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Electrophysiological characterization of BRL-32872 in canine Purkinje fiber and ventricular muscle
Effect on early after-depolarizations and repolarization dispersion

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Abstract

Amongst the different pharmacological approaches to the treatment of cardiac arrhythmias, compounds with multiple electrophysiological activities appear to exhibit a reduced adverse effect profile. BRL-32872 (*N*-(3,4-dimethoxyphenyl)-*N*-[3[[2-(3,4-dimethoxyphenyl) ethyl] propyl]-4-nitrobenzamide hydrochloride) is a typical example of an antiarrhythmic agent with combined K^+ and Ca^{2+} blocking actions. In this study, we investigated the effects of BRL-32872 on early after-depolarizations and on dispersion of repolarization. Action potentials were recorded either in canine cardiac Purkinje fibers alone or in preparations containing both ventricular muscle and the attached Purkinje fibers. In Purkinje fibers, BRL-32872 (0.3–10 μM) induced a bell-shaped concentration-dependent increase in action potential duration. At 90% of repolarization, the action potential was prolonged at concentrations up to 1 μM and was shortened when the concentration of BRL-32872 was further increased. In all 17 experiments, BRL-32872 did not cause early after-depolarizations in Purkinje fibers. On the contrary, BRL-32872 (3 μM) systematically suppressed early after-depolarizations induced by clofilium (4-chloro-*N*,*N*-diethyl-*N*-heptylbenzenebutanaminium tosylate, 1 μM), a selective inhibitor of the delayed rectifier K^+ current. A similar effect was observed once with 1 μM BRL-32872, a concentration able to prolong Purkinje fiber action potentials. Simultaneous recording of action potentials in ventricular and Purkinje preparations showed that increasing concentrations of BRL-32872 (0.3–10 μM) induced a limited increase in the difference of repolarization time between the two tissues. The selective K^+ channel inhibitor E-4031 (*N*-(4-(1-[2-(6-methyl-2-pyridyl) ethyl]-4-piperidyl)-carbonyl] phenyl) methanesulfonamide dihydrochloride dihydrate) exhibited a significant concentration-dependent increase in dispersion of repolarization. We conclude from the present results that the Ca^{2+} blocking activity of BRL-32872 (i) prevents the occurrence of early after-depolarizations associated with action potential prolongation and (ii) limits an excessive increase in action potential duration heterogeneity. These electrophysiological features might represent the basis for antiarrhythmic compounds with reduced proarrhythmic profile.

Keywords: Antiarrhythmic, Class III; Proarrhythmic profile; K^+ channel antagonist; Ca^{2+} channel antagonist; Clofilium; E-4031

1. Introduction

Class III antiarrhythmic compounds are defined as compounds able to prolong cardiac action potential duration. Most of the developed class III antiarrhythmics are specific inhibitors of the rapid component of the delayed rectifier K^+ current (I_{Kr}). These agents induce a dramatic reduction of the incidence of atrial and ventricular arrhythmias. However they also exhibit proarrhythmic effects which have been described extensively (Hondeghem and Snyders, 1990; Hohnloser and Singh, 1995; Bril, 1996; Lazzara, 1996; Roden, 1996) and which are characterized in patients by the occurrence of torsades de pointe, a particular type of polymorphic ventricular tachycardia. Whereas, the underlying mechanisms are not fully elucidated, it is generally assumed that two characteristics of known K^+ channel blockers may account for their proarrhythmic potential. First, these compounds demonstrate a reverse rate-dependent effect, i.e., they are more effective at prolonging cardiac action potential at slow than at fast heart rates. This property is responsible for the excessive prolongation of cardiac repolarization at slow rates, favoring the occurrence of early after-depolarizations and torsades de pointe. Second, the sensitivity of the different

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cardiac tissues to these compounds is heterogeneous (Lathrop, 1985; Gwilt et al., 1991; Abrahamsson et al., 1993; Antzelevitch et al., 1995). In particular, most known K^+ channel inhibitors increase action potential duration more markedly in Purkinje fibers than in ventricular tissue (Lathrop, 1985; Li et al., 1990; Abrahamsson et al., 1993). This effect, which leads to an increase in the dispersion of repolarization, may favor the occurrence of re-entry and therefore provoke cardiac arrhythmias. Both induction of early after-depolarizations and increased heterogeneity in refractoriness are suggested to be, at least in part, responsible for the relatively high incidence of proarrhythmic events during therapy with known class III antiarrhythmic drugs (Surawicz, 1989; Roden, 1993).

We and others have recently proposed that compounds with multiple electrophysiological activities could exhibit potent antiarrhythmic efficacy associated with less proarrhythmic risk than selective inhibitors of the delayed rectifier K^+ channel (Singh, 1994; Bril, 1996; Bril et al., 1997; Anyukhovsky et al., 1997; Banyasz et al., 1999). Among these agents, BRL-32872 (*N*-(3,4-dimethoxyphenyl)-*N*-[3[[2-(3,4-dimethoxyphenyl) ethyl] propyl]-4-nitrobenzamide hydrochloride, Fig. 1) has been shown to inhibit the delayed rectifier K^+ current with an additional inhibitory effect on the Ca^{2+} current, resulting in a bell-shaped concentration-dependent increase in action potential duration in guinea-pig preparations (Bril et al., 1995). The dual electrophysiological effect of BRL-32872 appeared to be responsible for the potent antiarrhythmic efficacy associated with a reduced proarrhythmic activity when the drug was investigated in vivo (Bril et al., 1995, 1996; Gout et al., 1995). In previous studies, we showed that, in contrast to selective inhibitors of the delayed rectifier K^+ current, BRL-32872 prolonged action potential duration in a neutral frequency-dependent manner, i.e., the stimulation rate did not influence the ability of BRL-32872 to prolong cardiac action potential (Bril et al., 1998). This property, which was confirmed in patients (Kosar et al., 1997), was suggested to result from the additional inhibition of the Ca^{2+} current induced by the compound (Bril et al., 1998).

The in vitro effects of BRL-32872 have been mainly investigated in guinea-pig preparations (Bril et al., 1995, 1998; Faivre et al., 1998). BRL-32872 has been shown in vivo to be a potent antiarrhythmic compound in canine models of arrhythmias (Bril et al., 1996). Therefore, the objective of the present study was to investigate the electrophysiological profile of BRL-32872 in canine preparations with particular attention being paid to early after-depolarizations and dispersion of repolarization, which are both involved in the proarrhythmic behavior of inhibitors of the delayed rectifier K^+ current.

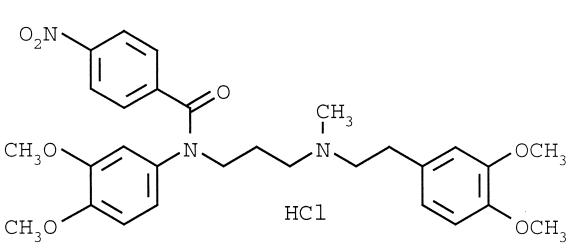

Fig. 1. Chemical structure of BRL-32872. Molecular weight: 574.07 (as monohydrochloride salt), 537.61 (as free base); pK_a: 8.52; log *P*: 2.79.

2. Material and methods

2.1. Canine ventricular tissue and Purkinje fiber preparations

Purpose bred Mongrel dogs of either sex (≈ 30 kg; Marshall Europe, Lyon, France) were maintained in accordance with National Institute of Health (NIH) guidelines (publication no. 85-23) for animal care. After deep anesthesia was induced with pentobarbital (30 mg/kg), the chest was opened and the heart was rapidly excised and immersed in a modified Tyrode solution, maintained at 4°C and previously saturated with 95% O_2/5% CO_2. The composition of the modified Tyrode solution was as follows (mM): NaCl 125, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, $NaHCO_3$ 24, NaH_2PO_4 0.9, glucose 11 (pH 7.4). A piece of either right or left ventricle containing free Purkinje fibers was removed and fixed to the silastic bottom of an organ bath perfused with the same solution maintained at 37°C.

2.2. Electrophysiological recordings

Transmembrane action potentials were recorded by means of a dual set of conventional microelectrodes filled with KCl 3 M and connected to a high input impedance Biologic VF 180 amplifier (Biologic, Claix, France). External stimuli (2 ms, twice threshold, 1 Hz or 0.25 Hz) were delivered by a Pulsar 6i stimulator (Frederick Haer, Brunswick, ME) via bipolar platinum electrodes applied at one end of the preparation. Signals were monitored on a storage 20 MHz oscilloscope (Gould 1604, Ballainvilliers, France) and simultaneously recorded on a digital tape recorder (Biologic DTR 1200). Action potentials were acquired and analyzed with a microcomputer equipped with a 12 bit analog–digital DAS50 converter.

2.3. Experimental protocols

After a stabilization period of at least 2 h, action potentials were recorded either from Purkinje fibers alone or simultaneously from Purkinje fibers and ventricular muscle. In the latter case, two microelectrodes were impaled simultaneously, one in the Purkinje fiber and one in the ventricular tissue. Only impalements that exhibited action potentials typical of Purkinje fibers or ventricular tissue were considered. Action potential stability was assessed during a further 30-min stabilization period. The preparation was then superfused with increasing concen-

trations of either E-4031 (*N*-(4-(1-[2-(6-methyl-2-pyridyl)ethyl]-4-piperidyl)-carbonyl]phenyl, successively 0.01, 0.03, 0.1 and 0.3 μM) or BRL-32872 (successively 0.3, 1, 3 and 10 μM). To assess the concentration-dependent effects of E-4031 and BRL-32872, each concentration was applied for 30 min, a duration that was considered as the time necessary to reach steady-state. In some experiments clofilium (1 μM, 4-chloro-*N*,*N*-diethyl-*N*-heptylbenzenebutanaminium tosylate) was superfused either alone or in the presence of BRL-32872 (1 μM or 3 μM) to determine the occurrence of early after-depolarizations. In all cases, measurements were made of the following: Resting membrane potential, action potential amplitude, maximum rate of depolarization (V_{max}) and action potential duration measured at 50% and 90% of repolarization (APD_{50} and APD_{90}, respectively).

2.4. Drugs

BRL-32872 (SmithKline Beecham, Saint-Grégoire, France; Fig. 1), clofilium (Lilly, synthesized at SmithKline Beecham, Saint-Grégoire, France) and E-4031, methanesulfonamide dihydrochloride dihydrate, (Eisai, synthesized at SmithKline Beecham, Saint-Grégoire, France) were prepared daily as stock solutions in water (2.5 mM) and further dilutions were made in the modified Tyrode solution. All other chemicals were of analytical grade.

2.5. Statistics

Results are presented as means ± S.E.M. The effects of increasing concentrations of BRL-32872 or E-4031 on ventricular or Purkinje preparations were analyzed using repeated measures analysis of variance followed by multiple comparisons according to the Sidak procedure. To compare the effects of E-4031 or BRL-32872 on the two tissues, statistical analysis was performed using a two factor repeated measures analysis of variance. The effect of compound concentrations on a given tissue and the influence of the tissue on the response to each compound concentration were assessed to compare the effects of BRL-32872 or E-4031 on Purkinje fibers and ventricular muscle for each concentration. The design of the analysis of variance consisted of a between-group comparison to analyze the effect of the tissue where the activity was measured and a within group comparison to analyze the effect of the different drug concentrations. For parameters in which a simple main effect of drug concentration was found to be significant, further comparisons of the effects of drugs on a given tissue were made using a within group analysis of variance followed by multiple comparisons using the Sidak procedure (Ludbrook, 1994). Because the group sizes considered in this study were equal, the Greenhouse–Geisser adjustment was used to calculate the *P*-value (Ludbrook, 1994). All statistical analyses were performed by means of the microcomputer statistical program CRUNCH 4.0 (Crunch Software, Oakland, CA). A *P*-value of less than 0.05 was considered as statistically significant.

3. Results

3.1. Effect of BRL-32872 in canine Purkinje fibers

BRL-32872 has been reported to induce a bell-shaped concentration-dependent increase in action potential duration in guinea-pig ventricular cells (Bril et al., 1995). In order to evaluate its effects in canine Purkinje fibers, BRL-32872 was applied in a concentration-dependent manner according to a protocol similar to that used in guinea-pig ventricular preparations (Bril et al., 1995). The control trace shown in Fig. 2A illustrates a typical action potential recorded from canine Purkinje fibers at a stimulation frequency of 1 Hz. The addition of increasing concentrations of BRL-32872 to the superfusion solution resulted in a dual modification of action potential duration (Fig. 2 and Table 1). For concentrations up to 1 μM, BRL-32872 induced a significant increase in APD_{90}; this action potential prolongation was reduced when the concentration was further increased to 3 μM and 10 μM. Thus, BRL-32872 induced a bell-shaped concentration-dependent increase in Purkinje fiber action potential duration (Fig. 2B), an effect which was qualitatively similar to that reported in guinea-pig papillary muscles (Bril et al., 1995). The resting membrane potential, action potential amplitude and upstroke velocity were not significantly changed by increasing the concentration (from 0.3 to 10 μM) of BRL-32872 (Table 1).

3.2. Effect of BRL-32872 on early after-depolarizations induced by a selective inhibitor of the delayed rectifier K^+ current in canine Purkinje fibers

Among the 17 experiments performed with BRL-32872 in Purkinje fibers, early after-depolarizations were not observed despite a significant increase in action potential duration. To study further the profile of BRL-32872 in vitro, its effects on fibers in which early after-depolarizations were induced by a selective K^+ channel antagonist were investigated. For these experiments, clofilium was chosen because this compound is one of the most effective K^+ channel blockers to induce early after-depolarizations in vivo (Buchanan et al., 1993a) as well as in vitro (Gough and El-Sherif, 1989). On this basis, clofilium (1 μM) was applied to Purkinje fibers continuously stimulated at a slow stimulation rate (0.25 Hz) in order to favor the occurrence of early after-depolarizations. In all experiments ($n = 9$), clofilium was found to increase markedly action potential duration and this prolongation resulted in the development of early after-depolarizations. The original recording obtained from one of these experiments is illustrated in Fig. 3. In this experiment, early after-de-

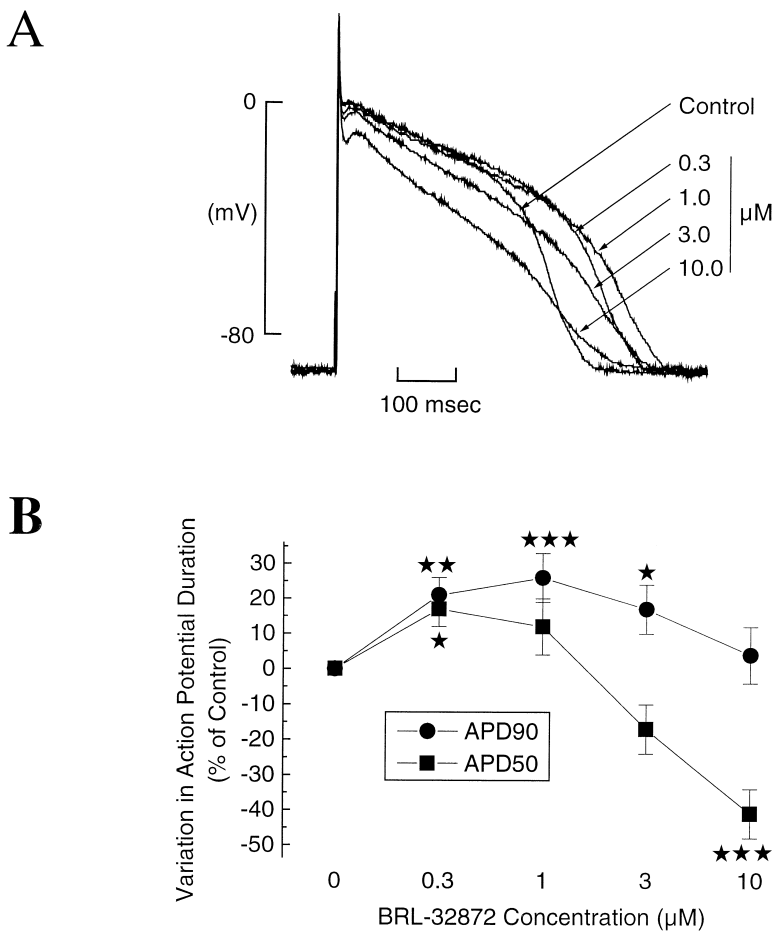

Fig. 2. Effect of BRL-32872 on the action potential of Purkinje fibers. (A) Effect of increasing concentrations of BRL-32872 on the action potential obtained from a fiber stimulated at 1 Hz. Each concentration was applied for 30 min before the recording was made. (B) Effect of increasing concentrations of BRL-32872 on action potential duration measured at 50% and 90% of repolarization (APD_{50} and APD_{90}, respectively). Each value represents the mean ± S.E.M. of eight experiments conducted at a stimulation frequency of 1 Hz. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control values.

polarizations occurred, 25 min after clofilium was added to the superfusion medium (Fig. 3, first panel). The early after-depolarization frequency increased progressively and then degenerated into bursts of spontaneous activity 12 min later (Fig. 3, second panel). At this time, BRL-32872 (3 μM) was added to the superfusion medium in the continued presence of clofilium. As illustrated in the third panel of Fig. 3, BRL-32872 inhibited early after-depolarizations within less than 10 min. When BRL-32872 was removed from the superfusion medium, the action potential duration increased again (Fig. 3, fourth panel). Although more commonly observed with 3 μM of BRL-32872, as illustrated in Fig. 3, a similar result was obtained with 1 μM of BRL-32872. These results demonstrate that BRL-32872 does not promote early after-depolarizations in Purkinje fibers and can actually suppress them when induced by selective inhibitors of the delayed rectifier K^+ current.

Table 1
Effect of BRL-32872 on action potential parameters recorded from canine Purkinje fibers ($n = 7$)
APA: Action potential amplitude; RMP: Resting membrane potential; V_{max}: Maximal upstroke velocity; APD_{50} and APD_{90}: Action potential duration measured at 50% and 90% of repolarization, respectively.

		BRL-32872 (μM)			
	Control	0.3	1.0	3.0	10.0
APA (mV)	125 ± 1	127 ± 2	126 ± 1	125 ± 1	122 ± 2
RMP (mV)	−90 ± 1	−91 ± 1	−90 ± 1	−89 ± 1	−89 ± 1
V_{max} (V/s)	439 ± 37	424 ± 31	454 ± 33	443 ± 27	398 ± 28
APD_{50} (ms)	291 ± 29	338 ± 29	322 ± 28	236 ± 21	165 ± 16[a]
APD_{90} (ms)	392 ± 32	470 ± 36[b]	491 ± 37[c]	451 ± 30	397 ± 25

[a] $P < 0.001$ vs. control value.
[b] $P < 0.05$.
[c] $P < 0.01$.

3.3. Effect of BRL-32872 on repolarization dispersion in Purkinje-ventricle preparations. Comparison with E-4031

The action potential duration is physiologically shorter in the ventricle than in Purkinje fibers and inhibitors of the delayed rectifier K^+ current are more effective in prolong-

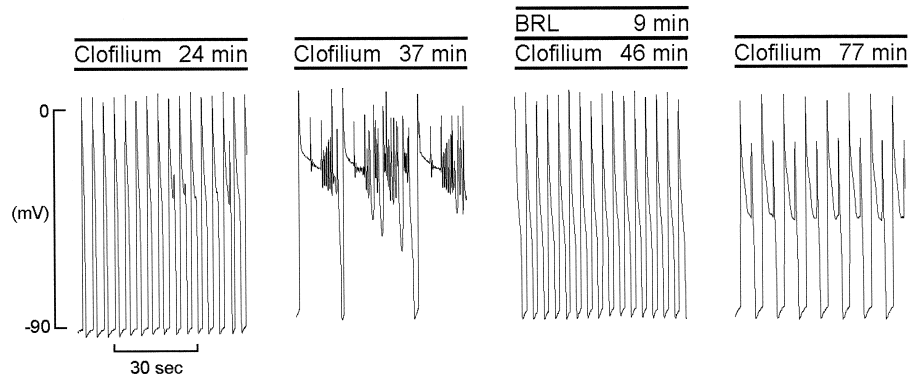

Fig. 3. Effects of BRL-32872 on early after-depolarizations in dog Purkinje fibers. The four experimental traces correspond to the same experiment and were recorded in the 24th, 37th, 46th and 77th minutes of 1 μM clofilium application. BRL-32872 3 μM was added during the 38th minute of the clofilium response. During the whole experiment, the preparation was stimulated at a frequency of 0.25 Hz.

ing the action potential in Purkinje fibers than in ventricular tissue (Lathrop, 1985; Li et al., 1990; Abrahamsson et al., 1993). Because of these characteristics, known class III antiarrhythmic agents lead to an increase in heterogeneity of action potential duration, principally when they are administered at high concentrations. To evaluate whether BRL-32872 affects the dispersion of repolarization, canine Purkinje-ventricle preparations were superfused with increasing concentrations of BRL-32872 and the results were compared to those observed with E-4031, a specific blocker of the rapid component of the delayed rectifier K^+ current (I_{Kr}) also known as the E-4031-sensitive current (Sanguinetti and Jurkiewicz, 1990). Experiments were conducted at a stimulation frequency of 1 Hz. Changes in action potential duration measured in Purkinje fibers and ventricular muscle after addition of increasing concentrations of BRL-32872 or E-4031 are shown in Fig. 4. When tested at 0.03 and 0.3 μM, E-4031 induced a concentration-dependent increase in APD_{90} both in Purkinje fibers and in ventricular muscle (Fig. 4, lower panel). At both concentrations, the prolongation observed with E-4031 was more pronounced in Purkinje fibers than in the ventricle, resulting in a concentration-dependent increase in dispersion of repolarization between the two tissues. This feature was not observed with BRL-32872 (Fig. 4, upper panel). Although the action potential duration was increased in a concentration-dependent manner in ventricular tissue, the increase in APD_{90} measured in Purkinje fibers was not more pronounced at 3 μM than at 0.3 μM. As a consequence, the dispersion in repolarization between Purkinje fibers and ventricular muscle was not increased, but instead reduced, when the concentration of BRL-32872 was increased (Fig. 4, upper panel).

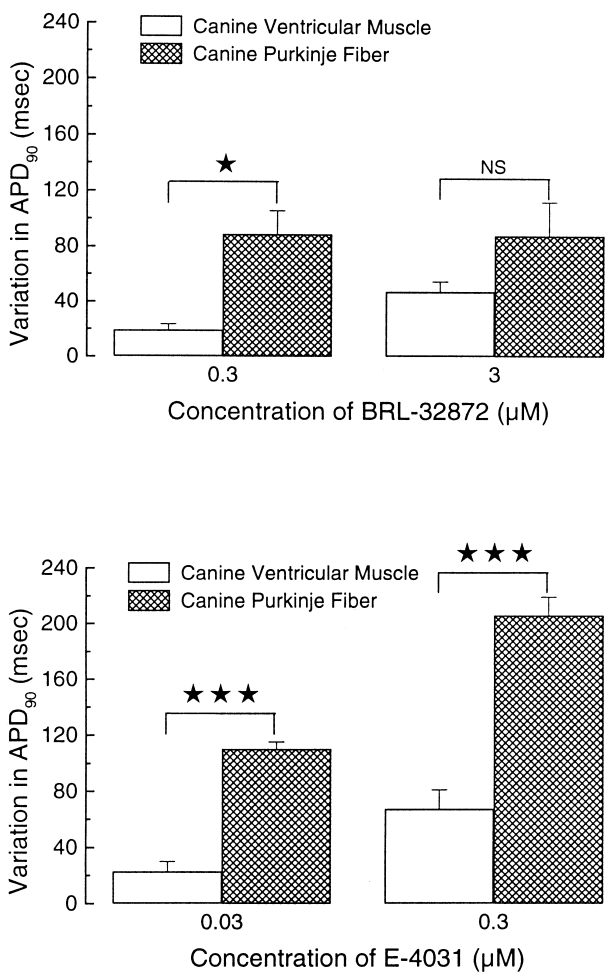

Fig. 4. Variation in APD_{90} induced by increasing concentrations of BRL-32872 and E-4031 in Purkinje fibers and ventricular muscles stimulated at 1 Hz. $^{*}P < 0.05$, $^{***}P < 0.001$.

4. Discussion

The efficacy of selective inhibitors of the delayed rectifier K^+ current on various experimental models of rhythm disturbances has been extensively demonstrated (Katoh et

al., 1990; Black et al., 1991; Chi et al., 1991; Zuanetti and Corr, 1991; Friedrichs et al., 1995). However, these compounds are associated with proarrhythmic adverse effects: they prolong cardiac repolarization in a reverse rate-dependent manner and thus favor the occurrence of torsades de pointe. They also increase dispersion of repolarization and thus favor the development of re-entry arrhythmias (Lathrop, 1985; Hondeghem and Snyders, 1990; Li et al., 1990; Gwilt et al., 1991; Abrahamsson et al., 1993; Buchanan et al., 1993b; Antzelevitch et al., 1995). Consequently, there is a need for alternative approaches aimed at identifying efficient antiarrhythmic compounds with low proarrhythmic profile. Amongst the different approaches that can be used to decrease the proarrhythmic risk associated with a class III antiarrhythmic mechanism, we and others have recently suggested that compounds with multiple electrophysiological activities may be required (Singh, 1994; Bril et al., 1997) and therefore we proposed that a combination of K^+ and Ca^{2+} blocking activities may fulfill such requirements. BRL-32872 has been described as a prototype compound whose mechanism of action is related to inhibition of I_{Kr} with an additional I_{Ca-L} blocking action (Bril et al., 1995). When investigated in animal models of arrhythmias, BRL-32872 was shown to be as efficient as typical class III antiarrhythmic agents but with a lower proarrhythmic profile (Gout et al., 1995; Bril et al., 1996). Class III antiarrhythmic effectiveness is thought to result from the ability of BRL-32872 to inhibit I_{Kr} whereas the low proarrhythmic profile may result from inhibition of I_{Ca-L}. It might be argued that inhibition of the Ca^{2+} current could also depress myocardial contractility and affect atrio-ventricular conduction time. However, we have shown previously that the antiarrhythmic efficacy of BRL-32872 was observed at concentrations which did not depress cardiac function assessed by mean arterial pressure, left ventricular pressure or $(+)dP/dt$ max and did not increase PR interval. It thus appears that a Ca^{2+} current inhibition limited enough not to cause cardiodepression is sufficient to provide BRL-32872 with a low proarrhythmic profile.

Three distinct effects can be suggested to explain the limited proarrhythmic profile of compounds combining K^+ and Ca^{2+} channel blocking activities such as BRL-32872. First we recently showed that because of this dual electrophysiological mechanism of action, the stimulation rate did not influence the ability of BRL-32872 to increase action potential duration. In other words, BRL-32872 is not reverse rate-dependent in contrast to selective inhibitors of the delayed rectifier K^+ channel (Bril et al., 1998). We further suggested that amongst the numerous mechanisms that may have a role in the rate-dependence of action potential prolongation, a suitable balance of K^+ and Ca^{2+} blocking activities may represent a strategy for neutral frequency dependence both in experimental animals (Bril et al., 1998) and in humans (Kosar et al., 1997). Second, the results of the present study clearly show that in canine cardiac preparations, BRL-32872 did not induce early after-depolarizations and was able to suppress them when induced by a selective inhibitor of the delayed rectifier K^+ current. Third, we show in the present study that whereas BRL-32872 can increase dispersion of repolarization to some extent at low concentrations (between 0.3 and 1 μM), this effect is self-limiting when the concentration is further increased.

BRL-32872 has been shown in guinea-pig papillary muscle to induce a bell-shaped concentration-dependent increase of action potential duration (Bril et al., 1995). A similar result was observed with canine cardiac Purkinje fibers. The limitation in action potential duration prolongation observed in Purkinje fibers at high BRL-32872 concentrations might explain why after-depolarizations are less likely to occur. Such early after-depolarizations at the Purkinje fiber level may trigger action potentials at the ventricular level and may generate ectopic foci of arrhyth-

Table 2
Effects of BRL-32872 and E-4031 on action potential parameters recorded from canine Purkinje/ventricular preparations. Values are means ± S.E.M. from *n* experiments. Values were analyzed using analysis of variance followed by multiple comparison with the Sidak procedure
APA: Action potential amplitude; RMP: Resting membrane potential; APD_{50} and APD_{90}: Action potential duration measured at 50% and 90% of repolarization, respectively.

Tissue	Parameter	E-4031 (μM)					BRL-32872 (μM)				
		n	Control	0.01	0.03	0.1	0.3	*n*	Control	0.3	1.0	3.0	10.0
Purkinje	APA (mV)	4	117 ± 5	117 ± 5	116 ± 5	114 ± 5	115 ± 5	5	123 ± 2	123 ± 1	121 ± 2	119 ± 2	117 ± 2
	RMP (mV)	4	−90 ± 0	−90 ± 0	−91 ± 0	−91 ± 0	−91 ± 0	5	−92 ± 1	−92 ± 1	−92 ± 1	−92 ± 1	−91 ± 1
	APD_{50} (ms)	4	240 ± 10	279 ± 10[a]	320 ± 9[b]	364 ± 7[b]	384 ± 9[b]	5	295 ± 28	346 ± 29	325 ± 25	244 ± 10	162 ± 15[b]
	APD_{90} (ms)	4	322 ± 12	380 ± 10[b]	432 ± 12[b]	496 ± 12[b]	528 ± 12[b]	5	402 ± 28	491 ± 35[a]	518 ± 38[b]	490 ± 33[a]	423 ± 22
Ventricle	APA (mV)	4	113 ± 2	112 ± 1	113 ± 1	111 ± 0	111 ± 1	5	119 ± 2	119 ± 2	119 ± 2	117 ± 2	115 ± 2
	RMP (mV)	4	−88 ± 2	−87 ± 1	−87 ± 3	−87 ± 3	−86 ± 3	5	−91 ± 1	−92 ± 1	−92 ± 1	−92 ± 1	−92 ± 1
	APD_{50} (ms)	4	186 ± 9	193 ± 8	202 ± 11	215 ± 13[c]	225 ± 14[a]	5	203 ± 10	214 ± 12	223 ± 13	220 ± 13	218 ± 13
	APD_{90} (ms)	4	237 ± 10	247 ± 9	261 ± 10	284 ± 12[b]	304 ± 12[b]	5	268 ± 16	287 ± 19	306 ± 20[c]	315 ± 20[a]	327 ± 18[b]

[a] $P < 0.01$ vs. control value.
[b] $P < 0.001$ vs. control value.
[c] $P < 0.05$.

mias (Kupersmith and Hoff, 1985). Because Ca^{2+} current inhibition has been reported to be able to suppress triggered arrhythmias (Lazzara, 1993), it seems reasonable to assume that the Ca^{2+} inhibitory effect of BRL-32872 (Bril et al., 1995) is responsible for this feature.

When investigated in ventricular muscle, it appeared that BRL-32872 induced a monotonic increase in APD_{90} up to the highest concentration studied. We have no definitive evidence to account for this behavior. One possible hypothesis is that the sensitivity to I_{Ca-L} inhibition of the canine ventricular APD_{90} is less than that of the Purkinje fiber. Up to 10 μM, the recorded effect on APD_{90} might still be located in the ascending arm of the bell-shaped concentration-dependent curve of BRL-32872 in ventricular tissue. The fact that APD_{50}, characterizing action potential duration at the plateau potential where the Ca^{2+} current is more prominent, is increased by BRL-32872 according to a bell-shaped concentration–response curve reinforces this hypothesis (Table 2). A similar tissue-specific effect has recently been demonstrated with nibentan (HE-11) (Anyukhovsky et al., 1997). The mechanism for this tissue specificity has not been identified. Although nibentan has been shown to antagonize the effect of isoproterenol (Anyukhovsky et al., 1997), a role for a β adrenergic mechanism is unlikely in the present case because BRL-32872 has been shown not to inhibit the effect of isoproterenol (Faivre et al., 1998).

Finally, most class III antiarrhythmic agents are known to be more effective in lengthening the action potential in Purkinje fibers than in ventricular preparations (Surawicz, 1989; Roden, 1993). Lathrop reported that the effects of sotalol were markedly more pronounced in Purkinje strands than in isolated trabeculae and proposed that this differential effect may explain the occurrence of sotalol-induced torsades de pointe (Lathrop, 1985). Similarly, Abrahamsson and coworkers described that E-4031 induced a linear concentration-dependent increase in APD_{90} which was more pronounced in Purkinje fibers than in ventricular muscle (Abrahamsson et al., 1993). The results obtained with E-4031 in the present study confirm this characteristic. This differential tissue sensitivity leads to an increase in dispersion of repolarization which thus represents an underlying mechanism for the proarrhythmic effect of class III antiarrhythmic compounds (Kuo et al., 1983, 1985; Surawicz, 1989). Because of the bell-shaped concentration-dependent effect of BRL-32872 on action potential duration in Purkinje fibers, the dispersion in repolarization obtained with BRL-32872 is self-limiting. Therefore, BRL-32872, in contrast to selective inhibitors of the delayed rectifier K^+ current, only moderately increased the heterogeneity of repolarization even at high concentrations.

In conclusion, we have previously shown that a combination of inhibition of K^+ and Ca^{2+} currents can be involved to explain the absence of a reverse rate-dependent increase in action potential duration observed with some compounds showing multiple electrophysiological activities (Bril et al., 1998). Taken together with the absence of early after-depolarization induced by these compounds and with the reduced heterogeneity of repolarization they generate, the results of the present study reinforce the hypothesis that compounds with combined K^+ and Ca^{2+} channel antagonistic activities may exhibit a reduced incidence of adverse effects.

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