ELSEVIER

European Journal of Pharmacology 294 (1995) 319–327

ejp

The cardiac electrophysiological effects of sparteine and its analogue BRB-I-28 in the rat

Michael K. Pugsley [a,*], David A. Saint [b], Eric Hayes [a], K. Darrell Berlin [c], Michael J.A. Walker [a]

[a] *Department of Pharmacology and Therapeutics, Faculty of Medicine, University of British Columbia, 2176 Health Sciences Mall, Vancouver, B.C., Canada, V6T 1Z3*

[b] *Protein Dynamics Unit, Department of Chemistry, The Faculties, Australian National University, Canberra, A.C.T. 0200, Australia*

[c] *Department of Chemistry, Oklahoma State University, Stillwater, OK 74078, USA*

Received 18 May 1995; revised 17 August 1995; accepted 25 August 1995

Abstract

This study compares the cardiovascular and antiarrhythmic effects of sparteine and a 3,7-diheterobicyclo[3.3.1]nonane analogue of sparteine, BRB-I-28, in pentobarbitone-anaesthetized rats subjected to left-ventricle electrical stimulation and occlusion of the left anterior descending coronary artery. Sparteine and BRB-I-28 produced a dose-dependent reduction in heart rate and blood pressure over the dose range 1–64 μmol/kg/min. As well, the P-R and Q-aT intervals of the electrocardiogram (ECG) were prolonged. The thresholds for induction of premature beats and ventricular fibrillation were dose-dependently increased and both drugs increased refractoriness. While sparteine and BRB-I-28 (at 16 and 64 μmol/kg/min, respectively) did not change the incidence of premature beats or ventricular tachycardia with coronary occlusion, both drugs equally reduced the incidence of ventricular fibrillation. We characterized the actions of sparteine and BRB-I-28 on cardiac Na^+, transient outward and sustained outward plateau K^+ currents of rat myocytes using the whole-cell patch-clamp. Sparteine and BRB-I-28 produced a concentration-dependent reduction in Na^+ current with EC_{50} values of 110 and 230 μM, respectively. Both drugs produced hyperpolarizing shifts of 8 and 11 mV, respectively, for Na^+ channel inactivation while neither produced a change in channel activation. Both drugs produced a concentration-dependent block of the sustained plateau K^+ current and increased the rate of decay of the transient outward K^+ current. Thus, sparteine and BRB-I-28 possess Na^+ and K^+ channel blocking properties which may account for their antiarrhythmic actions against electrical and ischaemic arrhythmias.

Keywords: Antiarrhythmic activity; Ischemic arrhythmia; Sparteine; BRB-I-28; Patch-clamp

1. Introduction

Sparteine is a heterobicyclononane plant alkaloid (Binnig, 1974) possessing antiarrhythmic actions in many models of cardiac arrhythmia. In isolated guinea pig preparations sparteine, at concentrations between 10–1000 μM, prolonged the action potential duration, and reduced conduction velocity and the maximum rise rate of action potentials (Senges and Ehe, 1973). As well, it reduced the incidence of ventricular tachycardia and fibrillation produced by aconitine in rats and ischaemia in dogs (Raschack, 1974).

A compound structurally related to sparteine, BRB-I-28 (a 3,7-diheterobicyclo[3.3.1]nonane), was developed as a novel antiarrhythmic agent (Thompson et al., 1987; Smith et al., 1990; Chen et al., 1994). In dogs BRB-I-28 was more effective at suppressing sustained re-entrant ventricular tachycardia than lidocaine (Scherlag et al., 1988). In isolated cardiac tissue BRB-I-28 (at 3.2 and 10 mg/l) reduced the maximum rise rate (V_{max}), and conduction velocity in ventricular fibers, suggesting that BRB-I-28 blocks Na^+ channels (Patterson et al., 1991). BRB-I-28 showed a rapid onset and offset from rate-dependent block consistent with fast drug-bound receptor recovery from the inactive to resting state, a feature shared by class Ib antiarrhyth-

* Corresponding author. Department of Pharmacology and Therapeutics, Faculty of Medicine, University of British Columbia, 2176 Health Sciences Mall, Vancouver, B.C., Canada, V6T 1Z3. Tel.: 1.604.822 2239; fax: 1.604.822 9578; e-mail: RSDAA@unixg.ubc.ca.

SSDI 0014-2999(95)00551-X

mic drugs (Patterson et al., 1991). BRB-I-28 also selectively depressed conduction, prolonged refractoriness and produced marked tonic and use-dependent conduction block in ischaemic fibers at low drug concentrations (Patterson et al., 1993). In dogs, BRB-I-28 showed low proarrhythmic activity and less cardiodepressant actions than lidocaine (Fazekas et al., 1993) while in rats preliminary results suggest that BRB-I-28 reduces ischaemic arrhythmia incidence (Pugsley et al., 1992).

We investigated the haemodynamic and ECG actions of sparteine and BRB-I-28 in pentobarbitone-anaesthetized rats and effects on sensitivity to electrical stimulation of the left ventricle. Effectiveness against ischaemic arrhythmias was also tested and characterized in terms of effect on Na^+ and sustained plateau and transient outward K^+ currents of isolated rat cardiac myocytes using the whole-cell patch-clamp. These antiarrhythmic and electrophysiological studies provide information which characterizes the molecular basis of the antiarrhythmic actions of these drugs.

2. Materials and methods

2.1. General

All experiments conducted in Canada were performed according to guidelines established by the Animal Care Committee of the University of British Columbia. Male Sprague-Dawley rats (U.B.C. Animal Care Centre) (200–300 g) were used for all whole animal studies conducted in Canada. Isolated cardiac myocyte studies were conducted in Australia with male Wistar rats (200–300 g) according to guidelines enforced by the Australian National University.

2.2. Animal preparation

Intact rats were anaesthetized with pentobarbitone (60 mg/kg i.p.) and the trachea cannulated for artificial ventilation at a stroke volume of 10 ml/kg and at a rate of 60 strokes/min. A rectal thermometer was used to record body temperature which was kept between 36–37°C using a heating lamp.

The right jugular vein and left carotid artery were cannulated for administration of drugs and recording of blood pressure, respectively. The ECG was recorded according to the method of Penz et al. (1992).

2.3. Sparteine and BRB-I-28 dose-response curves

Dose-response curves were constructed for effects of sparteine and BRB-I-28 in pentobarbitone-anaesthetized, artificially ventilated rats ($n = 5$). Cumulative doses of drug (1.0–64.0 μmol/kg/min i.v.) were infused over a 5 min period, and recordings were made at the end of the infusion time immediately prior to the next dose.

Heart rate was calculated from the R-R interval of the ECG recorded on a Grass polygraph (model 7D, Quincy, MA, USA) while the P-R, QRS, and Q-aT intervals were measured directly. The Q-aT interval, measured as the distance from the onset of the Q-wave to the peak of the T-wave, was not corrected for heart rate since, in the rat, this interval does not change with rate (Hayes et al., 1994). RSh, an index of Na^+ channel blockade in the rat, was also measured (Penz et al., 1992).

2.4. Electrical stimulation studies

Using intact rats, as above, electrical stimulation of the left ventricle was performed using two Teflon-coated silver wire stimulating electrodes which were inserted through the chest wall and implanted into the left ventricle (Walker and Beatch, 1988). This placement technique usually produced an inter-electrode distance of less than 2 mm. Square-wave stimulation was used to determine threshold current (i_t – μA) and pulse-width (t_t – ms) for induction of extrasystoles, ventricular fibrillation threshold (VF_t – μA), maximum following frequency (MFF – Hz) and effective refractory period (ERP – ms) according to the method described by Walker and Beatch (1988). Either drug was infused according to a random and blind protocol at the doses described above. Electrical stimulation measures were taken 3 min after increasing the dose of the drug by doubling the rate of infusion for each new dose.

2.5. Coronary artery occlusion studies

The surgical procedures used are based upon those described by Au et al. (1979). Briefly, rats were anaesthetized and ventilated as described above. The left carotid artery cannula was used to record blood pressure and remove blood samples for determination of serum K^+ concentrations (Ionetics K^+ Analyzer). A left thoracotomy between the 5–6 ribs exposed the heart and a polyethylene occluder was placed around the left main coronary artery. The chest was closed and the animal allowed to recover for 20 min after surgical preparation.

Prepared animals were given a random and blind infusion of either vehicle control (at a rate of 0.016 ml/min), sparteine (at 16 μmol/kg/min) or BRB-I-28 (at 64 μmol/kg/min). Blood pressure and ECG records were taken 5 min after beginning infusion. A blood sample (0.25 ml) was taken, just prior to coronary artery occlusion, for determination of serum K^+ levels.

The ECG, arrhythmias, blood pressure, heart rate, and mortality were monitored for 30 min after occlusion. Arrhythmias, consisting of ventricular premature beats, ventricular tachycardia, and ventricular fibrillation were recorded and summarized by the arrhythmia score of Curtis and Walker (1988). At the end of the 30 min observation period a second blood sample was taken from surviving animals. Hearts were then removed and perfused via the Langendorff technique with cardiogreen dye (1 mg/ml) to reveal the underperfused occluded zone (zone-at-risk). Experimental design and analyses were performed according to the Lambeth Conventions (Walker et al., 1988).

2.6. Isolation of adult rat cardiac myocytes

Enzymatic isolation of cardiac myocytes was performed according to the method of Farmer et al. (1983). Briefly, male Wistar rats (200–300 g) were given an injection of heparin (2000 U i.p.) and killed by cervical dislocation 25 min later. Hearts were removed, washed in ice-cold, oxygenated, Ca^{2+}-free Tyrode solution for 5 min before being perfused, via an aortic cannula, with the same Ca^{2+}-free Tyrode solution warmed to 37°C. The Tyrode solution contained (mM): NaCl 134; TES (*N*-tris-(hydroxymethyl)methyl-2-aminoethanesulphonic acid) 10; KCl 4; $MgCl_2$ 1.2; NaH_2PO_4 1.2; glucose 11, and adjusted to pH 7.4 with 1.0 M NaOH. After 5 min of washing, the heart was enzymatically dissociated in Tyrode solution supplemented with protease (0.1 mg/ml, Sigma type XIV), collagenase (1.0 mg/ml, Worthington CLS II), fetal calf serum (1.0 μg/ml) and 25 μM Ca^{2+}. Approximately 35–40 min later, the heart became pale and flaccid. The ventricles were removed, cut in fresh 25 μM Ca^{2+}-Tyrode solution and triturated to dissociate myocytes. Cell suspensions were centrifuged, washed and resuspended in a 200 μM and 1 mM Ca^{2+}-Tyrode solution and 1 h later plated onto glass coverslips.

2.7. Solutions and drugs

All experiments were performed at room temperature (22–24°C) in a bath solution containing (mM): NaCl 70; TES 10; KCl 5.4; $MgCl_2$ 1.0; $CaCl_2$ 2; $CoCl_2$ 5; CsCl 5; glucose 10; choline Cl 60, and adjusted to pH 7.4 with 1.0 M NaOH. The pipette solution allowed for the recording of Na^+ and K^+ currents. It contained (mM): KF 140; TES 10; $MgCl_2$ 1; K-EGTA 10; $CaCl_2$ 2; ATP-Na_2 10 and was adjusted to pH 7.4 with 1.0 M KOH.

BRB-I-28 (7-benzyl-3-thia-7-azabicyclo[3.3.1]nonane hydroperchlorate) was initially dissolved in 40% ethanol while (−)-sparteine [7*S*-(7α,7aα,14α,14aβ)]-dodecahydro-7,14-methano-2*H*,6*H*-dihydro[1,2-*a*:1′,2′-*e*]-[1,5]-diazocine sulphate was dissolved in distilled water prior to final dissolution in either saline (for infusion into intact rats) or the external bath solution (for isolated cardiac myocyte studies). BRB-I-28 was synthesized by a procedure outlined previously (Bailey et al., 1984) while sparteine was purchased from Sigma Chemical Co., St. Louis, MO, USA.

2.8. Electrophysiological recording

Experiments were performed in a tissue bath and cells continuously perfused at a rate of 0.5–1.0 ml/min. Electrodes were prepared from borosilicate glass using a two-stage puller (Narishige Scientific Instruments, Tokyo, Japan) and had resistances between 1–5 MΩ when containing the pipette solution. Myocyte currents were recorded 10 min after achieving whole-cell configuration (Hamill et al., 1984). All currents were measured using an Axopatch 200A amplifier (Axon Instruments) which allowed for compensation and reduction of capacitance transients and leak currents from computer-generated voltage commands. Final capacitance and leak compensation was performed at the time of analysis by subtraction of the current produced by a 20 mV hyperpolarizing current pulse which always preceded the test pulse. Currents were filtered at 5 kHz, sampled at 10 kHz using a 12-bit A-D converter and saved on the hard drive for analysis.

3. Results

3.1. The haemodynamic and electrocardiographic effects of BRB-I-28 and sparteine

Blood pressure and heart rate were stable in all vehicle control animals over the duration of the drug infusion period. Sparteine produced a marked dose-dependent reduction in both blood pressure and heart rate. At a dose of 32 μmol/kg/min sparteine reduced blood pressure from a pre-drug value of 118 ± 7 to 68 ± 4 mm Hg and heart rate from 389 ± 11 to 212 ± 7 beats/min (data not shown). BRB-I-28, at an equivalent dose, reduced blood pressure by half as much (from 121 ± 10 to 89 ± 12 mm Hg) and reduced heart rate (data not shown).

ECG measures were influenced in a dose-related manner by both compounds. Sparteine prolonged the P-R and Q-aT intervals to a greater extent than did BRB-I-28. At the highest dose infused (32 μmol/kg/min), sparteine produced a 49% increase in the P-R interval (from 55 ± 2 to 82 ± 1 ms) while BRB-I-28 produced only a 21% increase (from 56 ± 2 to 68 ± 1 ms) (Fig. 1A) at 64 μmol/kg/min. Neither drug effected QRS width. Fig. 1B shows that sparteine, at doses greater than 4 μmol/kg/min, produced marked, dose-dependent, increases in the Q-aT inter-

val. Sparteine (32 μmol/kg/min) increased RSh from 0.74 ± 0.06 to 0.96 ± 0.05 mV while BRB-I-28 increased it from 0.76 ± 0.08 to 0.98 ± 0.10 mV at the same infused dose (data not shown).

3.2. Electrical stimulation studies

To study the antiarrhythmic nature of the drugs we examined their effectiveness against electrically induced arrhythmias. Fig. 2A and B shows that both drugs dose-dependently increased the threshold for induction of ventricular fibrillation and prolonged effective refractory period. Sparteine was approximately 2–3 times more potent in increasing thresholds than was BRB-I-28 ($n = 6$). Changes in threshold pulse width, t_t, were not consistent; however, threshold current for capture, i_t, was dose-dependently increased (data not shown).

ERP was significantly prolonged by sparteine at 8 μmol/kg/min and by BRB-I-28 at 16 μmol/kg/min (Fig. 2B). Sparteine (32 μmol/kg/min) and BRB-I-28 (64 μmol/kg/min) reduced MFF from 16 ± 1 and 17 ± 1 Hz in controls to 11 ± 0.5 and 13 ± 1 Hz, respectively (data not shown). The vehicle control values for electrical stimulation measures were constant over the treatment period.

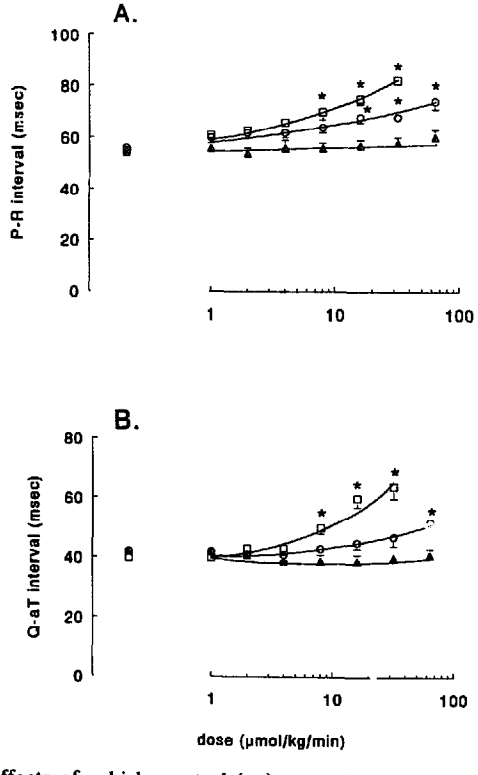

Fig. 1. Effects of vehicle control (▲), sparteine (□) and BRB-I-28 (○) on electrocardiographic measures. (A) shows the effects of drugs on the P-R interval while effects on Q-aT can be seen in (B). All values are mean ± S.E. for $n = 5$ animals/group. * Significant difference ($P < 0.05$) from control.

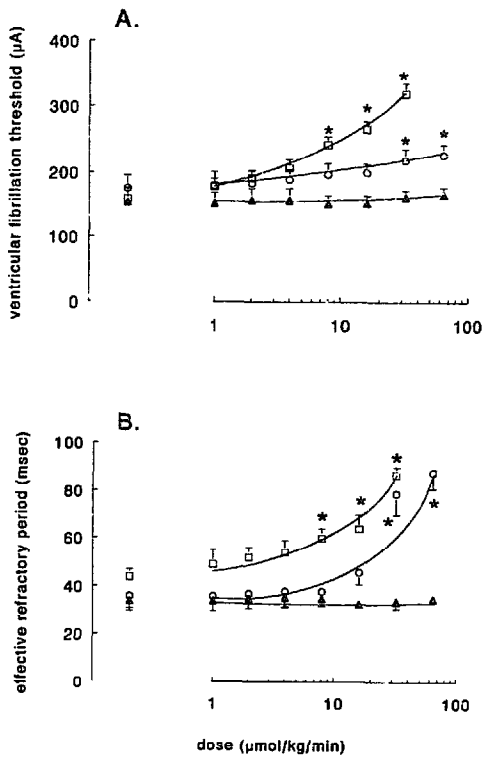

Fig. 2. The effects of vehicle control (▲), sparteine (□) and BRB-I-28 (○) on (A) ventricular fibrillation threshold (VFt) and (B) effective refractory period in pentobarbitone-anaesthetized rats. All values are mean ± S.E. for $n = 6$ animals/group. * Significant difference ($P < 0.05$) from control.

3.3. Ischaemic arrhythmia study

The coronary occlusion arrhythmia study (Table 1) shows that sparteine and BRB-I-28, infused at doses which produced significant changes in haemodynamic, ECG, and electrical stimulation measures, significantly reduced the incidence of ventricular fibrillation. The 16 μmol/kg/min dose of sparteine was the highest which could be safely given without producing fatal

Table 1
The antiarrhythmic properties of infused doses of sparteine and BRB-I-28 on arrhythmia incidence in pentobarbitone-anaesthetised rats subjected to coronary artery occlusion

Drug dose (μmol/kg/min)	log PVC	VT incidence	VF incidence	Arrhythmia score
Vehicle-control	2.1 ± 0.16	100	100	5.2 ± 0.4
Sparteine (16)	1.9 ± 0.11	100	25 [a]	4.0 ± 0.3 [a]
BRB-I-28 (64)	1.7 ± 0.10 [a]	100	38 [a]	2.9 ± 0.2 [a]

Values are expressed as either mean ± S.E., for arrhythmia score and log PVC (the $\log_{10}$ transformation for normalization of the number of premature ventricular contractions) or percent of animals in each group ($n = 8$) experiencing particular arrhythmias during 30 min post-occlusion. VT and VF are the group incidences of ventricular tachycardia and ventricular fibrillation, respectively. [a] Significant difference from vehicle-control at $P < 0.05$.

Table 2
The occluded zone (OZ) size and serum K^+ levels in pentobarbitone-anaesthetised rats subject to coronary artery occlusion

Drug dose (μmol/kg/min)	OZ (%)	Serum K^+ levels (mM)	
		Pre-occlusion	Post-occlusion
Vehicle-control	36 ± 2.0	3.6 ± 0.22	3.9 ± 0.30 (4)
Sparteine (16)	34 ± 1.7	3.5 ± 0.10	4.0 ± 0.10 (6)
BRB-I-28 (64)	37 ± 1.5	3.7 ± 0.10	3.7 ± 0.15 (7)

$n = 8$ animals/group except in the post-occlusion serum K^+ groups where n was reduced (to values in parentheses) after occlusion due to non-reversible ventricular fibrillation. Values are mean ± S.E.

cardiac output failure upon occlusion. This dose produced a similar reduction in the incidence of ventricular fibrillation as did the 64 μmol/kg/min dose of BRB-I-28; however, neither drug reduced the incidence of ventricular tachycardia. Neither drug significantly reduced premature ventricular contraction incidence.

The reduction in arrhythmia incidence could not be ascribed to either occlusion zone size (zone-at-risk) or post-occlusion serum K^+ levels. Table 2 shows that there were no significant differences between group occluded zone size, and hence the arrhythmic insult to the myocardium was assumed to be the same. Similarly, serum K^+ levels were not influenced by drug treatment.

3.4. Isolated cardiac myocytes

Both sparteine and BRB-I-28 reduced the magnitude of Na^+ current in isolated rat myocytes. Fig. 3 shows data obtained from an experiment in which Na^+ currents were evoked by a voltage step to −40 mV from a conditioning potential of −150 mV. These voltage steps were given at 3 s intervals and peak current amplitude measured. Sparteine (5–1500 μM) was added to the bath solution and produced a readily reversible concentration-dependent block of Na^+ current. Similar results were obtained for BRB-I-28 over the same concentration range (data not shown). Fig. 4, panel A, shows dose-response data ($n = 4$) for Na^+ current block obtained with sparteine while panel B shows that for BRB-I-28. The half-maximal Na^+ current block (EC_{50}) was 110 μM for sparteine and 230 μM for BRB-I-28 using the best fits to the equation $I_{Na} = 100/1 + (K_A/A)$.

The block of Na^+ current by sparteine and BRB-I-28 was examined by determining whether or not changes in voltage-dependence of inactivation or activation of the current occurred ($n = 4$), as shown for sparteine in Fig. 5. A voltage-step to −40 mV was given from various pre-pulse potentials between −130 mV and −50 mV. These studies revealed that sparteine produced channel blockade by changing the steady-state

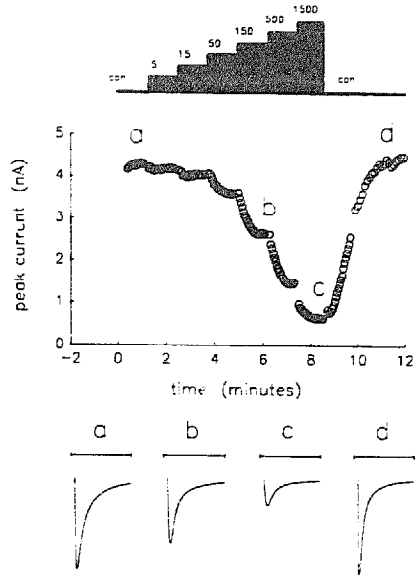

Fig. 3. Sparteine block of the rat cardiac Na^+ current. Na^+ currents were evoked by a voltage-step to −40 mV from a pre-pulse potential of −150 mV, at 3 s intervals. Sparteine was added to the bath solution for 90 s at concentrations indicated in the upper panel, and the peak Na^+ current measured. Examples of Na^+ currents at various applied concentrations are shown in the lower panel. All currents are plotted on the same axes and the bar above each trace indicates 10 ms duration. Similar results were obtained for BRB-I-28 (data not shown).

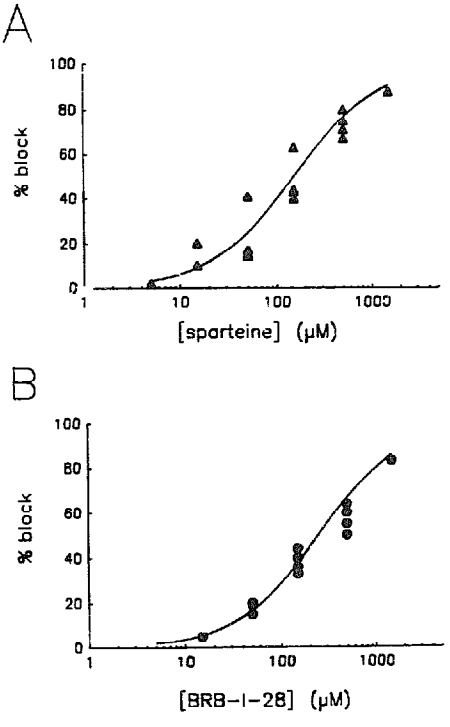

Fig. 4. Concentration-response curves of Na^+ current block by sparteine and BRB-I-28. Data are shown as percent block of Na^+ current for either sparteine (panel A) or BRB-I-28 (panel B) ($n = 4$). The lines are the best fits of the equation $I_{Na} = 100/1 + (K_A/A)$ to the data. The K_A values are 230 μM for BRB-I-28 and 110 μM for sparteine.

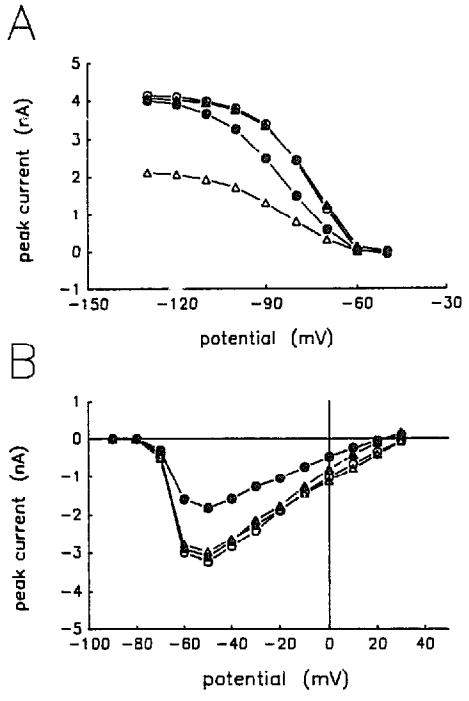

Fig. 5. The effect of sparteine on Na^+ channel inactivation (panel A) and the current-voltage relationship (panel B) ($n = 4$). In panel A Na^+ currents were evoked by a voltage step to −40 mV from pre-pulse potentials which varied between −130 mV and −50 mV in the absence (○) and presence (△) of 150 μM sparteine. The data are shown by the best-fit line using the Boltzmann equation $I_{Na} = I_{max}/1+\exp((V-V')/k)$. Data in the presence of sparteine scaled to control maximum (●) reveal an 8 mV hyperpolarizing shift. Recontrol data obtained after 5 min of recovery are shown by the filled triangles. Panel B shows the current-voltage relationship for activation of Na^+ currents. Currents were evoked by a voltage-step to potentials which varied between −90 mV and +30 mV from a fixed pre-pulse potential of −150 mV for control data (○) and in the presence of 150 μM sparteine (●). Conductance (G_{Na}) was calculated using the Boltzmann equation $I_{Na} = \{G_{max}/1+\exp((V-V')/k)\}\times(V-E_{rev})$. The sparteine curve was scaled to the control maximum (△) without a shift in activation kinetics. The re-control curve is shown by the filled triangles.

voltage-dependent inactivation kinetics of the current as shown in Fig. 5, panel A. A Boltzmann equation ($I_{Na} = I_{max}/1 + \exp(V - V')/k$, where I_{Na} is the maximal Na^+ current, V' is the voltage at which I_{Na} is half-maximal and k is the slope factor indicating the steepness of the slope or voltage dependence of inactivation) was used to derive a curve for the data obtained under control conditions and during the application of 150 μM sparteine. In the presence of this drug concentration the resulting Na^+ current block was accompanied by an 8 mV hyperpolarizing shift in voltage dependence. As well, sparteine increased the slope factor, k, from 7 mV in control to 9 mV per e-fold change in I_{Na}. The maximal current amplitude was reduced with 150 μM sparteine, even at very negative values for the pre-pulse potential, which indicates that the shift in the voltage dependence of inactivation may not be the principal means by which current block is produced. The average reduction in peak Na^+ current was 56 ± 7% in rat cardiac myocytes exposed to 150 μM sparteine ($n = 4$) (see Fig. 5). In cells exposed to 250 μM BRB-I-28, the average reduction in current amplitude was 54 ± 8% ($n = 4$) accompanied by an 11 mV hyperpolarizing shift and a comparable increase in the slope factor k (data not shown). Thus, at the concentrations examined the drugs blocked a fixed fraction of channels regardless of the membrane potential.

The effect of 150 μM sparteine was examined on the current-voltage relationship for activation of the Na^+ current. A voltage-step to various test potentials between −90 mV and +30 mV was given from a fixed pre-pulse potential of −150 mV. The peak current amplitude in the absence and presence of 150 μM sparteine is shown in Fig. 5 panel B. To examine effects of the drug on activation kinetics we defined the peak Na^+ conductance (G_{Na}) versus the reversal potential (E_{rev}) over the potential range of −100 to +40 mV. Conductance (G_{Na}) was calculated using a Hodgkin-Huxley model ($I_{Na} = G_{Na}(V - E_{rev})$ and approximated by a Boltzmann equation fit producing the relationship $I_{Na} = \{G_{max}/1 + \exp(V - V')/k)\} \times (V - E_{rev})$ where G_{max} is the maximal channel conductance for Na^+, V' is the voltage at which G_{Na} is half-maximal, k is the slope factor, and E_{rev} is the reversal potential for Na^+. In rat ventricular myocytes the peak I_{Na} amplitude was 3.3 ± 0.5 nA ($n = 4$). The G_{max} was 40 ± 5, with a V' of −62 ± 6 mV, a slope factor (k) of 1.5 ± 0.5 mV per e-fold change in I_{Na} and a reversal potential (E_{rev}) of 23 ± 1.4 mV. After exposure to 150 μM sparteine the values were, −62 ± 5 mV, 1.5 ± 0.3 mV per e-fold change in I_{Na} and E_{rev} of 26 ± 1.5 mV. Thus, sparteine reduces I_{Na} by decreasing G_{max}. These experiments were repeated for BRB-I-28 (250 μM) with similar results (data not shown).

In addition to effects on Na^+ currents, both drugs also blocked K^+ currents in myocytes. Fig. 6 shows representative current traces. Na^+ currents (panels A and C) were evoked as above while K^+ currents (panels B and D) were evoked by a voltage-step to +60 mV from a potential of −120 mV. Currents were recorded under control conditions and with 50, 150 and 500 μM concentrations of either sparteine (upper panels) or BRB-I-28 (lower panels) present in the bath ($n = 6$). These concentrations produced Na^+ current block as above, and a concentration-dependent blockade of the sustained plateau K^+ current. Fig. 6B and D shows that the EC_{50} values for sparteine and BRB-I-28 blockade of the sustained plateau K^+ current are 150 μM and 50 μM, respectively. Both drugs produced a concentration-dependent increase in the rate of decay of the transient outward K^+ current. Construction of a line tangential to the current decay curve approxi-

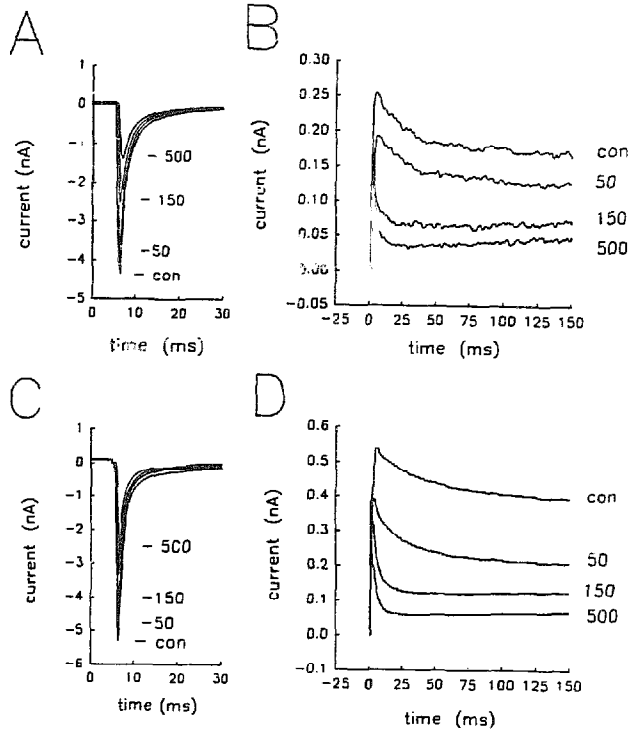

Fig. 6. Effect of sparteine and BRB-I-28 on Na^+ and K^+ currents. Panel A shows Na^+ currents evoked by a voltage-step to −40 mV from a potential of −150 mV in the absence (con) or presence of sparteine as indicated (μM). Panel B shows K^+ currents evoked by a voltage-step to +60 mV from a potential of −120 mV in the absence (con) or at the concentration of sparteine indicated (in μM). Recovery is not shown for clarity. Panels C and D show Na^+ and K^+ currents recorded in the absence and presence of BRB-I-28.

mated the drug effect on current inactivation. We assumed a monoexponential decay of evoked outward K^+ current (Josephson et al., 1984). In Fig. 6B sparteine (150 μM) increased the rate of current inactivation by 5.3-fold and at 500 μM by 9.8-fold. At equivalent concentrations, BRB-I-28 produced an 8.1-fold and 11.2-fold increase in the rate of inactivation.

4. Discussion

4.1. Effects of sparteine and BRB-I-28 on haemodynamics and the ECG

Both sparteine and BRB-I-28 produced a dose-dependent reduction in blood pressure and heart rate in anaesthetized rats. These findings are in contrast to those of Scherlag et al. (1988) who observed a consistent increase in blood pressure with BRB-I-28 in dogs. The myocardial depressant effects of sparteine in the rat are wholly consistent with findings in dogs by Schmidt et al. (1986). The cardiovascular depressant action of these agents in intact rats suggests that they may possess a myocardial depressant action similar to class I antiarrhythmic drugs.

Sparteine and BRB-I-28 have comparable actions on the ECG of the rat. We show that both sparteine and BRB-I-28 have a similar efficacy, in vivo, for blockade of cardiac Na^+ and K^+ channels, but are not equipotent. Measurements used to assess the blocking effects of these agents on Na^+ channels in vivo include prolongation of the P-R interval, and increased thresholds for capture and ventricular fibrillation. In addition to actions on Na^+ channels in vivo, there were effects on K^+ channels. In vivo measures indicative of K^+ channel blockade include prolongation of the Q-aT interval, reduction in MFF and an increase in ERP. Since both drugs produce dose-dependent changes in these measures it is difficult to differentiate preferential drug blockade of Na^+ or K^+ channels. The K^+ channel measures indicative of channel blockade reflect global morphological changes in cardiac repolarization processes and as such do not enable us to differentiate between the effect of the drugs on the many K^+ channels involved. However, in the rat the major repolarizing current is the transient outward K^+ current (Josephson et al., 1984) thus prolongation of repolarization in this species is dominated by blockade of this current.

The structural modifications made to BRB-I-28 do not significantly increase the potency of this compound towards blockade of cardiac channels since it was administered at doses 2-fold greater than sparteine without producing similar significant cardiodepressant actions. This difference in potency however does not reduce drug effectiveness against ischaemic arrhythmias. The antifibrillatory properties of BRB-I-28, especially towards high-frequency arrhythmias such as ventricular fibrillation, were equivalent to those of sparteine. The benefit of potency vs. selectivity for antiarrhythmic agents has been discussed at great lengths by Hondeghem (1991). The conclusion that potency is of less importance than selectivity for antiarrhythmic agents is seen with BRB-I-28 and sparteine.

4.2. An electrophysiological basis for in vivo actions of sparteine and BRB-I-28

Studies involving patch-clamp of isolated cardiac myocytes provided for an investigation of drug effects on ionic currents. A pharmacological profile for sparteine has not been fully elucidated and our study is the first to examine the effects of this agent on cardiac channels. However, BRB-I-28 has been well characterized (Chen et al., 1994), therefore we will confine the remainder of our discussion to results obtained with BRB-I-28 and relate, as best as possible, the proposed mechanisms of action for this agent to sparteine.

The current electrophysiological basis for observed in vivo ECG and antiarrhythmic actions of BRB-I-28 was developed from studies involving canine cardiac preparations. From its actions in intact canine hearts (Scherlag et al., 1988; Fazekas et al., 1993) and from recent studies involving models of injury to Purkinje and ventricular tissue (Patterson et al., 1991, 1993), it has been suggested that BRB-I-28 blocks Na^+ channels. Justification was based on the observation that BRB-I-28 reduced the maximum rate of depolarization of action potentials and produced tonic and use-dependent block of Na^+ channels (Patterson et al., 1993).

Our in vivo electrophysiological measures of a direct blockade of ionic currents are substantiated by patch-clamp studies and provide a firm basis for the conclusions by Patterson et al. (1993). According to the Modulated Receptor Hypothesis, antiarrhythmic drugs can interact in a voltage- and time-dependent manner with either of the three states of the Na^+ channel (resting, open, or inactive) (Hille, 1984; Hondeghem and Katzung, 1984). The non-conducting, drug-associated channels (resting or inactive states) have hyperpolarized voltage-dependent inactivation properties. Both BRB-I-28 and sparteine shift the inactivation curve for Na^+ channels in a hyperpolarizing direction indicating that the drug may preferentially bind to the inactive state of the Na^+ channel (Hille, 1984). However, the modest shift produced by both drugs could not account for the Na^+ channel block at the concentrations examined. Rather, a large component of block is likely due to tonic channel block. Studies with many antiarrhythmic agents suggest a concentration-dependent reduction in Na current at very negative pre-pulse potentials and low rates of stimulation (Bean et al., 1983; Hondeghem and Katzung, 1984). This may lead one to assume that the drug has a high affinity for the resting state of the Na^+ channel. When we examined drug effects at concentrations which were antiarrhythmic (low therapeutic concentrations) we did not see a similar tonic block to that observed at high concentrations. These observations recapitulate the findings of Bean et al. (1983) with lidocaine whereby tonic block is a minimal blocking component of BRB-I-28 and sparteine block.

Patch-clamp studies suggest that sparteine and BRB-I-28 may have a greater affinity for the active (open) or inactive (closed) channel than for the rested (closed) channel, as postulated by Patterson et al. (1991,1993). In vivo confirmation of the patch-clamp results suggests that at the high heart rates associated with ventricular fibrillation, both drugs effectively abolished the arrhythmia. Thus, the predicted fast kinetics of drug block for BRB-I-28 (Scherlag et al., 1988) implies that the antiarrhythmic properties of this drug, and sparteine, may be due to accumulated channel block at high rates, an event similar to that seen with lidocaine (Snyders and Hondeghem, 1990). While our experiments cannot confirm open vs. inactive channel block, this is rather an irrelevant point since only the amount of channel blockade which develops with each action potential is of any importance (Hondeghem, 1987).

Patch-clamp studies confirm in vivo observations that both drugs block at least two repolarizing K^+ currents found in rat hearts, the dominant transient outward (Josephson et al., 1984) and the sustained delayed rectifier plateau K^+ current (Wang et al., 1993). Both drugs increase the rate of inactivation of the transient outward current and block the sustained plateau component in a concentration-dependent manner. Depression of these K^+ currents prolongs refractoriness and in conjunction with the rapid recovery times consistent with drug-bound inactivated receptor blockade of Na^+ channels (Scherlag et al., 1988) suggests that the drug effectively may reduce or eliminate arrhythmias. Results from occlusion experiments confirm that those drug doses which produce marked actions on the ECG, indicative of Na^+ and K^+ channel blockade, reduced arrhythmia incidence.

The mixed action of these drugs on cardiac tissue is not unusual for antiarrhythmic agents. Quinidine, a class Ia antiarrhythmic, has been shown to possess both Na^+ and K^+ channel blocking activity (Snyders and Hondeghem, 1990). Sparteine and BRB-I-28 exhibit these same properties.

Regardless of the exact ion channel mechanism of blockade, both sparteine and BRB-I-28 are antiarrhythmic at doses producing both Na^+ and K^+ channel blockade and do not fit neatly into the present classification scheme of antiarrhythmics. Subclassification of these compounds within the class I scheme is difficult since they display properties similar to lidocaine, a class Ib antiarrhythmic agent but also prolong action potential duration in a manner similar to quinidine. Studies involving determination of the voltage dependence of both tonic and frequency-dependent components of block and rates of block development for these drugs should be examined.

Acknowledgements

We gratefully acknowledge the support of the British Columbia Health Care Research Foundation (M.J.A.W.), the National Health and Medical Research Council of Australia (D.A.S.), the Oklahoma Centre for Advanced Science and Technology/Presbyterian Foundation (ARO-015) (K.D.B.), and Rhythm Search Developments, Ltd. (E.H.). M.K.P. is a recipient of a Personnel Award from the Heart and Stroke Foundation of B.C. and Yukon and a G.R.E.A.T. Award from the Science Council of B.C.

References

Au, T.L.S., G.A. Collins, C.J. Harvie and M.J.A. Walker, 1979, The actions of prostaglandins I_2 and E_2 on arrhythmias produced by occlusion in rat and dog. Prostaglandins 18, 707.

Bailey, B.R., K.D. Berlin, E.M. Holt, B.J. Scherlag, R. Lazzara, J. Brachmann, D. Van Der Helm, D.R. Powell, N.S. Pantaleo and P.C. Ruenitz, 1984, Synthesis, conformational analysis and antiarrhythmic properties of 7-benzyl-3-thia-7-aza-bicyclo[3.3.1]nonane-9-one, 7-benzyl-3-thia-7-azabicyclo[3.3.1]nonane-9-one hydroperchlorate, and 7-benzyl-9-phenyl-3-thia-7-azabicyclo[3.3.1]nonane-9-ol hydroperchlorate and derivatives: single crystal X-ray diffraction analysis and evidence for chair-chair and chair-boat conformers in the solid state, J. Med. Chem. 27, 758.

Bean, B.P., J. Cohen and R.W. Tsien, 1983, Lidocaine block of cardiac Na^+ channels, J. Gen. Physiol. 81, 613.

Binnig, V.F., 1974, Zur Chemie des Sparteines, Arzneim.-Forsch. 24, 752.

Chen, C.L., S. Sangiah, K.D. Berlin, B. Scherlag, E. Patterson and R. Lazzara, 1994, BRB-I-28: a novel class Ib antiarrhythmic agent, Cardiovasc. Drug Rev. 12(3), 237.

Curtis, M.J. and M.J.A. Walker, 1988, Quantification of arrhythmias using scoring systems: an examination of seven scores in an in vivo model of regional myocardial ischaemia, Cardiovasc. Res. 22, 656.

Farmer, B.B., M. Mancina, E.S. Williams and A.M. Watanabe, 1983, Isolation of calcium tolerant myocytes from adult rat hearts: review of the literature and description of a method, Life Sci. 33, 1.

Fazekas, T., B.J. Scharlag, P. Mabo, K.D. Berlin, G.L. Garrison, C.L. Chen, S. Sangiah, E. Patterson and R. Lazzara, 1993, The comparative antiarrhythmic and proarrhythmic activity of a 3,7-dihetero bicyclo[3.3.1]nonane, BRB-I-28, and lidocaine in the 1–4-day old infarcted dog heart, Acta Physiol. Hung. 81(3), 289.

Hamill, O.P., A. Marty, E. Neher, B. Sakmann and F.J. Sigworth, 1984, Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches, Pflüg. Arch, 391, 85.

Hayes, E., M.K. Pugsley, W.P. Penz, G. Adaikan and M.J.A. Walker, 1994, Relationship between Q-aT and RR intervals in rats, guinea pigs, rabbits, and primates, J. Pharmacol. Toxicol. Methods 32, 201.

Hille, B., 1984, Mechanisms of block, in: Ionic Channels of Excitable Membranes, ed. Sunderland (Sinauer) p. 272.

Hondeghem, L.M., 1987, Antiarrhythmic agents: modulated receptor applications, Circulation 75(3), 514.

Hondeghem, L.M., 1991, Ideal antiarrhythmic agents: chemical defibrillators, J Cardiovasc. Electrophysiol. 2, 169.

Hondeghem, L.M. and B.G. Katzung, 1984, Antiarrhythmic agents: The modulated receptor mechanism of action of sodium and calcium channel-blocking drugs, Annu. Rev. Pharmacol. Toxicol. 24, 387.

Josephson, I.R., J. Sanchez-Chapula and A.M. Brown, 1984, Early outward current in rat single ventricular cells, Circ. Res. 54, 157.

Patterson, E., B.J. Scherlag, K.D. Berlin and R. Lazzara, 1991, Electrophysiological actions of BRB-I-28 in canine myocardial tissues, J. Pharmacol. Exp. Ther. 259, 558.

Patterson, E., B.J. Scherlag, K.D. Berlin and R. Lazzara, 1993, Electrophysiologic actions of BRB-I-28 in ischemically injured canine myocardium, J. Cardiovasc. Pharmacol. 21, 637.

Penz, W.P., M.K. Pugsley, M.Z. Hsieh and M.J.A. Walker, 1992, A new ECG measure (RSh) for detecting possible sodium channel blockade in vivo in rats, J. Pharmacol. Toxicol. Methods 27, 51.

Pugsley, M.K., M.J.A. Walker, G.L. Garrison, P.G. Howard, R. Lazzara, E. Patterson, W.P. Penz, B.J. Scherlag and K.D. Berlin, 1992, The cardiovascular and antiarrhythmic properties of a series of novel sparteine analogs, Proc. West. Pharmacol. Soc. 35, 87.

Raschack, V.M., 1974, Wirkung von Sperteine und Spar ..inderivaten auf Herz und Kreislauf, Arzneim.-Forsch. 24, 753.

Scherlag, B.J., E. Patterson, R. Lazzara, B.R. Bailey, M.D. Thompson and K.D. Berlin, 1988, Comparative electrophysiological and hemodynamic actions of BRB-I-28 and lidocaine in the normal and infarcted dog heart, J. Electrophysiol. 2, 461.

Schmidt, H.D., D. Padeken and L. Beck, 1986, Cardiovascular effects of sparteine in anaesthetised dogs with and without blockade of cardiac autonomic nerves, Arzneim.-Forsch. 36(10), 1481.

Senges, J. and L. Ehe, 1973, The effects of sparteine on membrane potential and contraction of mammalian heart muscle, Naunyn-Schmied. Arch. Pharmacol. 280, 253.

Smith, G.S., M.D. Thompson, K.D. Berlin, E.M. Holt, B.J. Scherlag, E. Patterson and R. Lazzara, 1990, A study of the synthesis and antiarrhythmic properties of selected 3,7-diheterabicyclo[3.3.1]nonanes with substituents at the 2,4-positions and at the 9-position, Eur. J. Med. Chem. 25, 1.

Snyders, D.J. and L.M. Hondeghem, 1990, Effects of quinidine on the sodium current of guinea pig ventricular myocytes, Circ. Res. 66, 565.

Thompson, M.D., G.S. Smith, K.D. Berlin, E.M. Holt, B.J. Scherlag, D. Van der Helm, S.W. Muchmore and K.A. Fidelis, 1987, Synthesis and antiarrhythmic properties of novel 3-selena-7-azabicyclo[3.3.1]nonanes and derivatives. Single-crystal X-ray diffraction analysis of 7-benzyl-3-selena-7-azabicyclo[3.3.1]nonan-9-one and 7-benzyl-3-selena-7-azabicyclo[3.3.1]nonane hydrochloride, J. Med. Chem. 30, 780.

Walker, M.J.A. and G. Beatch, 1988, A new method for electrical stimulation of the heart in rats, Proc. West. Pharmacol. Soc. 31, 167.

Walker, M.J.A., M.J. Curtis, D.J. Hearse, R.W.F. Campbell, M.J. Janse, S.M. Cobbe, S.J. Coker, J.B. Harness, D.W.G. Harron, A.L. Higgins, D.G. Julian, M.J. Lab, A.S. Manning, B.J. Northover, J.B. Parratt, R.A. Riemersma, E. Riva, D.C. Russell, D.J. Sheridan, E. Winslow and B.W. Woodward, 1988, The Lambeth Conventions: guidelines for the study of arrhythmias in ischæmia, infarction, and reperfusion, Cardiovasc. Res. 22, 447.

Wang, Z., B. Fermini and S. Nattel, 1993, Sustained depolarization-induced outward current in human atrial myocytes. Evidence for a novel delayed rectifier current similar to Kv1.5 cloned channel currents, Circ. Res. 73, 1061.