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Sodium cromoglycate, verapamil and nicardipine antagonism to leukotriene D_4 bronchoconstriction

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1 The effects of nicardipine, verapamil and sodium cromoglycate (SCG) on the increase in pulmonary airway resistance(R_{Aw}) and decrease in pulmonary dynamic compliance (C_{Dyn}) induced by leukotriene D_4 (LTD_4) 0.5 $\mu g\,kg^{-1}$ and acetylcholine (ACh) 3 $\mu g\,kg^{-1}$ were investigated in anaesthetized guinea-pigs. The effects of these three agents on the contractile effects of LTD_4 and ACh were tested on isolated tracheal preparations of the guinea-pig.

2 Nicardipine and verapamil (0.3 and 1 $mg\,kg^{-1}$), as well as SCG (3 and 10 $mg\,kg^{-1}$), partially but significantly inhibited the effects of LTD_4 on R_{Aw}. Partial inhibition of the effect of LTD_4 on C_{Dyn} was only observed with verapamil (0.3 and 1 $mg\,kg^{-1}$). Nicardipine and verapamil had no effect on ACh-induced bronchoconstriction *in vivo*.

3 In concentrations higher than 10^{-5} M, nicardipine and verapamil inhibited the contractile effects of LTD_4 and ACh on guinea-pig isolated trachea. SCG had no effect on this preparation.

4 These results suggest that nicardipine, verapamil and SCG partially reduce the component of bronchoconstriction associated with stimulation of irritant receptors by LTD_4. However, the site and mechanism of action of Ca^{2+}-entry antagonists remain uncertain.

Introduction

It has recently been reported that two calcium antagonists, nifedipine and verapamil, prevent exercise-induced bronchospasm in man (Barnes, Wilson & Brown, 1981; Cerrina, Denjean, Alexandre, Lockhart & Duroux, 1981; Patel, 1981). Nifedipine has also been shown to inhibit partially the contractile actions of histamine and acetylcholine (ACh) in guinea-pigs (Fanta, Venugopalan, Lacouture & Drazen, 1982). It was therefore of interest to investigate the effects of verapamil (Fleckenstein, Grün, Tritthart & Byon, 1971) and of another calcium antagonist, nicardipine (Takenaka, Usuda, Nomura, Maeno & Sado, 1982), on the bronchoconstrictor action of one of the major mediators of allergic bronchospasm in man, leukotriene D_4 (LTD_4) (Drazen, Austen, Lewis, Clark, Goto, Marfat & Corey, 1980; Piper, Samhoun, Tippins, Williams, Palmer & Peck, 1981), and to compare the effects of these agents with those of sodium cromoglycate (SCG), especially since studies on rat peritoneal mast cells suggest that SCG might act as a calcium antagonist (Foreman, Hallett & Mongar, 1977).

The effects of nicardipine, verapamil and SCG on LTD_4 and ACh-induced increase in pulmonary airway resistance and decrease in pulmonary dynamic compliance were studied *in vivo* in anaesthetized guinea-pigs. The effects of the same agents on the contractile effects of LTD_4 and ACh were tested *in vitro* on the isolated trachea of the guinea-pig.

Methods

Anaesthetized guinea-pigs

Male guinea-pigs weighing from 0.4 to 0.6 kg were anaesthetized with urethane (1.25 $g\,kg^{-1}$, i.p.). A cannula was inserted into the trachea and the animals were allowed to breathe spontaneously. Pulmonary airway resistance (R_{Aw}) and dynamic compliance (C_{Dyn}) were determined according to the methods of Amdur & Mead (1958) and Advenier, Boissier & Giudicelli (1972). Transpulmonary pressure was measured by needle pleural puncture, and airflow and tidal volume by plethysmography. All values were continuously recorded on a 7700 Hewlett Packard recorder.

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In the first series of experiments, the animals were initially tested for sensitivity to acetylcholine (ACh) 25 $\mu g\,kg^{-1}$; only those guinea-pigs that responded with bronchospasm were included in the study. Preliminary experiments had shown that each of three successive intravenous injections of LTD_4 0.5 $\mu g\,kg^{-1}$ given at 20 min intervals induced reproducible bronchospasm in guinea-pigs and that this bronchoconstrictor effect of LTD_4 was abolished by FPL 55712 (1 $mg\,kg^{-1}$) injected 1 min before LTD_4. Each of the guinea-pigs under study therefore received a control intravenous dose of LTD_4 (0.5 $\mu g\,kg^{-1}$) followed either by a second dose of LTD_4 preceded by pretreatment with propranolol or hexamethonium, or two other doses of LTD_4 preceded by two cumulative doses of verapamil, nicardipine, SCG or atropine. In the case of atropine, the drug was first injected alone, then followed by one dose of verapamil or nicardipine. Thus, the effects of nicardipine and verapamil were investigated on two groups of animals. Pretreatments were administered 10 min before the next injection of LTD_4. The dose of atropine selected totally antagonized the effect of ACh 30 $\mu g\,kg^{-1}$, while that of propranolol totally antagonized the effect of isoprenaline 1 $\mu g\,kg^{-1}$. The dose of hexamethonium was chosen according to published data (Severinghaus & Stupfel, 1955).

In the second series of experiments, the effects of verapamil, nicardipine and SCG on ACh-induced bronchoconstriction were investigated. ACh was given intravenously in doses of 3.0 $\mu g\,kg^{-1}$. One dose of ACh was injected on two successive occasions, then repeated after intravenous administration of verapamil or nicardipine in cumulative doses of 0.3 and 1 $mg\,kg^{-1}$ or SCG (3 and 10 $mg\,kg^{-1}$). Preliminary experiments had shown that the effects of ACh injections were reproducible.

Guinea-pig isolated trachea

Tracheal spirals containing 2 to 4 cartilaginous rings were obtained from male guinea-pigs (250–350 g) that had been anaesthetized with urethane (1.25 $g\,kg^{-1}$) and were equilibrated under an initial tension of 1.2 g in a physiological solution maintained at 37 °C and gassed with O_2. Tension was measured isometrically with a Ugo Basil strain gauge and was displayed on a Ugo Basil channel pen recorder. The initial tension ensured that after a 1.5 h equilibration period the resting tension was between 0.3 and 0.7 g. Under these conditions responses to agonists are reproducible and maximal (Stephens, 1970).

The composition of the physiological solution was (mM): NaCl 139.2, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.49, $NaHCO_3$ 11.3, Na_2HPO_4 0.4 and glucose 5.5. The preparations were contracted to maximal tension with ACh 6.6×10^{-5}M and tested for maximal relaxation with isoprenaline 3.9×10^{-6}M.

Verapamil, nicardipine or SCG were added to the solution in incremental concentrations until the submaximal contraction induced by ACh 2×10^{-5}M or LTD_4 1×10^{-7}M was inhibited, the preparations being thoroughly washed after each addition of these agents. Results are expressed in percentage inhibition of the initial control preparation.

The experiments were performed on groups of at least four preparations.

Drugs

The agents used in the experiments were: verapamil HCl (Biosédra, Paris), nicardipine HCl (Sandoz, Basle), propranolol HCl (I.C.I.), FPL 55712 and sodium cromoglycate (Fisons Ltd), atropine sulphate, hexamethonium diiodide and acetylcholine HCl. Leukotriene D_4 was synthesized by J. Rokach (Merck Frosst Laboratories, Kirkland, Quebec, Canada).

All agents (including LTD_4, which was initially a solution in methanol preserved at − 40 °C) were dissolved in 0.9% w/v NaCl solution. FPL 55712 (sodium 7 - [3(4 - acetyl - 3 - hydroxy - 2 - propylphenoxy)-2-hydroxy propoxy]-4-oxo-8-propyl-4H-1-benzo-pyran-2-carboxylate) was dissolved in distilled water.

Statistical analysis of results

Statistical analysis of the results was performed using Student's *t* test for paired data.

Results

Effects of the agents tested on leukotriene D_4-induced bronchoconstriction in guinea-pig

The effects of LTD_4 on pulmonary airway resistance (R_{Aw}) and dynamic compliance (C_{Dyn}) before and after pretreatment with the six agents tested are given in Tables 1 and 2. Basal values of R_{Aw} ($0.45 \pm 0.09\,cmH_2O\,ml^{-1}\,s^{-1}$, $n = 54$) and C_{Dyn} ($0.38 \pm 0.08\,ml\,cmH_2O^{-1}$, $n = 54$) were unmodified by the different pretreatments, except propranolol, which increased R_{Aw} by $37.0 \pm 12.7\%$ ($n = 4$, $P < 0.05$).

Nicardipine, verapamil, SCG and atropine partially but significantly reduced the increase in R_{Aw} induced by LTD_4, atropine being the most active, no matter whether administered alone or together with verapamil or nicardipine.

A significant reduction of the decrease in C_{Dyn} induced by LTD_4 was only observed with verapamil (0.3 and 1 $mg\,kg^{-1}$) and atropine (1 $mg\,kg^{-1}$). The effect of atropine was not modified when this agent was associated with verapamil or nicardipine.

Hexamethonium and propranolol potentiated the

Table 1 Effects of six different agents on the increase in pulmonary airway resistance induced by leukotriene D_4 0.5 $\mu g\,kg^{-1}$ in anaesthetized guinea-pigs

Agents		*Increase in pulmonary airway resistance (%)*		
($mg\,kg^{-1}$)	n	*Before treatment*	*After treatment*	P
Nicardipine 0.03	4	59± 9.3	57±13.3	>0.05
Nicardipine 0.1	5	84±21.2	46±10.9	>0.05
Nicardipine 0.3	6	77±16.1	42±13.7	<0.05
Nicardipine 1.	6	48± 7.1	31± 6.5	<0.01
Verapamil 0.03	4	79±26.2	72±33.8	>0.05
Verapamil 0.1	4	79±26.2	86±35.2	>0.05
Verapamil 0.3	6	66±20.5	29± 7.2	>0.05
Verapamil 1	6	66±20.5	19± 5.7	<0.01
SCG 1	4	171±80.6	154±64.8	<0.05
SCG 3	4	171±80.6	89±44.8	<0.01
Atropine 1	10	82±10.7	19± 5.6	<0.001
Atropine 1 + nicardipine 1	5	85±17.1	14± 4.6	<0.001
Atropine 1 + verapamil 1	5	79±14.5	13± 2.4	<0.001
Hexamethonium 10	5	106±20.6	234±86.2	<0.001
Propranolol 1	4	77±47.5	657±99.8	<0.05

Each value represents the mean percentage increase ± s.e.mean. n = number of guinea-pigs in each group.

effects of LTD_4 on R_{Aw} and C_{Dyn}. However, the increase in R_{Aw} and decrease in C_{Dyn} were both considerably augmented by propranolol, whereas hexamethonium only had a significant potentiating effect on the increase in R_{Aw}.

Effects of nicardipine, verapamil and sodium cromoglycate on acetylcholine-induced bronchoconstriction in guinea-pigs

Verapamil, nicardipine and SCG administered in doses which partially antagonized the bronchoconstrictor effects of LTD_4 in guinea-pigs had no effects on changes in R_{Aw} and C_{Dyn} induced by ACh 3.0 $\mu g\,kg^{-1}$ in these animals (Table 3).

Effects of nicardipine, verapamil and sodium cromoglycate on contracted guinea-pig trachea

In concentrations higher than 10^{-5}M, verapamil and nicardipine inhibited the contractile effects of ACh and LTD_4 on guinea-pig isolated trachea. The $-\log EC_{50}$ of these agents against ACh and LTD_4 were 4.75±0.14 (n = 5) and 4.10±0.16 (n = 4) respectively for verapamil and 4.76±0.09 (n = 5) and 4.65±0.09 (n = 4) respectively for nicardipine.

SCG did not inhibit the tracheal ring contractions induced by ACh or LTD_4 (Figure 1).

Table 2 Effects of six different agents on the decrease in pulmonary dynamic compliance induced by leukotriene D_4 0.5 $\mu g\,kg^{-1}$ in anaesthetized guinea-pigs

Agents		*Decrease in pulmonary dynamic compliance (%)*		
($mg\,kg^{-1}$)	n	*Before treatment*	*After treatment*	P
Nicardipine 0.03	4	54± 8.3	55± 6.9	>0.05
Nicardipine 0.1	5	74± 7.7	65± 5.7	>0.05
Nicardipine 0.3	6	74± 5.0	72± 4.3	>0.05
Nicardipine 1	6	68± 2.8	58± 9.2	>0.05
Verapamil 0.03	4	64± 9.8	71± 5.9	>0.05
Verapamil 0.1	4	64± 9.8	75± 6.8	>0.05
Verapamil 0.3	6	63± 4.9	51± 4.8	<0.05
Verapamil 1	6	63± 4.9	41± 6.1	<0.05
SCG 1	4	78± 8.3	74±13.0	>0.05
SCG 3	4	78± 8.3	66±12.8	>0.05
Atropine 1	10	70± 5.7	42± 3.5	<0.001
Atropine 1 + nicardipine 1	5	74± 6.9	34± 6.9	<0.001
Atropine 1 + verapamil 1	5	66± 9.5	53± 6.2	<0.001
Hexamethonium 10	5	73± 6.8	74± 8.0	>0.05
Propranolol 1	4	47±11.5	95± 0.6	<0.05

Each value represents the mean percentage decrease ± s.e.mean. n = number of guinea pigs in each group.

Table 3 Effects of nicardipine, verapamil and sodium cromoglycate (SCG) on the increase in pulmonary airway resistance (R_{Aw}) and decrease in pulmonary dynamic compliance (C_{Dyn}) induced by acetylcholine (3 $\mu g\,kg^{-1}$) in anaesthetized guinea-pig

Pulmonary airway resistance				
Agents		*Increase in R_{Aw} (%)*		
($mg\,kg^{-1}$)	n	*Before treatment*	*After treatment*	P
Verapamil 0.3	8	+165±24.3	+155±34.3	>0.05
Verapamil 1	8	+165±24.3	+112±35.4	>0.05
Nicardipine 0.3	8	+ 87±12.7	+ 97±15.5	>0.05
Nicardipine 1	8	+ 87±12.7	+ 82±11.9	>0.05
SCG 3	8	+122±34.7	+ 84±16.2	>0.05
SCG 10	8	+122±34.7	+ 81±24.1	>0.05
Dynamic compliance				
Agents		*Decrease in C_{Dyn} (%)*		
($mg\,kg^{-1}$)	n	*Before treatment*	*After treatment*	P
Verapamil 0.3	8	−67±3.4	−63±4.7	>0.05
Verapamil 1	8	−67±3.4	−51±9.1	>0.05
Nicardipine 0.3	8	−57±2.7	−62±1.6	>0.05
Nicardipine 1	8	−57±2.7	−53±2.3	>0.05
SCG 3	8	−63±4.1	−55±7.7	>0.05
SCG 10	8	−63±4.1	−50±7.9	>0.05

n = number of guinea-pigs in each group.

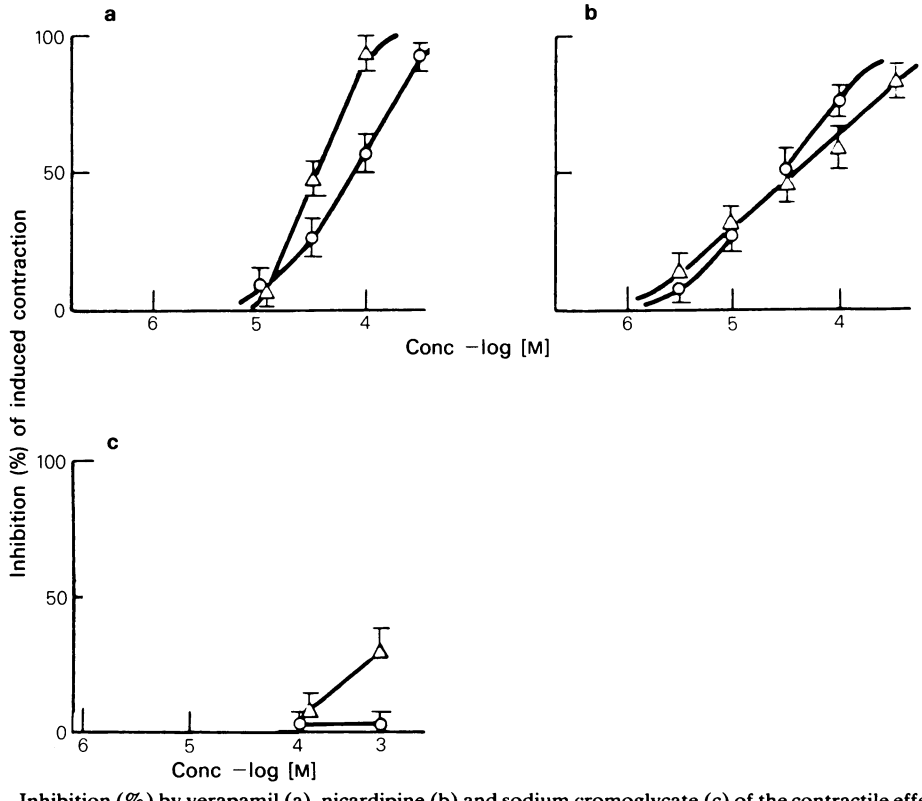

Figure 1 Inhibition (%) by verapamil (a), nicardipine (b) and sodium cromoglycate (c) of the contractile effects of acetylcholine (ACh, 2×10^{-5}M, O) and leukotriene D_4 (LTD_4, 1×10^{-7}M, △) on guinea-pig isolated tracheal rings. Values are expressed as mean with s.e.mean shown as vertical lines. Experiments were performed on groups of at least 4 preparations.

Discussion

The effects of propranolol, atropine and hexamethonium in the *in vivo* experiments show that the bronchoconstriction induced by LTD_4 in guinea-pigs involves at least two reactions. Propranolol considerably potentiated the increase in R_{Aw} and decrease in C_{Dyn} produced by LTD_4, thus pointing to an adrenergic reaction, possibly associated with catecholamine release from the lung (O'Donnell & Saar, 1973) or the adrenal medulla (James, 1967). Atropine significantly reduced the LTD_4-induced bronchoconstriction, as already reported with slow reacting substance of anaphylaxis (SRS-A, Drazen & Austen, 1975), thus demonstrating that LTD_4 induces a cholinergic reflex, probably by stimulation of bronchial irritant receptors, resulting in release of ACh which adds its effects to those of LTD_4.

The effects of LTD_4 on R_{Aw} was significantly reduced by nicardipine, verapamil and SCG, but its effect on C_{Dyn} was only reduced by verapamil. However, these substances were less potent than atropine, and showed no cumulative effect with the muscarinic antagonist.

Do calcium antagonists and SCG act by blocking the direct effects of LTD_4 on bronchial muscle receptors, or by inhibiting the LTD_4-induced vagal reflex? Some answers to this question are provided by comparing the effects of nicardipine, verapamil and SCG on the contractile actions of ACh and LTD_4 on guinea-pig isolated trachea.

SCG did not antagonize LTD_4 *in vitro*, which indicates that SCG acts on the vagal reflex; moreover, its site of action must be different from that of atropine, since it has no antagonistic effect on muscarinic receptors. Inhibition of the vagal reflex by SCG is also suggested by experiments in dogs. In this species, SCG reduced the bronchospasm induced by histamine aerosols (Jackson & Richards, 1977), as well as the frequency of electrical discharges induced by capsaicin in sensory nerve endings of the lung (Dixon, Jackson & Richards, 1980), but had no effect on the bronchoconstriction produced by stimulation of the distal end of the pneumogastric nerve (Jackson & Richards, 1977). SCG has also been reported to inhibit bronchospasms induced by inhalation of histamine, methacholine and SO_2 in asthmatic patients (Kerr, Govindaraj & Patel, 1970; Woenne, Kattan & Levison, 1979; Sheppard, Nadel & Boushey, 1981).

Since verapamil and nicardipine inhibited the contractile actions of LTD_4 and ACh on guinea-pig isolated trachea, direct antagonism between these two agents and LTD_4 and/or ACh at bronchial muscle receptor level may be suspected. However, this inhibitory effect only occurred at very high concentrations ($> 10^{-5}$M). Furthermore, in *in vivo* experiments verapamil and nicardipine (0.1 to 1 mg kg^{-1}) did not significantly alter the effects on R_{Aw} and C_{Dyn} of ACh. It therefore seems reasonable to conclude that verapamil and nicardipine have no direct action *in vivo* on LTD_4 receptors in bronchial smooth muscle. As these agents are unable to antagonize the direct actions of LTD_4 and of concomitantly released ACh on their respective bronchial muscle receptors, it may be hypothesized that they act by blocking the irritant receptor pathway. However, it is impossible to determine whether these products act on the efferent or afferent component of the irritant receptor pathway, and the only point established is that muscarinic receptors are not involved. The blockade might take place at irritant receptor level and may be related to a local anaesthetic action, as recently demonstrated at least with verapamil (Hay & Wadsworth, 1982). In addition, inhibition by verapamil, nicardipine and SCG of mast cell degranulation, as suggested for nifedipine (Patel & Al-Shamma, 1982), or of the production (or effects) of cyclooxygenase products under the influence of LTD_4 (Piper *et al.*, 1981) cannot be excluded.

Thus, the precise site and mode of action of these agents remain uncertain, Verapamil and nicardipine probably act by blocking calcium movements, but such a mechanism is questionable in the case of SCG, for which such an action has only been suggested in relation to its effects on rat peritoneal mast-cells (Foreman *et al.*, 1977).

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