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PRELIMINARY COMMUNICATIONS

CALCIUM-ANTAGONISTIC EFFECTS OF L 9394 * ON VASCULAR AND VENTRICULAR MUSCLE

Peter Polster and Michel de Clavière

S.A. LABAZ N.V. 1, avenue de Béjar 1120 Brussels

L 9394 (4-[3-(dibutylamino)propoxy]phenyl] (2-ethyl-3-indolizinyl)-methanone.monohydrochloride has both competitive and non-competitive antagonistic effect on noradrenaline-induced contraction of the isolated aorta of rat ; it also inhibits the response to potassium-induced depolarisation (1). It therefore probably counteracts the intracellular penetration of calcium. This is why we have examined the antagonistic effect of L 9394 in relation to calcium in vascular smooth muscle and cardiac muscle.

METHODS

1) Experiments on the isolated aorta of rat

The thoracic aorta of male rats weighing about 300 g. is cut spirally and placed in a modified Krebs solution, oxygenated, and kept at 37°C. An initial tension of 2 g.is applied to the organ and its contractions are recorded by means of an isometric force transducer. After a 90-minute stabilisation period, the preparation is bathed successively in a modified Krebs solution without calcium for 30 minutes, then in a potassium solution for 10 minutes. Calcium chloride is then injected into the potassium solution and its contractile effects are recorded in the form of a cumulative curve. Two cumulative curves are made with an interval of 120 minutes, L 9394 being added to the bathing medium 40 minutes before recording the second curve ; each preparation is used only for the study of a single concentration of L 9394. Preliminary tests showed that the period of 40 minutes is sufficient for development of the maximal activity of L 9394. As contracting effects of calcium are reproducible in our conditions, they are expressed in percentage of the maximal effect obtained at the time of examination of the first dose-action curve.

2) Experiments on an isolated right ventricular strip of guinea-pig

A strip, about 15 mm long and 2 mm wide, is excised from the right ventricular wall of the heart of guinea-pigs weighing about 430 g. It is placed at 32°C in a modified Locke solution, oxygenated, and supplemented with different calcium concentrations. An initial tension of 500 mg is applied to the preparation which, during the whole experiment, is stimulated electrically at a frequency of 1.5 Hz by rectangular pulses of 5 ms. and a double voltage of the threshold voltage. The contractions are recorded by means of an isometric force transducer. After a period of 60 minutes intended to ensure stabilisation of the preparation, the strength of the contractions is measured ; then L 9394 is added to the bathing medium which is left in contact with the preparation for 60 minutes.

* *Butoprozine Labaz*

At the end of this period the strength of the contractions is again recorded and the new value obtained is expressed in percentage of that obtained before the addition of L 9394. Each preparation is used for the study of a single concentration of $CaCl_2$ and L 9394.

COMPOSITION OF SOLUTIONS

- Modified Krebs (in mmol) NaCl 122 ; $NaHCO_3$ 15 ; KCl 5.9 ; $NaCl_2$ 1.25 ; $MgCl_2$ 1.25 ; Glucose 11 ; distilled water q.s.p. 1000 ml.
- Depolarising potassium solution (in mmol) NaCl 22 ; $NaHCO_3$ 15 ; KCl 100 ; $MgCl_2$ 1.25 ; Glucose 11 ; distilled water q.s.p. 1000 ml.
- Modified Locke solution (in mmol) NaCl 125 ; KCl 5.6 ; $NaHCO_3$ 25 ; Glucose 11 ; and $CaCl_2$ in different concentrations ; 1.08 ; 1.80 ; 2.16 ; 3.60 ; 5.40 ; 8.10 ; distilled water q.s.p. 1000 ml.

STATISTICAL ANALYSIS

For each series of measurements, the means and their standard error are calculated ; comparisons of means are made by Snedecor " F " test or by Student's " t " test. Regression lines were traced using the method of least squares. The comparison of slopes of regression lines is made by Student's " t " test, applied to the comparison of two observed regression coefficients (2).

RESULTS

1) The effect of L 9394 on calcium contractile response in isolated rat aorta depolarised by potassium

The results illustrated by figure 1 showed that calcium induced contraction is less in the presence than in the absence of L 9394. The calcium dose-response curves had the shape of a stretched sigmoid with a maximum between 10 and 20 mM of Ca^{++}. Beyond that, any administration of calcium was followed by a depression of the mechanical response, due to an auto-inhibitory effect of heavy concentrations at the level of the membrane permeability of cells for this ion (3,4). Increasing doses of L 9394 induced parallel shift of the dose-response curves of Ca^{++} to the right.

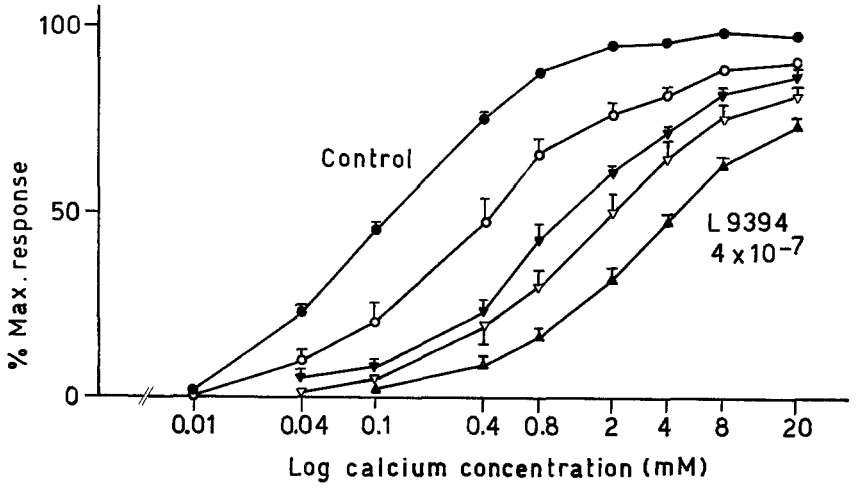

Fig. 1. Cumulative $CaCl_2$ dose-response curves in isolated rat aortic strips incubated in depolarizing Krebs solution (100 mM K^+) in the presence of L 9394. Key : (●—●) control ; (o—o) 4 x 10^{-8}M L 9394 ; (▼—▼) 10^{-7}M L 9394 ; (▽—▽) 2 x 10^{-7}M L 9394 ; (▲—▲) 4 x 10^{-7}M L 9394. Each point represents the mean value ± S.E.M. for 4 to 7 determinations.

Comparison of regression lines obtained for the different doses of L 9394 showed that the slopes were not significantly different (the comparison relates to the linear part of the dose-response curves, between 20 % and 80 %). These regression lines were therefore parallel, suggesting the existence of an antagonism of the competitive type between calcium and L 9394. This was confirmed by the fact that the means of the pA_2 parameter (5) calculated for each experiment were not significantly different from one concentration of L 9394 to another. Moreover, according to Arunlakshana and Schild (6), calculation of the weighted log (X - 1) regression line with reference to the negative logarithm of the molar concentrations of L 9394, showed that the line obtained ($y = 1.0023\ x + 7.903$) had a slope ($- 1.0023 \pm 0.106$) which was not significantly different from - 1 (X represents the ratio of equiactive doses of calcium in the presence and absence of L 9394). Thus the antagonism of L 9394 with calcium may be regarded as being of the competitive type in the aorta of rat.

2) Action of L 9394 on an isolated right ventricular strip of guinea-pig stimulated electrically at a constant frequency

L 9394, for a specific concentration of calcium in the medium reduced the contractile force of an isolated strip of the right ventricle of guinea-pig, stimulated electrically at a constant frequency (fig. 2). In addition, figure 2 showed that the negative inotropic effect of L 9394 was more pronounced at the lower calcium levels.

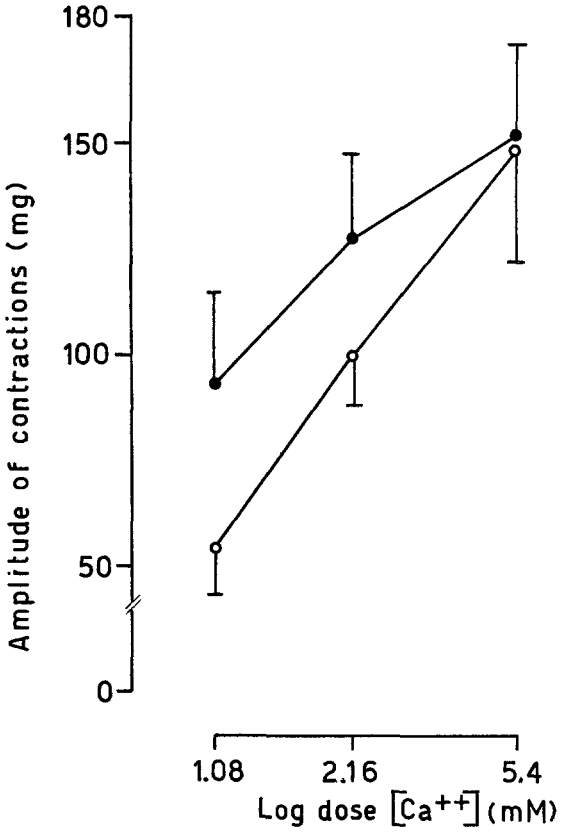

Fig. 2. Effect of increasing $[Ca^{++}]_o$ on the amplitude of contractions in the isolated right ventricular strip of the guinea-pig stimulated at 1.5 Hz in the absence (●——●) and the presence of L 9394 (10^{-5}M) (o——o). Each point represents the mean value ± S.E.M. for 3 to 6 determinations.

The L 9394 dose response curves were shifted in a parallel manner to the right in the presence of increasing concentrations of calcium. Comparison of the slopes of the regression lines showed that they were not significantly different. Regression lines were therefore parallel, which suggested the existence of an antagonism of the competitive type between L 9394 and calcium ions in the cardiac muscle of guinea-pig (fig. 3).

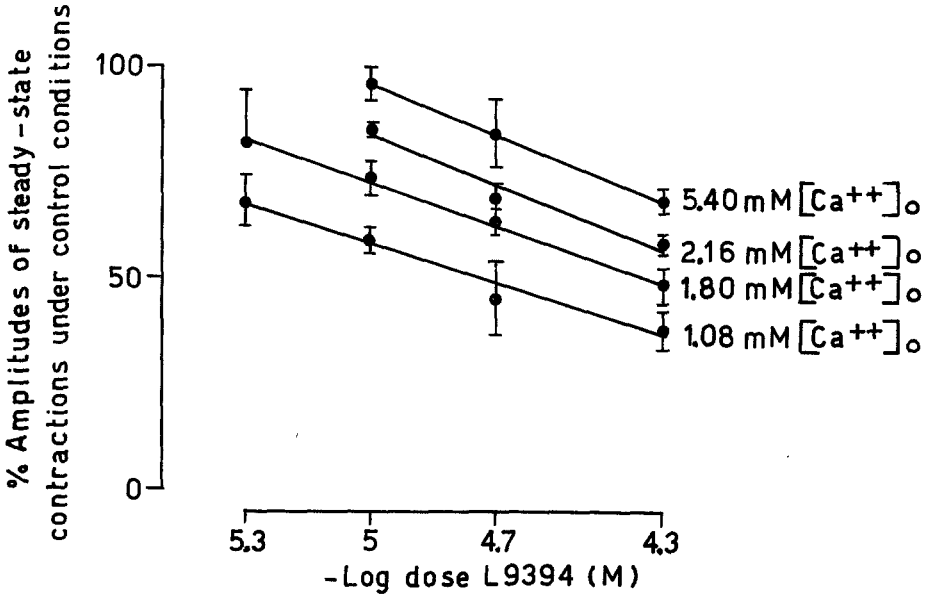

Fig. 3. Contractile force of isolated right ventricular strips of the guinea-pig stimulated at 1.5 Hz at varying extracellular calcium concentrations and at different L 9394 concentrations. Each point represents the mean value ± S.E.M. for 3 to 6 determinations.

DISCUSSION

Our results show that L 9394 has an antagonistic activity on calcium-induced vascular smooth muscle contraction. Moreover its negative inotropic action on cardiac muscle can be overcome by increasing the concentration of calcium in the bathing medium. Since, in the two preparations, the mechanical response is proportional to the intracellular concentration of ionized calcium, itself function of the availability of extracellular calcium (7, 8, 9, 10, 11), L 9394 probably acts on cell membranes by producing a conformational change which alters either the calcium transport or calcium storage capacity of these membranes. In this way it reduces the amount of calcium that enters the cell during the pleateau phase of the action potential and is required for the activation of the mechanical response. A similar activity has been described for drugs of the Verapamil type (12). Thus L 9394 can be considered as a " calcium antagonist ". This property may be of interest in angina pectoris and supraventricular tachycardias as proved with drugs of the Verapamil and Nifedipine type. The haemodynamic profile of L 9394 (13) is that of amiodarone (a classical drug in angina pectoris and arrhythmia) but amiodarone is devoid of any appreciable effect on contractile response to K^+ depolarization in rat aortic strip and cannot be considered as a calcium antagonist (14).

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