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Effect of levamisole hydrochloride on the guinea-pig atrium*

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Abstract

The effects of levamisole on the guinea-pig atrial preparation were determined. At 3 µg/ml, levamisole and lignocaine prevented electrically induced arrhythmia in 3 and 5 out of 5 preparations respectively. It was concluded that levamisole at the therapeutic anthelmintic dose would not abolish clinical atrial arrhythmia. Levamisole even at 3 µg/ml had definite salutary effect on the hypodynamic state induced by continuous electrical stimulation. The dose-related positive inotropic effect of levamisole 5-200µg/ml was not antagonised by practolol but was absent in atria from reserpinised animals. Therefore, cAMP may not be involved in the positive inotrópic effect. Levamisole antagonised verapamil-induced negative inotropic effect and no positive inotropic effect was observed when the Ca^{2+} content of the Ringer-Locke solution was below normal. These suggested that Ca^{2+} must be involved in the inotropic effect. The negative chronotropic effect due to levamisole was not antagonised by hexamethonium but was antagonised by atropine, thus indicating that stimulation of M_1 or M_2 receptors in the atria may be responsible.

Extrait

On a déterminé les effets due Levamisole sur une préparation de l'orifice de l'oreillette d'un cochon d'Inde. A une dose de 3µg/ml de Levamisole et la Lignocaine ont prévenu une arythmie produite électriquement dans trois cas sur cinq et cinq cas sur cinq respectivement. On a conclu que le Levamisole, à une dose thérapeutique, d'anthelmitique no supprimerait pas une arythmie clinique de l'orifice de l'oreillette. Même à une dose de 3µg/ml, le Levamisole à un effet salutaire sur un état hypodynamique provoqué par une excitation électrique continue. L'effet inotropique positif provoqué par une dose de Levasimole de 5-200µg/ml na pas ete oppose par le Practolol mais n'apparaissait pas à l'orifice de l'oreillette d'animaux "reserpinised". Cependant, cAMP ne peut jouer un rôle dans l'effet inotropique positif. Le Levamisole s'est opposé à l'effet inotropique négatif provoqué par le verapamil et aucun effet inotropique positif n'a été observé quand le contenu en Ca2+ de la solution de Ringer-Locke était en dessous de la normale. On peut penser que Ca2+ peut jouer un rôle dans l'effet inotropique. L'Hexamethonium ne s'est pas opposé a l'effet chronotropique négatif provoqué par le Levamisole contrairement à l'atropine, ce qui indique que la stimulation des recepteurs M1 or M2 de l'orifice de l'oreillette peut être responsable.

Introduction

Levamisole, a standard anthelmintic, was shown to have antiarrhythmic effect on ventricular dysrhythmia induced with ouabain in the toad or with $BaCl_2$ in the rat[1]. Its effect on experimental atrial arrhythmia was however, not investigated. The present study therefore investigated in the first instance the effect of levamisole on electrically-induced atrial arrhythmia and later, the effect of the drug on the contractility of the atrium in the spontaneously beating and electrically driven guinea-pig atrial preparations.

Materials and methods

Ninety-nine guinea-pigs of either sex weighing between 200 and 400g were used. The animal was killed by a blow on the head and the heart was quickly removed and placed in a dish containing Ringer-Locke (RL) solution aerated with oxygen and of the following composition (g/litre) NaCl, 9.0; KCl, 0.4; $CaCl_2$, 0.24; $NaHCO_3$, 0.5; dextrose, 2.0. The atria were dissected free from fat and other tissues and then attached to an aerator at one end and suspended in a 50ml organ bath containing RL

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solution maintained at 30-31°C and aerated with oxygen. The other end of the atrial preparation was then attached to a Starling writing lever. The weight on the tissue was 0.5g. A period of 30 min was allowed for equilibration before drugs were added.

The following studies were done:

1. *Antiarrhythmic effects of levamisole and lignocaine on electrically-induced arrhythmia*
Twenty-five preparations were used: 5 for each drug concentrations. The preparation was mounted in such a way as to allow electrical stimulation of the tissue through electrodes attached to the lower end of the preparation. After the period of equilibration and a constant height and rate was attained, the tissue was then stimulated for 20 sec at 2-4Hz, 5msec, 5-7.5 volts and the contractions recorded. The bathing fluid was then changed to one containing 1/4 of the K^+ concentration of the normal RL solution. The tissue was then incubated for 10 min after which it was stimulated for 20 sec. If arrhythmia was not induced, the tissue was discarded but if arrhythmia was induced, the bath was drained and then refilled with normal RL solution. When the contraction became regular, the tissue was allowed to rest for 10 min before changing the bathing fluid once again to the low K^+ solution. Levamisole or lignocaine was added to the bath to give a drug concentration of 1, 3 or 10μg/ml. The tissue was then incubated for 10 min after which it was stimulated electrically as described to see if that drug concentration would prevent the electrically-induced arrhythmia. Each tissue was used once and for one drug concentration only. In each experiment, the voltage and frequency were kept constant.

2. *Effect of levamisole (3 or 50μg/ml) on hypodynamic state (atrial muscle fatigue) induced by continuous electrical stimulation.*
Ten preparations were used; 5 for each concentration. The preparation was continuously stimulated at 2Hz, 5msec and 5 volts. After 30-60 min of stimulation, severe hypodynamic state occurred and while still stimulating, levamisole was added to the RL solution to give a drug concentration of 3 or 50μg/ml. Its effect on the hypodynamic state was then observed over a 30 min period with the electrical stimulation still in progress.

3. *Effect of levamisole on spontaneously beating preparation.*
(a) The effect of various concentrations of levamisole on the force and rate of atrial contractions were studied in 5 preprations and log dose-response curves were constructed. Each drug concentration was allowed to act for 2 min but 5 min recovery time was allowed before studying the effect of the next concentration. In another 5 preparations, the effect of practolol 1μg/ml on the positive inotropic effect of levamisole was then studied. To satisfy ourselves that the sample of practolol was still potent and for comparison, the effect of practolol on adrenaline-induced inotropic effect was studied on the same preparation.
The cumulative dose-responses in 5 atrial preparations from reserpinised guinea-pigs were then examined. The guinea-pigs were injected intraperitoneally with reserpine (serpasil) 4mg/kg 24 hr before sacrifice. This procedure according to Crout *et al.*[2] and Carrier and Jurevice[3] would deplete the heart of its catecholamine stores.
Next, the effects of hexamethonium 0.2μg/ml (5.5×10^{-7}M) or atropine 0.2μg/ml (2.88×10^{-7}M) on the cumulative dose-responses due to levamisole were studied in 10 preparations; each preparation was used as its own control. For comparison and to verify the potency of the sample of hexamethonium its effect at (0.2μg/ml) on nicotine-induced increases in the height of contraction was studied.

(b) Effect of varying calcium concentrations and verapamil on levamisole-induced contractions — The effect of varying the Ca^{2+} content of the RL solution on the cumulative dose-responses due to levamisole was also studied in 24 preparations. Eight preparations each were studied in Ca^{2+} free or normal RL solutions; 4 preparations each were in RL solution with 1/4 or 1/2 Ca^{2+} content.
Cumulative dose responses were studied in 5 preparations. Each concentration was allowed 5 min contact time and without draining the bath, more levamisole was added to give higher and higher concentrations so that the effect of

cumulative doses could be studied. Next, the effect of cumulative dosing with levamisole on verapamil (0.1μg/ml)-induced negative inotropic effect was studied in another 5 preparations. Verapamil was allowed to act for 10 min before adding levamisole.

Averages were expressed as arithmetic means ± standard error of the mean (SEM). Statistical analysis was performed using students' *t*-test (paired).

Results

Effect of levamisole and lignocaine on electrically induced arrhythmia

Arrhythmia was considered to have been induced if one or more of the following were present: irregularity in the pattern of atrial contractions; irregularity in the height of contractions; and irregularity in the rhythm of contractions. During stimulation in normal RL solution, the rate of contractions was the same as the rate of stimulation and there were no irregularities in the height or pattern or rhythm of the contractions. When the bathing fluid was changed to low K^+ RL solution, stimulation in the absence of any drug resulted in gross irregularity in the height, pattern and rhythm of the contractions.

Lignocaine (1μg/ml) prevented the induction of arrhythmia in 2 out of the 5 preparations studied. However, when the concentration of lignocaine was 3μg/ml it was not possible to induce arrhythmia in the 5 preparations studied. It was not possible to induce arrhythmia in the presence of levamisole (10μg/ml) in the 5 preparations studied (Fig. 1). At 3μg/ml, levamisole prevented the induction of arrhythmia in 3 out of the 5 preparations studied and at 1μg/ml it was successful in only 2 out of the 5 preparations studied.

Fig. 1: Effect of levamisole 2μg/ml (Panel A) and 10μg/ml (Panel B) on electrically- induced arrhythmia. Time scale refers to fast drum speed.

Effect of levamisole on hypodynamic state (fatigue) induced by continuous electrical stimulation

Levamisole even at 3μg/ml had salutary effect on the severe hypodynamic state induced by continuous electrical stimulation in spite of continuing the electrical stimulation. The height of contraction at equilibrium was attained within 25min of adding the drug. Fig. 2 shows the fairly rapid complete reversal of the hypodynamic state by levamisole 50μg/ml.

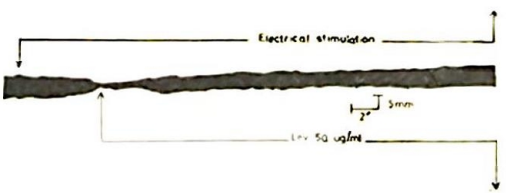

Fig. 2: Effect of levamisole 50μg/ml on atrial muscle fatigue induced by continuous electrical stimulation of the isolated guinea-pig atrium. The electrical stimulation was continued throughout the period of the experiment.

Spontaneously beating atria

Levamisole induced concentration-dependent responses in the height of contractions. At 10μg and 50μg/ml it caused respectively, average percentage increases of 62.50 ± 23.9 and 115 ± 69.9 above the value at equilibration. The average percentage increase at 200μg/ml was however slightly lower than the value at 50μg/ml. At much higher concentrations, dose-dependent reduction in height resulted. Table 1 shows the effect of various concentrations on the height of contraction. On rate, levamisole induced concentration-dependent reductions. At 10μg/ml, it caused an average percentage reduction of 7.1 ± 0.8 and at 600μg/ml the average percentage reduction from the value at equilibration was 35.1 ± 3.1. Nearly complete asystole however occurred at 1mg/ml. Practolol (1μg/ml), did not inhibit the inotropic effect of levamisole (50μg/ml) but abolished the inotropic effect due to adrenaline (Fig. 3).

Table 1: Isolated guinea-pig atria preparation: Effect of various concentrations of levamisole on the height of contraction. Results are expressed as percentage change in height over the height at equilibration. $n = 5$

Levamisole μg/m	% change in height 1	2	3	4	5	Average ± SEM
10	0	+100	+50	+100	+63	+62.50 ± 18.54
50	-40	+300	+100	+100	+115	+115.00 ± 54.18
200	-	+66.7	-50	+150	+55	+55.48 ± 31.77
600	-	-45	-75	-60	-60	-60.0 ± 8.66
1000	-	-100	-100	-100	-100	-100.0 ± 0.0

Fig 3: Effect of practolol 1μg/ml on levamisole and adrenaline-induced positive inotropy.

Levamisole did not produce any positive inotropic effect in the 5 atrial preparations from reserpinised guinea-pig. Instead, it induced dose-dependent reductions in the height of contractions. However, as in non-reserpinised hearts, levamisole reduced the rate of contractions, dose-dependently. Fig. 4 shows the lack of inotropic action of levamisole even at 50μg/ml in a reserpinised heart.

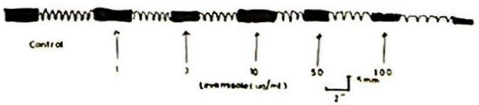

Fig 4: Absence of the positive inotropic effect from cumulative dosing with levamisole in a guinea-pig atrial preparation from animal pretreated with reserpine 4mg/kg i.p. 24 hr earlier. Time scale refers to fast drum speed.

Atropine 0.2μg/ml while not having an effect of its own on atrial rate reduced the degree of bradycardia induced by levamisole. The differences between the values in the presence and absence of atropine were however not statistically significant. Hexamethonium 0.2μg/ml had no effect on changes in the rate and height of atrial contractions induced by levamisole. However it reduced the average percentage increase in height due to nicotine from 270.0 ± 57.9 to 53.3 ± 34.6 ($P < 0.05$).

Cumulative concentrations of levamisole induced concentration- dependent increases in the height of contraction, reaching its maximum at 50μg/ml with an average percentage increase of 108% above the value at equilibration, but at 1mg/ml complete asystole almost invariably resulted. There was also progressive reduction in rate of atrial contraction as the concentration rose. It was observed that levamisole at 200μg/ml and above, induced arrhythmia almost invariably. Fig. 5 shows the results in a typical experiment and shows the arrhythmia and the very slow rates induced at 200μg/ml and the almost complete asystole at 1mg/ml.

On the effect of the Ca^{2+} concentration in the RL solution on the cumulative dose responses to levamisole, it was found that levamisole was unable to produce any inotropic effect in the atria suspended in Ca^{2+} free, 1/4 Ca^{2+} or 1/2 Ca^{2+} RL solution. Table 2 shows the results obtained at different Ca^{2+} concentrations.

Fig. 5: Effect of cumulative dosing with levamisole on the height, rate and rhythm of contraction of the spontaneously beating isolated guinea-pig atria preparation.

Table 2: Isolated guinea-pig atria preparation: Effect of varying calcium concentrations on cumulative dose-responses (in mm) due to levamisole. Results are expressed as means ± SEM. *Statistically significant difference from control value ($P < 0.05$). n = number of preparations.

Ca^{2+} Concentration	Levamisole (μg/ml)					
	0	1	3	10	50	100
Ca^{2+} Free	0.32	0.15	0.15	0.15	0.15	0.07
$n = 8$	±0.16	±0.08	±0.08	±0.08	±0.08	±0.04
1/4Ca^{2+}	0.80	0.38	0.25	0.25	0.50	0.25
$n = 4$	±0.45	±0.38	±0.25	±0.25	±0.29	±0.25
1/2 Ca^{2+}	1.63	1.25	1.00	0.75	1.00	0.88
$n = 4$	±1.46	±1.25	±1.00	±0.75	±0.54	±0.43
Full Ca^{2+}	2.18	2.5	2.34	2.44	3.81	4.94*
$n = 8$	±0.38	±0.50	±0.42	±0.49	±0.65	±1.09

The spontaneous atrial contractions were abolished in the presence of verapamil (0.1μg/ml) but when levamisole was added to the bathing fluid, the contractions became recordable when the concentration of levamisole was as low as 10μg/ml. Fig. 6 shows the effect of various concentrations of

levamisole on the depressed spontaneous atrial contractions induced by verapamil (0.1μg/ml).

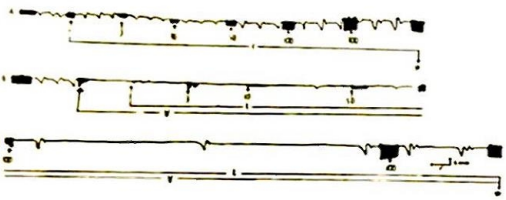

Fig. 6: Effect of cumulative dosing with levamisole (L) in μg/ml, on verapamil 0.1μg/ml (V) induced negative inotrpic effect in the isolated spontaneously beating guinea-pig atria preparation. Panel A shows the concentration-dependent positive inotropic effect of levamisole. Panels B and C show the concentration-related reversal of the cardiac depressant action of verapamil by levamisole. Time scale refers to fast drum speed.

Discussion

Levamisole (10μg/ml) and lignocaine (3μg/ml) prevented the genesis of electrically- induced atrial arrhythmia in isolated guinea-pig atria suspended in very low K^+ concentration RL solution. However, the therapeutic plasma concentration of lignocaine in the treatment of ventricular arrhythmia in man is between 1- 3μg/ml. At this plasma concentration, lignocaine does not revert atrial arrhythmia to sinus rhythm. Therefore, levamisole even at 10μg/ml will not be expected to have salutary effects on atrial arrhythmia in clinical practice. A single oral dose of levamisole of 150mg or 450mg which would yield 86 and 100% cure rates respectively[4] would be expected to produce peak plasma levels of 0.5 and 1.5μg/ml respectively[5]. Therefore, even at the highest therapeutic anthelmintic dose, levamisole would not be expected to have any antiarrhythmic effect on atrial arrhythmia. However, the salutary effect of levamisole at 3μg/ml on the hypodynamic or fatigued atrial muscle as demonstrated in the present study is noteworthy. If this positive inotropic effect is also demonstrated on ventricular muscle, then levamisole at the oral anthelmintic therapeutic dose would have some potential value in the treatment of myocardial failure associated with atrial arrhythmia especially as some concomitant drug-induced fall in atrial and therefore ventricular rate would be expected to occur.

Levamisole at lower concentrations (3-200μg/ml) induced dose dependent positive inotropic effect. Pretreatment with practolol at a concentration which completely inhibited the positive inotropic effect of adrenaline did not affect this positive inotropic effect of levamisole. This would indicate that β adrenoceptor stimulation is not responsible for the positive inotropic effect. However, levamisole was shown in the present study, not to have any positive inotropic effect in the reserpinised guinea-pig at a concentration that would have produced very considerable increases in the force of contractions in non-reserpinised atrial. It has been shown by Crout *et al.*[2]. Hudgins and Flemming[6] that reserpinised hearts are depleted of their catecholamine stores. Such reserpinised hearts and vascular tissues may exhibit supersensitivity to exogenous adrenaline but respond poorly (i.e. subsensitivity) to indirect acting sympathomimetics[6,7]. It can thus be concluded that catecholamine release may be involved in the positive inotropic action of levamisole but that cAMP is not involved. Alpha-adrenoceptors are known to be present in the heart[8] but their stimulation induce both positive inotropic and chronotropic responses with inotropic responses being more predominant[9]. However, the inotropic response is not associated with increase in cAMP level in the heart[10]. It is also of some interest that Hadden *et al.*[11] showed that levamisole increases intracellular cGMP levels in lymphocytes. Studies of the effect of alpha-adrenoceptor antagonists on levamisole-induced positive inotropic effect and the effect of levamisole on cAMP and cGMP levels in the heart may therefore yield useful information.

In the spontaneously beating atrial, levamisole at 3μg and 1000μg/ml caused concentration dependent reduction in rate which was not antagonised by hexamethonium but to some extent, was antagonised by atropine, suggesting that levamisole either stimulated M_1 (pre-ganglionic) receptors in the cholinergic ganglia located in the atria or stimulated the post-ganglionic muscarinic (M_2) receptors in the atria.

Levamisole was unable to produce any positive

inotropic effect in atria suspended in Ca^{2+} free RL solution or in solutions with 1/4 or 1/2 Ca^{2+} concentration, indicating that external calcium is required for its inotropic effect. The fact that levamisole restored normal contractile activity to atria severely depressed by verapamil would suggest that levamisole opened Ca^{2+} channels blocked by verapamil. In other words, levamisole could facilitate entry of Ca^{2+} into the heart muscle thereby inducing positive inotropic response.

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