and Hartley strains.

In vivo *protocols*

Animals were randomly divided into groups and subjected to one of the following treatments. One group of control animals ($n = 6$) did not receive any injections. Other groups were injected either with Tyrode solution ($n = 4$), Tyrode solution containing ascorbic acid (0.1 mM; $n = 36$) or isoprenaline prepared in Tyrode solution with ascorbic acid (0.4 μmol, $n = 29$;

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4.0 μmol, $n = 14$; 40.0 μmol, $n = 20$; three times a day for 21 days, subcutaneously). The isoprenaline was prepared immediately before administration and the desired dose was given in a volume per unit body weight. The ascorbic acid was used to delay catecholamine auto-oxidation. In another series of experiments animals were injected with indomethacin (30 mg/kg i.p.; $n = 10$) subsequent to chronic treatment (see above). The indomethacin was administered in two doses; one injection 5 h after the last injection of chronic treatment and the second 12 h later, that is, 1 h before the animal was exsanguinated. Untreated guinea-pigs ($n = 4$) and vehicle-injected animals ($n = 4$) were similarly treated with indomethacin and served as controls.

In vitro *protocols*

Tracheas were cut as spirals and either set up as a complete tracheal preparation or divided into two equal parts, caudal portion (CP) and distal portion (DP). Complete spiral preparations were placed under a 4 g initial load while half spiral tissues were equilibrated under a 3 g load. In either case the preparations were equilibrated for 90 min at 37°C (gassed with 5% CO_2 in O_2). Isometric measurements were made with an Apelab strain gauge (model 05.7004) and an Apelab I recorder. The composition of the Tyrode solution was (mM): NaCl 139.2, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.5 and ascorbic acid 0.1, pH 7.3.

Contractile agents

Histamine concentration-effect curves were produced in complete tracheal spirals or paired portions of the same trachea both before and after a 30 min incubation with indomethacin (1.7 μM). Similarly, the responses to histamine were produced in complete tracheal preparations taken from animals that had been chronically treated *in vivo* with isoprenaline (40.0 μmol, s.c.; three times daily for 21 days). Subsequent to each experiment the tissues were dried in an oven (65°C) for 12 h and weighed.

Concentration-effect curves were produced by adding graded concentrations of histamine, in a volume less than 0.5 ml, in random order, to the tissue bath. When the response to an agonist dose reached a plateau, the bath fluid was exchanged for fresh Tyrode solution. The preparations returned passively to their resting tone. In order to compare the responses produced among tissues, the force was determined from the recordings and was normalized for the dry weight of the preparations. Since the dry weight is a good estimate of the muscle mass of the tissues the ratio of force/dry weight for guinea-pigs of the same age is constant. Contractions produced by histamine were expressed as a percentage of the maximal force developed per mg tissue dry weight. Mean concentration-effect curves were calculated from responses to fixed concentrations of agonist and pD_2 values ($-\log_{10} EC_{50}$) for histamine were interpolated from each curve.

Relaxant agents

Cumulative concentration-effect curves to relaxant agonists were produced in paired half spiral preparations. Each preparation was tested with various concentrations of histamine in order to select a concentration of this agonist which was maximal but not supramaximal. This concentration of histamine ranged 2.5 fold (2 to 5 μM). When the induced tension reached a plateau, the relaxant agonist (isoprenaline or theophylline) was added to the bath. The relaxant concentration-effect curves were repeated after a 60 min re-equilibration period during which the preparations were washed every 15 min with Tyrode solution. pD_2 values of the relaxant drugs were derived from individual concentration-effect curves. Relaxations produced by the drugs were expressed as a percentage of reduction of the maximal force (g) induced by histamine per mg tissue dry weight. Average concentration-effect curves for the data were determined from the mean concentrations of relaxant drugs which induced 20, 40, 50, 60 and 80% relaxation respectively. In some cases, the average concentration-effect curves were calculated as the means of responses to fixed concentrations of relaxants.

The change in basal tone of each preparation was analysed and an equilibration ratio (R_E) was determined, that is, the ratio of the resting tone (g/mg tissue dry weight) at the end of the equilibration period to the initial tone of the preparations. The drugs used were histamine dihydrochloride, (±)-isoprenaline (Merck Laboratories) and (−)-ascorbic acid, theophylline and indomethacin (Sigma Chemical Company). The results are shown as means ± s.e. mean; an analysis of variance and Student's t test for paired or unpaired variates were used for statistical evaluation.

Results

Histamine concentration-effect curves were produced before and after indomethacin (1.7 μM) in complete tracheal spirals from control (Figure 1a) and chronically treated animals (Figure 1b). The pD_2 value of control tracheal preparations was 5.55 ± 0.15 and after indomethacin (1.7 μM) was 5.41 ± 0.14. Tracheal preparations from chronically treated animals showed a pD_2 value of 5.48 ± 0.18 and subsequent to indomethacin (1.7 μM) 5.44 ± 0.16. When the pD_2 values of control preparations were compared

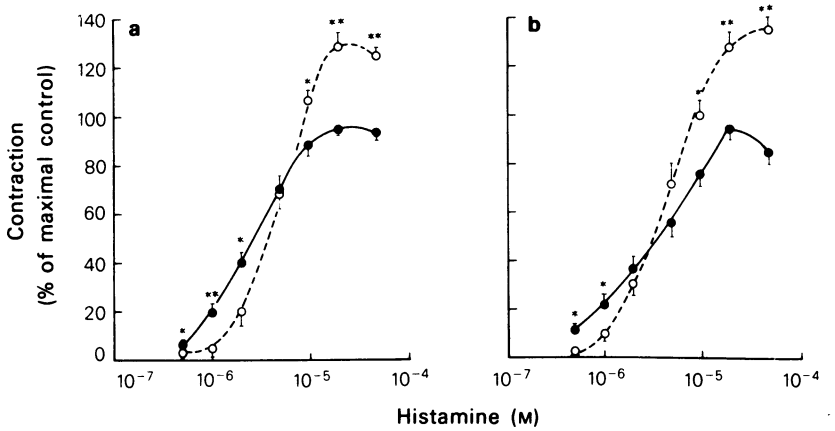

Figure 1 Histamine concentration-effect curves produced in complete tracheal spiral preparations from normal (a) and chronically treated guinea-pigs (b) before (●) and after (○) a 30 min incubation with indomethacin (1.7 μM). Mean values are presented; vertical lines show s.e.mean. $*P < 0.05$ and $**P < 0.001$.

to preparations taken from chronically treated animals, they were not significantly different (before, $P > 0.05$; after, $P > 0.05$; $n = 6$). The maximal response to histamine in control preparations was 0.19 ± 0.04 g/mg tissue dry weight and after indomethacin (1.7 μM), 0.24 ± 0.09 g/mg tissue dry weight. Preparations from chronically treated animals exhibited a significant reduction in maximal response; before 0.14 ± 0.07 g/mg tissue dry weight and after indomethacin (1.7 μM), 0.18 ± 0.09 g/mg tissue dry weight ($P < 0.05$ and $P < 0.05$, respectively; $n = 6$). While the sensitivities of these preparations were unchanged, the responses to low concentrations of histamine were significantly reduced while high concentrations of the agonist were significantly potentiated (Figure 1). Paired caudal and distal portions of the trachea exhibited similar responses (Figure 2) which were not significantly different from complete tracheal preparations. Figure 2(a) shows the caudal portion (CP) of the tracheal spirals (pD_2 values; before, 5.52 ± 0.09 and after indomethacin (1.7 μM), 5.34 ± 0.10) while panel (b) shows the distal portion (DP) of the tracheal preparation's (pD_2 values; before 5.55 ± 0.07 and after indomethacin (1.7 μM), 5.47 ± 0.08). The histamine maximal response for the CP preparation was 0.14 ± 0.08 g/mg tissue dry weight and 0.17 ± 0.09 g/mg tissue dry weight, before and after indomethacin, respectively.

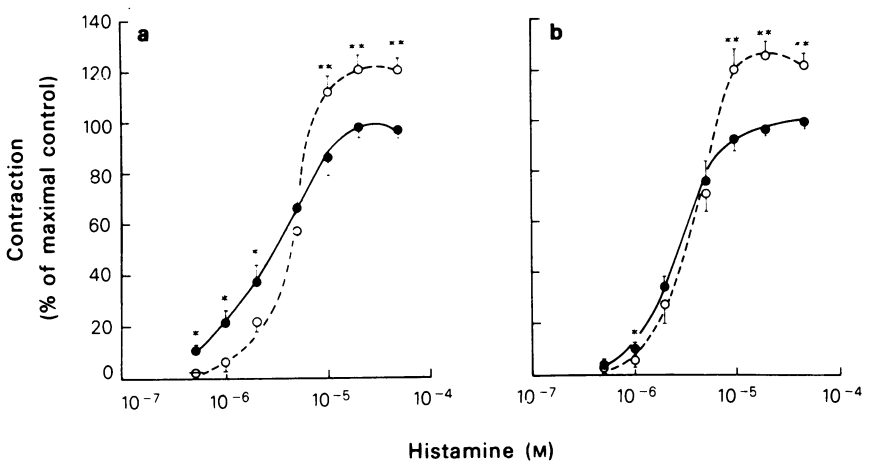

Figure 2 Histamine concentration-effect curves produced in paired tracheal spiral preparations, before (●) and after (○) indomethacin (1.7 μM). (a) Shows the caudal portion (CP) and (b) shows the distal portion (DP) of the trachea. Mean values are shown; vertical lines indicate s.e. mean $*P<0.05$ and $**P < 0.001$.

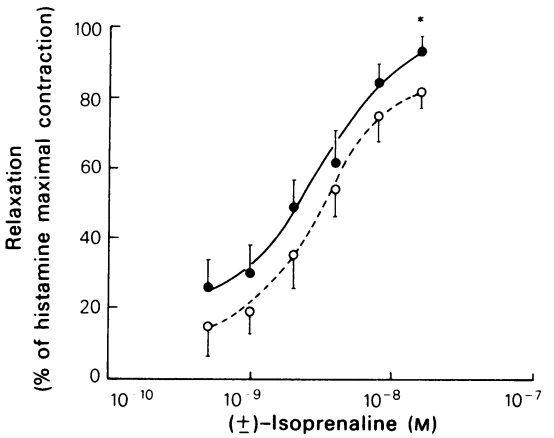

Figure 3 Isoprenaline concentration-effect curves produced in caudal portions (○) and distal portions (●) of tracheal spiral preparations, contracted maximally with histamine. Mean values are presented; vertical lines show s.e.mean. $*P < 0.05$.

The DP spirals exhibited a maximal response to histamine of 0.15 ± 0.04 g/mg tissue dry weight and 0.18 ± 0.06 g/mg tissue dry weight, before and after incubation with indomethacin (1.7 μM). Maximal responses of CP and DP were not significantly different from each other (before: $P>0.05$; after: $P>0.05$; $n = 6$).

When concentration-effect curves to relaxant agonists were produced in the paired portions of the trachea, the isoprenaline sensitivities were not significantly different (pD_2 values: CP, 8.60 ± 0.11; DP, 8.50 ± 0.10; $P > 0.05$; $n = 6$ Figure 3). The histamine maximal response was CP, 0.14 ± 0.06 and DP, 0.15 ± 0.05 ($P > 0.05$; $n = 6$). Paired half portions of the trachea exhibited the same sensitivity to theophylline (pD_2 values: CP, 4.30 ± 0.09; DP, 4.38 ± 0.06, $P > 0.05$).

Since the pD_2 values to isoprenaline of control groups were not significantly different (one way analysis of variance gave an F value of 0.972) they were combined. The pD_2 values for this relaxant agonist in preparations taken from chronically treated animals were also combined (one way analysis of variance gave an F value of 1.629) and both are shown in Figure 4. There was a significant shift in the isoprenaline concentration-effect curves when control preparations were compared with preparations from treated animals (pD_2 values; control, 8.36 ± 0.04; $n = 56$; treated, 7.87 ± 0.02; $n = 36$; $P < 0.0001$). Theophylline concentration-effect curves were not significantly different among the control groups (Table 1) and were combined (Figure 4). Theophylline relaxation curves in the paired half tracheal preparations from chronically treated animals (0.4 μmol isoprenaline) were not significantly different from appropriate control preparations (Table 1). However, the paired half portions from guinea-pigs which received higher doses of isoprenaline (4.0 μmol and 40 μmol) exhibited a reduced sensitivity to theophylline when compared to controls (Table 1). This reduced theophylline sensitivity was not altered by indomethacin treatment *in vivo*. Indomethacin treatment (30 mg/kg, i.p.) effectively reduced the isoprenaline desensitization induced by chronic treatment (0.4 μmol isoprenaline) but was ineffective in animals that

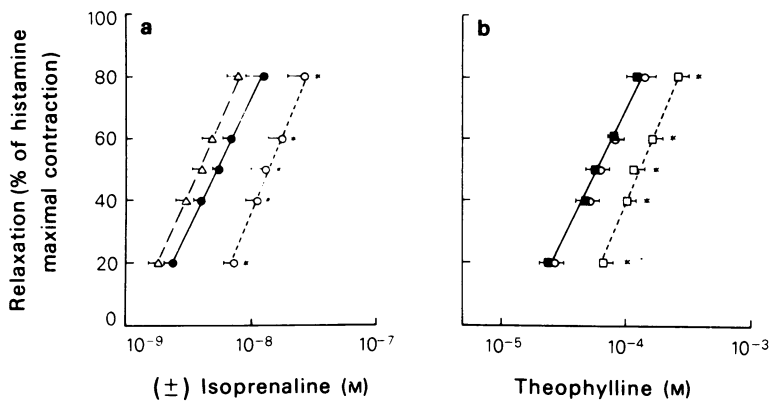

Figure 4 Comparison of concentration-effect curves to relaxant agonists in paired half tracheal preparations taken from control and chronically treated animals. (a) Concentration-effect curves to isoprenaline. Control (●; $n = 56$) and preparations taken from animals treated with isoprenaline (40.0, 4.0 and 0.4 μmol: (○; $n = 36$)). Chronic treatment with isoprenaline (0.4 μmol) followed by two injections of indomethacin (△; $n = 15$). (b) Concentration-effect curves to theophylline in control preparations (■; $n = 24$) and preparations from animals chronically treated with isoprenaline (40.0 and 4.0 μmol. □: $n = 15$; 0.4 μmol. ○: $n = 7$). Mean values are shown, horizontal lines indicate s.e. mean. $*P < 0.001$.

Table 1 A comparison of the sensitivities of guinea-pig paired half tracheal preparations to isoprenaline and theophylline from control and chronically treated animals

Treatment	*Isoprenaline* pD_2 *value*	*Theophylline* pD_2 *value*
Control†		
I	8.21 ± 0.13 (6)	4.27 ± 0.10 (6)
II	8.31 ± 0.03 (4)	4.41 ± 0.17 (4)
III	8.36 ± 0.06 (36)	4.28 ± 0.05 (36)
IV	8.48 ± 0.06 (10)	4.32 ± 0.09 (10)
Isoprenaline‡		
0.4 μmol	7.81 ± 0.06 (14)*	4.23 ± 0.07 (9)
4.0 μmol	7.97 ± 0.04 (8)*	3.85 ± 0.04 (8)*
40.0 μmol	7.88 ± 0.05 (14)*	3.90 ± 0.04 (7)*
Isoprenaline/Indomethacin††		
0.4 μmol	8.47 ± 0.06 (15)[a]	4.27 ± 0.10 (7)[c]
4.0 μmol	8.06 ± 0.10 (6)*[b]	3.94 ± 0.05 (6)*[b]
40.0 μmol	7.93 ± 0.08 (6)*[b]	3.92 ± 0.07 (6)*[b]

Mean ± s.e. mean are shown. (n) = number of preparations.
†Control: I No injections: II Tyrode solution (three times daily for 21 days, s.c.); III Vehicle and ascorbic acid (three times daily for 21 days, s.c.); IV Tyrode solution containing ascorbic acid (three times daily for 21 days, s.c.) followed by two injections of indomethacin (see Methods).
‡ Isoprenaline: chronic treatment with isoprenaline (three times daily for 21 days, s.c.).
††Isoprenaline/Indomethacin: chronic treatment with isoprenaline (three times daily for 21 days, s.c.) followed by two injections of indomethacin. *$P < 0.001$ (when compared to appropriate control† preparations)
[a]Not significantly different from appropriate controls† but different from isoprenaline treatment; [b]significantly different from appropriate controls† but not significantly different from isoprenaline treatment; [c]not significantly different from either appropriate control† or isoprenaline treatment.

received the higher doses of isoprenaline (4.0 μmol and 40.0 μmol: Table 1).

There was a significant increase in basal tone in preparations from chronically treated animals as well as a significantly reduced maximal response (g/mg tissue dry weight) to the contractile agonist (Table 2).

Discussion

In preparations of airway muscle from animals chronically treated with bronchodilator agent *in vivo* there was a significantly reduced response (force/mg tissue dry weight, Table 2) while there was no significant alteration in sensitivity (pD_2 values, Figure 1) to histamine. Since the pD_2 values were unchanged there may have been no alteration of the histamine receptors suggesting that the reduced responsiveness may be due to biochemical events either subsequent to the drug-receptor complex or a change in production of mediators, for example, prostaglandins which can modify the tissues responsiveness to contractile agents.

Pace-Asciak (1972) found an increase in prosta-

Table 2 Resting tone and histamine-maximal response (force (g)/mg tissue dry weight) of guinea-pig half tracheal spiral preparations from control and chronically treated animals.

Treatment (n) (in vivo)	R_E†	*Histamine maximum response*
Controls III (18)	0.76 ± 0.06	0.15 ± 0.01
Isoprenaline		
0.4 μmol (10)	1.16 ± 0.26*	0.11 ± 0.08*
4.0 μmol (10)	1.05 ± 0.18*	0.10 ± 0.04*
40.0 μmol (11)	1.05 ± 0.14*	0.11 ± 0.05*

Mean ± s.e.mean are shown. (n) = number of preparations. R_E† = the ratio of the resting tone (g/mg tissue dry weight) at the end of the equilibration period to the initial tone of the preparation.

glandin production in rat fundus strips exposed to noradrenaline. Gryglewski & Ocetkiewicz (1974) demonstrated increased prostaglandin production in mesenteric arteries exposed to noradrenaline. Such an increase in endogenous prostaglandins may modulate the response of the tissue rather than alter its sensitivity to contractile agonists, as occurs with exogenously applied prostaglandins. Douglas *et al.* (1978) showed that chronic treatment of guinea-pigs with isoprenaline *in vivo* results in a loss of sensitivity to histamine *in vivo*. Recently, Brink, Ridgway & Douglas (1978) have demonstrated that this reduced sensitivity to histamine *in vivo* may be related to altered prostaglandin production since the reduced histamine sensitivity was reversed by a single injection of indomethacin. In addition, the reduced response to antigen as reported by Wieczorek, Assem & D'Mello (1979) subsequent to chronic stimulation with isoprenaline in sensitized animals may also be explained by a modulatory role of prostaglandins and/or their precursors. The loss of histamine sensitivity *in vivo* (Brink *et al.*, 1978) and the altered responsiveness to histamine *in vitro* (Orehek, Douglas & Bouhuys, 1975) appears, therefore, to be related to this mechanism of action. The observation in this paper that guinea-pig tracheal preparations from animals pretreated with the sympathomimetic agent *in vivo* exhibited an increased basal tone (Table 2) which was unaffected by the cyclo-oxygenase inhibitor indomethacin *in vitro* (unpublished observations) does not necessarily preclude the possibility that other products resulting from metabolism of arachidonic acid, for example slow reacting substance of anaphylaxis (SRS-A), may be affecting the tone of the airway muscle. Whether chronic use of bronchodilator agents in man can result in increased biogenic tone is unknown. This observation may be of significance in patients with bronchial asthma where airway narrowing may result in an asthma attack or sudden death (Bateman & Clarke, 1979).

Another hypothesis that has been used to explain the loss of responsiveness to bronchodilator drugs has focused attention on the β-adrenoceptor and those biochemical events associated with this entity (Conolly *et al.*, 1971; Avner & Jenne, 1977; El-Fellah & Turnbull, 1978). In several studies the evaluation of such drugs has required the induction of tone in tissues in order to assess the potency of the sympathomimetic amines. Benoy *et al.* (1975) induced tone in perfused lung preparation with histamine and others used similar contractile agonists in isolated airway preparations (Anderson & Lees, 1976). Recently Buckner & Saini (1975) pointed out that the relative potency of a relaxant drug on airway muscle is dependent upon the tone induced in the preparations. For example, in the study of Benoy *et al.* (1975) it was not clear whether perfusion pressures or basal flow rates were similar in control and pretreated preparations. The desensitization reported could therefore be attributed to estimates of bronchodilator potency in preparations where the tone induced with histamine was variable. In this paper a reduced response to the contractile agent was observed. From the observatons of Buckner & Saini (1975) an increased sensitivity to the relaxant agonist would have been predicted. However, the fact that the isoprenaline concentration-effect curves shifted to the right indicates a slight but significant β-adrenoceptor tolerance.

The mechanism by which β-stimulants relax airway muscle is not well understood but probably involves several biochemical events. One or more of these processes may be modified when the tissue is chronically exposed to sympathomimetic agents. The observation in this paper that prolonged exposure *in vivo* to high doses of isoprenaline altered theophylline concentration-effect curves indicates that the chronic stimulation *in vivo* probably affected chemical events subsequent to the β-receptor. In addition, prostaglandins or their precursors may be involved in the desensitization process since indomethacin treatment was effective in reversing β-adrenoceptor tolerance in guinea-pigs that had been exposed to low doses of isoprenaline. While a chronic *in vivo* treatment with a sympathomimetic agent slightly alters the sensitivity of airway muscle preparations to the β-stimulant, it apparently affects other processes as well, making it difficult to elucidate the exact mechanism of smooth muscle desensitization.

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