British Journal of Pharmacology (1996) 117, 377-383

Cardiotoxicity of emetine dihydrochloride by calcium channel blockade in isolated preparations and ventricular myocytes of guinea-pig hearts

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1 The cardiotoxic effects of emetine dihydrochloride on mechanical and electrical activity were studied in isolated preparations (papillary muscles, sinoatrial and atrioventricular nodes, ventricular myocytes) of the guinea-pig heart.

2 Force of contraction was measured isometrically, action potentials and maximum rate of rise of the action potential were recorded by means of the intracellular microelectrode technique. Single channel L-type calcium current (Ba^{2+} ions as charge carrier) was studied with the patch-clamp technique in the cell-attached mode.

3 Emetine dihydrochloride (8–256 μM) reduced force of contraction in papillary muscles and spontaneous activity of sinoatrial and atrioventricular nodes concentration-dependently; the negative inotropic effect was abolished when the extracellular Ca^{2+} concentration was increased.

4 Maximum diastolic potential, action potential amplitude, maximum rate of rise of the action potential and the slope of the slow diastolic depolarization were decreased by emetine in sinoatrial as well as atrioventricular nodes, while action potential duration was prolonged in both preparations (1–64 μM).

5 The amplitude of the L-type calcium single channel current was not altered by emetine dihydrochloride, while average open state probability was decreased concentration-dependently (10, 30 and 60 μM).

6 The most prominent effect of emetine dihydrochloride on single channel current was an increase of sweeps without activity.

7 At 60 μM, emetine dihydrochloride caused a decrease of the mean open time and an increase of the mean closed time. The number of openings per record and number of bursts per record were reduced.

8 It is concluded that emetine dihydrochloride produces an L-type calcium channel block which might contribute to its cardiac side effects.

Keywords: Emetine; L-type calcium channel current; cardiotoxicity; heart muscle preparations; cardiomyocyte

Introduction

Emetine, a constituent of *Cephaelis ipecacuanha* BROT. and *Cephaelis acuminata* KARST., is effective against invasive intestinal amoebiasis and amoebic liver abscess (Stuiver & Visser, 1993). The most relevant side effect of emetine is its well documented cardiotoxicity (see Marino *et al.*, 1990; Combs & Acosta, 1990). Thus, because of its high toxicity emetine is used therapeutically only in severe, recurrent and life-threatening cases, when other drugs are ineffective or contraindicated (Stuiver & Visser, 1993). Besides these indications, emetine is still used as an expectorant and emetic (Steinegger & Hänsel, 1992) in preparations of Radix ipecacuanhae. Furthermore, emetine is given in the form of ipecac syrup in the treatment of certain oral intoxications (Adler *et al.*, 1980; Palmer & Guay, 1985) and as an aversive conditioning agent in the therapy of chronic alcoholism (Mateer *et al.*, 1985) because of its emetic effect. Apart from its therapeutic use, emetine is abused by persons suffering from either anorexia nervosa or bulimia (Palmer & Guay, 1985; Thyagarajan *et al.*, 1993).

Intoxication with ipecac syrup is reported to induce severe cardiac side effects such as ventricular tachycardia and fibrillation (Adler *et al.*, 1980; Friedman, 1984; Schiff *et al.*, 1986), and reversible myopathy (Mateer *et al.*, 1985; Palmer & Guay, 1985; Thyagarajan *et al.*, 1993). Moreover hypotension, negative inotropy and changes in the electrocardiogram (Bianchi *et al.*, 1965; Brink *et al.*, 1969; Kuntzer *et al.*, 1989) as well as negative chronotropy (Salako & Durotoye, 1971) and cardiomyopathy (Dresser *et al.*, 1993) have been reported.

Thus it was of interest to study the effects of emetine dihydrochloride in isolated cardiac preparations and cardiomyocytes of guinea-pigs to elucidate the mechanisms underlying its cardiotoxicity.

Methods

Guinea-pigs of either sex (340–500 g) were killed by a blow to the neck. After excision of the heart, papillary muscles were dissected from the right ventricle for contractility measurements. The right atria were separated from the ventricles and were further cut into pieces to obtain preparations containing the sino-atrial (SA-) or atrio-ventricular (AV-) node. Ventricular myocytes were isolated enzymatically for patch-clamp experiments.

Contractility experiments

An experimental set-up described by Reiter (1967) was used for the isometric measurement of force of contraction in electrically stimulated papillary muscles using an AE 875 force transducer (Aksjeselkapet Mikro-Elektronikk, Horten, Norway) under a resting tension of 3.92 mN. The preparations (diameter < 0.8 mm) were bathed in Krebs-Henseleit solution

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with the following composition (in mM): NaCl 114.9, KCl 4.73, $CaCl_2$ 3.2, $MgSO_4$ 1.18, $NaHCO_3$ 24.9, KH_2PO_4 1.18 and glucose 10; at pH 7.2–7.4. The bathing solution was continuously gassed with 95% O_2/5% CO_2 at 35±1°C. Papillary muscles were electrically driven with an Anapulse Stimulator Model 301-T and an Isolation Unit Model 305-T (WP-Instruments, Hamden, CT, U.S.A.) at a rate of 1 Hz and a pulse duration of 3 ms, 10% above threshold intensity. Signals were recorded with a dual beam oscilloscope Type RM 565 (Tektronix Inc., Beaverton, OR, U.S.A.). Photos from the screen were taken every 5 min (Grass Camera Model C45, Grass Instruments Co., Quincy, MA, U.S.A.). Stock solutions of the drug were prepared in distilled water on the day of experiment and further diluted to the required concentrations. The drug concentration was increased cumulatively (duration of exposure: 30 min each).

Electrophysiology experiments

Recording of action potentials and spontaneous activity of SA- and AV-node cells Small pieces of right atria of about 10–20 mm^2 including the SA- or AV-node cells were fixed in a lucite chamber (volume 1.5 ml), which was continuously superfused (1.6–2.1 ml min^{-1}) with a gassed (95% O_2/5% CO_2) bathing solution of the following composition (in mM): NaCl 136.9, KCl 5.4, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42, $CaCl_2$ 1.8, glucose 5.55; at pH 7.2–7.4. Experiments were performed at 37±1°C. Action potentials were differentially recorded by means of the intracellular microelectrode technique, and were electrically differentiated for measurement of the maximum rate of rise of the action potential (V_{max}). Each concentration was added to the bathing solution for 45 min, followed by a wash-out period of 1 h. For recording two microprobe systems (Model M 701, WP-Instruments, Hamden, CT, U.S.A.) and a D13 dual beam storage oscilloscope Type 5103N (Tektronix, Beaverton, OR, U.S.A.) were used. Photos were taken every 5 min with a Nihon Kohden Camera Model PC-2A (Tokyo, Japan) and action potential parameters were measured after magnification.

Single channel recording Single ventricular myocytes were enzymatically dissociated by a method similar to that described by Mitra & Morad (1985). Experiments were performed at room temperature (22±1°C). After isolation, the cells were stored in a solution of the following composition (in mM): NaCl 140, $CaCl_2$ 1.8, KCl 5.4, $MgCl_2$ 2, HEPES 10, titrated with NaOH to pH 7.4. During the experiments a bathing solution was used which contained (in mM) potassium aspartate 140, $MgCl_2$ 2, EGTA 10, ATP 2, HEPES 10, titrated with KOH to pH 7.4. Cells were depolarized by this extracellular solution to about 0 mV. The pipette solution contained 110 mM $BaCl_2$ and 10 mM HEPES, titrated with $Ca(OH)_2$ to a pH of 7.2. Ba^{2+} was used as the charge carrier, because under these conditions L-type calcium channels are bursting throughout the whole sweep (Droogmans & Nilius, 1989), which ensures superior single channel analysis. Single channel currents were measured in the cell-attached mode of the patch-clamp technique (Hamill *et al.*, 1981). Borosilicate pipettes (Corning, WPI) with a resistance of about 1–3 MΩ were coated with Sylgard. Depolarizing steps of 100 or 300 ms were delivered from a holding potential of −50 or −40 mV to a test potential of 0, +10 and +20 mV by a pulse generator at a frequency of 1 Hz during the control period and in the presence of the drug. About 5 min after gigaseal formation, control recordings started, followed by the addition of the drug at concentrations of 10, 30 and 60 μM. Steady-state effects of the drug were reached in less than 10 min. In a few experiments reversibility of the drug effect was studied during a wash-out period of 15 min. The currents were filtered at 5 kHz with a Kemo Variable Filter VBF/8 (Kemo Limited, Beckenham, Kent, U.K.), and digitized with an analog-to-digital converter (TL-1, Axon Instruments, Foster City, CA, U.S.A.) which was connected with a PC. Data acquisition and analysis were controlled by pCLAMP (Axon Instruments, Foster City, CA, U.S.A.) and ASCD (Droogmans, Laboratorium voor Fysiologie, KU Leuven, Belgium).

Drug

Emetine dihydrochloride (Sigma, U.S.A.) was used.

Statistics

Quantitative results are given as arithmetic means±standard error of the mean (s.e.mean) of n experiments. $P<0.05$ was considered as statistically significant (Student's t test, paired observations).

Results

Contractility

At a rate of 1 Hz, emetine dihydrochloride reduced force of contraction (f_c) in papillary muscles concentration-dependently ($n=3-10$, Figure 1). The effective concentration of the drug for 50% reduction of the contractility (EC_{50}) was graphically estimated to be 45±3 μM ($n=10$). The decrease of f_c was significant at 16 μM ($P<0.01$, $n=10$) and higher concentrations; at 512 μM emetine hydrochloride contractility was completely suppressed. Time of peak force, relaxation time, maximum rate of force development and maximum rate of force relaxation were concentration-dependently decreased.

An increase of the $CaCl_2$ concentration in the bathing solution from 3.2 mM to 4.3 mM (15 μM emetine dihydrochloride, $n=4$), 6.1 mM (32 μM emetine dihydrochloride, $n=4$) and 9.4 mM $CaCl_2$ (128 μM emetine dihydrochloride, $n=5$), respectively, abolished the negative inotropic effect of emetine dihydrochloride completely (Figure 1).

SA- and AV-node cells

The spontaneous rate of activity of SA- ($n=5$) and AV-node cells ($n=5$) was concentration-dependently reduced by emetine dihydrochloride. The EC_{50} for negative chronotropy was estimated graphically to be 47±4 and 41±5 μM, respectively (Figure 2).

In action potentials of SA- and AV-node cells, emetine dihydrochloride caused a concentration-dependent decrease of the maximum diastolic potential, the action potential ampli-

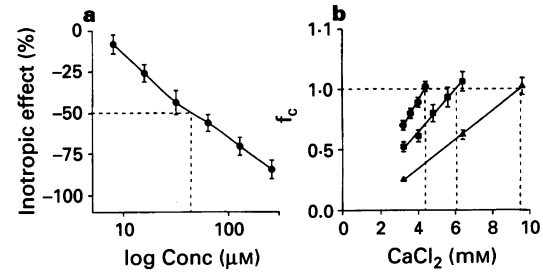

Figure 1 In (a) the concentration-response curve for the negative inotropic effect of emetine dihydrochloride in papillary muscles is shown. The EC_{50}-value was graphically estimated to be 45±3 μM ($n=10$). The symbols represent mean values with s.e.mean. In (b) the abolition of the negative inotropic effect of emetine dihydrochloride by $CaCl_2$ is illustrated. The reduction of contraction force by 16 (●, $n=4$), 32 (■, $n=4$) and 128 μM (▲, $n=5$) emetine dihydrochloride is attenuated by increasing the extracellular calcium concentration. Regression lines were calculated to estimate the calcium concentration needed for complete abolition of the negative inotropic effect. Mean values are shown with vertical lines indicating s.e.mean. Force of contraction (f_c), controls=1.

tude, $\dot{V}_{max}$ and slope of slow diastolic depolarization, while action potential duration was concentration-dependently prolonged in both preparations (Figure 3, Table 1, Table 2). Most of the significant changes occurred in SA-node cells already at a lower concentration than in AV-node cells. The effects of emetine dihydrochloride on the action potential and spontaneous rate of activity were not completely reversible after a wash-out period of 1 h.

Single channel current

From 39 cell-attached patches, four showed single channel activity. In these four patches no overlapping events were seen throughout the experiments. Ba^{2+} (110 mM) was used as the charge carrier, because under these conditions L-type calcium channels are bursting throughout the whole sweep. Figure 4 shows some representative records and the ensemble averaged current of Ba^{2+}-currents flowing through the L-type calcium channel during control and after drug addition at concentrations of 30 and 60 μM emetine dihydrochloride.

The amplitude of the single channel current at a test potential of 0 mV was 1.94 ± 0.11 pA ($n=4$). Emetine dihydrochloride did not significantly change the amplitude of the unitary current (10 μM: 1.87 ± 0.09 pA, $n=3$; 30 μM: 1.86 ± 0.12 pA, $n=4$; 60 μM: 1.90 ± 0.08 pA, $n=4$), but decreased average open state probability. From a control value of 0.47 ± 0.07 ($n=4$) emetine dihydrochloride reduced average open state probability to 0.36 ± 0.09 ($n=4$) at a concentration of 30 μM; the reduction to 0.16 ± 0.03 ($n=4$) by 60 μM emetine dihydrochloride was significant ($P<0.001$). A depressant effect of the drug on the current was confirmed by the decrease of the mean current per record (Figure 5). The mean current decreased concentration-dependently with an EC_{50} of 48 ± 3 μM ($n=4$), which is in the range of the EC_{50} values for the negative chronotropic effect in SA- and AV-node cells and the negative inotropic activity in papillary muscles. The extent of reduction in the time integral of the current from 144 ± 21 pA × ms to 111 ± 25 (30 μM) and 33 ± 15 pA × ms (60 μM) was in the same concentration-range. The decrease of the ensemble averaged current at the beginning of a 100 ms depolarizing pulse was the same as at the end of the pulse (see Figure 4). The most striking effect of emetine dihydrochloride was a concentration-dependent increase of the number of sweeps without activity (see Figure 4). At a concentration of 10 μM the number of empty sweeps was not changed notably (1.2 fold, $n=3$), but at 30 μM

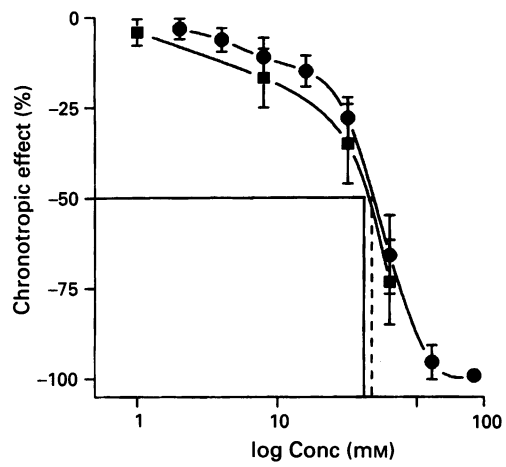

Figure 2 The concentration-dependent negative chronotropic effect of emetine dihydrochloride in SA-node cells (●, $n=5$) and AV-node cells (■, $n=5$) is shown. The EC_{50} was graphically estimated with 47 ± 4 and 41 ± 5 μM, respectively. The symbols represent mean values with vertical lines indicating s.e.mean.

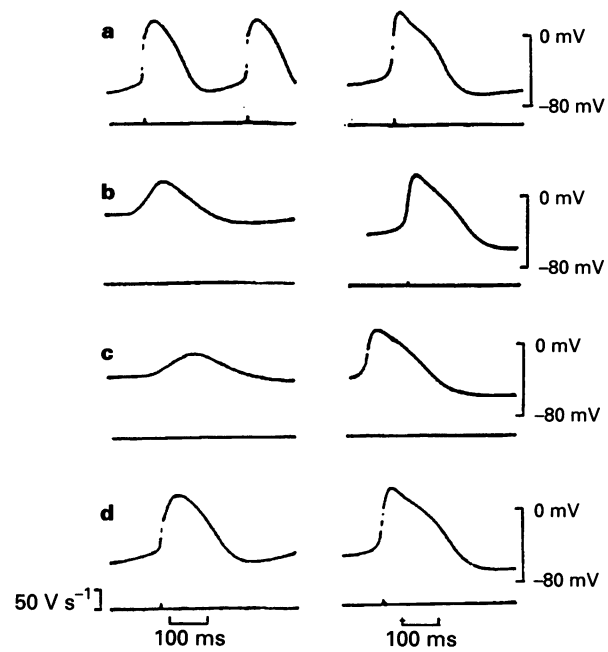

Figure 3 Original recordings from spontaneously beating SA- and AV-node cells. Action potentials and $\dot{V}_{max}$ recorded from SA-node cells (left panel) and AV-node cells (right panel) are shown during control (a), with 32 (b) and 64 μM (c) emetine dihydrochloride, and after wash-out (d).

Table 1 Effects of emetine dihydrochloride on action potential parameters and spontaneous rate of activity of SA-node cells

Conc. (μM)	*MDP* (mV)	*APA* (mV)	$\dot{V}_{max}$ (Vs^{-1})	$APD_{20\%}$ (ms)	$APD_{50\%}$ (ms)	$APD_{90\%}$ (ms)	*SSDD* (mVs^{-1})	*f* (beats min^{-1})
0	59±4	78±3	7±4	48±5	85±6	142±9	112±11	198±11
1	55±5	71±5	5±2	50±4	87±5	143±11	111±10	199±12
8	51±5	66±6	3±2	47±6	93±6	161±9*	87±15	162±12
32	40±8	47±5§	1±1*	53±6	98±9	206±16§	44±7†	95±17§
65	30±7*	27±5†	0.6±1§	55±4	147±15§	282±14†	12±2†	45±6†

$n=5$

Abbreviations: drug concentration (Conc.), maximum diastolic potential (MDP), action potential amplitude (APA), maximum rate of rise of the action potential ($\dot{V}_{max}$), time to 20 –, 50 – and 90% repolarization ($APD_{20\%}$, $APD_{50\%}$, and $APD_{90\%}$), slope of slow diastolic depolarization (SSDD), spontaneous rate of activity (f).

*$P<0.05$; §$P<0.01$; †$P<0.001$

Table 2 Effects of emetine dihydrochloride on action potential parameters and spontaneous rate of activity of AV-node cells

Conc. (μM)	*MDP* (mV)	*APA* (mV)	$\dot{V}_{max}$ (Vs^{-1})	$APD_{20\%}$ (ms)	$APD_{50\%}$ (ms)	$APD_{90\%}$ (ms)	*SSDD* (mVs^{-1})	*f* (beats min^{-1})
0	63±3	92±6	11±4	42±6	74±7	126±9	63±8	88±12
1	62±6	92±5	10±5	45±4	74±5	128±11	65±10	89±14
8	61±7	93±6	9±6	47±6	81±8	146±8*	67±11	73±17
32	58±8	89±7	7±5	50±5	83±12	162±10*	39±15	55±9*
64	55±8	80±8	3±1*	53±6	108±18*	195±5†	37±17	20±12§

$n=5$
Abbreviations: see Table 1

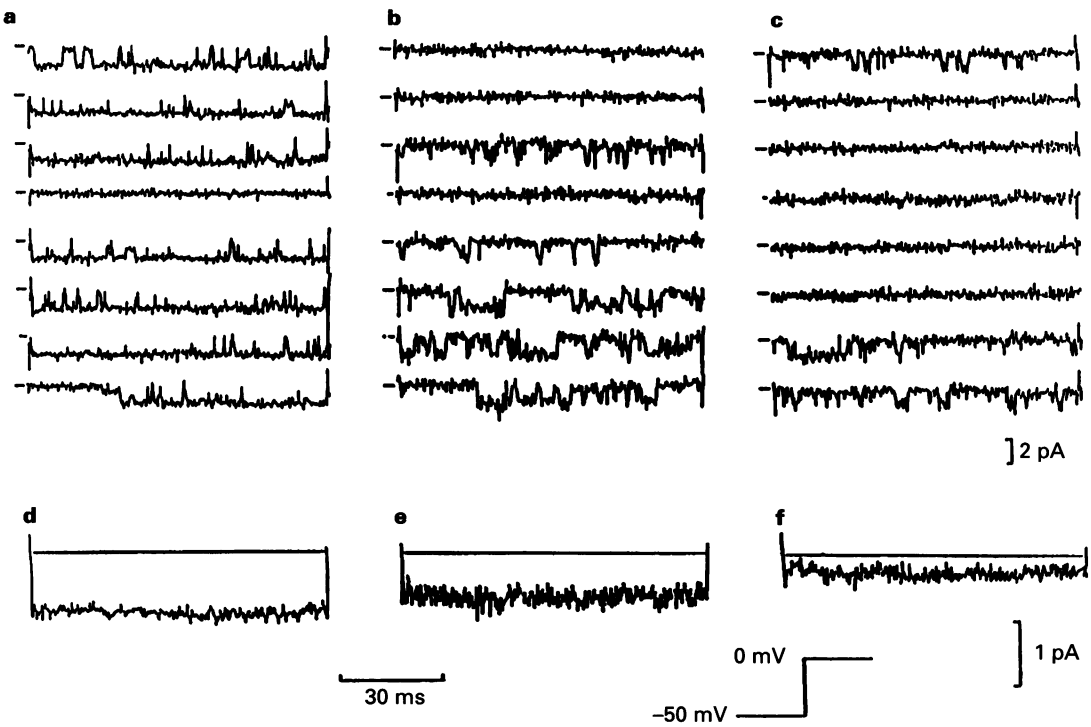

Figure 4 Effect of emetine dihydrochloride on single channel current. Eight consecutive sweeps, recorded from an L-type calcium channel of a cell-attached patch are shown during control (a) and after addition of emetine dihydrochloride (b: 30 μM, c: 60 μM) to the bathing solution; (d), (e) and (f) represent the corresponding averaged currents from 202 sweeps each. Downward deflections represent channel openings. The mark at the side of the records, preceding each current trace, indicates the baseline level (closed channel states). Depolarizing pulses of 100 ms duration to 0 mV were applied from a holding potential of −50 mV at a rate of 1 Hz.

the augmentation was already 3.7 fold ($n=4$). At 60 μM emetine dihydrochloride the effect was prominent with a 5.2 fold increase ($n=4$) of records without single channel openings.

Analysis of the data not only revealed an effect of the drug on the availability of the L-type calcium channel, but also showed an influence on the single channel kinetics. The effect of emetine dihydrochloride on single channel current kinetics was studied at a concentration of 30 and 60 μM. The open and closed time histograms could be best fitted with one and two exponentials, respectively. At 30 μM emetine dihydrochloride did not change the mean open time and increased the mean closed time; however, the change was not significant. At 60 μM emetine dihydrochloride, the time constant of the open time histogram non-significantly decreased from 0.7±0.2 ms to 0.5±0.1 ms ($n=4$), while time constants τ_1 and τ_2 of the mean closed time significantly increased from 0.6±0.1 ms to 1.4±0.3 ms ($P<0.05$, $n=4$) and from 12.9±4.5 ms to 23.5±4.3 ms ($P<0.05$, $n=4$) (Figure 6). The latency for the first opening of a single channel during a depolarizing pulse was slightly prolonged by 60 μM emetine dihydrochloride from 33±13 ms to 42±11 ms ($n=4$).

Burst analysis revealed a non-significant shortening of the burst duration at both tested drug concentrations. At 30 μM emetine dihydrochloride this effect was attended by a small increase in the number of bursts per record without any change in the number of openings per record. At the higher drug concentration, however, emetine dihydrochloride caused a significant reduction in the number of bursts per record from 1.6±0.7 to 0.5±0.2 ($P<0.01$, $n=4$). Concomitant with a small shortening of the burst duration that effect resulted in a significant decrease of the number of openings per record from 6.4±3.0 to 1.9±0.7 ($P<0.05$, $n=4$).

Discussion

Emetine may cause cardiac side effects such as negative inotropic (Bianchi *et al.*, 1965, Brink *et al.*, 1969) and negative chronotropic (Salako & Durotoye, 1971) effects.

The effects of emetine on contractility, spontaneous activity, action potential parameters and single channel currents in different cardiac preparations provide evidence that calcium L-type channel block is involved in the cardiotoxicity of the drug: emetine (a) reduced the force of contraction and this negative

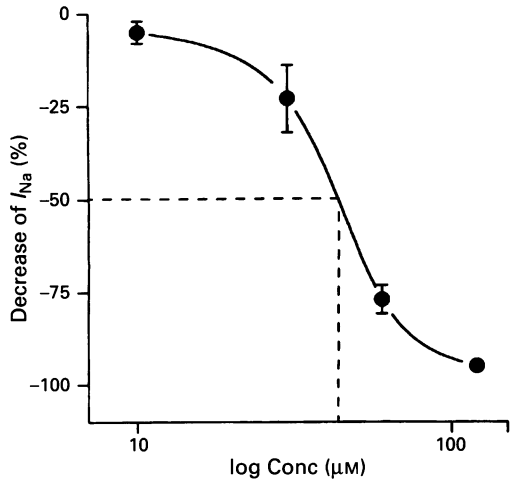

Figure 5 Concentration-response curve for the blockade of calcium channel current by emetine dihydrochloride. Data were obtained from cell-attached patches which were clamped from a holding potential of −50 mV to a test potential of 0 mV. The EC_{50}-value for the decrease of the mean current per record was graphically estimated to be 48±3 μM (n=4). The symbols represent mean values with s.e.mean.

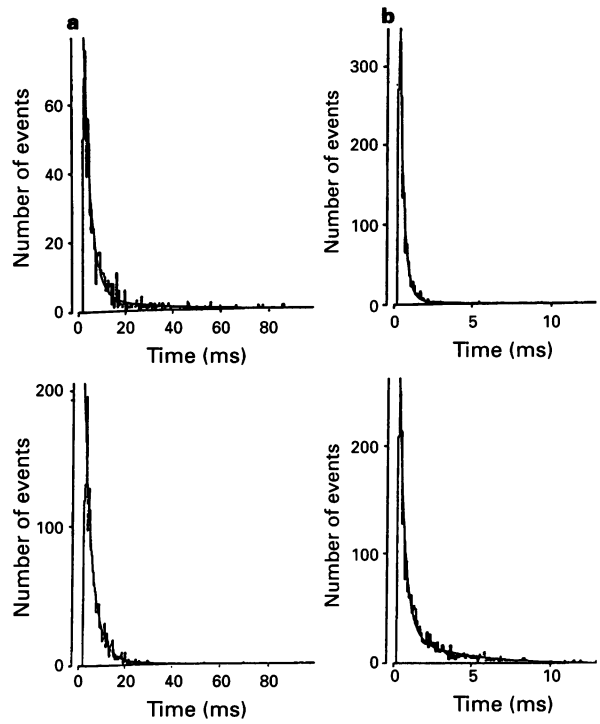

Figure 6 Effect of emetine dihydrochloride on single channel kinetics. From the same experiment as that shown in Figure 4 histograms of the mean open (a) and mean closed (b) time distribution in control (upper row) and with 60 μM emetine dihydrochloride (lower row) are illustrated. For time constants of the fits see text.

inotropic effect was antagonized by increasing the extracellular calcium concentration, (b) reduced spontaneous rate of activity in SA- and AV-node cells, (c) depressed action potential amplitude and $\dot{V}_{max}$ of SA- and AV-node cells, and (d) decreased the current through the L-type calcium channel in cell-attached patches of ventricular myocytes.

Emetine exerted a concentration-dependent negative inotropic effect in guinea-pig papillary muscles comparable to the effect of the calcium-antagonistic drugs, nifedipine (Bayer *et al.*, 1977), gallopamil and verapamil (Bayer *et al.*, 1975) in ventricles of various species. The first link between depression of contractility and decrease of slow inward current was shown by Kohlhardt *et al.* (1971) and Beeler & Reuter (1977). The calcium influx through the L-type calcium channel triggers the calcium release from the sarcoplasmic reticulum (Näbauer *et al.*, 1989) and therefore plays an important role in the mechanical activity of the heart. The negative inotropic effect of calcium antagonists in papillary muscles of mammalian hearts can be antagonized by increasing the extracellular calcium concentration (Nakajima *et al.*, 1975; Fleckenstein, 1977). Also for emetine dihydrochloride it could be shown that the negative inotropic effect is abolished by raising extracellular calcium concentration. Both the negative inotropic activity and the reduction of the slow calcium inward current by verapamil and nifedipine could be attenuated by a high extracellular calcium concentration (Kohlhardt, 1983). Lee & Tsien (1983) compared the inhibitory effects of gallopamil, diltiazem and nitrendipine in guinea-pig ventricular myocytes in the presence of 3 mM and 30 μM Ca, and in each case elevation of the extracellular calcium concentration significantly increased the percentage of unblocked calcium channels.

Further support for the assumption that emetine blocks the calcium inward current, are the effects of the drug on $\dot{V}_{max}$ and amplitude of action potentials and spontaneous activity of SA- and AV-node cells. The $\dot{V}_{max}$ of the action potentials of SA- and AV-node cells can be regarded as a measure for the slow inward current during the depolarization phase, mainly caused by calcium ions (Hachisu & Pappano, 1983). A reduction of the rate of spontaneous activity, action potential amplitude and $\dot{V}_{max}$ was shown for the slow channel inhibitor verapamil (Cranefield *et al.*, 1974), nifedipine and diltiazem (Kawai *et al*, 1981). Similar results were obtained with emetine dihydrochloride, which gives additional evidence for a possible calcium-antagonistic effect of the drug. An obvious effect of calcium channel blockade is a reduction in the action potential duration. In action potentials of spontaneously beating Purkinje fibres the time to 20% repolarization was decreased by emetine dihydrochloride (not shown); in SA- and AV-node cells action potential duration was increased. The prolongation of the action potential duration by emetine dihydrochloride could secondarily be caused by the reduced calcium inward current during the plateau phase, because the subsarcolemmal calcium concentration exerts an inhibitory influence on the repolarizing potassium outward current (McGuigan & Bassingthwaighte, 1974). This effect was also observed e.g. for the calcium antagonists verapamil and gallopamil (Cranefield *et al.*, 1974). Kass (1982, 1984) demonstrated that the effect of calcium channel blockers on I_K is not secondary to an effect on I_{Ca}. In guinea-pig ventricular cells there is evidence for modulation of I_K by intracellular calcium concentrations ≥10 nM, while I_K remained unchanged at concentrations ≤1 nM (Tohse, 1990).

Since verapamil, nifedipine and diltiazem exert similar changes on contractility, spontaneous activity and action potentials of SA- and AV-node cells to emetine, it was reasonable to assume that emetine blocks the calcium inward current. Therefore, the effect of emetine on the current flowing through the L-type calcium channel was studied in cell-attached patches of ventricular myocytes. The size of the calcium current is dependent on the open state probability, primarily determined by the activation process and the availability of the channel determined by the slow state transitions. It has been suggested that the activation kinetics of calcium channels can be described by a simple three-state reaction scheme involving two non-conducting and one conducting state (Fenwick *et al.*, 1982). Emetine reduced the L-type calcium current in ventricular myocytes of guinea-pigs mainly by decreasing single channel availability, while the amplitude of the unitary current remained unchanged. Such an effect was also reported for e.g. nitrendipine (Kawashima & Ochi, 1988). As with this drug, the

prolongation of the unavailable states initiated by emetine binding during depolarizing steps can be interpreted as the main mechanism of the channel blockade. Unlike nitrendipine (Lee & Tsien, 1983 ; Hess *et al.*, 1984), but like diltiazem (Lee & Tsien, 1983) emetine did not lead to an acceleration of the time course of inactivation of the ensemble averaged current. A faster decay of the averaged current is commonly interpreted as open channel block. Thus, it is assumed that emetine preferentially binds to a closed (inactivated) state of the channel or that binding to the channel in the open state occurs very fast. The fast gating process was little affected by emetine dihydrochloride, since the mean open time was almost the same in the absence and presence of the drug. Closed time histograms were fitted by double-exponential curves, reflecting the occurrence of bursts (Fenwick *et al.*, 1982); closed times were prolonged by emetine. The fast component with time constant τ_1 corresponds to the brief closures of the channel during the bursts. The slow component of the closed time distribution with time constant, τ_2 represents the longer closing periods between bursts, and was prolonged by emetine. At the higher drug concentration this increase was pronounced, reflecting longer closures between bursts which resulted in a reduction in the number of bursts per sweep.

In conclusion one can deduce from the results obtained on isolated papillary muscles, SA- and AV-node cells, and ventricular myocytes of the guinea-pig heart that the blockade of the L-type calcium channel by emetine contributes to the cardiotoxicity of the drug.

We are grateful to P. Saleh for technical assistance and to Dr J. Vereecke for his critical comments on the manuscript.

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(Received June 16, 1995
Revised September 1, 1995
Accepted September 21, 1995.)