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Effects of five different organic calcium antagonists on guinea-pig isolated trachea

C. Advenier, J. Cerrina*, P. Duroux**, Anne Floch & Annick Renier

Laboratoire de Pharmacologie, Faculté de Médecine Paris-Ouest et Institut Biomédical des Cordeliers, 15, rue de l'Ecole de Médecine, F-75270 Paris Cédex 06, Centre Chirurgical Marie-Lannelongue*, 133, avenue de la Résistance, F-92350 Le Plessis-Robinson and Service de Pneumologie**, Hôpital Antoine-Béclère, 157, rue de la Porte de Trivaux, F-92140 Clamart, France

1 The relaxant effects of five organic calcium antagonists (nicardipine, diltiazem, PY 108068, verapamil and bepridil) on guinea-pig isolated trachea were tested against contractions induced by acetylcholine, histamine, 5-hydroxytryptamine, potassium chloride (KCl) and tetraethylammonium (TEA) in a medium containing the normal amount of calcium and against calcium dose-response curves in a calcium-free, potassium-enriched medium. These effects were compared with those of spasmolytic or specific acetylcholine or histamine antagonists.

2 In contrast to the other drugs tested, organic calcium antagonists exerted a specific inhibitory effect on KCl- and TEA-induced contractions. Their degree of activity was in the order: nicardipine > diltiazem > PY 108068 > verapamil > bepridil. All calcium antagonists inhibited calcium dose-response curves at similar concentrations.

3 Organic calcium antagonists therefore seem to exert a specific inhibitory effect on depolarizing agents in the guinea-pig isolated trachea, unlike other tissues, notably some vascular smooth muscles.

Introduction

A relaxant effect of organic calcium antagonists on bronchial smooth muscle has been demonstrated both clinically in exercise-induced bronchospasm (Cerrina *et al.*, 1981; Barnes *et al.*, 1981; Patel, 1981) and experimentally against the bronchoconstriction induced by histamine, acetylcholine and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or by inhalation of antigen in sensitized animals (Fanta *et al.*, 1982; Malo *et al.*, 1982; Advenier *et al.*, 1983; Drazen *et al.*, 1983; Brugman *et al.*, 1983; Cerrina *et al.*, 1983).

The characteristics of individual organic calcium antagonists in their effects on bronchial smooth muscle i.e. relative activity, specificity of action against depolarizing agents, responsiveness of bronchial smooth muscle as opposed to other tissues, notably vascular smooth muscle, do not appear clearly from the published data; nor can they be inferred from experiments on non-bronchial preparations, since tissues are known to differ widely in their response to calcium antagonists (Van Nueten & Vanhoutte, 1981; Andersson *et al.*, 1983; Cauvin *et al.*, 1983).

In a attempt to determine these characteristics, we have investigated the relative activities of five different organic calcium antagonists, viz. verapamil (Fleckenstein *et al.*, 1971), diltiazem (Ito *et al.*, 1978; Fujiwara *et al.*, 1982), nicardipine (Takenaka *et al.*, 1976; Fujiwara & Kuriyama, 1983), PY 108068 (Hof, 1983) and bepridil (Labrid *et al.*, 1979), against contractions of the guinea-pig isolated trachea induced by histamine, acetylcholine (ACh), 5-hydroxytryptamine (5-HT), potassium chloride (KCl) and tetraethylammonium (TEA). In addition, we have compared, in the same preparation, the relaxant effects of calcium antagonists with those of a β-adrenoceptor agonist (isoprenaline), phosphodiesterase inhibitors (theophylline, amrinone; Meisheri *et al.*, 1980; Martorana *et al.*, 1982), parasympatholytic drugs (atropine), anti-histaminics (mepyramine, ketotifen), terfluzine and lanthanum chloride. Terfluzine has been reported to inhibit calmodulin (Stoclet *et al.*, 1981) and lanthanum chloride to antagonize calcium (Takata *et al.*, 1966; Foreman & Mongar, 1973).

Methods

Experimental procedure

Tracheal spirals were obtained from male guinea-pigs (250–350g) anaesthetized with urethane (1.25 g kg^{-1}, i.p.) and were equilibrated under an initial tension of 1.50g in a Tyrode solution at 37°C gassed with O_2. After 1.25 h equilibration, the resting tension was between 0.4 and 0.6 g. Under these conditions responses to agonists were reproducible (Stephens, 1970). Tension was measured isometrically with a Ugo Basil strain gauge and was displayed on Ugo basil pen recorder.

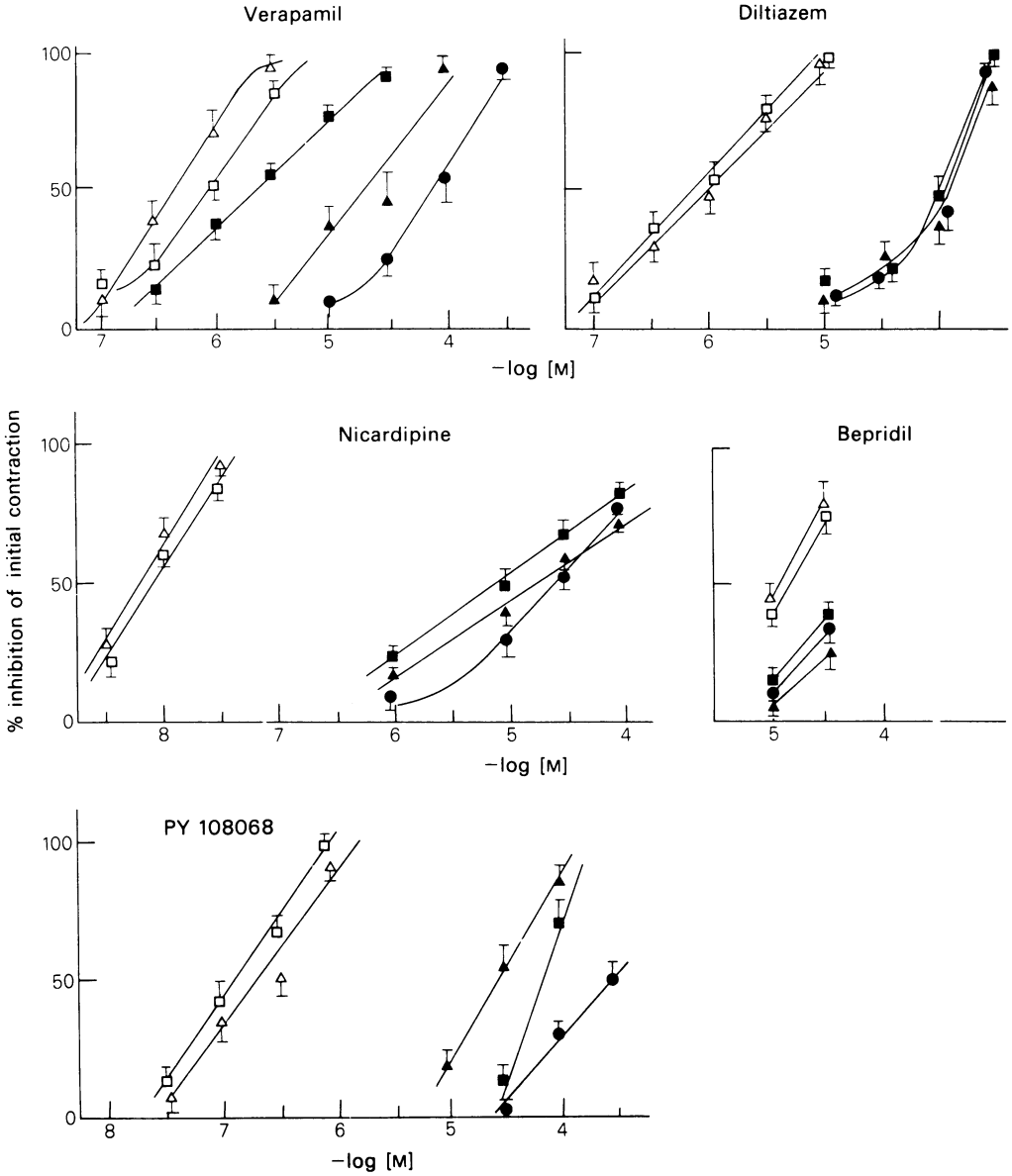

Figure 1 Inhibition by different organic calcium antagonists of the contractile effects of acetylcholine 2×10^{-5} M (●), histamine 2×10^{-5} M (▲), 5-hydroxytryptamine, 2×10^{-5} M (■), potassium chloride 3×10^{-2} M (□) or tetraethylammonium 10^{-2} M (△) on guinea-pig isolated trachea. Experiments were performed on groups of at least 5 preparations. Points represent mean with s.e.mean shown by vertical lines.

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, Na_2HPO_4 0.4 and glucose 5.5 (pH 7.46). Preparations were contracted to 90–100% of maximal tension with acetylcholine 2×10^{-5} M, histamine 2×10^{-5} M, 5-HT 2×10^{-5} M, TEA 10^{-2} M or potassium chloride 3×10^{-2} M.

With the exception of isoprenaline, each dose of the drugs tested was added to the bath 15 min before addition of a new dose of the contracting agent; 10–15 min were allowed for the response curve to plateau. Isoprenaline was added after the contractile effect had stabilized. The results are expressed as percentage inhibition of the contraction induced.

Ca^{2+} dose-response curves were established according to Godfraind *et al.*, (1968). Tracheal spirals were incubated for 1 h in a solution similar to the one just described but without $CaCl_2$, then for 15 min in $CaCl_2$-free solution in the presence of ethylenediaminetetraacetic acid 10^{-3} M. The preparations were washed at intervals of 15 min. In a second stage, the spirals were incubated in a calcium-free solution with additional K^+. The composition of the potassium-enriched solution was (mM): NaCl 109, KCl 30, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, Na_2HPO_4 0.4, and glucose 5.5 (pH 7.46). After incubation the dose-response curves of Ca^{2+} 0.01 to 3 mM were determined by cumulative addition. The drugs tested were added to the bath 15 min before addition of Ca^{2+}.

Drugs

The drugs used were: verapamil HCl (Biosédra, Paris), diltiazem HCl (Synthélabo, Paris), nicardipine HCl (Sandoz, Basel), PY 108068 (4-(2, 1, 3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethylpyridine-3, 5-dicarboxylic acid diethylester) (Sandoz, Basel), bepridil HCl (CERM, F-Riom), theophylline as sodium anisate (Bruneau, Paris), isoprenaline sulphate (Winthrop, Paris), amrinone (Winthrop, Paris), terfluzine di-HCl (Théraplix, Paris), lanthanum chloride (Sigma, St. Louis, USA), atropine sulphate (Prolabo, Paris), mepyramine maleate (Spécia, Paris), ketotifen fumarate (Sandoz, Paris), acetylcholine di-HCl (Lematte & Boinot, Paris), KCl (Prolabo, Paris), tetraethylammonium bromide (Sigma, St. Louis, USA), histamine HCl (Prolabo, Paris), 5-hydroxytryptamine as creatinine sulphate (Schuchardt, München) and calcium chloride (Prolabo, Paris).

Isoprenaline (Isuprel) and theophylline (Theophylline Bruneau) were used as proprietary injectable solutions. Amrinone and PY 108068 solutions were diluted in 90° ethanol and the other drugs in sterile water. Dilutions were made with the incubation fluid.

Statistical analysis of results

Statistical analysis of the results obtained was performed using Student's *t* test. All values in the text and table are expressed as mean ± s.e.mean.

Results

The effects of organic calcium antagonists on contractions induced by histamine, ACh, 5-HT, KCl or TEA in medium containing normal calcium

Table 1 $-\log IC_{50}$ values of 13 different drugs against the contractile effects of acetylcholine (ACh), histamine, 5-hydroxytryptamine (5-HT), potassium chloride (KCl) and tetraethylammonium (TEA) on guinea-pig isolated trachea

Drug	*ACh* 2×10^{-5} M	*Histamine* 2×10^{-5} M	*5-HT* 2×10^{-5} M	*KCl* 3×10^{-2} M	*TEA* 10^{-2} M	Ca^{2+} 10^{-3} M
Verapamil	4.17 ± 0.11	4.73 ± 0.16	5.74 ± 0.23	6.05 ± 0.06	6.34 ± 0.07	6.55
Diltiazem	3.91 ± 0.05	3.91 ± 0.03	4.02 ± 0.04	6.94 ± 0.07	7.05 ± 0.10	6.60
Nicardipine	4.53 ± 0.13	4.69 ± 0.06	5.02 ± 0.26	7.99 ± 0.06	8.26 ± 0.10	7.63
PY 108068	3.45 ± 0.33	4.50 ± 0.21	4.15 ± 0.09	6.50 ± 0.08	6.44 ± 0.14	–
Bepridil	< 4	< 4	< 4	4.81 ± 0.16	4.85 ± 0.06	–
$LaCl_3$	2.27 ± 0.03	2.22 ± 0.01	2.41 ± 0.09	2.28 ± 0.02	2.28 ± 0.04	–
Isoprenaline	6.81 ± 0.11	7.66 ± 0.11	7.31 ± 0.08	7.02 ± 0.08	–	7.59
Theophylline	3.51 ± 0.06	4.08 ± 0.10	3.69 ± 0.15	2.94 ± 0.08	2.89 ± 0.09	3.61
Amrinone	3.50 ± 0.11	4.48 ± 0.24	5.00 ± 0.14	4.32 ± 0.18	4.45 ± 0.15	–
Terfluzine	4.19 ± 0.11	5.19 ± 0.11	4.79 ± 0.05	5.05 ± 0.11	4.72 ± 0.11	–
Atropine	8.30 ± 0.16	4.42 ± 0.23	4.19 ± 0.26	4.57 ± 0.15	4.21 ± 0.24	4.16
Mepyramine	3.92 ± 0.16	8.87 ± 0.12	5.01 ± 0.21	5.34 ± 0.24	4.97 ± 0.05	5.05
Ketotifen	4.98 ± 0.11	8.56 ± 0.09	7.21 ± 0.08	5.22 ± 0.13	4.91 ± 0.05	–

Experiments were performed on groups of at least 5 preparations. Results are expressed as mean ± s.e.mean values, except for Ca^{2+} against which only one dose of each drug per trachea was tested.

(normocalcic) are depicted in Figure 1. The $-\log IC_{50}$ values calculated from inhibition curves are given in Table 1. The effects of the spasmolytic drugs tested and of lanthanum chloride ($LaCl_3$) on contractions induced by the same agents were investigated by the same method; $-\log IC_{50}$ values are also given in Table 1.

Calcium antagonists appeared to inhibit selectively KCl- or TEA-induced contractions, their relative activities being: nicardipine > diltiazem > PY 108068 > verapamil > bepridil. Much higher concentrations of these drugs were required to inhibit ACh-, histamine or 5-HT-induced contractions. The highest selectivity was observed with nicardipine, PY 108068 or diltiazem which were 100 to 1000 times more active against KCl or TEA than against ACh, histamine or 5-HT. In contrast, lanthanum, isoprenaline, theophylline, amrinone and terfluzine had similar inhibitory activities on the effects of the five contracting agents. The selective anti-acetylcholine effect of atropine and the selective antihistaminic effects of mepyramine and ketotifen were particularly clear on guinea-pig isolated tracheal spirals.

The effects of three calcium antagonists (verapamil, nicardipine and diltiazem) and those of theophylline, atropine and mepyramine on the calcium dose-response curves in the potassium-enriched medium are shown in Figure 2 and the corresponding $-\log IC_{50}$ values are given in Table 1. Calcium antagonists displaced dose-response curves to the right, but response maxima were depressed suggesting non-competitive antagonism. However, $-\log IC_{50}$ values were comparable to those measured for KCl- and TEA-induced contractions in the normocalcic medium.

Theophylline, mepyramine and atropine also displaced the calcium dose-response curves to the right and again, maximal responses were not sustained. $-\log IC_{50}$ values of these three drugs were similar to those calculated for the contractile action of the other agents in the case of theophylline, or corresponded to concentrations at which selectivity was lost in the case of atropine and mepyramine.

Discussion

One result of this study is to show that on guinea-pig isolated trachea the five organic calcium antagonists tested exert a highly specific inhibitory effect on the contractile action of depolarizing agents, such as KCl or TEA (Kroeger & Stephens, 1975), an action known to be strictly dependent on extracellular Ca^{2+} (Coburn, 1977; Kirkpatrick, 1975; Foster *et al.*, 1973; Cerrina *et al.*, 1983). In this respect, calcium antagonists clearly differ from spasmolytic agents,

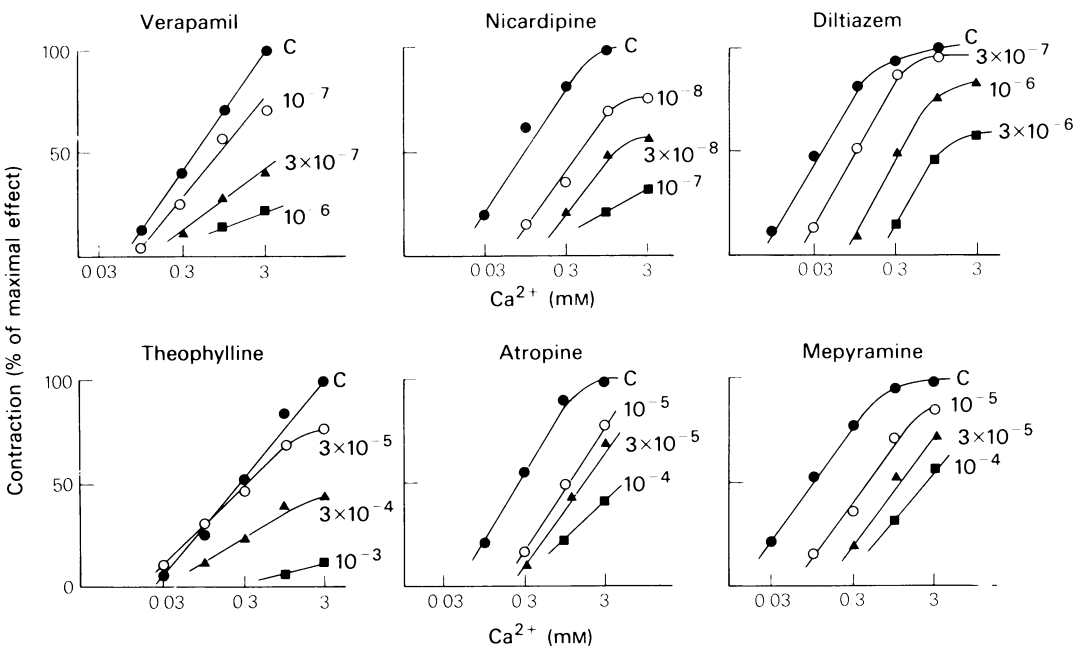

Figure 2 Influence of 6 different drugs (molar concentrations indicated) on calcium dose-response curves in guinea-pig tracheal spirals in a potassium-rich solution (30 mM) (Godfraind *et al.*, 1968, method). Experiments were performed on groups of at least 5 preparations. Points represent means; standard errors were less than 12.5% of mean in every case.

such as isoprenaline, theophylline or amrinone, and from terfluzine, which at similar concentrations inhibited the contractile actions of all agents used in this study; they also clearly differ from specific antagonists of ACh (atropine) or histamine (mepyramine, ketotifen).

These highly selective effects of calcium antagonists on KCl- and TEA- induced contractions were observed at concentrations which displaced the Ca^{2+} dose-response curves to the right in depolarizing medium. However, in contrast to the results obtained by Fleckenstein (1981) on heart muscle, the reduction of maximal responses indicated non-competitive antagonism. Non-competitive antagonism between calcium antagonists and Ca^{2+} has also been reported by Schümann *et al.*, (1975) and by Godfraind (1983) working on vascular smooth muscle of rabbits and rats and has been explained by an inhibitory effect of calcium itself in high concentrations on smooth muscle contraction. In our study, calcium was antagonized by spasmolytic drugs at the same concentrations which inhibited the contractile actions of the other agents, and by ACh and histamine antagonists at concentrations so high that these drugs had lost their specificity.

The specificity of calcium antagonists against KCl, TEA and Ca^{2+} as opposed to other contracting agents was highest with nicardipine, diltiazem and PY 108068, moderate with verapamil and low with bepridil.

The inhibition by calcium antagonists of contractions induced by histamine, 5-HT or ACh may be ascribed to an action of these drugs on other sites or movements of Ca^{2+} ions, notably intracellular Ca^{2+}, as has been suggested for diltiazem by Saida & Van Breemen (1983) for skinned rabbit mesenteric artery.

Numerous studies have shown that responses to calcium antagonists vary from one tissue to another. Our results suggest that the response of guinea-pig tracheal muscle to these drugs is similar to the response of those vascular muscles for which a selective effect on KCl-induced contraction has been demonstrated, e.g. rat aorta and mesenteric artery, dog coronary and mesenteric arteries and rabbit aorta and basilar artery (Brockaert & Godfraind, 1979, Shimizu *et al.*, 1980; Kondo *et al.*, 1980; Cauvin *et al.*, 1983), but differs from other vascular muscles in which calcium antagonists do not act selectively on KCl-induced contraction, also inhibiting contractions induced by noradrenaline, $PGF_{2\alpha}$ or 5-HT, e.g. rat basilar and renal arteries, dog cerebral artery, rabbit renal artery and rabbit and human mesenteric artery (Cauvin *et al.*, 1983; Mikkelson *et al.*, 1978).

However, the potency of calcium antagonists on guinea-pig tracheal muscle appears to differ from that observed in vascular smooth muscle. Thus, verapamil seems to be less active against KCl in guinea-pig trachea ($-\log IC_{50} = 6.05$) than in dog coronary artery ($-\log IC_{50} = 6.70$, Shimizu *et al.*, 1980). Similarly, $-\log IC_{50}$ values of 8.96, 8.20 and 8.51, respectively, have been reported with nicardipine in dog coronary artery, mesenteric artery and inferior vena cava (Eglen *et al* 1983), as against 7.99 in our study.

Comparisons between the five calcium antagonists tested by us showed that their order of activity was: nicardipine > PY 108068 > diltiazem > verapamil > bepridil. The rank order seems to be different in other experimental models, verapamil, for instance, being more active than diltiazem in cat cerebral and mesenteric arteries (Andersson *et al.*, 1983).

Although described as a (mineral) calcium antagonist (Takata *et al.*, 1966; Foreman & Mongar, 1973), in our experiments lanthanum chloride had none of the specific inhibitory effects on depolarizing agents observed with organic calcium antagonists. Moreover, it acted only in concentrations much higher than those used, for example, in guinea-pig vas deferens or taenia coli (Weiss & Goodman, 1969; Hay & Wadsworth, 1982). The lack of selectivity against KCl- or TEA-induced contraction of the tracheal muscle suggests that lanthanum chloride exerts a non-specific action on Ca^{2+} movements across the cell membrane or within the cell.

To conclude, it appears from this study that in the guinea-pig isolated trachea, organic calcium antagonists behave differently from spasmolytics, calmodulin inhibitors or specific ACh or histamine antagonists. In particular, their effect on KCl- or TEA-induced contraction closely correlates with their effect on extracellular Ca^{2+}. In addition, our study demonstrates that their smooth muscle relaxant activity is less pronounced in guinea-pig trachea than in other preparations, thus confirming that tissues vary widely in their response to this category of drug.

Reprint requests to C.A. please.

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