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European Journal of Pharmacology 382 (1999) 197–205

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Cicletanine prevents the excitation–conduction blocks induced by terfenadine in ischemic myocardium

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Received 22 March 1999; received in revised form 19 August 1999; accepted 24 August 1999

Abstract

Terfenadine, a histamine H_1 receptor antagonist, has been associated with clinical ventricular arrhythmias and in vitro excitation–conduction blocks, whereas anti-ischemic and antiarrhythmic effects have been shown with cicletanine, a prostacyclin generation stimulator. We aimed at determining in vitro if cicletanine can protect the ischemic myocardium from excitation–conduction blocks and specifically those induced by terfenadine. In a double-chamber bath, isolated guinea pig ventricular strips were partly exposed to normoxia and partly to ischemic, then reperfused, conditions, in the presence of 10 μM terfenadine, 10 μM indomethacin (prostacyclin generation blocker) or the solvent (dimethylsulfoxide 1:100, control) randomly allocated, and thus either in the absence ($n = 20$) or presence ($n = 21$) of 10 μM cicletanine during the total protocol duration. The multivariate Cox's model was used to predict the excitation–conduction block events and to assess the estimated survival of preparations (excitation–conduction block-free rate). Cicletanine protected the preparations (relative risk = 0.08, $t = -3.28$) from the ischemia-induced excitation–conduction blocks (estimated survival = 0.83 versus 0.30 in control), and this effect was abolished by indomethacin (estimated survival = 0.35). Terfenadine enhanced 3.58-fold the risk of occurrence of excitation–conduction blocks during ischemia ($t = 2.10$) and this effect was inhibited by cicletanine pretreatment (estimated survival = 0.40 versus 0.10 in untreated preparations). In conclusion, these in vitro findings have provided evidence for (1) protective effects of cicletanine against ischemia-induced excitation–conduction blocks, possibly related to its stimulating activity on local prostacyclin generation, and (2) efficacy of cicletanine to prevent excitation–conduction blocks induced by terfenadine in ischemic cardiac tissue.

Keywords: Myocardium; Ischemia; Electrophysiology; Conduction; Prostaglandin; Cicletanine; Terfenadine

1. Introduction

Terfenadine, a widely prescribed nonsedating antihistamine, is known as generally safe and effective. However, in some clinical settings, this specific histamine H_1 receptor antagonist has been associated with QTc prolongation and serious cardiac ventricular arrhythmias (Davies et al., 1989; Monahan et al., 1990), when elevated plasma concentrations are reached (Honig et al., 1993). In addition, in an in vitro study aimed at clarifying the role of prostacyclin (prostaglandin I_2) and histamine in the emergence of excitation–conduction blocks in ischemic cardiac muscle, terfenadine and indomethacin, a prostacyclin generation blocker, favored conduction disturbances induced by ischemia, increasing 793- and 94-fold respectively the risk of excitation–conduction blocks incidence (Monti et al., 1991). These findings thus suggest that strategies aimed at stimulating local prostacyclin production might be judicious to prevent ischemia-induced excitation–conduction blocks and eventually excitation–conduction blocks induced by terfenadine in ischemic ventricular tissue.

Otherwise, some cardioprotective activities of prostacyclin have been shown in ischemic cardiac tissue (Araki and Lefer, 1980; Ribeiro et al., 1981), for example, antiarrhythmic (Au et al., 1979) and antifibrillatory effects (Starnes et al., 1982). In this context, several studies have investigated the electrophysiological effects of cicletanine, an antihypertensive agent that has several properties, e.g., a natriuretic effect (Malherbe et al., 1988), an antihis-

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00617-2

tamine effect (Schoeffter et al., 1987; Schoeffter and Godfraind, 1988) and a stimulating action on prostacyclin generation (Dorian et al., 1984, 1988). Experimental evidence has also been provided for antiarrhythmic and/or antifibrillatory effects of cicletanine during reperfusion after coronary artery occlusion in anesthetized dogs (Jouve et al., 1986), in anesthetized rabbits (Burton et al., 1992) and in isolated rat hearts (Tosaki et al., 1990). Cicletanine might also exert anti-ischemic effects (Szilvassy et al., 1993; Ferdinandy et al., 1995) and antiarrhythmic efficacy during the early phase of ischemia, as demonstrated during coronary ligation in rabbits (Burton et al., 1992). The mechanisms underlying these antiarrhythmic effects in ischemic myocardium are however unclear. Considering the main role the myocardial conduction abnormalities play in the emergence of arrhythmias during myocardial ischemia–reperfusion (Picard et al., 1998a), examination of cicletanine effects on excitation conduction in ischemic-reperfused cardiac tissue might be helpful for the understanding of its antiarrhythmic properties.

We aimed therefore at determining, in an in vitro model of myocardial ischemia–reperfusion, whether cicletanine might confer some protection against ischemia-induced excitation–conduction blocks, particularly those induced by terfenadine in ischemic myocardium. We also tried to find if these protective effects are related to its stimulating action on local prostacyclin generation.

2. Materials and methods

Animals received humane care in compliance with the ''Principles of Laboratory Animal Care'' formulated by the U.S. National Society for Medical Research and the ''Guide for the Care and Use of Laboratory Animals'' prepared by the Institute of Laboratory Animal Resource and published by the U.S. National Institutes of Health (NIH Publication No. 86-23, revised 1985).

2.1. Materials

Female guinea-pigs (Morini, Reggio Emilia, I) weighing 400 to 500 g were killed, after brief anesthesia with ether, by cervical dislocation and exsanguination. The hearts were quickly removed and placed in oxygenated Tyrode's solution at room temperature. A thin myocardium strip was longitudinally dissected from the free wall of the right ventricle and pinned, endocardial surface upward, in a special perfusion double-chamber bath (Rouet et al., 1989; Bélichard et al., 1991; Picard et al., 1998a,b). This bath (5 ml) is bisected by a thin latex membrane containing a centrally located hole allowing the preparation to be passed carefully through and divided into two zones. The two chambers were independently superfused at the rate of 2 ml/min with Tyrode's solution oxygenated with 95% O_2 and 5% CO_2. The composition of the Tyrode's solution was (in mM): Na^+: 135; K^+: 4; Ca^{2+}: 1.8; Mg^{2+}: 1; $H_2PO_4^-$: 1.8; HCO_3^-: 25; Cl^-: 117.8 and glucose: 5.5. The pH was 7.35 ± 0.05; the temperature was maintained at 37°C by a thermostated water circulation (Polystat 86602, Bioblock, Illkirch, France). pO_2 and pCO_2 were 510 ± 20 and 34 ± 2 mmHg, respectively (BG Electrolytes Instrumentation Laboratory, Milano, Italy). At the end of each experiment, absence of leakage of the latex membrane was verified by a dye injection (methylene blue) into one chamber.

2.2. Stimulation and recordings

The preparations were stimulated at a cycle length of 450 ms, using a bipolar Teflon-coated steel wire electrode positioned on the ventricular muscle in chamber 2 (chamber remaining under normal conditions during the protocol duration). The 450-ms cycle length was chosen on the basis of a previous frequency–relationship study showing an increased incidence of electrical disturbances at high stimulation rates in this model of ischemia–reperfusion (Schiariti et al., 1994). Stimuli were rectangular pulses of 1 ms in duration and twice the diastolic threshold intensity (1–2 mA) delivered by an orthorhythmic stimulator (Explorer 1000, VPA Medical, Paris, France). Transmembrane potentials were recorded using glass microelectrodes filled with 3 M KCl and the tip resistance ranged from 10 to 30 MΩ. The microelectrode was coupled to the input stages of a capacitance-neutralizing amplifier (Electro 705, World Precision Instruments, New Haven, USA). The action potentials were displayed on a digital memory oscilloscope (Tektronix TDS 210, Tektronix, Beaverton, USA) and recorded with a polygraph (Gould RS3400, Gould Recording System Div, Cleveland, USA) allowing precise measurements of time to onset of excitation–conduction blocks during the experimental phases. The signal was concomitantly acquired and treated by Lab Windows and the following action potential characteristics were measured: resting membrane potential (RMP), action potential amplitude (APA), action potential duration at 50% of repolarization (APD_{50}), and at 90% repolarization (APD_{90}) and maximal upstroke velocity (V_{max}). Excitation–conduction blocks were defined when the ventricular preparation failed to conduct the signal from the stimulated zone (normal zone) to the adjacent one (ischemic-reperfused zone). Excitation–conduction blocks were either partial, i.e., 1–2, 1–3, etc. blocks when action potentials were recorded in the ischemic region every 2, 3, etc. stimulations, or complete when the signal elicited by each stimulation failed to reach the ischemic zone. Statistical analysis was applied to the occurrence of complete blocks.

2.3. Experimental protocol

During a 75-min equilibration period the two chambers were perfused with normal Tyrode's solution (Fig. 1).

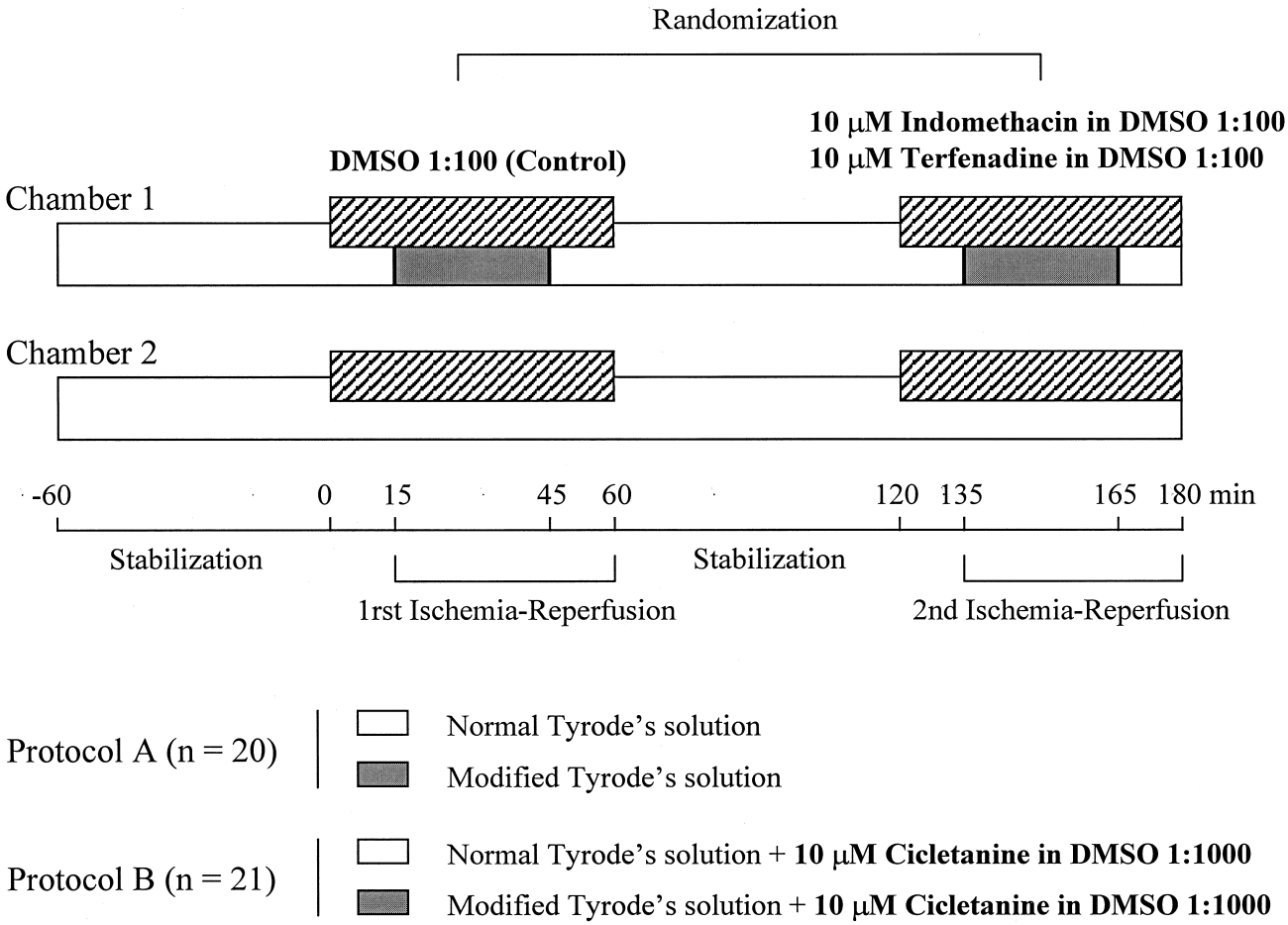

Fig. 1. Experimental protocol. Superfusion with DMSO 1:100 and drugs (10 μM indomethacin or 10 μM terfenadine) was randomized between the first and the second ischemia–reperfusion period, for the 41 preparations used in the study. Both compartments (chamber 1 and 2) of the double-chamber bath were superfused simultaneously according to the randomized drug protocol.

Thereafter, simulated ischemia was induced and maintained for 30 min in one compartment (chamber 1: ischemic zone) by superfusion with a modified Tyrode's solution while the other compartment remained under normal conditions (chamber 2: normal zone). The modified Tyrode's solution differed from normal by an elevated K^+ concentration (from 4 to 12 mM), decreased HCO_3^- concentration (from 25 to 9 mM) leading to a decrease in pH (from 7.35 ± 0.05 to 7.00 ± 0.05), decrease in pO_2 (from 510 ± 20 to 80 ± 10 mmHg) by replacement of 95% O_2 and 5% CO_2 with 95% N_2 and 5% CO_2 and withdrawal of glucose. As previously reported (Rouet et al., 1989; Bélichard et al., 1991; Picard et al., 1998a,b) the present modifications combining hypoxia, hyperkalemia, acidosis and lack of substrates are similar to those reported by Morena et al. (1980), which reproduced in vitro the electrical abnormalities induced in vivo by ischemia. Chamber 1 was then returned to superfusion with the normal Tyrode's solution for 15 min (reperfusion phase). After a consecutive 75-min equilibration period, the preparations were subjected to a second ischemia–reperfusion phase.

From 15 min before the initiation of ischemia until the end of reperfusion, the preparations were superfused in both regions with dimethylsulfoxide (DMSO) 1:100 (control) and 10 μM indomethacin or 10 μM terfenadine, both diluted in DMSO 1:100, in random order between the first and the second ischemia–reperfusion periods. This randomized protocol was applied to 20 untreated ventricular strips (protocol A) and to 21 preparations treated with 10 μM cicletanine diluted in DMSO 1:1000 during the total experiment duration (protocol B). DMSO, indomethacin and terfenadine were obtained from Sigma and cicletanine from IPSEN (Paris, France).

2.4. Multivariate Cox's analysis

The data in text and table are expressed as means ± standard deviation (SD). The statistical analysis of data was performed using an exponential model of risk prediction for complete excitation–conduction block occurrence during simulated ischemia, as continuous monitoring allowed precise measurement of the time of onset of com-

plete excitation–conduction block. Indeed, the time to occurrence of a given event, even during brief observation periods as in experiments on acute coronary occlusion in dogs, is a crucial element for the use of a predictive exponential model considered more appropriate for the analysis of predictive variables (risk factors) than the logistic model (Puddu et al., 1988, 1989). Using the BMDP-2L program (forced Cox's model) the following covariates were considered in the analysis: ischemia number (1 = first, 2 = second), guinea pig weight (in g), differences (Δ) in action potential parameters between values measured after 15 min pretreatment with either DMSO 1:100, 10 μM indomethacin or 10 μM terfenadine and basal values, and presence of 10 μM cicletanine or 10 μM terfenadine. Patterns of estimated survival during ischemia, namely the proportion of cardiac preparations still devoid of complete excitation–conduction block, were also built: first, for pooled groups of each protocol, A and B, in order to investigate the contribution of cicletanine treatment to excitation–conduction block occurrence, second, for each group in order to determine the contributory role of indomethacin and terfenadine to excitation–conduction block occurrence in untreated (protocol A) and treated (protocol B) ventricular strips.

3. Results

As illustrated in Fig. 2, ischemic conditions induced slowing of signal conduction (Fig. 2E) leading to partial (Fig. 2E–F) then total (Fig. 2G) blocks between both normal and ischemic zones. All excitation–conduction blocks were thereafter removed by reperfusion, and action the potentials parameters previously modified by ischemia returned to close to their values measured before initiation of the ischemia–reperfusion phase (Fig. 2I versus B).

Basal action potential parameters measured before DMSO or drug pretreatment for the 41 preparations were

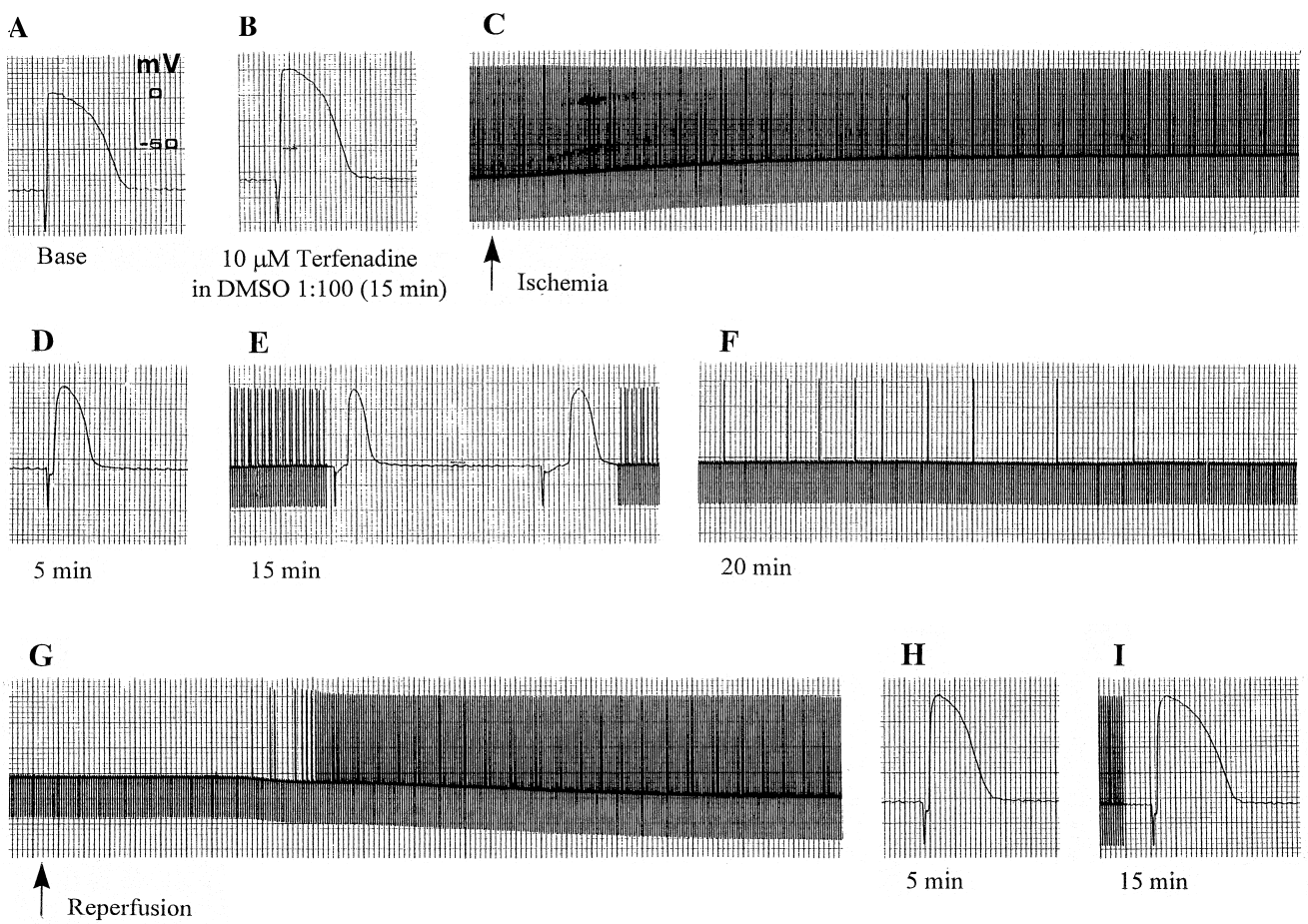

Fig. 2. Representative recordings of excitation–conduction block during simulated ischemia–reperfusion. Action potentials were recorded in the ischemic zone (chamber 1) while the ventricular strips were stimulated in the normal zone (chamber 2). Action potentials were recorded with a polygraph at 100 mm/s (A, B, D, E, H, I) or at 1 mm/s (C, E, F, G, I) in the presence of 10 μM terfenadine in DMSO 1:100. Please note slight depolarisation induced by 15 min with terfenadine (B versus A). Ischemic conditions altered action potentials shape (D, E) and slowed signal conduction (E: increased time interval between the stimulus artifact and the action potential) until partial (E: block 3:1, panel F) then total (G) excitation–conduction block occurred. Thereafter, reperfusion allowed removal of excitation–conduction block (G) and rapid membrane repolarisation (H). At the end of the reperfusion phase (I), action potentials were similar to those recorded before initiation of ischemia (B).

APA 109 ± 8 mV, RMP -85 ± 8 mV, APD_{50} 99 ± 21 ms, APD_{90} 134 ± 23 ms, V_{max} 206 ± 30 V/s. In the control (protocol A), reperfusion allowed recovery of the action potential parameters previously altered by ischemia, to values close to the basal ones: APA 115 ± 9 ms versus 113 ± 7 ms, RMP -84 ± 8 ms versus -86 ± 8 ms, APD_{50} 108 ± 11 ms versus 106 ± 17 ms, APD_{90} 142 ± 15 ms versus 138 ± 20 ms, V_{max} 212 ± 43 V/s versus 213 ± 25 V/s. Fifteen minutes superfusion with DMSO 1:100 alone did not modify significantly the action potential parameters, whereas cicletanine shortened APD_{50} and APD_{90} by 9.7 ± 22.2 ms and 9.3 ± 25.2 ms, respectively, indomethacin reduced APD_{50} and APD_{90} by 7.0 ± 21.8 ms and 8.5 ± 19.0 ms, respectively, and terfenadine depolarized the cardiac cell membrane by 2.2 ± 9.0 mV, although these variations were not statistically significant as compared to effects in the DMSO 1:100 group (control, protocol A). After reperfusion in the presence of terfenadine, APD_{50} and APD_{90} were lengthened as compared to their basal values: $+7.0 \pm 16.2$ ms and $+6.5 \pm 19.4$ ms, respectively, and V_{max} was decreased by 30.7 ± 3.3 V/s versus a decrease of 1.1 ± 38.8 V/s in DMSO 1:100 group (protocol A); again these variations were not statistically significant.

The covariates used in the forced multivariate analysis of the predictive Cox's model are described in Table 1.

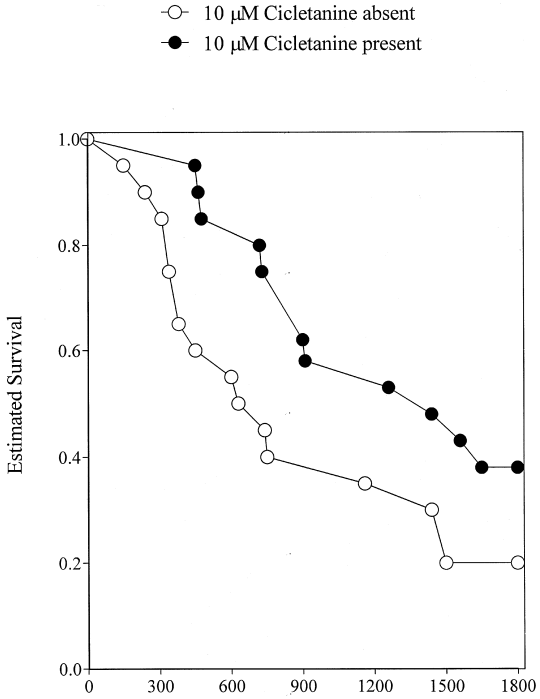

Fig. 3. Estimated survival for excitation–conduction blocks during ischemia, based on Cox's model. Cumulative estimated survival curves are given for pooled groups of protocol A (Fig. 1): Cicletanine absent ($n = 20$) and protocol B (Fig. 1): 10 μM cicletanine present ($n = 21$).

Table 1
Descriptive statistics for the 41 experiments of the study and prediction of excitation–conduction blocks during ischemia, based on the forced multivariate Cox's model

Δ: Difference between values measured after drug pretreatment before initiation of ischemia (Fig. 1: 15 min and 135 min) and basal values (Fig. 1: 0 min and 120 min).

Abbreviations: APA, action potential amplitude; RMP, resting membrane potential; $APD_{50,90}$, action potential duration measured at 50% and 90% of repolarization respectively; V_{max}, Maximal upstroke velocity of action potential; *df*, degree of freedom. $|t| > 1.96: P < 0.05$.

Variables	Mean ± SD	Coefficient	*t*	Relative risk
Ischemia 1 versus 2[a]	0.46 ± 0.50	0.520	1.08	1.68
Weight (g)	442 ± 165	−0.004	−1.93	1.00
Presence of cicletanine[b]	0.51 ± 0.51	−2.487	−3.28	0.08
ΔAPA (mV)	2.68 ± 8.00	−0.058	−1.50	0.94
ΔRMP (mV)	−1.93 ± 8.14	0.057	1.96	1.06
ΔAPD_{50} (ms)	−8.37 ± 20.04	0.049	1.53	1.05
ΔAPD_{90} (ms)	−8.17 ± 18.43	−0.045	−1.16	0.96
ΔV_{max} (V/s)	1.49 ± 23.88	0.004	0.44	1.00
Presence of terfenadine[b]	0.49 ± 0.51	1.275	2.10	3.58
Loglikelihood	−83.98			
Global χ^2	20.68			
df	9			
P value	0.0141			

[a] Coded 0 (1st Ischemia) or 1 (2nd Ischemia).
[b] Coded 0 (absent) or 1 (present).

Covariates showed a significant global contribution in the prediction of the occurrence of excitation–conduction blocks during simulated ischemia (loglikelihood = −83.98; $\chi^2 = 20.68$; $P = 0.0141$). Neither the variable used for the randomization (ischemia number) nor the animal weight and the covariates measured before and after drug pretreatment (ΔAPA, ΔRMP, ΔAPD_{50}, ΔAPD_{90}, ΔV_{max}) were significantly related to the occurrence of excitation-conduction blocks. The pretreatment with 10 μM cicletanine showed significant protective effects against excitation–conduction blocks ($t = -3.28$), and increased the estimated surviving rate (Fig. 3), i.e., the proportion of preparations free of excitation–conduction blocks, from 0.20 (in the 20 preparations of protocol A) to 0.38 (in the 21 preparations of protocol B) at the end of the ischemic phase. Conversely, the presence of 10 μM terfenadine during ischemia–reperfusion favored the excitation–conduction blocks ($t = 2.10$), enhancing more than threefold the risk of their occurrence [exp(coeff) = 3.58].

The effects of the drugs used in the randomized protocol (indomethacin and terfenadine) on the occurrence of excitation–conduction blocks, in the absence or presence of 10 μM cicletanine, are detailed on Fig. 4, which shows the estimated survival of ventricular preparations for each group of protocols A and B. The presence of 10 μM cicletanine enhanced the estimated surviving rate of preparations from 0.30 to 0.83 (Fig. 4: Controls) after 30 min of

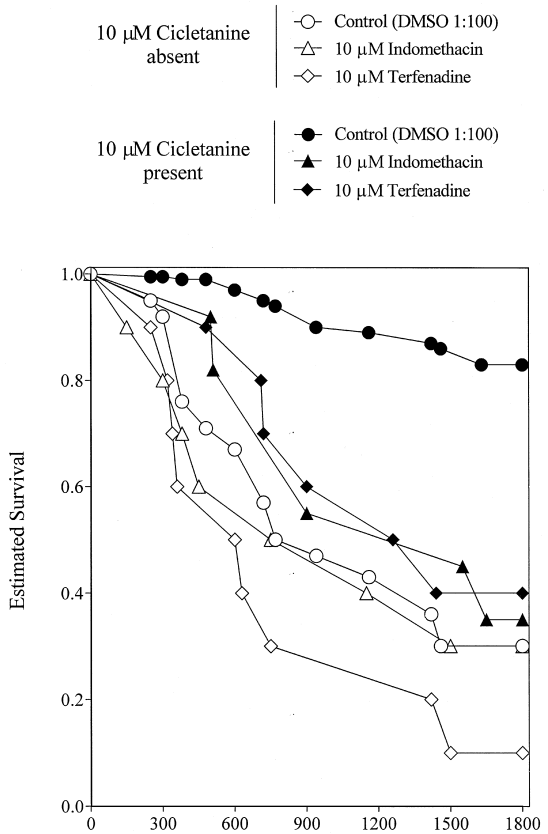

Fig. 4. Estimated survival for excitation–conduction blocks during ischemia, based on Cox's model. Estimated survival curves are given for each group: control (DMSO 1:100), 10 μM indomethacin and 10 μM terfenadine, in the absence of cicletanine (Fig. 1: protocol A, $n = 20$) and in preparations pretreated with 10 μM cicletanine (Fig. 1: protocol B, $n = 21$).

ischemia, and this protective effect was antagonized by 10 μM indomethacin (estimated survival = 0.35). When used alone, indomethacin did not modify the proportion of preparations free of excitation–conduction blocks at the end of the ischemic period (estimated survival = 0.30 versus 0.30 for control in protocol A). In ventricular preparations not pretreated with cicletanine, 10 μM terfenadine favoured the occurrence of excitation–conduction blocks, decreasing the estimated survival of preparations from 0.30 (control) to 0.10 after 30 min of simulated ischemia. This promoting effect of terfenadine on excitation–conduction blocks was abolished by pretreatment with 10 μM cicletanine, leading to an estimated survival of preparations (0.40, Terfenadine group in protocol B) similar to that of the control in the absence of the drugs (0.30, control in protocol A) after 30 min of simulated ischemia.

4. Discussion

The main findings of this in vitro study were: (1) cicletanine protects the ventricular myocardium from the occurrence of excitation–conduction blocks during simulated ischemia; (2) this protection was abolished by indomethacin, suggesting that benefits of cicletanine against the emergence of conduction disturbances might be related to its stimulating action on local prostacyclin generation, and (3) cicletanine pretreatment is efficient to prevent the excitation–conduction blocks induced by terfenadine in the ischemic cardiac muscle.

In this in vitro model of partial ischemia–reperfusion, cicletanine has shown a protective efficacy against the occurrence of excitation-conduction blocks between normal and ischemic ventricular tissues. These findings might explain, at least in part, some antiarrhythmic effects obtained with cicletanine during coronary artery ligation in anesthetized rabbits (Burton et al., 1992). Indeed, myocardial conduction disturbances including excitation–conduction blocks and slow retrograde conduction are required to initiate re-entrant circuits and facilitate the emergence of arrhythmias. By prevention of excitation–conduction blocks between the normal and ischemic cardiac regions, cicletanine may thus inhibit re-entry movements and show an antiarrhythmic efficacy in cardiac muscle rendered partly ischemic by coronary artery occlusion. Our in vitro results may be relevant for the understanding of in vivo antiarrhythmic effects, considering that, as previously discussed (Rouet et al., 1989; Picard et al., 1998a,b), the experimental conditions used to simulate myocardial ischemia are able to reproduce in vitro the electrophysiological alterations observed in animal models during acute myocardial ischemia (Janse and Wit, 1989; Janse et al., 1979).

The effects of cicletanine against the ischemia-induced excitation–conduction blocks were abolished by indomethacin, suggesting that the protection afforded by cicletanine may be due to its stimulating action on the local endogenous prostacyclin generation within the cardiac muscle. Based on an in vivo model of coronary artery occlusion–reperfusion in anesthetized dogs, Jouve et al. (1986) had suggested a correlation between an increased local prostacyclin production and antiarrhythmic effects of cicletanine. Tosaki et al. (1991) also reported an increased endogenous 6-keto prostaglandin $F_{1\alpha}$, a stable metabolite of prostacyclin, in isolated rat heart before ischemia. Although the ventricular preparations used in our model were superfused, local prostacyclin generation might occur in our preparations, in accordance with a previous finding of significant effects of indomethacin on the occurrence of excitation–conduction blocks (Monti et al., 1991). Otherwise, it cannot be ruled out that cicletanine may prevent the excitation–conduction blocks during ischemia also by interfering with the myocardial ionic homeostasis. There are several reports that cicletanine may stimulate K^+ movements across the human erythrocyte membrane (Garay et al., 1984) and may block voltage-dependent Ca^{2+} channels (Noack et al., 1991) and/or open K^+ channels (Ebeigbe and Cabanie, 1991) in vascular cells. Ca^{2+} channel blocking and K^+ channel opening in cardiac muscle

can explain the slight action potential shortening observed in our preparations superfused with cicletanine. In rat cardiac muscle also, cicletanine attenuates the ion shifts (Na^+ and Ca^{2+} gains and K^+ loss) induced by ischemia–reperfusion (Koltai et al., 1992). Further investigations are now needed to determine the precise mechanisms by which cicletanine prevents the excitation–conduction blocks in ischemic ventricular tissue.

Although the role of histamine in arrhythmia genesis in the ischemic myocardium is still controversial, the hormone has been associated with ventricular arrhythmias in various animal models (Trzeciakowski and Levi, 1982; Gaide et al., 1984; Gross et al., 1984; Dai, 1987) and in human atrial fibers (Levi et al., 1981). Some histamine receptor agonists have been closely correlated with impaired atrio-ventricular conduction in guinea pig isolated hearts (Levi et al., 1975), suggesting that the pro-arrhythmicity induced by histamine might arise from alterations of cardiac excitation conduction. The arrhythmogenic effects of the hormone have thus aroused a certain interest in the potential antiarrhythmic benefits of antihistaminic agents (Giotti and Zilletti, 1976). The promoting effect of terfenadine on the occurrence of excitation–conduction blocks in our model of myocardial ischemia is therefore in contrast with the abovementioned studies. Nevertheless, this in vitro finding may provide some explanation for the pro-arrhythmic action of this H_1 receptor antagonist, observed more recently in some clinical settings (Davies et al., 1989; Monahan et al., 1990), although caution is required when confronting in vitro observations with clinical data.

The mechanisms by which terfenadine favoured the excitation–conduction blocks in ischemic myocardial tissue now need to be elucidated. Patch–clamp studies have demonstrated the ability of terfenadine to block the delayed rectifier K^+ current (Rampe et al., 1993), the inward rectifier K^+ current (I_{K1}) and the transient outward currents (I_{to}) (Berul and Morad, 1995) and also the adenosine triphosphate-sensitive K^+ current (Nishio et al., 1998). Blocking effects of terfenadine have also been found on L-type Ca^{2+} channels (Liu et al., 1997) and Na^+ channels (Ming and Nordin, 1995; Lu and Wang, 1999), thus reducing V_{max} in cardiac cells (Lang et al., 1993). These terfenadine effects on K^+ and Na^+ channels can explain the RMP, APD_{50}, APD_{90} and V_{max} changes observed in the present study, although these variations were not statistically significant, likely due to a too short terfenadine superfusion period preceding the ischemia phase. Indeed, we superfused the preparations with terfenadine only for 15 min in order to avoid dramatic V_{max} reduction and eventual excitation–conduction block onset before initiation of the ischemic phase. The cell membrane depolarization induced by terfenadine might modify excitation conduction in the cardiac tissue, according to the lessened availability of the Na^+ channels in depolarized cells, enhancing the terfenadine-induced Na^+ blockade. This hypothesis is supported by our present finding of a promoting tendency, although only borderline statistically significant ($t = +1.96$), of membrane depolarization (ΔRMP variable in Table 1) on excitation–conduction blocks occurrence. It is also unclear if the preventive action of cicletanine on the emergence of terfenadine-induced excitation–conduction blocks during ischemia involves its stimulating effects on K^+ current, counteracting the K^+ blockade induced by terfenadine, thus maintaining Na^+-channel availability and preventing the occurrence of conduction blocks, or its antihistaminic properties which might somehow compete with terfenadine at the cell membrane H_1 receptors. Further investigations would be necessary to clarify the electrophysiological interaction between these two agents.

An important finding of this in vitro study was the efficacy of cicletanine to prevent the excitation–conduction blocks related to myocardial ischemia and also those induced by the antihistamine H_1 receptor antagonist, terfenadine, in ischemic ventricular tissue. These results might open interesting perspectives regarding the clinically observed proarrhythmic effects of terfenadine and the benefits of cicletanine against the conduction abnormalities associated with ischemia and terfenadine. The relevance of the model used was already discussed (Rouet et al., 1989; Schiariti et al., 1994; Picard et al., 1998a,b) and the adequacy of the electrophysiological approach was supported by the stability of the isolated preparations used, as illustrated by the recovery of action potential parameters after 15-min reperfusion. It should, however, be kept in mind that an in vitro model using superfused isolated ventricular tissue under simulated ischemic-reperfused conditions remains distant from both clinical and in vivo ischemic conditions. As discussed well by Rosen (1988), an in vitro study combining basic electrophysiology, mathematical modeling of event prediction and clinically addressed simulation of pathological conditions, may provide interesting information on the limits of a cell's ability to perform in a simulated controlled environment rather than its usual behavior in a healthy or a diseased heart. Our findings should thus be confirmed in vivo and, cicletanine, used in the treatment of hypertension, needs to be shown to be clinically effective against cardiac electrical disorders in ischemic myocardium, especially when conduction disturbances are associated with drug administration, as is the case with terfenadine.

Acknowledgements

The authors thank Alberto Lanza, Roberto Frioni, Anna Di Mario, Ernesto Evangelista and Giovanni Pulvirenti from the Laboratory of Cardiovascular Pharmacology for technical assistance during the experiments. This work was supported in part by Cardioricerca, Rome, Italy.

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