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Characterization of the interaction of R 56865 with cardiac Na- and L-type Ca channels

[1]D. Wilhelm, *H. Himmel, *U. Ravens & T. Peters

Janssen Research Foundation, Raiffeisenstraße 8, D-4040 Neuss 21 and *Pharmakologisches Institut, Universität-Gesamthochschule Essen, Hufelandstraße 55, D-4300 Essen 1, Germany

1 In isolated cardiac muscle, submicromolar concentrations of R 56865 (N-[1-[4-(4-fluorophenoxy)-butyl]-4-piperidinyl]-N-methyl-2-benzothiazolamine) have been shown to attenuate the toxicity of cardiac glycosides.

2 We studied the influence of R 56865 on calcium and sodium currents in single isolated ventricular cardiomyocytes. The effect of R 56865 on action potential and contractile force in the presence of increased sodium load was also tested by exposing papillary muscles to veratridine or *Anemonia sulcata* toxin ATX II.

3 The calcium current was not affected by R 56865 as assessed in slow action potentials of papillary muscles and current measurements in ventricular cardiomyocytes.

4 In papillary muscles, R 56865 (1 μmol l^{-1}) abolished veratridine-induced aftercontractions and afterdepolarizations without affecting the profound prolongation of the action potential. When pretreated with R 56865, the occurrence of afterdepolarizations was prevented and the decline of the resting membrane potential was attenuated.

5 Pretreatment with R 56865 (1 μmol l^{-1}) did not counteract the ATX II-induced prolongation of the action potential.

6 The sodium current (Na_o 30 mmol l^{-1}) was concentration-dependently decreased by R 56865 (0.1–10 μmol l^{-1}). The blocking effect was more pronounced at less negative holding potentials.

7 Our results demonstrate that the protective effect of R 56865 against veratridine-induced electrical and mechanical oscillations is not due to a direct effect on the calcium current. A potential-dependent inhibition of the sodium current may contribute. Additional sites of action, like interference with intracellular calcium release and inhibition of potassium currents, remain to be investigated.

Keywords: Ca and Na currents; R 56865; veratridine; guinea-pig heart

Introduction

R 56865 (N-[1-[4-(4-fluorophenoxy)-butyl]-4-piperidinyl]-N-methyl-2-benzothiazolamine) has been found to prevent electrical and mechanical signs of cardiac glycoside toxicity in the guinea-pig papillary muscle (Vollmer *et al.*, 1987). The compound attenuates the glycoside-induced shortening of the action potential duration and the decrease in action potential amplitude and prevents the occurrence of delayed afterdepolarizations. With respect to changes of mechanical parameters induced by a toxic concentration of ouabain, R 56865 hinders the development of a 'contracture' (rise in diastolic and decline in systolic tension) and the occurrence of arrhythmias and aftercontractions. These effects of R 56865 cannot be explained by an interference with the Na-K-ATPase or by a direct interaction with binding of ouabain at the receptor level (Heers *et al.*, 1988). From these results it may be speculated that R 56865 inhibits the cardiac glycoside-induced gain in sodium and calcium. In order to test this hypothesis the influence of R 56865 was studied on calcium-dependent slow action potentials and on the calcium current in ventricular myocytes. The possible interference of R 56865 with the sodium influx was tested indirectly by exposing the cardiac tissue to either veratridine or *Anemonia sulcata* toxin ATX II, compounds known to increase the intracellular sodium load (Honerjäger & Reiter, 1975; Ravens, 1976). Furthermore, the effect of R 56865 on the sodium current was assessed in isolated ventricular myocytes of the guinea-pig.

[1] Author for correspondence.

Methods

Measurements in isolated papillary muscles

Experimental set-up Right ventricular papillary muscles of about 1–2 mm in diameter were obtained from guinea-pig hearts. The muscles were fixed horizontally in a 2.5 ml tissue chamber between the lever of a force transducer (Gould U 2 cell) and a teflon clamp. In order to initiate action potentials, the muscles were electrically stimulated at 1 Hz with square wave pulses of 1 ms duration and 20% above threshold voltage via two platinum electrodes close to the muscle base. The organ bath was continuously perfused with modified Tyrode solution (8 ml min^{-1}), the temperature was maintained at 35 ± 0.2°C. The composition of the Tyrode solution (in mmol l^{-1}) was as follows: NaCl 124.0, KCl 4.0, $CaCl_2$ 0.9, $MgCl_2$ 1.1, $NaHCO_3$ 25.0, NaH_2PO_4 0.42, glucose 11.0 gassed with 95% O_2 and 5% CO_2 (pH 7.3–7.5). Tension was measured via a force transducer and documented on a pen recorder (WeKaGraph 1100 R). Resting tension was adjusted to about 5 mN in order to obtain maximal contractile force. The intracellular potential was measured against a silver/silver chloride electrode in the organ bath with conventional glass electrodes (10–20 MOhm), filled with KCl 3 mol l^{-1}. The potential difference was measured by a preamplifier (Hugo Sachs Elektronik) with a capacitance compensated, high impedance input and displayed on the screen of a storage oscilloscope (Tektronix 7D20). The recordings were collected by an analog-digital converter and transferred to an IBM host computer. The parameters resting membrane potential (RMP), action potential amplitude (APA), action potential duration (APD), maximal rate of rise ($\dot{V}_{max}$) and contractile

force (CF) were calculated by means of computer programmes developed in our department.

Experimental protocol After an equilibration period of 90 min in normal Tyrode solution, the perfusion solution was changed to veratridine-containing Tyrode solution (0.8 or 2.5 μmol l^{-1}). The influence of veratridine on electrical and mechanical parameters was observed for 60 min before R 56865 (1 μmol l^{-1}) was added in the continuous presence of veratridine and 45 min allowed for incubation. Additionally, experiments were carried out with veratridine in the absence of R 56865 for 105 min to correct for the effect of veratridine over time.

In another set of experiments, papillary muscles were pretreated with R 56865 (1 μmol l^{-1}) for 45 min during the equilibration period and then veratridine (2.5 μmol l^{-1}) was added for 105 min.

Furthermore, the effect of ATX II (0.01 μmol l^{-1}) was studied in the papillary muscle for 60 min. In case of pretreatment, R 56865 (1 μmol l^{-1}) was added to the Tyrode solution during the equilibration period for 45 min and then ATX II (0.01 μmol l^{-1}) was administered for 60 min.

Slow action potentials were induced by the following experimental protocol: after an equilibration period of at least 90 min during which normal action potentials were recorded, the extracellular KCl concentration was increased to 25 mmol l^{-1}. In the presence of isoprenaline (1 μmol l^{-1}) calcium-dependent slow action potentials were recorded. R 56865 was added to the superfusion medium at concentrations ranging from 0.01 to 10 μmol l^{-1}.

The data are expressed as means ± s.e.mean ($n = 6$). Statistical significance was evaluated by the Mann-Whitney U test and is indicated with an asterisk; $P < 0.05$ was taken as significant.

Measurements in single cardiomyocytes

Cell isolation and experimental set-up Guinea-pig ventricular cardiomyocytes were isolated by enzymatic dissociation according to the method described by Bendukidze *et al.* (1985). In order to measure the membrane currents by means of the 'whole-cell patch-clamp' technique (Hamill *et al.*, 1981), with a List EPC-7 amplifier, isolated cardiomyocytes were transferred into a small superfusion chamber mounted on the stage of an inverted microscope (details in Cragoe *et al.*, 1987). The liquid junction potential was corrected for electronically before the seal was established. After disruption of the membrane, the series resistance was compensated to a level just before 'ringing' (> 50%), the current signals were filtered at 3 kHz. At the beginning of each experiment, a depolarizing ramp pulse (4 V s^{-1}, 5 ms duration) was applied to determine the membrane capacity. Assuming a specific membrane capacitance C_s of 1 μF cm^2 (Marty & Neher, 1983), the surface area A of the cell was calculated according to $A = I/[(dU/dt)C_s]$. All membrane currents were normalized to the cell surface.

Measurement of calcium current I_{Ca} Cardiomyocytes with a mean surface area (± s.e.mean) of 11,792 ± 1596 μm^2 ($n = 6$) were chosen. The microelectrodes used had a mean resistance of 3.3 ± 0.3 MOhm, the mean series resistance was 9.7 ± 0.9 MOhm. The pipette solution consisted of (in mmol l^{-1}) CsCl 140.0, $MgCl_2$ 4.0, EGTA 10.0, HEPES 10.0, Na_2ATP 4.0.

The superfusion solution contained (in mmol l^{-1}): NaCl 150.0, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 2.5, HEPES 10.0 and glucose 10.0. With a frequency of 0.5 Hz, clamp pulses of 400 ms duration from −40 mV to +10 mV were applied subsequent to a 100 ms prepulse from the holding potential of −80 mV to −40 mV to inactivate the sodium channels. After 6 min of stabilization, each cell was exposed to one concentration of R 56865 for 6 min followed by a wash-out period of at least 6 min. At the end of each period, we determined the current-voltage relation in the range of −40 to +50 mV.

Measurement of sodium current I_{Na} The cardiomyocytes chosen ($n = 24$) had a mean surface area (± s.e.mean) of 10,140 ± 1940 μm^2. The microelectrodes used had a mean resistance of 2.2 ± 0.3 MOhm, the mean series resistance was 6.0 ± 1.2 MOhm. The experiments were carried out at room temperature (20–22°C) using medium-size cardiomyocytes and microelectrodes with large tip openings in order to keep the voltage errors of the clamp as low as possible (Marty & Neher, 1983). The pipette solution consisted of (in mmol l^{-1}): CsCl 140.0, $MgCl_2$ 4.0, EGTA 10.0, HEPES 10.0 and Na_2ATP 4.0. The superfusion solution contained (in mmol l^{-1}): NaCl 30.0, CsCl 107.0, $CaCl_2$ 0.5, $CdCl_2$ 0.05, $MgCl_2$ 2.5, HEPES 10.0, glucose 10.0. Clamp pulses were applied at a frequency of 0.5 Hz from a holding potential of −80 mV to −20 mV (pulse duration 16 ms). These pulses activated the inwardly directed, TTX-sensitive I_{Na}. Each cell was maintained for 5 min under control conditions, then exposed to one concentration of R 56865 for 5 min, followed by a washout period. At the end of each of these periods, the dependence of I_{Na} on the holding potential V_H was determined by running the following pulse protocol: a 500 ms conditioning pulse from the holding potential of −80 mV to various conditioning potentials V_{con} ranging from −90 to −70 mV with a subsequent test clamp step to −20 mV (16 ms); in time-matched controls, this protocol was run in intervals of 2 min.

Data processing of I_{Ca} *and* I_{Na} The data shown are expressed as mean values ± s.e.mean of n experiments. Concentration-response curves were roughly estimated by fitting a four parameter logistic equation to the mean values (computer program GraphPAD, Motulsky, 1985). Thereby three of the four parameters were set constant, i.e. maximum = 1, minimum = 0, and slope = −1. The concentration at which half-maximum effects occur were expressed as pD_2-values. The non-parametric H-test was used in order to detect significant differences ($P < 0.05$) between grouped observations (in: Sachs, 1984).

Compounds and stock solutions

Analytical grade chemicals (Merck, Darmstadt, F.R.G.), dissolved in demineralized water, were used throughout. Compounds tested in the present study were as follows: veratridine (Sigma Chemie GmbH, Deisenhofen, F.R.G.) and R 56865 (=(N-[1-[4-(4-fluorophenoxy)-butyl]-4-piperidinyl]-N-methyl-2-benzothiazolamine) (Janssen Pharmaceutica, Beerse, Belgium). Stock solutions of veratridine and R 56865 (both 1 mmol l^{-1}) were prepared by using acidified water (10 ml l^{-1}, 1 N-HCl).

ATX II was kindly provided by Dr Béress (Inst. f. Toxokologie, Kiel, F.R.G.). The toxin was dissolved in demineralized water.

Results

Effect of R 56865 on slow action potentials of guinea-pig isolated papillary muscles

In order to investigate a possible interference of R 56865 with the slow inward current, experiments were performed in guinea-pig right ventricular papillary muscles partially depolarized by exposure to KCl 25 mmol l^{-1} which inactivates the fast sodium current. In the presence of isoprenaline (1 μmol l^{-1}), slow action potentials could be elicited from a resting potential of −45 ± 5 mV. Addition of R 56865 in concentrations ranging from 0.01–10 μmol l^{-1} did not influence the slow action potentials. The respective parameters are listed in Table 1.

Table 1 Slow action potentials in the presence of 25 mmol l^{-1} KCl and of isoprenaline (control) and the influence of R 56865

	Control	*R 56865* (10^{-8} mol l^{-1})	*Control*	*R 56865* (10^{-7} mol l^{-1})	*Control*	*R 56865* (10^{-6} mol l^{-1})	*Control*	*R 56865* (10^{-5} mol l^{-1})
RMP (−mV)	45 ± 4	48 ± 5	42 ± 5	45 ± 2	47 ± 3	45 ± 4	44 ± 1	50 ± 5
APA (mV)	54 ± 2	58 ± 4	56 ± 5	63 ± 7	63 ± 5	64 ± 7	65 ± 1	65 ± 1
APD_{50} (ms)	151 ± 13	161 ± 9	146 ± 6	159 ± 8	147 ± 8	157 ± 8	154 ± 10	155 ± 15
APD_{90} (ms)	174 ± 12	177 ± 8	175 ± 7	185 ± 5	171 ± 6	179 ± 6	175 ± 8	171 ± 6

RMP, resting membrane potential; APA, action potential amplitude; APD_{50} and APD_{90}, action potential duration at 50% and 90% repolarization. Mean values ± s.e.mean of 4–5 experiments.

Effect of R 56865 on the calcium current I_{Ca} in isolated ventricular cardiomyocytes of the guinea-pig

I_{Ca} was separated from other ionic currents by prepulse inactivation of the sodium channels and by replacement of K^+ by Cs^+ in order to reduce the potassium currents. Because of the lack of effect on slow action potentials in guinea-pig papillary muscles, only one concentration of R 56865 (10 μmol l^{-1}) was used in the single cell experiments. Figure 1a shows original current tracings obtained during a clamp step from −40 to +10 mV for 400 ms in the absence and presence of R 56865.

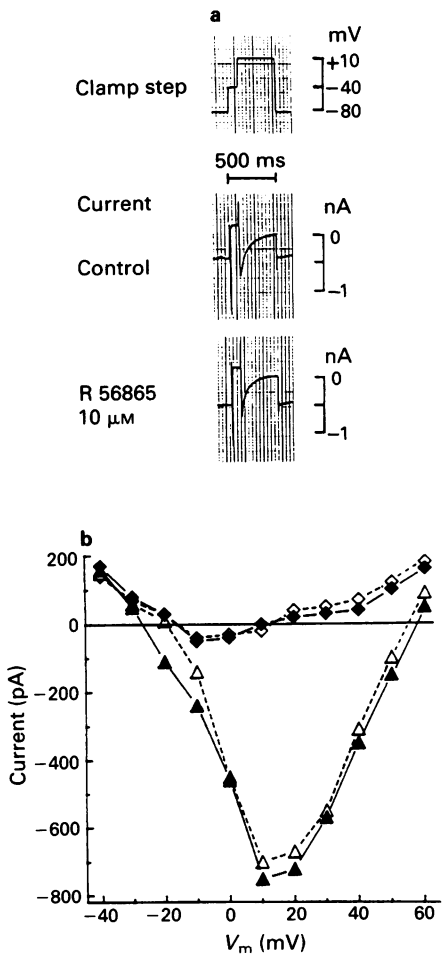

Figure 1 Effects of R 56865 on the calcium current I_{Ca}. (a) Original current tracings from a representative experiment. The clamp step was from −40 mV to +10 mV (400 ms) subsequent to a prepulse from the holding potential of −80 mV to −40 mV (100 ms). The current tracings were recorded at the end of the control period and after 6 min of exposure to R 56865 (10 μmol l^{-1}), respectively. (b) Current-voltage relation for I_{Ca} under control conditions (filled symbols) and in the presence of 10 μmol l^{-1} of R 56865 (open symbols). Peak currents (triangles) and currents measured at the end of the 400 ms clamp step (diamonds) from the same experiment as in (a) and plotted as a function of the clamp potential V_m. Similar results were obtained in 2 other experiments with cardiomyocytes from different hearts.

Neither peak currents nor currents measured at the end of the clamp step were affected by R 56865 as shown in the current-voltage relationship in Figure 1b.

Effect of R 56865 on veratridine-induced electrical and mechanical alterations in guinea-pig papillary muscles

A possible influence of R 56865 on the cellular sodium load was investigated by experiments with two compounds known to lead to an increased sodium influx, ATX II and veratridine. After 105 min of exposure, veratridine (2.5 μmol l^{-1}) caused a profound prolongation of APD_{90} from 195 ± 8 ms to 715 ± 35 ms (n = 6), a depolarization from −82 ± 4 mV to −62 ± 6 mV, a decrease in APA from 99 ± 5 mV to 64 ± 5 mV, and the occurrence of early afterdepolarizations associated with aftercontractions (Figure 2a). Veratridine (2.5 μmol l^{-1}) strongly increased force of contraction from 0.2 ± 0.1 mN to 1.7 ± 0.3 mN after 60 min of exposure (Figure 3). Toxicity manifested itself in the occurrence of extrasystoles and aftercontractions, by an increase in diastolic tension by 0.4, 0.6, and 0.4 mN in 3 out of 6 experiments, and by a decline in systolic tension (final value: 1.3 ± 0.2 mN; Figures 3 and 4).

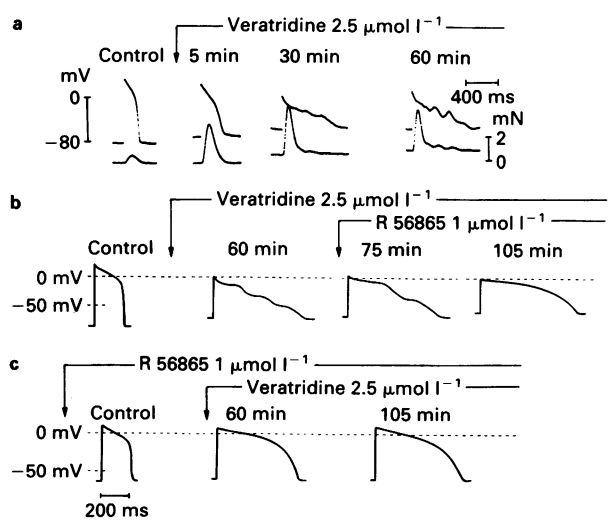

Figure 2 Redrawn recordings of action potentials (a–c) and contractile force (a) of a guinea-pig papillary muscle. Stimulation rate was 1 Hz: (a) shows alterations of the action potential configuration and force of contraction in the presence of veratridine (2.5 μmol l^{-1}). Action potential duration is increased accompanied by a rise in force of contraction. After 60 min of exposure to veratridine, electrical and concomitant mechanical oscillations and a shift of the resting membrane potential are prominent. The inhibitory effect of R 56865 (1 μmol l^{-1}) on veratridine-induced afterdepolarizations is shown in (b). Pretreatment with R 56865 (1 μmol l^{-1}) prevents the development of afterdepolarizations and the shift of the resting membrane potential (c). R 56865 has no effect on the prolongation of the action potential duration by veratridine.

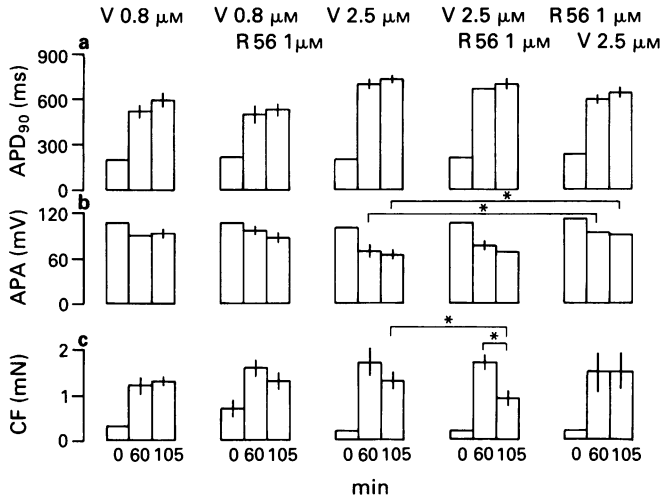

Figure 3 Effect of 60 and 105 min of exposure to veratridine (V 0.8 μmol l^{-1}, 1st and 2nd data set arranged vertically) on action potential duration (APD_{90}, a), action potential amplitude (APA, b), and contractile force (CF, c). The influence of R 56865 (R 56 1 μmol l^{-1}) given post or prior veratridine is shown in the 2nd and 4th, and the 5th data set arranged vertically, respectively. Statistical significance of differences versus veratridine value is indicated by asterisks (*$P < 0.05$). The veratridine-induced significant differences versus control value are not indicated for sake of clarity.

Addition of R 56865 (1 μmol l^{-1}) on top of veratridine after 60 min resulted in the abolition of the early afterdepolarizations and a decline in force of contraction (final value: 0.9 ± 0.2 mN; $P < 0.05$) (Figure 2–Figure 4). Early afterdepolarization occurred in all experiments with veratridine (2.5 μmol l^{-1}) and were abolished by R 56865 in 5 out of 6 experiments. R 56865 was not able to counteract the veratridine-induced alterations in APD_{90} and APA (Figure 3). A lower concentration of veratridine (0.8 μmol l^{-1}) exerted a sustained positive inotropic effect accompanied by a prolongation of the action potential duration from 195 ± 11 ms to 592 ± 40 ms after 105 min ($n = 6$). The action potential amplitude declined over the time course of the experiment (Figure 3). No change in resting membrane potential occurred: control: -87 ± 1 mV versus veratridine: -87 ± 5 mV (105 min). Addition of R 56865 (1 μmol l^{-1}) on top of veratridine (0.8 μmol l^{-1}) did not affect the alterations by veratridine of the action potential duration and action potential amplitude. R 56865 did not impair the positive inotropic response to veratridine (0.8 μmol l^{-1}) (Figure 3).

R 56865 alone (1 μmol l^{-1}) did not affect the APD_{90} (control: 195 ± 8 ms; R 56865: 207 ± 14 ms; $n = 6$) the APA (control: 99 ± 4 mV; R 56865: 104 ± 1 mV) or the rate of rise (control: 270 ± 4 V s^{-1}; R 56865: 260 ± 8 V s^{-1}). In myocytes, 1 μmol l^{-1} R 56865 reduced I_{Na} to 70% of the control at a holding potential of -90 mV. This seeming discrepancy can be explained by differences in Na_0 and drug concentration and distribution in myocytes and papillary muscles. Furthermore, inhibition of I_{Na} and reduction of the rate of rise are not strictly parallel (Walton & Fozzard, 1979). Exposure to R 56865 prior to veratridine (2.5 μmol l^{-1}) prevented the occurrence of early afterdepolarizations and attenuated the decline in action potential amplitude (Figure 2). R 56865 did not influence the effect of veratridine on action potential duration (Figures 2 and 3). Force of contraction of untreated papillary muscles was 0.2 ± 0.1 mN which was not affected by R 56865 (1 μmol l^{-1}; 0.2 ± 0.1 mN). Pretreatment with R 56865 allowed the development of the positive inotropic response to veratridine without signs of extrasystoles and aftercontractions (1.5 ± 0.4 mN; 105 min) (Figure 3 and 4).

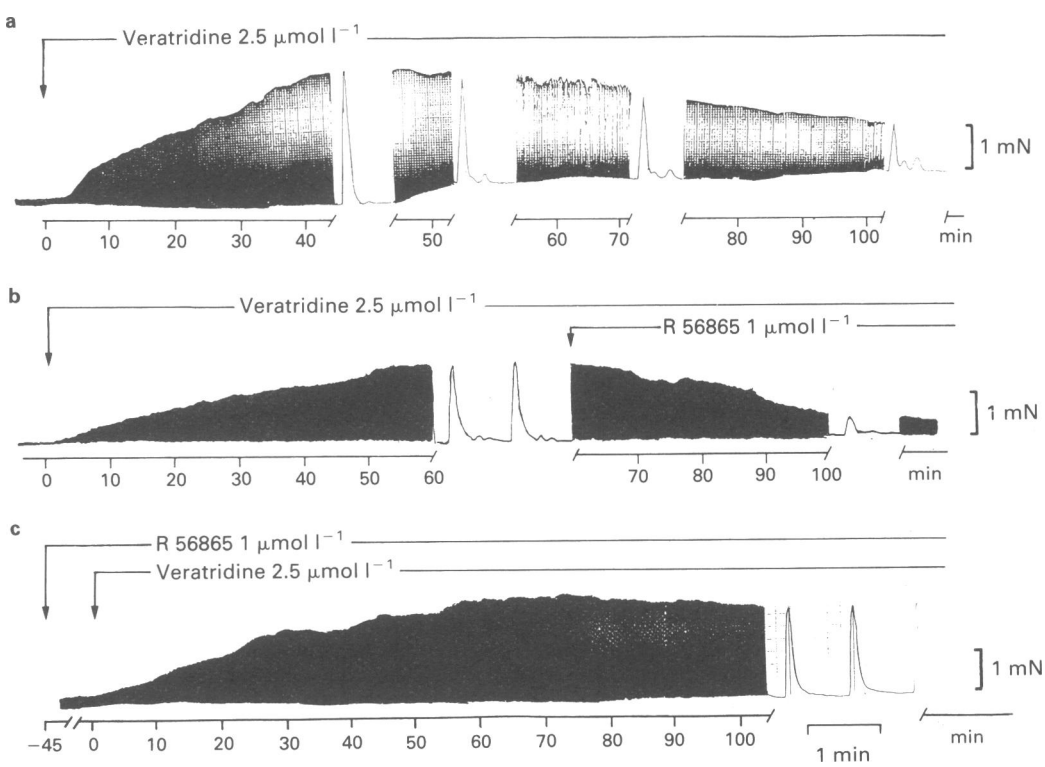

Figure 4 Force of contraction of the guinea-pig papillary muscle (stimulation rate: 1 Hz). (a) Mechanical recordings showing the positive inotropic effect of veratridine (2.5 μmol l^{-1}) accompanied by extrasystoles, aftercontractions, and an increase in diastolic tension. (b) R 56865 (1 μmol l^{-1}), given after 60 min of exposure to veratridine (2.5 μmol l^{-1}), causes a decline of contractile force accompanied by a marked reduction of aftercontractions. (c) Effect of pretreatment with R 56865 (1 μmol l^{-1}) on the veratridine-induced increase of contractile force. Aftercontractions did not occur. Time scale in lower right corner for recordings at higher paper speed which were redrawn for clarity.

Table 2 Electrophysiological characteristics of guinea-pig papillary muscles under the influence of *Anemonia sulcata* toxin (ATX II) and R 56865

	Control	*ATX II* (10^{-8} mol l^{-1})	*R 56865* (10^{-6} mol l^{-1}) *plus* *ATX II* (10^{-8} mol l^{-1})
RMP (−mV)	87 ± 5	88 ± 4	89 ± 5
APA (mV)	106 ± 7	110 ± 4	113 ± 5
APD_{50} (ms)	192 ± 4	221 ± 4*	227 ± 5*
APD_{90} (ms)	222 ± 5	251 ± 7*	265 ± 8*

RMP, resting membrane potential; APA, action potential amplitude; APD_{50} and APD_{90}, action potential duration at 50% and 90% repolarization.
Mean values ± s.e.mean of 4–5 experiments. *$P < 0.05$ to control.

Effect of R 56865 on ATX II-induced electrical alterations in guinea-pig papillary muscles

The *Anemonia sulcata* polypeptide ATX II (0.01 μmol l^{-1}) increased the action potential duration and augmented force of contraction (Ravens, 1976). Neither action potential amplitude nor resting membrane potential were altered by application of ATX II and pretreatment with R 56865 (1 μmol l^{-1}) did not counteract the effect of ATX II on action potential duration (Table 2).

Effect of R 56865 on the sodium current I_{Na} in isolated ventricular cardiomyocytes of the guinea-pig

The sodium current I_{Na} was isolated by blocking Ca^{2+} channels with Cd^{2+} (Rosenberg *et al.*, 1988) and by using Cs^+ instead of K^+ in order to inhibit the K^+ currents (Isenberg, 1976). Furthermoré, the extracellular sodium concentration was reduced to 30 mmol l^{-1} in order to facilitate potential control. At the end of the control period, the mean amplitude of I_{Na} was 3.5 ± 0.9 nA ($n = 24$), and the mean sodium current density was 343 ± 75 mA m^{-2}.

In control experiments ($n = 6$), we could demonstrate that I_{Na} was subject to a substantial, time-dependent decline in amplitude. The rate of this 'run-down' depended on the conditioning potential V_{con} (data not shown).

R 56865 (1 μmol l^{-1}) clearly reduced I_{Na} (inset, Figure 5). The extent of this reduction depended on both the concentration of R 56865 and the conditioning potential V_{con}: the sodium channel blocking effect was more pronounced at less negative conditioning potentials (see Figure 5).

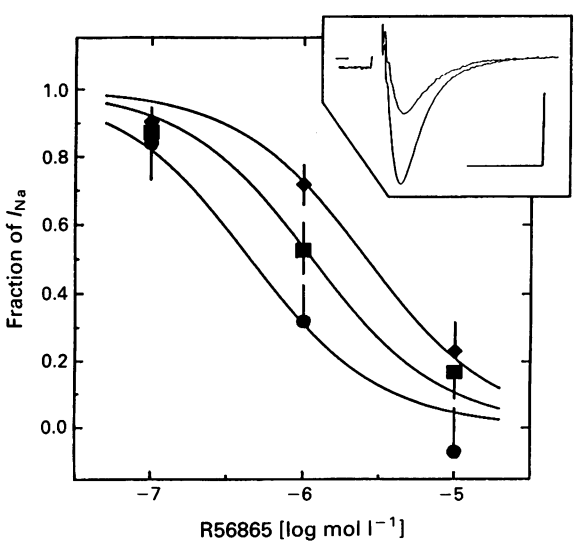

Figure 5 Potential- and concentration-dependence of the effects of R 56865 on the sodium current I_{Na}. Concentration-response relation for the effect of R 56865 on I_{Na} measured from various conditioning potentials V_H. The test clamp steps to −20 mV were preceded by 500 ms clamp steps to −90 mV (◆), −80 mV (■), and −70 mV (●), respectively. The currents were normalized to their respective amplitudes at the end of the control period (5 min) and corrected for the mean time-dependent decrease in I_{Na} occurring in time-matched controls. The application of a four parameter logistic equation to the data resulted in pD_2-values of 6.4 (V_H − 70 mV), 6.0 (V_H − 80 mV), and 5.6 (V_H − 90 mV) with the three other parameters assumed to be constant (maximum = 1, minimum = 0, slope = −1). The amplitudes of I_{Na} in the presence of 1 μmol l^{-1} R 56865 measured with clamp steps from −70 mV, −80 mV, or −90 mV do not originate from one single totality (H-test, $P < 0.05$). Mean from 5–6 experiments per concentration; vertical lines show s.e.mean. Inset: Original superimposed tracings of I_{Na} under control conditions and after 5 min exposure to R 56865 (1 μmol l^{-1}). Holding potential was −80 mV between stimuli (0.5 Hz); calibration bars: 3 ms, 3 nA; the small horizontal bar indicates the zero current level.

Discussion

The benzothiazolamine derivative R 56865 has been found to protect cardiac muscle against cardiac glycoside intoxication (Vollmer *et al.*, 1987; Schneider *et al.*, 1988), the electrical signs of which, e.g. action potential shortening and afterdepolarizations, and mechanical signs, e.g. rise in diastolic tension and aftercontractions, are thought to be due to intracellular sodium and/or calcium accumulation (Wit & Rosen, 1986). Therefore, R 56865 could elicit its protective effect by several mechanisms, including an interaction with the inhibitory effect of ouabain on the Na-K-ATPase; an influence on the Na-Ca-exchanger; a direct inhibition of the sodium and/or calcium influx via potential-dependent channels; or an interference with the calcium release from cellular stores. An interaction of R 56865 with ouabain at the Na-K-ATPase could be excluded since R 56865 does not modify the enzyme activity of a crude cardiac membrane Na-K-ATPase preparation (Heers *et al.*, 1988), nor does it influence the ouabain-induced inhibition of the sodium pump in human red blood cells (Finet *et al.*, 1989). In sarcolemmal vesicles prepared from bovine cardiac tissue, a direct influence of R 56865 on the Na-Ca-exchange was observed only with concentrations above 10 μmol l^{-1} (Heers, unpublished results). Theoretically, R 56865 could reduce intracellular calcium accomulation in the presence of a toxic concentration of cardiac glycosides (Wier & Hess, 1984) by directly inhibiting the calcium current. However, we have no experimental evidence for this possibility, even at the high concentration of 10 μmol l^{-1}.

In rat left atria, pretreatment with R 56865 prevented a rise in the cellular sodium content after exposure to a toxic concentration of ouabain (Wilhelm *et al.*, 1990). An inhibitory action of R 56865 on the sodium current could indirectly relieve the sodium load in an ouabain-intoxicated cardiac muscle. In our experiments with cardiomyocytes, R 56865 reduced the sodium current; however, the concentrations necessary were 2 to 3 orders of magnitude larger than those previously found to be protective (Vollmer *et al.*, 1987). The inhibition of the sodium current by R 56865 can nevertheless contribute to the protective effect because of its marked potential dependence. In the papillary muscle, R 56865 did

not affect the enhanced action potential duration in the presence of a non-toxic concentration of veratridine or ATX II. Under both conditions the resting membrane potential was unaltered. In the cardiomyocytes, a conditioning potential, less negative to the normal resting potential by only 20 mV, shifted the concentration-response curve of sodium current reduction to the left by almost one order of magnitude. If such a shift continues at even less negative potentials, it may be speculated that in the potential range of the action potential plateau, R 56865 may become effective at nanomolar concentrations. However, in this case, one would expect that veratridine could not maintain a prolonged plateau. This discrepancy suggests that possibly additional effects on potassium currents are involved. In a previous study, however, no effect of R 56865 on quasi steady-state potassium current was found (Himmel *et al.*, 1990). In any case, further experiments are needed to clarify this point.

One of the most prominent findings of our present study was the abolition of afterdepolarizations and aftercontractions by R 56865. In order to explain this effect, a brief outline of the mechanism of oscillations seems useful. Early and late afterdepolarizations occur under conditions that induce a large increase in the cytosolic calcium concentration with which the sarcoplasmic reticulum is unable to cope. Both ouabain and veratridine induce a marked sodium load albeit by different actions. The elevation of the intracellar sodium activity leads to an increase in the intracellular calcium concentration via the Na-Ca-exchange (Reuter & Seitz, 1968). This extra calcium is taken up by cellular stores from which it can be released upon following excitations. If the capacity limit of the stores to sequester calcium is reached, they become unstable and may release calcium spontaneously by an as yet unknown mechanism. The subsequent rise in the intracellular calcium concentration may induce a contractile response and a transient depolarization due to electrogenic calcium efflux via the sodium-calcium exchange (e.g. Noble, 1984; Arlock & Katzung, 1985; Mechmann & Pott, 1986; Wettwer & Ravens, 1991). It should also be mentioned that the contribution of non-specific leaky channels has been discussed (e.g. Kass *et al.*, 1978 a,b; Colquhoun *et al.*, 1981; Cannell & Lederer, 1986; Ehara *et al.*, 1988). According to this model, R 56865 could suppress potential and contractile oscillations by interference with the intracellular release mechanism of calcium; however, at present, we have no experimental evidence for a positive proof.

In addition to suppression of veratridine-induced early afterdepolarizations, R 56865 shifted the plateau of the action potential to more positive potentials indicating additional changes in net membrane current. Since inward currents were either inhibited (I_{Na}) or unaffected (I_{Ca}), this finding strongly suggests that R 56865 inhibits outward current. We have shown previously that the quasi steady-state potassium current is not altered (Himmel *et al.*, 1990), but no further positive evidence as to which potassium channel may be involved is at present available. A likely candidate is the sodium-activated potassium channel (Luk & Carmeliet, 1990).

Contractile force of untreated guinea-pig papillary muscles is not impaired by R 56865. Likewise, if veratridine is applied in a low concentration leading to a positive inotropic effect and a prolongation of the action potential duration without changing the resting membrane potential, R 56865 does not diminish the augmented force of contraction when added after veratridine. In contrast, the positive inotropism brought about by veratridine in the concentration of 2.5 $\mu mol\,l^{-1}$ is reduced when R 56865 is given on top of veratridine. The reason for the decline of contractile force is not clear; it may be speculated that a high concentration of veratridine changes the process of contraction, making it more susceptible to attenuation by R 56865.

In conclusion, our results demonstrate that the protective effect of R 56865 against veratridine-induced electrical and mechanical oscillations is not caused by a direct inhibitory effect on the calcium current. A potential-dependent inhibition of the sodium current may, however, contribute. As additional sites of action, the compound may interfere with the calcium release mechanism and inhibit potassium currents, but direct evidence for the latter two mechanisms awaits further investigations.

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