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Different Ability of Trifluoperazine to Inhibit Agonist-induced Contraction of Lung Parenchyma Strips from Control and Sensitized Guinea-pigs

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Abstract—There is increasing interest in the therapeutic potential of calcium antagonists in asthma. Among them the use of calmodulin antagonists deserves consideration. In the present work the effect of trifluoperazine on contractions generated by different mechanisms ($CaCl_2$, KCl, acetylcholine, histamine and 5-hydroxytryptamine) in lung parenchyma strip isolated from control and actively sensitized guinea-pigs has been studied. Trifluoperazine produced both in unsensitized and sensitized lung strips, a concentration-dependent, right, downward displacement of the concentration-response curves to the agonists used, although the sensitization procedure resulted in a potentiation in the ability of trifluoperazine to inhibit agonist-induced contractions. The basis for this greater potency of trifluoperazine in sensitized tissues remains to be elucidated but raises attention to the future use of selective calmodulin antagonists in the management of asthma.

Although calcium antagonists are currently used in the treatment of several cardiovascular diseases (Flaim & Zelis 1981) only recently has attention been focused on their potential use as clinically efficacious agents for respiratory disorders such as bronchial asthma (Middleton 1980; Barnes 1983).

However, the clinical studies with calcium-entry channel blockers have shown a slight and variable protective effect against exercise-, cold air- or allergen-induced bronchoconstriction and no effect on resting bronchomotor tone (Fish 1984; Barnes 1985; Fanta 1985). These results and the findings from experimental studies (Advenier et al 1984; Burka 1984; Foster et al 1984; Ahmed et al 1985; Cortijo et al 1986b) suggest that voltage-dependent calcium entry into airway smooth muscle cells appears not to be substantial in the pathogenesis of asthmatic bronchoconstriction (Barnes 1985). Among the alternative approaches are agents which block receptor-operated calcium channels, inhibit the release of calcium from internal stores or antagonize the calcium binding protein calmodulin.

Trifluoperazine is a drug which inhibits calcium-induced contractions in 'skinned' taenia coli (Sparrow et al 1981; Spedding 1984; Cortijo et al 1987) and tracheal smooth muscle (Sparrow et al 1984; Cortijo et al 1986a) from guinea-pigs. In addition it has been found to inhibit histamine release from human basophils (Cumella et al 1983; Marone et al 1983) and able to reduce antigen-induced contraction or airway smooth muscle (Burka 1984). This drug has been characterized as a calmodulin antagonist (Weiss et al 1980).

Our aim was to analyse the inhibitory action of trifluoperazine on the contractions induced by various agonists ($CaCl_2$, KCl, acetylcholine, histamine and 5-hydroxytryptamine 5-HT) in lung parenchyma strip isolated from control and actively sensitized guinea-pigs to ascertain whether differences exist between these two situations with respect to the effects of this drug.

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Methods

Randomly bred, male, adult guinea-pigs, 300–400 g, were injected intraperitoneally with either 2 mL of saline (control group) of 0·1 mg g^{-1} of bovine serum albumin (BSA) dissolved in 1 mL saline plus 1 mL of Freund's complete adjuvant (sensitized group) and killed by stunning and exsanguination 15 days later.

Lung parenchyma strips were prepared according to Lulich et al (1976). In brief, 2-4 parenchyma strips, 20 mm in length with 3 × 3 mm cross section, were trimmed from the pleural edge of the right and left lower lobes of the lung and mounted in different organ baths containing a physiologic salt solution of the following composition (mM): NaCl, 118·0; KCl 4·7; $NaHCO_3$, 25·0; $CaCl_2$, 2·5; $MgSO_4$, 1·2; KH_2PO_4, 1·2; dextrose, 10·1. This solution maintained at 37 °C, was continuously gassed with 5% CO_2 in oxygen and had a pH of 7·4 ± 0·1. Changes in tone were recorded via isometric force transducers (Hewlett-Packard FTA 100-1, Hewlett-Packard, Corvallis, Oreg., or Grass FT 0·3 C. Grass Instrument Co., Quincy, Mass.) connected through carrier amplifiers Hewlett-Packard 8805B to a Philips PM-8222 pen recorder (Philips, Eindhoven, The Netherlands) or a Grass 7D polygraph. The strips were gently stretched up to 1 g of initial isometric force, and a 90 min equilibration period was permitted before the addition of any drug. At the end of the equilibration period, the lengths of the preparations were recorded. Agonists were added to the bath in a cumulative fashion to obtain concentration-response curves.

In those experiments in which $CaCl_2$ was the contractile agonist the following protocol was used. The lung parenchyma strip was initially set up in Tris-buffer solution of the following composition (mM): NaCl, 134·8; KCl, 4·7; $CaCl_2$, 2·5; $MgSO_4$, 1·2; Tris, 5·0; HCl 5·0; dextrose 10·1. After 1 h of equilibration this medium was changed for a

Ca^{2+}-free (neither Ca^{2+} nor a Ca^{2+} chelating agent was added) Tris solution and after a 30 min period of equilibration, $CaCl_2$ was added cumulatively to the bath. Tris-buffer solutions were aerated with pure oxygen.

After the concentration-response curve to one agonist on two strips from the same animal had been established, a predetermined concentration of trifluoperazine was added to one of the tissues, and after 30 min incubation the concentration-response curve was repeated on both strips, the second strip serving as control to monitor any time-related change in the sensitivity of the tissues to the agonists.

The concentration of the agonist producing 50% of its maximal effect, i.e., the effective concentration 50% (EC50), was calculated graphically from individual concentration-response curves. The antagonism by trifluoperazine of the agonists used was examined (i) by determining the concentration-ratios of the agonists, i.e., the ratio of equieffective concentrations of the agonists in the absence or presence of the antagonist trifluoperazine, and (ii) by determining its 50% inhibitory concentration (IC50), i.e. the concentration of trifluoperazine in the presence of which the maximal effect of $CaCl_2$, KCl, acetylcholine, histamine and 5-HT is reduced to 50% of the control value. Because of significant differences in the agonist-induced maximal responses between control and sensitized tissues (Morcillo et al 1984), an additional group of experiments was designed in which single equieffective concentrations of the agonist were used to calculate the IC50 values of trifluoperazine.

Lung parenchyma strips from actively sensitized animals were challenged at the end of the experiment with BSA 1 mg mL^{-1} to confirm the existence of an antigen-induced contraction. This was also done in some of the strips from non-sensitized animals to check the lack of responsiveness. Immediately after completion of the pharmacomechanical studies the tissue was removed from the bath, blotted, and weighed on a precision balance (±0·1 mg).

Unless otherwise indicated, the drug concentrations are expressed for the free acid or base as final molar (M) bath concentrations. The changes in force resulting from the addition of drugs were directly determined from recordings and were transformed into tension, i.e. force per unit cross-sectional area. The cross-sectional area of the preparations was determined by dividing the tissue wet weight by the tissue length. Data are reported as means ± s.e.m. An unpaired *t*-test was used to evaluate statistical differences in the data observed. Statistical significance was established at the 5% level.

Drugs and their sources were: acetylcholine chloride (Roche Products Ltd, Welwyn Garden, Herts, UK), bovine serum albumin (Sigma Chemical Co., St Louis, Mo), Freund's complete adjuvant (Difco Lab, Detroit, Mich.), histamine dihydrochloride (Sigma), 5-hydroxytryptamine (Sigma), trifluoperazine (Sigma).

Results

Effect of trifluoperazine on contractions evoked by $CaCl_2$

Addition of $CaCl_2$ (10^{-5} to 10^{-2} M) to the bath containing a depolarizing (K^+ 30 mM), Ca^{2+}-free Tris solution evoked concentration-dependent increases in tension in control and sensitized specimens with maximum effect and EC50 values shown in Table 1 and 2. Trifluoperazine produced a right, downward shift of the concentration-response curves to $CaCl_2$ (Fig. 1). The depression of the maximal response

Table 1. Maximal effects of $CaCl_2$, KCl, acetylcholine (ACh), histamine (HA) and 5-HT in the absence or presence of several molar concentrations of trifluoperazine (TFP) in lung parenchyma strips isolated from control and sensitized guinea-pigs.

	Control				Sensitized			
TFP	0	10^{-5}	10^{-4}	5×10^{-4}	0	10^{-5}	10^{-4}	5×10^{-4}
$CaCl_2$	82 ± 7 (32)	85 ± 5 (6)	29 ± 6* (6)	12 ± 4* (6)	174 ± 11 (32)	102 ± 11* (6)	78 ± 12* (6)	22 ± 11* (6)
KCl	130 ± 11 (32)	120 ± 11 (6)	102 ± 12* (6)	74 ± 14* (6)	211 ± 18 (32)	192 ± 10 (6)	141 ± 12* (6)	97 ± 17* (6)
ACh	87 ± 5 (18)	64 ± 6 (6)	47 ± 5* (6)	29 ± 7* (6)	168 ± 8 (18)	127 ± 6* (6)	97 ± 10* (6)	69 ± 11* (6)
HA	136 ± 14 (18)	148 ± 11 (6)	68 ± 12* (6)	27 ± 8* (6)	229 ± 29 (18)	111 ± 21* (6)	64 ± 15* (6)	20 ± 3* (6)
5-HT	68 ± 9 (18)	44 ± 7 (6)	24 ± 3* (6)	18 ± 4* (6)	112 ± 12 (18)	47 ± 4* (6)	31 ± 7* (6)	16 ± 3* (6)

Data are mean ± s.e.m. expressed in mg mm^{-2}. Figures in parentheses mean the number of experiments.
*$P < 0.05$ compared with the response in the absence of trifluoperazine.

Table 2. EC50 values of various agonists (as in Table 1) in the absence or presence of several molar concentrations of trifluoperazine (TFP) in lung parenchyma strips isolated from control and sensitized guinea-pigs.

	Control				Sensitized			
TFP	0	10^{-5}	10^{-4}	5×10^{-4}	0	10^{-5}	10^{-4}	5×10^{-4}
$CaCl_2$	2·60 ± 0·08	2·44 ± 0·10	2·00 ± 0·12*	1·47 ± 0·11*	2·84 ± 0·05	2·21 ± 0·06*	1·73 ± 0·13*	1·45 ± 0·09*
KCl	1·67 ± 0·03	1·75 ± 0·07	1·75 ± 0·03*	1·68 ± 0·03*	1·80 ± 0·03	1·67 ± 0·16	1·68 ± 0·01*	1·68 ± 0·01*
ACh	5·19 ± 0·14	5·45 ± 0·20	4·45 ± 0·19*	4·07 ± 0·13*	6·17 ± 0·17	4·75 ± 0·17*	4·24 ± 0·14*	4·17 ± 0·14*
HA	4·79 ± 0·13	4·77 ± 0·12	3·85 ± 0·24*	3·52 ± 0·21*	5·37 ± 0·11	3·91 ± 0·10*	3·44 ± 0·16*	3·22 ± 0·13*
5-HT	4·10 ± 0·09	3·82 ± 0·09*	3·61 ± 0·11*	3·58 ± 0·11*	4·45 ± 0·09	4·09 ± 0·13*	4·01 ± 0·21*	3·60 ± 0·12*

Data are mean ± s.e.m. of −log molar concentrations of the agonists. *$P < 0.05$ compared with the value in the absence of trifluoperazine. Number of experiments as in Table 1.

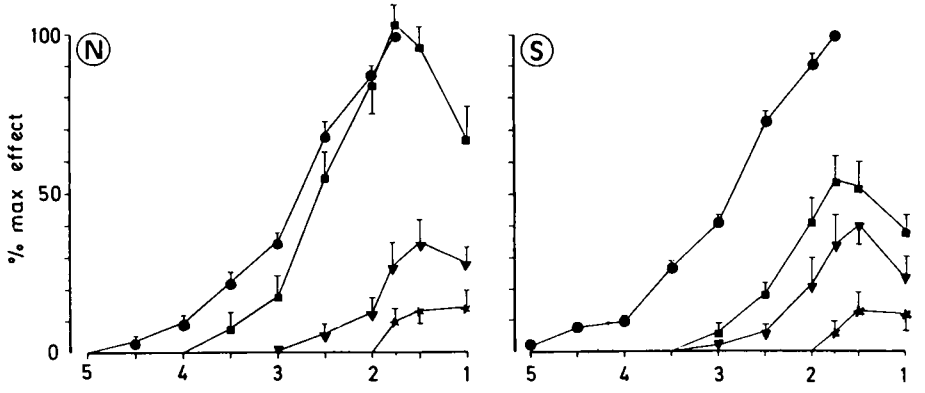

FIG. 1. Concentration-response curves for contraction of guinea-pig lung parenchyma strip by $CaCl_2$ in the presence of a depolarizing (K^+ 30 mM) Ca^{2+}-free Tris-physiological salt solution. In this and the following Figs, the concentration–response curves are shown in the absence (●) or presence of various concentrations of trifluoperazine (10^{-5} M ■; 10^{-4} M ▼; 5×10^{-4} M ★). Control (N) and sensitized (S) tissues are shown, and the bars represent standard errors of the mean (s.e.m.).

to $CaCl_2$ indicated that this antagonism is of a non-competitive nature. The concentration-ratios for $CaCl_2$ in the presence of trifluoperazine and the IC50 values for this antagonist as inhibitor of the maximal response to $CaCl_2$ are shown in Tables 3 and 4 (column A), respectively. When this antagonism was studied using single equieffective concentrations of $CaCl_2$ (10^{-2} M producing 83 ± 9 mg mm^{-2} in control, n = 6, and 10^{-3} M producing 94 ± 14 mg mm^{-2} in sensitized tissues, n = 6), the calculated IC50 values are shown in Table 4 (column B).

Effect of trifluoperazine on contractions evoked by KCl, acetylcholine, histamine, and 5-hydroxytryptamine

Addition of the agonists to the bath produced concentration-dependent contractions with maximal effects and EC50 values as displayed in Tables 1 and 2. Incubation with trifluoperazine resulted in a variable degree of a right, downward displacement of these concentration-response curves as shown in Figs 2 to 5. The concentration ratios for these agonists in the presence of trifluoperazine and the calculated IC50 values for both inhibition of the response to the maximal concentration used and inhibition of the response to single equieffective concentrations of KCl (2.5×10^{-2} M producing 105 ± 9 mg mm^{-2} in control, n = 6, and 10^{-2} M producing 101 ± 8 mg mm^{-2} in sensitized tissues, n = 6) acetylcholine (10^{-4} M producing 67·4 ± 4·5 mg mm^{-2} in control n = 6, and 10^{-7} M producing 63·2 ± 6·7 mg mm^{-2} in sensitized tissues, n = 6) histamine (10^{-4} M producing 109 ± 12 mg mm^{-2} in control, n = 6, and 5×10^{-6} M producing 121 ± 9 mg mm^{-2} in sensitized tissues, n = 6) and 5-HT (3×10^{-4} M producing 68 ± 16 mg mm^{-2} in control, n = 6, and 3×10^{-5} M producing 72 ± 10 mg mm^{-2} in sensitized tissues, n = 6) are shown in Tables 3 and 4.

Table 3. Concentration-ratios of various agonists (as in Table 1) in the presence of several concentrations of trifluoperazine (TFP).

	Control			Sensitized		
TFP	10^{-5}	10^{-4}	5×10^{-4}	10^{-5}	10^{-4}	5×10^{-4}
$CaCl_2$	0·19 ± 0·06	0·38 ± 0·21	0·90 ± 0·21	0·76 ± 0·05*	0·97 ± 0·15*	1·18 ± 0·16
KCl	−0·39 ± 0·22	−0·04 ± 0·07	−0·01 ± 0·05	0·13 ± 0·16	0·15 ± 0·04*	0·12 ± 0·03*
ACh	−0·20 ± 0·27	0·54 ± 0·14	0·87 ± 0·16	1·28 ± 0·17*	1·61 ± 0·21*	1·96 ± 0·06*
HA	−0·11 ± 0·23	0·98 ± 0·06	1·34 ± 0·13	1·36 ± 0·08*	1·76 ± 0·16*	2·12 ± 0·06*
5-HT	−0·15 ± 0·28	0·17 ± 0·12	0·31 ± 0·12	−0·13 ± 0·19	0·27 ± 0·22	0·79 ± 0·11*

Data are mean ± s.e.m. of log (concentration ratio) derived from at least 6 experiments.
*$P < 0.05$ compared with control.

Table 4. IC50 values of the inhibitory effect of trifluoperazine on guinea-pig lung parenchyma contractions induced by either maximal concentrations (column A) or single equieffective concentrations (column B) (see text) of different agonists. Results are expressed as −log molar concentrations of trifluoperazine.

	Control		Sensitized	
Agonist	A	B	A	B
$CaCl_2$	4·20 ± 0·16	4·28 ± 0·15	4·41 ± 0·21	>5
KCl	3·05 ± 0·08	3·18 ± 0·10	3·45 ± 0·13*	4·13 ± 0·17**
ACh	3·51 ± 0·09	3·09 ± 0·07	3·70 ± 0·12	4·03 ± 0·15**
HA	4·02 ± 0·15	4·11 ± 0·16	5·11 ± 0·23**	>5
5-HT	4·51 ± 0·13	4·33 ± 0·19	5·52 ± 0·26**	5·72 ± 0·25**

Data represent mean ± s.e.m. of 6 experiments. *$P < 0.05$, **$P < 0.01$ compared with control.

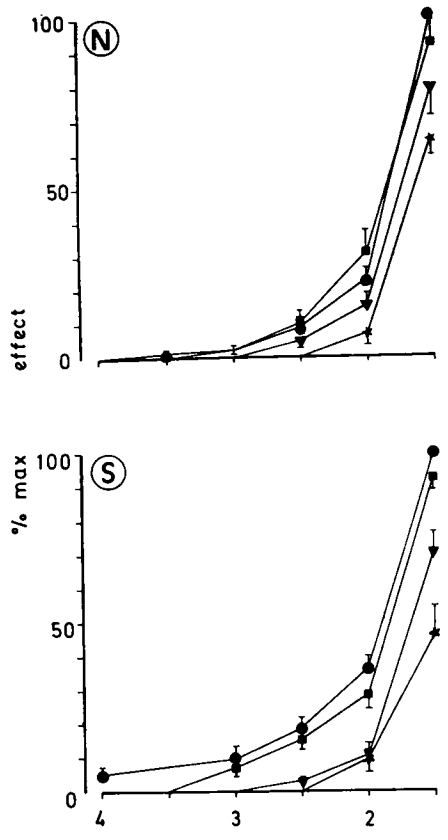

FIG. 2. Concentration-response curves for contraction of guinea-pig lung parenchyma strip by KCl in the absence or presence of several concentrations of trifluoperazine (as in Fig. 1).

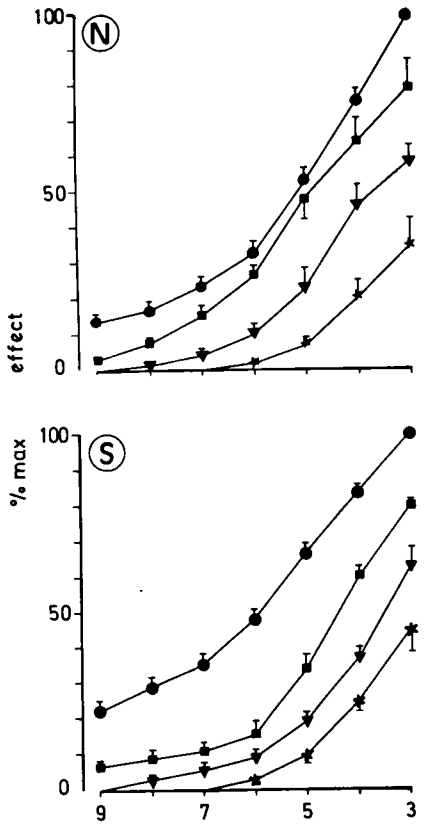

FIG. 3. Concentration-response curves to acetylcholine in the absence or presence of several concentrations of trifluoperazine (as in Fig. 1).

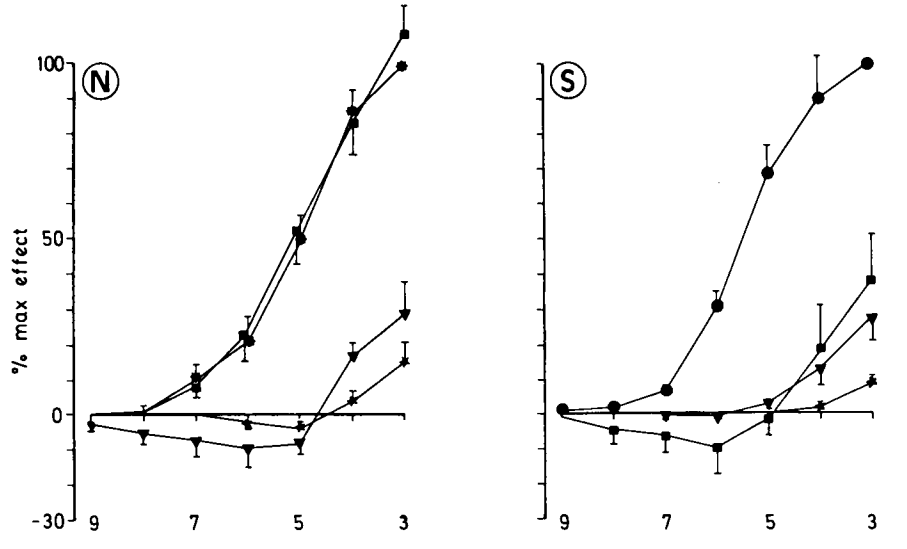

FIG. 4. Concentration-response curves to histamine in the absence or presence of several concentrations of trifluoperazine (as in Fig. 1).

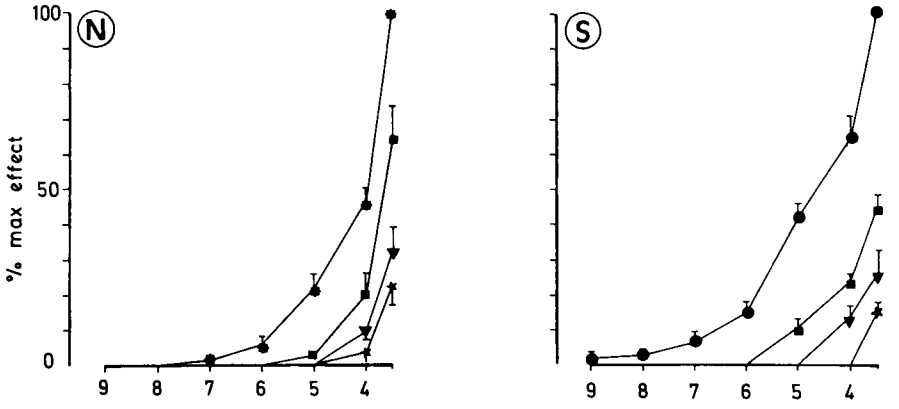

FIG. 5. Concentration-response curves to 5-hydroxytryptamine in the absence or presence of several concentrations of trifluoperazine (as in Fig. 1).

Discussion

The main finding of the present study is that active sensitization in guinea-pigs results in a potentiation in the ability of trifluoperazine to inhibit agonist-induced contractions of lung parenchyma strips. Three classes of agonistical procedures were selected: (i) exogenously applied Ca^{2+} acting on K^+-depolarized smooth muscle, (ii) K^+-induced depolarization leading to Ca^{2+} entry through voltage-dependent channels and (iii) agonists activating specific receptors and promoting Ca^{2+} entry through receptor-operated channels (Triggle 1985) or receptor-mediated intracellular Ca^{2+} release (Small & Foster 1986).

Triofluoperazine, consistently produced in the unsensitized tissues, a concentration-dependent, right (expressed as concentration-ratios) and downward (expressed as $-\log$ IC50) displacement of the concentration-response curves to the various agonists used in this study. The selective inhibitory effect (greater inhibition of the contraction induced by KCl compared with acetylcholine) reported for calcium entry channel blockers (Advenier et al 1984; Ahmed et al 1985; Cortijo et al 1986b) was not found with trifluoperazine. The absence of specificity against different agonistical procedures has been previously reported for other calmodulin antagonist in guinea-pig isolated trachea (Advenier et al 1984).

In the sensitized preparation trifluoperazine maintained its unselective inhibitory action but increased its potency, i.e. significantly greater inhibitions were seen for the same level of concentration of the antagonist. Conversely, the ability of verapamil to inhibit $CaCl_2$-, KCl-, acetylcholine- and histamine-induced contractions was substantially reduced in lung parenchyma strips from sensitized animals compared with control (Perpiñá et al 1986). Therefore, it appears that the inhibitory action of verapamil and trifluoperazine was affected in a quite different manner by the sensitization procedure.

This finding cannot be attributed to differences in the magnitude of the contractions to the agonists between unsensitized and sensitized states (Morcillo et al 1984) since, as shown in this and other study (Perpiñá et al 1986), the difference is maintained for both antagonists when the concentrations of the agonists were chosen to give contractions of approximately equal magnitude in control and sensitized tissues.

Spedding (1984) has demonstrated that increasing the negative surface charge of the membrane of smooth muscle of guinea-pig taenia caecum by a pharmacological procedure (incubation with salicylate) resulted in a reduction of the effectiveness of verapamil and an increase in that of the calmodulin antagonist W-7, which is in agreement with the findings herein reported. A modification of this nature in the characteristics of the membrane of airway smooth muscle cells may differentially affect the action of calcium antagonists through different mechanisms. Thus, an increase in the negative charge of the membrane diminishes the proportion of inactivated calcium-channels and the binding of verapamil (Shimoni 1981), reducing its effectiveness as a calcium entry channel blocker.

The greater potency of the clamodulin antagonist trifluoperazine could be explained in terms of an increase in its intracellular accumulation due to its enhanced passage through cell membrane (Spedding 1984). Another possibility is the existence of a change in the characteristics of calmodulin due to the sensitization procedure.

Considering the limitations shown by calcium channel blockers in the prophylaxis and treatment of asthma (Fish 1984; Barnes 1985; Fanta 1985) further attention should be paid to the role of selective calmodulin antagonists in the management of asthma.

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