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Pharmacological and toxicological activity of RSD921, a novel sodium channel blocker

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Abstract

Background: RSD921, the R,R enantiomer of the kappa (k) agonist PD117,302, lacks significant activity on opioid receptors.

Methods: The pharmacological and toxicological actions were studied with reference to cardiovascular, cardiac, antiarrhythmic, toxic and local anaesthetic activity.

Results: In rats, dogs and baboons, RSD921 dose-dependently reduced blood pressure and heart rate. In a manner consistent with sodium channel blockade it prolonged the PR and QRS intervals of the ECG. Furthermore, in rats and NHP, RSD921 increased the threshold currents for induction of extra-systoles and ventricular fibrillation (VFt), and prolonged effective refractory period (ERP). In rats, RSD921 was protective against arrhythmias induced by electrical stimulation and coronary artery occlusion. Application of RSD921 to voltage-clamped rat cardiac myocytes blocked sodium currents. RSD921 also blocked transient (i_{to}) and sustained (IKsus) outward potassium currents, albeit with reduced potency relative to sodium current blockade. Sodium

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Conflict of interest

The authors have neither financial nor personal relationships that may have biased conduct of the work contained in this manuscript and declare that we have no conflicts of interest to disclose.

channel blockade due to RSD921 in myocytes and isolated hearts was enhanced under ischaemic conditions (low pH and high extracellular potassium concentration). When tested on the cardiac, neuronal and skeletal muscle forms of sodium channels expressed in *Xenopus laevis oocytes,* RSD921 produced equipotent tonic block of sodium currents, enhanced channel block at reduced pH (6.4) and marked use-dependent block of the cardiac isoform. RSD921 had limited but quantifiable effects in subacute toxicology studies in rats and dogs. Pharmacokinetic analyses were performed in baboons. Plasma concentrations producing cardiac actions *in vivo* after intravenous administration of RSD921 were similar to the concentrations effective in the *in vitro* assays utilized.

Conclusions: RSD921 primarily blocks sodium currents, and possesses antiarrhythmic and local anaesthetic activity.

Keywords

RSD921; Sodium and potassium blocker; Antiarrhythmic; Toxicology; Electrophysiology; Pharmacokinetics; Myocyte; Langendorff; Neuromuscular; Local anesthetic

1. Introduction

Opioid receptors have been implicated in the genesis of arrhythmias due to myocardial ischemia [1–4], and a variety of experiments involving the use of both agonist and antagonist opiates have been used to support such claims [3–10]. However, one complication in the use of such drugs is that many also have ancillary pharmacological actions unrelated to opioid receptors. Thus, many opioid agonists and antagonists have antiarrhythmic actions which relate to actions on cardiac ion channels, particularly sodium channels [7,11–13], rather than actions on opioid receptors. We have found this to be the case for racemic kappa (k) opioid receptor agonists, and their respective optical enantiomers which lack potency on opioid receptors. Kappa agonism in this series of compounds resides predominantly in the S,S (−) enantiomers [14] whereas sodium current blockade and antiarrhythmic activity occurs in both S,S and R,R enantiomers [7]. In a previous study [7] we used enantiomeric pairs to establish that the antiarrhythmic actions were independent of κ receptor agonism. A systematic study of such enantiomers discovered that RSD921 (also known as PD123,497), the R,R (+) enantiomer of the racemic κ agonist, (±)PD117,302, originally developed by Clark et al. [15] was most potent in this regard.

Preliminary experiments suggested that RSD921 had a pharmacological profile similar to that of lidocaine, and that the profile was of potential therapeutic value as a local anaesthetic and/or antiarrhythmic drug. We employed pharmacological and toxicological studies to fully describe its actions both *in vivo* and *in vitro* in a variety of species. Our results demonstrate that RSD921 is a novel, potent sodium channel blocker with antiarrhythmic and local anaesthetic actions.

2. Methods

2.1. In vitro studies with RSD921

2.1.1. Binding studies—RSD921 was tested for binding to sodium channels and opioid receptors. In the opioid binding studies, RSD921 was compared to its racemate, RSD920 ((±) PD117,302).

2.1.2. Opioid receptor binding—The binding of RSD921 to mu (m), k and delta (d) opioid receptors and neuronal sodium channels was evaluated (Eurofins Pharma Discovery Services, Bothell, WA, USA) according to established radioligand binding study protocols. In order to study specific opioid binding, selective ligands were used. These were [^{3}H]U-69,593 (3 nM) for k receptors, [^{3}H](D-Ala^2, N-methyl-Phe^4, Gly-ol^5)-enkephalin (DAMGO) (2 nM) for m receptors, [^{3}H]D-Pen^3, D-Pen^5 enkephalin (DPDPE) (2 nM) for d receptors and [^{3}H] Batrachotoxinin (BTX, 5 nM) for sodium channels (site 2).

Membrane aliquots were incubated in duplicate (to a total assay volume of 1 mL) with either [^{3}H] DAMGO (0.5 nM) or [^{3}H] U-69593 (0.5 nM) and increasing concentrations of RSD921 or RSD920. Non-specific binding was determined in the presence of morphine (10 mM) or unlabeled U-69,593 for m and k receptors respectively. Veratridine (100 μM) was used for sodium channels. Incubations were performed at 25 °C for 60 min after which the reaction was terminated by filtration through glass fiber filter strips (Whatman GF/B). Approximately 24 h later the reactivity bound to the filters was quantified with liquid scintillation spectrometry performed at room temperature (25 °C). Data was analysed by linear regression analysis and expressed as IC_{50}.

2.1.3. Studies in isolated tissue preparations

2.1.3.1. Langendorff isolated rat hearts.: Rat hearts (n = 6) were perfused on a modified Langendorff apparatus [7,12] at an aortic root pressure of 100 mmHg with piperazine-*N*,N'-bis(ethanesulfonic acid) (PIPES) buffer at 35 °C oxygenated with 100% O_2. A non-compliant saline-filled balloon was used to produce an end-diastolic left-ventricular pressure of 10 mmHg. The peak left ventricular systolic pressure was recorded as well as the maximal rate of intraventricular pressure development ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$). The *in vitro* ECG was recorded using silver-ball wick electrodes placed on the left atrium and left ventricle.

Two buffer solutions were used in the isolated heart studies. The first, designated 'normal' buffer (pH = 7.4), was composed of the following (mM): NaCl 121, KCl 3.4, $MgSO_4$ 1.2, PIPES 13.9, Glucose 11.1, $CaCl_2$ 2.5. This allowed for examination of drug effects in a well standardized *in vitro* milieu. A second buffer, designated 'ischemia-like' because of the low pH (pH = 6.4) and high K + concentration, was composed of the following (mM): NaCl 115, KCl 10.1, $MgSO_4$ 1.2, PIPES 14.8, Glucose 11.1, and $CaCl_2$ 2.5. Use of these two solutions allowed for the actions of RSD921 to be determined in normal physiological milieu or in one which mimicked several of the conditions of myocardial ischemia.

Concentration-response curves for RSD921 were constructed in both buffers, and the concentrations required to elicit a 25% change from control measures ($C_{25\%}$) for the PR and

QRS intervals were estimated. An 'ischemia-like to normal' ratio (I:N) was calculated to provide an index for drug potency under ischemia-like versus normal conditions.

2.1.4. Isolated neuromuscular preparations—Rat phrenic nerve/diaphragm and hypogastric nerve/vas deferens neuromuscular preparations were prepared according to established methods [16,17]. Briefly, tissues were isolated with the corresponding nerve attached and suspended in tissue baths with resting tensions of 2 g (diaphragm) and 1 g (vas deferens). The tissues stabilized for 30 min prior to compound exposure. Control drugs included tetrodotoxin (TTX), naloxone and noradrenaline which were added to the bath and left in for 3 min. TTX (0.1 nM) immediately followed by noradrenaline (20μM) was given at both the beginning and the end of the vas deferens experiment, while for the diaphragm, TTX (1 nM) was given at the end of the experiment. All isolated preparations were bathed in Krebs-Henseleit solution (pH = 7.4) and aerated with carbogen (5% CO_2, 95% O_2). The composition (mM) of the Krebs-Henseleit solution was: NaCl, 118; KCl, 4.74; $CaCl_2$•$2H_20$, 2.5; KH_2PO_4, 0.93; $NaHCO_3$, 25; D-Glucose, 10; $MgSO_4$•$7H_20$, 1.2. Nerve stimulation was delivered at twice the threshold current (i_t) by silver bipolar electrodes placed on the phrenic and hypogastric nerves above the level of the bathing solution. Stimulation frequency was 20 Hz for the hypogastric and 0.2 Hz for the phrenic nerve. The vas deferens was challenged every 2 min with a concentration of noradrenaline eliciting a sub-maximal response. The effect of RSD921 on nerve-induced contractions was determined by cumulative additions at 3 min intervals until contractions could no longer be obtained. The EC_{50} values for inhibition of contraction were calculated for individual concentration-response curves.

2.1.5. Ion channel electrophysiology studies in isolated rat myocytes—Single cardiac myocytes were prepared by enzymatic digestion of isolated adult male rat hearts [12,18]. Isolated cells were settled onto a glass slide coated with poly-l-lysine and contained in a chamber with a bath solution flow rate of 1 mL/min. The bath solution (24 °C, pH = 7.4) was composed of the following (mM): NaCl 130, KCl 5.4, MgCl2 1.0, CaCl2 2.0, CoCl2 5.0, CsCl 5.0, tetraethylsulphonic acid 10.0, NaOH 5.0, glucose 10.0 at a pH of 7.4. Evoked currents were recorded by conventional whole-cell patch clamp techniques. The composition of the electrode solution (mM) was: CsF 50.0, NaF 70.0, K-EGTA 20, CaCl2 2.0, tetraethylsulphonic acid 10.0, ATP-Na2 5.0, ATP-Mg 5.0 at a pH of 7.4. While recording potassium currents, 50 μM TTX was added to the bath solution to block sodium channels. Microelectrode resistance was in the 5–10 MW range when containing solution, and cells were clamped using an Axopatch 200 A amplifier (Axon In-struments, Foster City, CA). Currents were filtered at 5 kHz, digitized at 10 kHz and stored in a microcomputer. Recordings were only done if at least 90% series resistance compensation could be achieved. Sodium currents were elicited every 3 s by a voltage step to 0 mV from a holding potential of −150 mV. The concentration-dependent effects of RSD921 (100 and 300μM) were examined on the transient outward (I_{to}) and sustained outward plateau (I_{Ksus}) potassium currents evoked by depolarisation to +50 mV from a pre-pulse potential of −150 mV for a duration of 300 ms. RSD921 was added either externally to the bathing solution or internally via addition to the electrode solution.

2.1.6. Ion channel electrophysiology studies in Xenopus laevis oocytes—

Electrophysiological studies were performed using heart ($rNa_v1.5$), brain ($rNa_v1.2$) and skeletal muscle ($rNa_v1.4$) wild-type isoforms of the sodium channel obtained from rat tissue [19]. Plasmid DNA containing the full-length sodium channel coding region for each isoform was linearized and the linearized DNA (1.0 mg) was used for *in vitro* transcription. Stage V oocytes were obtained from adult female *Xenopus laevis* frogs, defolliculated with collagenase, and injected with *in vitro* transcribed RNA (50 nL). Forty-eight hours later, currents (1–4 mA) were evoked to examine the effects of RSD921.

Electrophysiological recording involved use of the two-electrode voltage clamp technique. Oocytes were clamped at a holding potential of −100 mV for $rNa_v1.2$ and $rNa_v1.4$, and at −120 mV for $rNa_v1.5$, and currents were evoked by 20 ms depolarization to −10 mV. Capacitative transient and leak currents were corrected by a P/4 protocol. Data was obtained and analysed using pCLAMP and filtered online. Non-linear curve-fitting was performed using SigmaPlot™ (Systat Software, Inc., San Jose, CA) and the Hill equation, $y = X^n/(A^n + X^n)$, where y is fractional response, X is concentration, A is the EC_{50}, and n is the Hill coefficient [19]. All currents were allowed to recover from slow channel inactivation for at least 10 min prior to the conduct of any electrophysiological studies. Studies were conducted with RSD921 at pH 7.4 to obtain EC_{50} values. At a concentration of 30 μM, RSD921 block of sodium channel isoforms was also examined at pH 6.4 to determine whether there is an effect of pH on channel block. Frequency-dependent properties of RSD921 were examined on each sodium channel isoform using a 30-pulse stimulation protocol [19]. Cells were depolarized from a holding potential of −100 mV (−120 mV for $rNa_v1.5$) to −10 mV at a rate of 1 and 30 Hz. The effect of RSD921 on the sodium channels was examined by bath application [19] in a manner similar to that of lidocaine [20].

2.2. In vivo studies with RSD921

2.2.1. General in vivo methods—

Male (CD-1) albino mice (18–25 g), and male Sprague-Dawley rats (200–350 g) (Charles River Laboratories, Montreal, Quebec) were used for all experiments that were conducted at the University of British Columbia (U.B.C). Male mongrel dogs weighing (25–30 kg) were obtained from the Animal Care Facilities at the University of Ottawa, Canada. Baboons (*Papio annubis*) of both sexes were kept in open-range cages at the Animal Housing Facility at the National University of Singapore. Fifteen beagle dogs, 9 males (10–12 kg), and 6 females (8– 10 kg), were obtained from the Shanghai Medical University Breeding Unit at the Shanghai Medical University, China for experiments conducted at that University.

All experiments conducted at U.B.C. (mice, rats, humans) were performed according the guidelines outlined by the Animal Care Committee of the University of British Columbia and Research and those involving humans adhered to the principles of the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects, revised Nov 13, 2001. Informed consent was received from both volunteers and UBC IRB approval was waived. Those studies conducted in Singapore (baboons) were performed according to guidelines established by the National University of Singapore Animal Care Committee. Similarly, experiments conducted in Australia (rat ventricular

myocytes) were performed according to Animal Health & Welfare Regulations of the Australian National University. Studies approved by the University of Ottawa were performed there according to the guidelines of the Canadian Council on Animal Care. Studies in China were conducted according to approved University Animal Care Committee procedures. The study design and animal ethics conformed with ARRIVE [21] and more recent guidance on experimental design and analysis [22].

2.2.2. Cardiovascular studies in rats—Rats (n = 5) were anaesthetized with pentobarbital (60 mg/kg, i.p.) and artificially ventilated [23]. Body temperature was maintained at 36 ± 1 °C with a heating lamp. The left carotid artery and right jugular vein were cannulated for blood pressure measurements and administration of drugs respectively. Needle ECG electrodes were introduced with a lead II configuration. ECG variables measured included heart rate (HR), PR, QRS, QT intervals, as well as the ECG measure, RSh, introduced by Penz et al. [24].

2.2.3. Electrophysiological studies in rats, baboons and dogs

2.2.3.1. Electrical stimulation in rats.: Anaesthetized rats (n = 5) were prepared as described above and the left ventricle was stimulated using two Teflon™-coated silver wire electrodes inserted transthoracically [25]. Square wave stimulation was used to determine the threshold current for capture of the heart (i_t-mA), effective refractory period (ERP-ms), threshold current for the induction of VF (VF_t) and maximum following frequency (MFF-Hz) according to Walker and Beatch [26].

2.2.3.2. Electrical stimulation in baboons.: Male (18–28 kg) and female (10–15 kg) baboons were sedated with ketamine (10 mg/kg i.m.). Each animal was intubated and maintained with halothane (0.5–1.5 %) in humidified 100% oxygen and breathed spontaneously. The femoral vein and artery were cannulated percutaneously for the administration of saline or RSD921 and BP measurements, respectively. The external jugular vein was cannulated for introduction of a ($Ag/AgCl_2$) monophasic action potential (MAP) dual pacing and recording electrode (7.0 French, EP Technologies, Sunnyvale, CA) [27]. The ECG was recorded from a lead II configuration. Blood pressure, right ventricular MAP and ECG were recorded and analysed off-line using customized pre-amplifiers and GlobalLab™ data acquisition software [27].

Prior to administration of RSD921 the threshold current (i_t), threshold duration for capture (t_t), MFF (at twice i_t and t_t), and right ventricular ERP (S_1S_2; train of 8–10 beats, 300 and 500 ms cycle lengths) were determined. In addition, i_t was determined over a range of durations such that current amplitude (i_t) versus duration (t_t) curves could be plotted as an index of sodium channel availability. Determinations were repeated so as to obtain three consistent estimates.

Once stable pre-drug recordings were obtained, RSD921 (0.2–6.4 μmole/kg), given as a slow bolus dose, was cumulatively administered at 30 min intervals. ECG and other variables were measured continuously so that time-effect curves could be plotted. Electrophysiological variables were determined 3 min (i_t, t_t and i_t *vs* t_t) and at 10 min (ERP) after drug administration (n = 4).

2.2.3.3. Conduction velocity studies in dogs.: A four-lead 2 × 2 cm bipolar recording electrode array plaque was sewn onto the right ventricular epicardium of open-chest mongrel dogs (n = 3) anaesthetized with chloralose (10 mg/kg/hr) and fentanyl (75 mg/kg) [27]. The electrodes were used to determine conduction velocities for responses evoked by stimulation at 500 ms *via* an electrode that was placed at a corner of the electrode array plaque.

2.2.3.4. Ischemia-induced arrhythmias in rats.: Antiarrhythmic efficacy of RSD921 against occlusion-induced arrhythmia was examined in rats subjected to occlusion of a branch of the left anterior descending (LAD) coronary artery [6,28,29]. Animals were given 30 min. to recover after surgery prior to administration of RSD921. Five min after commencement of drug administration, ischemia was induced by ligature occlusion of the LAD artery. Arrhythmias were summarized as an arrhythmia score (AS) which was calculated using the occurrence after occlusion of premature ventricular contractions (PVC), the number and duration of ventricular tachycardia (VT), and ventricular fibrillation (VF) episodes according to the Lambeth Conventions [30,31] and as defined by Curtis and Walker [32]. Fifteen min after LAD occlusion, the animals were euthanized and the occluded zone (OZ or zone-at-risk) was determined [33] by perfusing the heart in Langendorff fashion with a Krebs-Henseleit solution containing 1 mg/ml Indocyanin (cardiac green) dye. Rats were excluded if their occluded zone (ischaemic ventricular mass) lay outside the range of 25–45% of the total ventricular mass. Two blood samples (0.5 ml each) were taken from the carotid artery for measurement of serum K^+ concentration prior to drug administration, and 15 min after occlusion. Potassium-selective electrodes (Accumet model 25 pH/ion meter, Fisher Scientific, Pittsburgh, PA) were used to determine serum K^+ concentration.

2.2.3.5. Local anaesthetic studies in mice, guinea pigs and humans.: The local anaesthetic actions of RSD921 were assessed in mice, guinea pigs and humans for regional and dermal block.

2.2.3.6. Regional block anesthesia

2.2.3.6.1. Tail anesthesia in mice.: Sixty mice were divided into 3 groups, each of which received one of RSD921, lidocaine or saline in two 20 ml injections close to the base of the tail. Concentrations of RSD921 solutions used were 0.05%, 0.1%, 0.2%, 0.4%, and 1.0%, and lidocaine concentrations were 0.1%, 0.2%, 0.5%, and 1.0%. Two min after injection, the pin prick test was conducted proximally and distally to the injection site with a positive response being a tail flick.

2.2.3.6.2. Sciatic nerve block in mice.: Sciatic nerve blockade by RSD921 was investigated using previously described methods [34]. To determine the onset time of regional anesthesia by RSD921 compared to lidocaine, thirty-six CD-1 female mice were given saline, RSD921 or lidocaine at 0.063% (1.6 mM), 0.125% (3.2 mM), and 0.25% (6.4 mM). These compounds (50 mL) were injected into the popliteal space, in the area surrounding the sciatic nerve. Any mouse that could not use the injected hindlimb to walk on top and on an inverted wire mesh was considered to have a positive response. The onset time was recorded as the time from injection when a mouse displayed a positive response.

To determine time to recovery from regional anesthesia, forty-eight CD-1 female mice were injected in the popliteal space with RSD921 or lidocaine at 0.063% (1.6 mM), 0.125% (3.2 mM), 0.25% (6.4 mM), 0.5% (12.8 mM) and 1.0% (25.6 mM) concentrations in a randomized blinded design. Recovery was defined as the ability to walk normally on top and on the inverted wire mesh for at least 15 s, and was evaluated every 5 min from the time of injection until full recovery was attained. Neuromuscular blockade was also evaluated and defined as the ability to walk normally on top of the wire mesh screen but not hang on to the inverted screen.

2.2.3.7. Intradermal anesthesia

2.2.3.7.1. Guinea pigs.: Intradermal toxicity and local anaesthetic actions of RSD921 were studied in guinea pigs. The backs of male guinea pigs (300–400 g) were shaved 24 h prior to the start of the experiment. Concentrations of 0.063%, 0.125%, 0.25%, 0.5%, and 1% RSD921 and lidocaine were injected subcutaneously in the dorsal area using 27 G needles. Guinea pigs received either the RSD921 concentration range (n = 5) or the lidocaine concentration range (n = 3) for a total of 6 injections per animal. To assess local anesthesia, a 10 g weighted needle was used to prick each bleb site 10 times at 1, 2, and 5 min, and every 5 min thereafter for a total of 60 min. Local anesthesia was determined by calculating the ratio of the number of positive responses (no flinch) to the number of negative responses (flinch).

2.2.3.7.2. Humans.: Local anaesthetic actions of RSD921 were compared with those of lidocaine by means of intradermal injections in two male volunteers. Sites of injection were marked on the ventral surfaces of the forearms. Various concentrations of RSD921 or lidocaine dissolved in distilled water were injected in a volume of 0.2 ml in a random and masked manner. The state of anesthesia at each injection site was assessed at 5, 10, 15, 20, 25, and 30 min after injection by pricking the area 10 times with a pin. In some experiments RSD921 and lidocaine solutions also contained naloxone.

2.3. Toxicology of RSD921

2.3.1. Acute toxicity in mice—Various doses (1.0–12.5 mg/kg) of RSD921 were administered via the tail vein. Mortality was recorded 24 h after administration, and the maximum tolerable dose, defined as the dose that resulted in mortality in 50% of the mice was determined along with its 95% confidence limits.

2.3.2. Sub-acute toxicity in rats and dogs—In a 14-day study, rats were injected with RSD921 (10 mmole/kg, 0.1 ml/kg, i.v.) in the tail vein, while control rats received corresponding volumes of saline. Injections were made with a 25G-Long Butterfly needle twice daily, at 8.30 a.m. and at 4.30 p.m. The rats were observed for physiological and behavioral changes at 1 min and 30 min subsequent to each injection. Blood samples (1 mL) were extracted from all animals prior to drug or saline exposure, mid-treatment (day 7) and post-treatment (day 14). On days 7 and 14, the samples were taken before the first injection. Blood samples were kept for blood cell count, peripheral (differential) smears and hematological analysis. After 15 days the animals were euthanized with carbon dioxide, and post mortem examinations conducted. Organs (consisting of a standard panel including

brain, heart, lung, liver, kidneys, and stomach) were removed, preserved in 10% neutral buffered formalin, and subjected to histopathological examination.

Toxicity tests in beagle dogs were preceded by a 7-day conditioning period, during which daily measurements of cardiovascular parameters (see below) were made. For a total of 14 days, dogs were given RSD921 (either 1 or 5 mmol/kg, i.v., BID) or appropriate volumes normal saline administered over 10 min. Observations were made according to a double blinded design during injection, at 5, 15, 30, and 60 min following injection, and included measurements of blood pressure, heart rate, ECG, gross behavior, and mortality. Body weight was measured daily. At day 7 and day 14 blood and urine samples were taken for hematological and biochemical analyses. Following 14 days of treatment, 60% of the dogs (2 males, 1 female per group) were euthanized, and subjected to gross pathological and histological examinations. The remaining 40% of the dogs (1 male, 1 female per group) recovered for 7 days to observe any potential delayed toxicological events and recovery characteristics. At day 21 blood and urine samples were drawn and the animals were sacrificed.

2.3.3. Pharmacokinetic studies in rats and baboons—A single tail vein injection of RSD921 (8 μmol/kg, i.v.) was given and the resulting tissue (brain, heart, liver, and skeletal muscle) and whole blood concentrations determined in rats. The results of this pharmacokinetic study were described by Walker et al. [35]. Brain, heart, liver and skeletal muscle (right hind leg) samples were removed and rinsed in cold saline. Briefly, samples were homogenized and stored frozen at −20 °C until analysis. Preparation for analysis involved organic extraction with methyl-t-butyl ether, back-extraction into sulfuric acid, and organic re-extraction. Purified samples were analyzed using reversed-phase high-performance liquid chromatography (HPLC) coupled with UV detection (215 nm). Tissue sample drug recovery from the extraction procedure ranged from 77 to 90% with a minimum detection limit of 80 ng/ml and maximum limit of 200 ng/ml per gram of tissue or mL of blood analyzed [35]. This is the first quantitative method described for measurement of an arylacetamide of this type in biological matrices [35].

Blood samples were collected from two male baboons (weighing 23 and 28 kg respectively) under halothane anesthesia at 5, 15, and 30 min after cumulative i.v. bolus doses of 0.2, 0.4, 0.8, 1.6, and 3.2 μmol/kg RSD921. Blood samples were collected from the neck area, mixed with heparin (1000 IU/mL), and diluted with saline. Samples were quantitatively analysed by high performance liquid chromatography (HPLC).

2.4. Statistical analysis

Unless otherwise indicated values are expressed as the mean ± standard deviation (s.d.) for n experiments. Statistical analyses were usually performed using an NCSS statistical package (NCSS LLC, Kaysville, Utah), and accepting an a-level of 0.05 as indicating statistical significance. A general linear model analysis of variance (ANOVA) followed by Duncan's test for critical differences between means were used. In arrhythmia studies some data was $\log_{10}$ transformed in order to normalize data for parametric statistical testing. Mainland's contingency tables [36] were used to determine significance between incidences of events.

In isolated heart studies, drug effects were expressed as percentage changes from control conditions. Generally, non-linear dose or concentration response curves were fitted by least squares approximation to the logic function of the form $y = X^n (A^n+X^n)^{-1}$ using GraphPad Prism (Win version 5.04, La Jolla, CA, USA).

2.5. Drugs

RSD921 ((+)-trans-*N*-methyl-N-[2-(1-pyrrolidinyl)cyclo-hexyl] benzo[b]thiophene-4-acetamide) and RSD920 ((±)-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclo-hexyl]benzo[*b*]thiophene-4-acetamide) were synthesized in the laboratories of Rhythm Search Developments Ltd, a wholly owned subsidiary of Nortran Pharmaceuticals Inc. Naloxone hydrochloride dihydrate ((5α)-4,5-Epoxy-3,14-dihydroxy-17-(2-propen-1-yl)morphinan-6-one) (PubChem CID:5464092), lidocaine hydrochloride (2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide) (PubChem CID 6314), noradrenaline tartarate (PubChem CID 439260) and piperazine-*N*,N'-bis(ethanesulfonic acid) (PIPES) buffer (PubChem CID 10193124) were purchased from Sigma Chemical Co. (St. Louis, MO) and tetrodotoxin (TTX) (PubChem CID 11174599) was purchased from Tocris Bioscience (Bristol, UK). All drugs were dissolved distilled water or in normal saline (0.9% NaCl) prior to i.v. injection or dissolution in the external bath solution of *in vitro* study preparations.

3. Results

3.1. In vitro actions of RSD921

RSD921 is a substituted arylbenzacetamide with IUPAC designation (1R,2R)-(+)-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzo[b]thiophene-4-acetamide monochloride. The molecular weight for the hydrated hydrochloride salt is 406 g/mol and it has a melting point of 147 °C with a lipid partition coefficient (log P) of 1.59.

3.2. Binding studies

3.2.1. Opioid receptor binding—Whereas RSD921 was equipotent on m receptors and sodium channels (at site 2, the BTX site), the concentrations required to displace the k-specific ligand were 10–20 fold lower than those required to displace m receptor and sodium channel radioligands. IC_{50} values were found to be 0.4 ± 0.05 mM, 7.6 ± 1.2 mM, and 6.8 ± 0.9 mM for the k receptor, m receptor, and sodium channel, respectively. The K_i values were 0.2 ± 0.02 mM for the k receptor, 0.9 ± 0.1 mM for the m receptor, and 6.1 ± 0.8 mM for the sodium channel. RSD921 had negligible effects on d receptors (data not shown).

3.2.2. A comparison to racemic RSD920—RSD920, the racemate containing the S,S enantiomer, possessed higher affinity for both m (80-fold) and k (1000-fold) receptors. The IC_{50} of RSD920 against m receptors in the same radioligand binding assay was 0.01 mM while that of RSD921 was 7.6 mM. The IC_{50} of RSD920 against k receptors in the same binding assay was 0.0004 mM while that of RSD921 was 0.4 mM. The fact that LD_{50} values derived from mice for both RSD920 and RSD921 were almost identical (10 mmol/kg for RSD920 and 23 mmol/kg for RSD921) strongly suggested that mortality was unrelated to opioid activity.

3.2.3. Studies in isolated tissue preparations

3.2.3.1. Isolated rat hearts.: In isolated rat hearts perfused with 'normal' buffer solution (pH 7.4 and 3.4 mM K^+) RSD921 prolonged the PR and QRS intervals of the *in vitro* ECG (Fig. 1A) and depressed heart rate but also increased left-ventricular peak systolic pressure (LVP) (Fig. 1B). When isolated hearts were perfused with an 'ischemia-like' buffer solution (pH 6.4 and 10.1 mM K^+), the potency of RSD921 was increased. An ischemia-like/normal ratio (which acts as an indicator of ischemic tissue efficacy) was calculated based on the concentrations of RSD921 required to produce an increase in the PR interval by 25% (C_{25}) in both buffer solutions (Fig. 2). The ischemia-like/normal ratio was 0.33 for the normal buffer C_{25} (3μM). This ratio ranged between 0.2–0.6 over the concentration range 0.03–30 μM.

3.2.4. Isolated neuromuscular preparations—In the rat diaphragm/phrenic nerve preparation, RSD921 produced a concentration-dependent inhibition of contraction (EC_{50} = 100 μM). Contractions were also inhibited concentration-dependently by RSD921 (EC_{50} = 15 μM) in the vas deferens/hypogastric nerve preparation. The two resulting curves showed remarkably different shapes. The curve for the diaphragm/phrenic nerve data had a very steep slope, while that for the vas deferens/hypogastric nerve data was much less steep (Fig. 3). In both preparations, the inhibition of nerve-or noradrenaline-induced contraction by RSD921 was not reversed by naloxone whereas it was abolished by TTX (EC_{50} ~0.001 μM).

3.3. Electrophysiological studies with RSD921

3.3.1. Actions in isolated rat myocytes—The bath application of RSD921 (1–30 μM) to isolated cardiac myocytes subject to whole-cell voltage clamp resulted in a concentration-dependent inhibition of evoked sodium currents (Fig. 4A). The IC_{50} was 3.8 ± 0.5μM (n = 3 cells) with a slope (Hill coefficient, n_H) of 1.3 ± 0.2 using the best fits of the equation $I_{Na} = 1/[1+(KA/[A])$. Naloxone (5μM) had no influence on the sodium current blocking activity of RSD921 (Fig. 4B).

Fig. 5 shows the effects of whole-cell recording with RSD921 (130μM) added to the pipette solution. Unlike the rapid blocking actions seen when the drug was bath perfused, very little block of current occurred when currents were evoked beginning at 4 min after patch rupture. After an additional 4 min of intracellular drug application the sodium current was only reduced by 18% (note that without drug in the pipette the current amplitude was 26 ± 7 nA, n = 3). In the presence of intracellularly applied RSD921 (130μM), bath applied RSD921 (130μM), produced an immediate, marked and readily-reversible inhibition of the sodium current (Fig. 5).

Under acid bath conditions (pH6.4) there was a limited increase (~15% greater block at 10 μM) in RSD921 sodium current blocking potency (data not shown). At concentrations ~25-fold higher than that required to block sodium currents, RSD921 also blocked potassium currents. RSD921 (100 and 300 μM) produced block of the peak component of I_{to} as well as I_{Ksus} as shown in the cell in Fig. 6.

3.3.2. Actions in Xenopus laevis oocytes expressing sodium current—

RSD921 produced a concentration-dependent blockade of sodium current evoked in oocytes at physiological pH 7.4 [19]. When the data were fit using a Hill equation for a concentration range of 1–1000 mM, the following EC_{50} values were obtained: 47 ± 3 mM for rNa $_v$1.5, 37 ± 4 mM for $rNa_v1.2$ and 35 ± 3μM for $rNa_v1.4$. Under similar study conditions, when peak sodium current was determined and fit using the Hill equation, the EC_{50} value for lidocaine was 563 ± 22 μM [20]. Thus, RSD921 has a 12–fold greater potency for cardiac sodium channel blockade than lidocaine at physiological pH values in the oocyte expression system.

The effect of RSD921 (30 μM) on channel block was also examined at pH6.4 on all sodium channel isoforms (n = 4–10 oocytes). At pH 7.4 RSD921 reduced sodium current to 60 ± 5% for $rNa_v1.5$, 48 ± 6% for $rNa_v1.2$ and 51 ± 2% for $rNa_v1.4$ while at pH6.4 channel block was 50 ± 1% for $rNa_v1.5$, 47 ± 7% for $rNa_v1.2$ and 34 ± 2% for $rNa_v1.4$. Thus, for both the cardiac and skeletal muscle isoforms a reduction in pH enhanced channel block.

RD921 produced a marked use-dependent block of cardiac currents. At a frequency of 30 Hz, RSD921 (100 mM) blocked 81 ± 4% of the evoked $rNa_v1.5$ current. In contrast, the same concentration of RSD921 produced a 40 ± 5% block of $rNa_v1.4$ currents and only a 24 ± 3% of $rNa_v1.2$ currents. RSD921 produced no significant use-dependent actions at 1 Hz [19].

3.4. In vivo results with RSD921

3.4.1. Cardiovascular actions in rats

3.4.1.1. Effects of RSD921 on blood pressure, heart rate and ECG in rats.: RSD921 given intravenously as a slow bolus produced dose-related decreases in blood pressure and heart rate in anaesthetized rats. The hypotensive and bradycardic responses resulting from intravenous doses of RSD921 are shown in Table 1. These doses also had effects on the ECG which was most marked in terms of an increase in the PR interval. At the peak dose (8 μmol/kg) these ECG effects were most pronounced. At this dose the PR interval was prolonged from a control saline value of 60 ± 2 ms to 112 ± 3 ms while the QRS interval was prolonged from 30 ± 1 ms to 39 ± 2 ms. The amplitude of RSh was increased from 0.40 ± 0.06 to 0.88 ± 0.03 mV. Similarly, this dose prolonged the QT interval from 48 ± 2 ms to 59 ± 3 ms (Table 1).

3.4.2. Cardiovascular and electrophysiological actions in rats, baboons and dogs

3.4.2.1. Effects on electrical stimulation in rats.: Slow bolus doses of RSD921 produced dose-dependent increases in current thresholds for induction of single extrasystoles (i_t) and ventricular fibrillation (VF_t) as well as an increase in the effective refractory period (ERP) of the rat myocardium (Fig. 7). The increased threshold currents suggest a concentration-dependent antiarrhythmic action against electrically-induced arrhythmia in the rat.

3.4.2.2. Effects on electrical stimulation in baboons.: In anaesthetized baboons (n = 4) the hemodynamic (Fig. 8A) and ECG effects (Fig. 8B) of RSD921 given i.v. were similar to

those seen in rats and occurred over approximately the same dose range. Notably lower predrug blood pressures were encountered in baboons anaesthetized with halothane (83 ± 5 mmHg) as compared to rats anaesthetized with pentobarbital (152 ± 6 mmHg). This initial low blood pressure appeared to potentiate the hypotensive effect of RSD921 in these animals. RSD921 produced dose-dependent increases in both the PR and QT intervals to maximum values of 69 ± 9% and 79 ± 5%, respectively at a dose of 6.4 mg/kg (Fig. 8B). Ventricular excitability curves (current thresholds: amplitude (i) versus duration (t)) in baboons were recorded 5 min after doses 0.2–3.2 μmol/kg. Fig. 9 shows that RSD921 produced a dose-dependent shift in the 'i vs. t' curve to the right. The effects of RSD921 on the baboon cardiac AP were studied at two dose levels: 0.25 mmol/kg/min infused for 10 min, and 0.5 mmol/kg/min infused for 15 min. As shown in Fig. 10, RSD921 prolonged AP duration (APD) at 25, 50 and 75% repolarization with the highest dose producing the greatest effect (n = 4).

3.4.2.3. Conduction velocity effects in dogs.: In open-chest dogs (n = 3), administration of RSD921 at doses of 1, 2 and 4 μmol/kg given every 5 min produced a dose-related decrease in conduction velocity in the left ventricular epicardium (Fig. 11). The decrease was accompanied by hemodynamic ECG changes similar to those seen in rats and baboons.

3.4.2.4. Actions on ischemia-induced arrhythmia in rats.: The antiarrhythmic efficacy of RSD921 was tested in rats subjected to coronary artery occlusion and RSD921 reduced in the incidence of PVC, VT, and VF (Table 2). At the 1.0 μmol/kg i.v. dose, VF incidence was reduced to 0% compared to 80% in control animals. Animals at the highest dose administered did not survive the observation period due to insufficient cardiac output resulting from coronary occlusion. RSD921 reduced arrhythmia incidence without changing the occluded zone (ischaemic zone) size or serum K^+ concentration.

Arrhythmia protection by RSD921 occurred at doses which were significantly lower than those which produced marked changes in ECG and BP measurements. The efficacy and potency of RSD921 in protecting against ischemia-induced arrhythmias was comparable to its efficacy and potency against electrically-induced arrhythmias (VF_t). RSD921 was also equipotent in producing hypotension in ischemia and electrical models of arrhythmia induction.

3.4.3. Local anaesthetic actions in mice and rats

3.4.3.1. Regional analgesia

3.4.3.1.1. Tail anesthesia in mice.: RSD921 produced concentration-dependent local anesthesia in mice. Its efficacy and potency were comparable to that of lidocaine. At 0.1, 0.2, 0.4, and 1% concentrations RSD921 produced local anesthesia in 33%, 100%, 60%, and 100% of the mice tested, respectively. Lidocaine at 0.1, 0.2, 0.5, and 1% concentrations produced local anesthesia in 17%, 80%, 17%, and 100% of the mice tested, respectively.

3.4.3.1.2. Sciatic nerve block in mice.: There was no sign of neuromuscular blockade by either RSD921 or lidocaine. At the same concentrations, RSD921 and lidocaine were not statistically different from each other in times to onset of anesthesia. The onset time of

RSD921 (0.063%) was 70 ± 24 s while that of lidocaine at the same concentration was 42 ± 3 s. The onset time for RSD921 (0.125%) was 62 ± 13 s, while that for lidocaine (0.125%) was 37 ± 24 s. RSD921 (0.25%) produced anesthesia at 25 ± 13 s, compared to the 37 ± 13 s taken by the same concentration of lidocaine. Recovery from anesthesia was concentration-dependent for both drugs, although the recovery time from RSD921 was much longer than that from lidocaine at the same concentrations. Table 3 shows the mean recovery times for RSD921 and lidocaine. It is interesting to note that whereas at least 83% of RSD921-treated mice were anaesthetized at all concentrations of RSD921 used, lidocaine produced anesthesia in only 50% of the animals at lower concentrations (0.063% and 0.125%); this suggests that RSD921 is at least 2-fold more potent than lidocaine in producing anesthesia

3.4.3.1.3. Intradermal anesthesia in guinea pigs.: The time to maximum measured response was approximately 1 min post injection and occurred at higher doses of lidocaine (0.5% and 1%) and RSD921 (0.5% and 1%). Times to peak response for lidocaine and RSD921 at lower concentrations (0.063%, 0.125% and 0.25%) were also similar and fell within 3–5 min post injection. RSD921 produced a longer duration of local anesthesia (approximately 2-fold), with concentrations of 0.063% and 0.5% lasting up to 55 min post-injection, as compared to 35 min duration for the same concentrations of lidocaine. Furthermore, RSD921 (1%) produced local anesthesia for a duration of 80–85 min, whereas recovery occurred at 40 min with the same concentration of lidocaine. The threshold concentrations for local anesthesia could not be determined at the range of RSD921 and lidocaine concentrations used in this study.

3.4.3.1.4. Intradermal analgesia in humans.: Intradermal injection of RSD921 produced a wheal and analgesia in both subjects. RSD921 was 4 times as potent as lidocaine in producing analgesia, since an 800 mM concentration of RSD921 produced analgesia comparable to 3200 mM lidocaine. In addition, the duration of anesthesia produced by RSD921 was longer than that produced by lidocaine. In experiments where RSD921 was administered with naloxone, all mixtures of the two drugs produced analgesia.

3.5. Toxicological effects of RSD921

3.5.1. Acute toxicity in mice and rats—Two modalities of death were observed after bolus dose injection of RSD921 in mice. Lower doses appeared to cause death from respiratory failure within 15 min of injection. Higher doses appeared to cause cardiac arrest within 20 s of injection. The maximum tolerable doses for such respiratory deaths were calculated to be 19 μmol/kg (95% confidence limits of 15–24 μmol/kg). The corresponding values for cardiac deaths were 87.3 μmol/kg (95% confidence limits of 48–160 μmol/kg). The approximate maximum tolerable dose for anaesthetized rats was 16 μmol/kg. In a limited number of studies, the maximum tolerated dose in conscious rats was 10 μmol/kg. This dose produced early preconvulsive signs (e.g., hypothermia, straub tail, hyperactivity and tremor). Animals given twice this dose died immediately from cardiac arrest as deduced from ECG evidence. Conscious dogs given 10 μmol/kg over a period of 10 min also showed signs of a pre-convulsive state (e.g., glazed visual response, aphasia, hyperactivity and tremor). Where comparisons were made, doses causing lethality in anaesthetised animals were also lethal in conscious animals.

3.5.2. Sub-acute toxicity in rats and dogs—Approximately one min after injection, rats receiving RSD921 (10 mmol/kg) were hypoactive compared to the controls. Hypoactivity included decreased locomotor activity, grooming, rearing, climbing, and increased stupor. RSD921 induced episodes of ataxia evidenced by an abnormal gait, and markedly flattened postures in the first min after injection. Ataxia was not observed in any animal 30 min after dosing was complete. No significant differences in food consumption, water intake, body weight and temperature between the RSD921-treated and control rats were observed throughout the study (data not shown). In terms of gross pathology, kidney discoloration (pale-white mottled patches) were apparent on the surface of 2 RSD921 treated rats and in one saline-treated rat. No microscopic changes were observed. Histopathological, biochemical and hematological analysis showed no significant differences between saline-treated controls and RSD921-treated animals (data not shown).

Sub-acute toxicity studies in dogs showed that RSD921 (5 mmol/kg) induced reversible convulsions, muscular spasms, tremor, salivation, and asthenia, symptoms which appeared during infusion, and which lasted about 10 min. Following infusion of RSD921 (5 mmol/kg), heart rate and blood pressure decreased for 5–10 min before slowly recovering. Apart from the prolonged R-R interval (heart rate), no changes in the ECG were observed. At 1 mmol/kg, the above responses were not observed, although the animals were quiet, early after injection. Histopathological examination of animals sacrificed after 14 days of drug administration (5 mmol/kg) revealed minor changes in the liver and kidneys; however, these changes were reversed in the 1-week recovery period in animals sacrificed at day 21. No such changes were observed in controls and animals that received 1 mmol/kg dose of RSD921.

3.5.3. Pharmacokinetic studies in rats and baboons—In a pharmacokinetic study, rats were given a single bolus dose of RSD921 (8.0 μmol/kg, i.v.) and the tissue and blood levels were quantified [33]. Analysis of the concentration-versus-time response curves (drug levels per gram tissue or mL of blood) showed that disposition of RSD921 was greatest in the heart (29 μg/g tissue) followed by blood (7.5 μg/mL), brain (6.5 μg/g tissue), skeletal muscle (2.6 μg/g tissue) and liver (0.5 μg/g tissue). Tissue recovery of RSD921 was highest in the heart at 85 ± 2.1% and lowest in the brain at 77 ± 0.7%. It was found that the $t_{½}$ value for the a phase (rapid phase of elimination) was 30 s and for the ß-phase (slow phase of elimination) was approximately 90 min. Determination of these tissue levels of the drug showed that elimination from the rat heart was followed by sequestration in the brain and liver [35].

Plasma concentrations of RSD921 were determined in baboons following cumulative bolus i.v. doses (0.2, 0.4, 0.8, 1.6 and 3.2 μmol/kg) and are shown in Fig. 12. Each cumulative dose of RSD921 produced a proportional increase in plasma concentration of RSD921 with the largest increase occurring after the 3.2 μmol/kg dose. The elimination half-life ($t_{½}$) for RSD921 was determined to be 12 min. Such trends in responses suggested that RSD921 redistributes within tissues and that this, and not metabolism, may be responsible for termination of the cardiac effects subsequent to bolus dose administration. Studies performed using rat liver microsomal preparations showed that there was no significant metabolism of RSD921 over a 32 min period (data not shown).

4. Discussion

The purpose of this series of studies was to determine the pharmacological profile of RSD921, a novel sodium and potassium current blocking drug, with reference to antiarrhythmic and local an-aesthetic actions. Where appropriate, the pharmacology of RSD921 was compared to that of lidocaine using data obtained in this study or data available in the literature. Based upon the profile of its cardiac actions *in vitro* and *in vivo* we suggest that sodium current blockade with RSD921 occurs from binding to an extracellular site on the channel pore protein. Blockade is rapid, reversible, and concentration (or dose) dependent. The drug is more selective for blockade of sodium currents than for at least two repolarizing cardiac potassium currents.

The evidence for sodium and potassium blocking actions is consistent from studies conducted investigating effects at the ion channel level up to changes in physiological responses in various animal species. Thus, RSD921 blocks the cardiac, neuronal and skeletal muscle forms of the sodium channel heterologously expressed in oocytes and blocks both sodium and potassium channels in myocytes. The actions of RSD921 on isolated hearts are consistent with findings in myocytes. In rats the effects of the drug on the ECG are consistent with findings in isolated hearts. Furthermore, RSD921 influences responses to electrical stimulation in rats in a manner which is also consistent with blockade of sodium and potassium currents. An increase in threshold current and duration for induction of electrical arrhythmias is to be expected to occur with sodium channel blockers while prolongation of the effective refractory period can occur with potassium channel blockers as well as sodium channel blockers, particularly those which are frequency dependent in their actions.

The electrophysiological findings in rat tissue are recapitulated in other species. Thus, in baboons RSD921 prolongs both the QRS and QT intervals and has a pattern of effects on electrical stimulation which is the same as that seen in rats. Furthermore, in dogs RSD921 prolongs conduction velocity in a manner expected of a sodium channel blocker [37–39].

The full scope of ion channel blockade produced by RSD921 was not determined in the current set of studies. However, the available evidence suggests that with respect to sodium channel blockade this might occur via actions at an extracellular binding site. However, neuronal and skeletal isoforms of the channel are also blocked by RSD921.

The major action of RSD921 on evoked sodium currents in isolated rat myocytes was to reduce the maximum sodium channel conductance; however, the drug only caused a minor hyperpolarizing shift in the kinetics of channel inactivation without changing activation (data not shown). Pugsley & Goldin [19] found that while RSD921 was a potent sodium channel blocker in all wild-type sodium channel isoforms it also produced open channel blockade of the IFMQ3 mutant channel. This mutant results in the loss of fast channel inactivation properties of the channel and is a useful tool to probe drug effects on the state dependence of block. In addition, a slow recovery kinetic assessment from open channel block of the IFMQ3 channel with RSD921 suggests that the interaction between RSD921 and the sodium channel occurs in the open state and can account for tonic as well as use-dependent block

[19]. Together, these studies suggest that the putative site of drug interaction for this arylacetamide may be in the outer vestibule region of the pore since intracellular application of the drug did not result in significant channel block until it was bath applied which supports the theory of drug-mediated open channel block at an extracellular binding site.

Two binding sites have been suggested for the sodium channel: an intracellular binding site [37–39], and an extracellular one [40–42]. The high efficacy of RSD921 in blocking sodium currents when applied from the bathing fluid favors an extracellular site of action on the sodium channel. The fast onset of action and rapid reversal of drug effect also provides support for the likelihood of an external site of blockade. The perfusion cannulas used ensured minimal delay to the time of exposure [18], thus the results clearly showed that, independent of drug concentration, half-maximal block by RSD921 occurred within 5–10 s after application. Similarly, the perfusion apparatus used in the isolated heart study allowed for very rapid (< 2 s) changes in perfusate to be made, therefore it was possible to observe the rapid onset and offset of blockade [43]. As was the case with isolated myocytes, blockade of sodium current occurred within 10 s of exposure in the isolated heart preparations, and the drug had the same wash-out rate in both preparations.

Additional data to support an external site of action comes from the enhanced action of the drug under ischaemic conditions both in myocytes, oocytes and isolated hearts. This is in agreement with earlier observations made by other laboratories that the external sodium channel site is pH-dependent [40,44–47]. It should be noted that an external site of action, as well as an increase in activity at low pH, have both been demonstrated for (±) PD117302, the active enantiomer of (+)PD123,497 [7]. Nevertheless, the extracellular site of action on the sodium channel ascribed to RSD921 is in marked contrast to standard Class I antiarrhythmics which act “internally” as open channel blockers [48,49].

The lack of effect of naloxone on the RSD921-induced blockade of sodium currents in the myocytes indicates that this effect of RSD921 was independent of opioid receptor activation. This is in full agreement with the lack of kappa opioid activity observed for RSD921 [49,50], and its low affinity for m opioid receptors [51,52].

RSD921 was found to inhibit nerve-induced contractions of the vas deferens and diaphragm without influencing contractions due to noradrenaline (vas deferens) and KCl (both preparations). Given that contractions induced by noradrenaline and KCl were not affected, and with the understanding that blockade of neuromuscular transmission can occur at a number of different loci, it would be reasonable to assume that such actions were related to nerve blockade, presumably neuronal sodium channel blockade. The concentrations required to inhibit contractions in this preparation were considerably higher (see below) than those required to block sodium currents in the myocytes or decrease ventricular pressure in the isolated heart, and this may indicate greater efficacy in blocking myocardial sodium channels than neuronal sodium channels. The ability of RSD921 to cause mortality both in mice and rats by way of cardiac arrest supports our view of direct depression of myocardial excitability by this compound.

The outward potassium currents examined in myocytes in this study have two components: a rapid transient phase, I_{to}, and a delayed, sustained phase, I_{Ksus} [12,53,54], of which the transient outward is the major repolarizing current in the rat heart [54–56]. At 100 and 300μM, concentrations that produced complete blockade of cardiac sodium currents, RSD921 attenuated the potassium currents during the transient phase, and reduced the magnitude of the delayed potassium current. Generally, the concentrations required to inhibit potassium currents were at least 10 times greater than those which inhibited the sodium current. With this respect the electrophysiological spectrum of RSD921 is similar to that characterized for Class Ia antiarrhythmic drugs.

The high selectivity for, and fast onset of, sodium current blockade observed in the isolated single ventricular cells was indirectly substantiated by experiments on isolated hearts. In isolated hearts RSD921 produced both PR and QRS prolongation. It is generally accepted that widening of the QRS interval reflects a depression of the phase 0 sodium currents and a reduction in ventricular conduction velocity [56]. PR interval prolongation in the hearts of small animals is primarily derived from sodium channel blockade (unlike larger mammals) and depression of conduction in atrial tissue [57]. Although it has been observed that L-type calcium channel blockers prolong the PR interval in the rat by way of depressing inward A–V nodal calcium current [58], this mechanism is unlikely to play a role in the case of RSD921 as it occurs at high doses of these drugs associated with non-selective channel block. In the isolated hearts there is no clearly definable T wave and therefore QT measurements reflecting potassium current blockade [59] were not made; however, the effect of RSD921 on this variable could be established in the intact rat where it prolonged PR, QRS and QT intervals. The marked PR interval prolongation, as compared to either QRS width or the QT measure, reflects the greater blocking action of RSD921 on sodium channels. As expected from its effects in prolonging PR and QRS intervals, RSD921 also affected RSh which is taken as a measure of sodium channel blockade in the rat [24]. While this measure seems to be valid for use in the rat, its significance may be questionable when it comes to humans [60]. In full agreement with the results obtained in rats, electrical stimulation studies both in rats and Baboons provide further evidence for sodium channel blockade by RSD921. In both animal species RSD921 shifted the position of ventricular excitability (i versus t) curves and reduced cardiac contractility. Slowed conduction velocity observed in dogs is entirely consistent with blockade of voltage and time dependent sodium channels. Thus, RSD921 appeared to exhibit a preferential sodium channel blockade in three mammalian species.

The hypotensive and bradycardic effects of RSD921 in animals (rats, dogs, baboons) are consistent with those observed with Class 1a anti-arrhythmic agents. Although kappa agonists depress contractions in various vascular tissues such as the rat tail artery [61] and porcine coronary circumflex artery [62] it is unlikely that RSD921 acts through this mechanism due to its lack of opioid activity. A more probable postulate is that the hemodynamic effects of RSD921 may derive from both peripheral vasodilatation and myocardial depression resulting from sodium and potassium blockade. It is of interest to note that a bradycardic response in rats was observed in the absence of a decrease in blood pressure and this can easily be explained by pertaining a direct myocardial depressant effect.

The argument favoring ion channel blockade as an underlying mechanism for RSD921 is further supported by other observations made in our laboratory and by others. For example, U-50,488H (the first κ-opioid receptor agonist developed) was shown to produce hemodynamic effects and possess antiarrhythmic actions in a manner consistent with sodium and potassium current blockade [6,12]. In a study further analyzing the actions of PD129,290, a potent kappa agonist and its R,R enantiomer (PD129,289) with only 1/1000th kappa agonist potency, it was clearly demonstrated that the antiarrhythmic, ECG and electro-physiological effects were related to channel blocking actions rather than to actions on opioid receptors [7]). The results with RSD921, an R,R enantiomer of the racemic kappa agonist PD117,302 are consistent with the above findings which have been reviewed by Pugsley et al. [63,64].

In order to determine the relationship between effects seen *in vitro* and those observed *in vivo* a pharmacokinetic analysis was performed. Doses of RSD921 associated with i.v. effects in intact animals were similar to those concentrations producing *in vitro* isolated heart and ventricular cell effects, but considerably lower than those inhibiting neuronally-induced smooth or skeletal muscle contraction.

The sodium channel blocking activity of RSD921 was found to establish marked antiarrhythmic activity both against ischemia-induced as well as electrically-induced arrhythmias. Based on the lack of effect on serum K^+ concentration or OZ size it is unlikely that RSD921 exerts its antiarrhythmic activity by modifying these parameters. The increases in i_t and VF_t produced by RSD921 were achieved at doses higher than those required to protect against occlusion-induced arrhythmias. This observation leads to the conclusion that the antiarrhythmic actions of RSD921 are more selective for occlusion-induced arrhythmias as compared to electrically-induced arrhythmias.

Regarding the sub-acute, chronic toxicity profile for RSD921, our studies in rats showed that RSD921 is rather non-toxic at relatively high doses (in the range of half LD_{50} values) under the conditions stated. Apart from the transient motor and autonomic disturbances noted at high doses in beagle dogs, no permanent histological changes were discovered in the brain during evaluation. The reversible toxicological effects observed in the liver and kidney at high doses and the absence of any obvious histopathological changes at low doses in dogs would suggest that RSD921 is relatively devoid of specific end-organ toxicities.

With respect to the assessment of local anaesthetic activity in mice, guinea pig and human studies, the potency and efficacy of RSD921 suggest it could be a good substitute for lidocaine.

A rapid pharmacokinetic distribution of RSD921 in rat blood and tissues following the administration of a single bolus dose was reported by Walker et al. (33). Similarly, based on data obtained from blood samples taken after dosing in baboons, the elimination half-life ($t_{½}$) was determined to be 12 min. Overall, rapid tissue uptake and subsequent elimination primarily occurs in the heart but is followed by sequestration in the brain and liver suggesting that redistribution, and not metabolism, may be responsible for termination of cardiac effects following injection of the drug.

5. Conclusions

In animals RSD921 dose-dependently reduced blood pressure and heart rate. RSD921 applied externally to patch-clamped myocytes blocked sodium currents independent of opioid activity. At higher concentrations RSD921 also produced potassium channel blockade. Sodium channel blockade exerted by RSD921 was expressed as prolongation of PR and QRS intervals of the ECG both in isolated preparations and in animals. Similarly, RSD921 increased the thresholds for electrical induction of extra-systoles and ventricular fibrillation and prolonged effective refractory periods in rats. RSD921-induced sodium channel blockade was enhanced under ischaemic conditions both in myocytes, oocytes and isolated hearts. The cardiac sodium channel isoform was found to be more sensitive to the blocking effect of RSD921 than the neuronal isoform. The toxicological effects of RSD921 appear to be related to excessive cardiac sodium current blockade.

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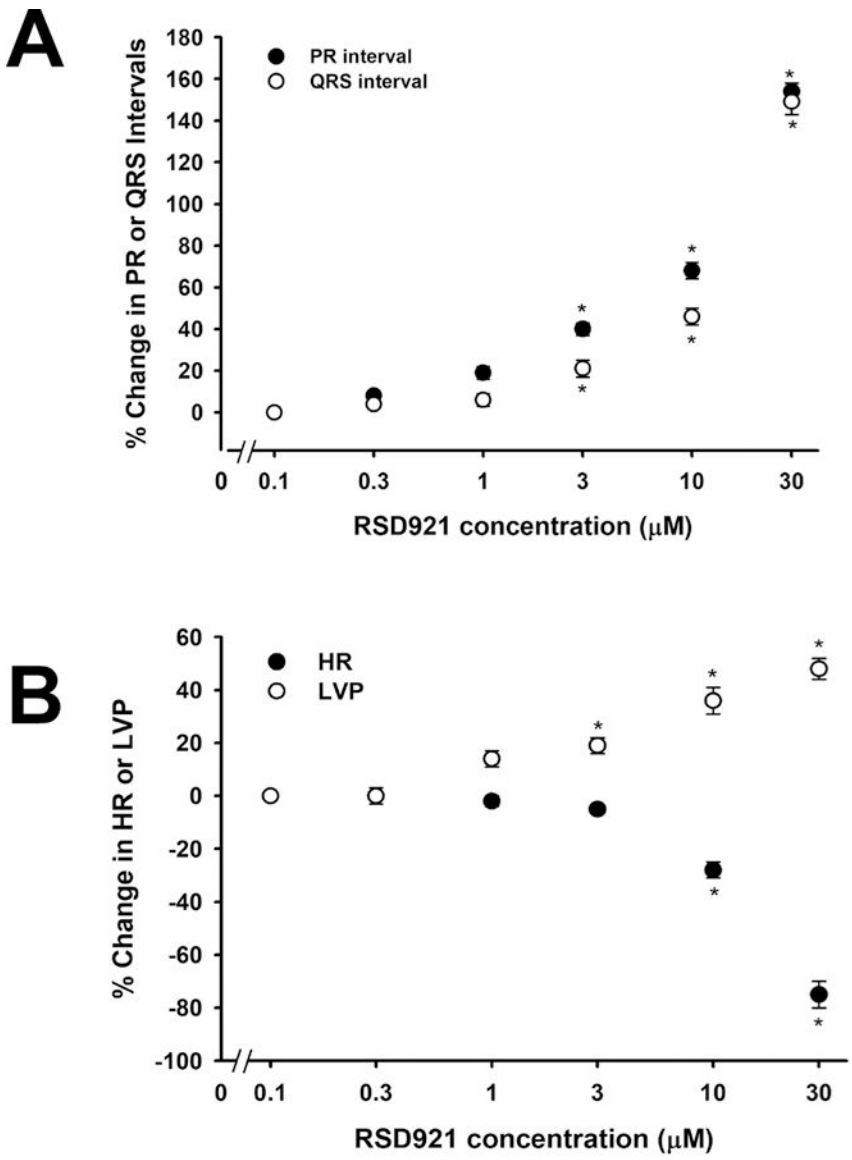

Fig. 1.

Concentration-response effects of RSD921 on the PR and QRS intervals of the ECG (A) and left ventricular pressure (LVP) and heart rate (HR) (B) in rat Langendorff hearts. Data shows the percent change from control after 5 min exposure to different concentrations of drug in a normal (pH7.4, K^+ 3.4 mM) PIPES buffer at 35 °C. Values are mean ± s.d. for n = 6. Using a nonlinear curve fit for the data, the correlation coefficient for the PR interval, HR and LVP was $p < 0.01$ and the QRS interval was $p < 0.001$.

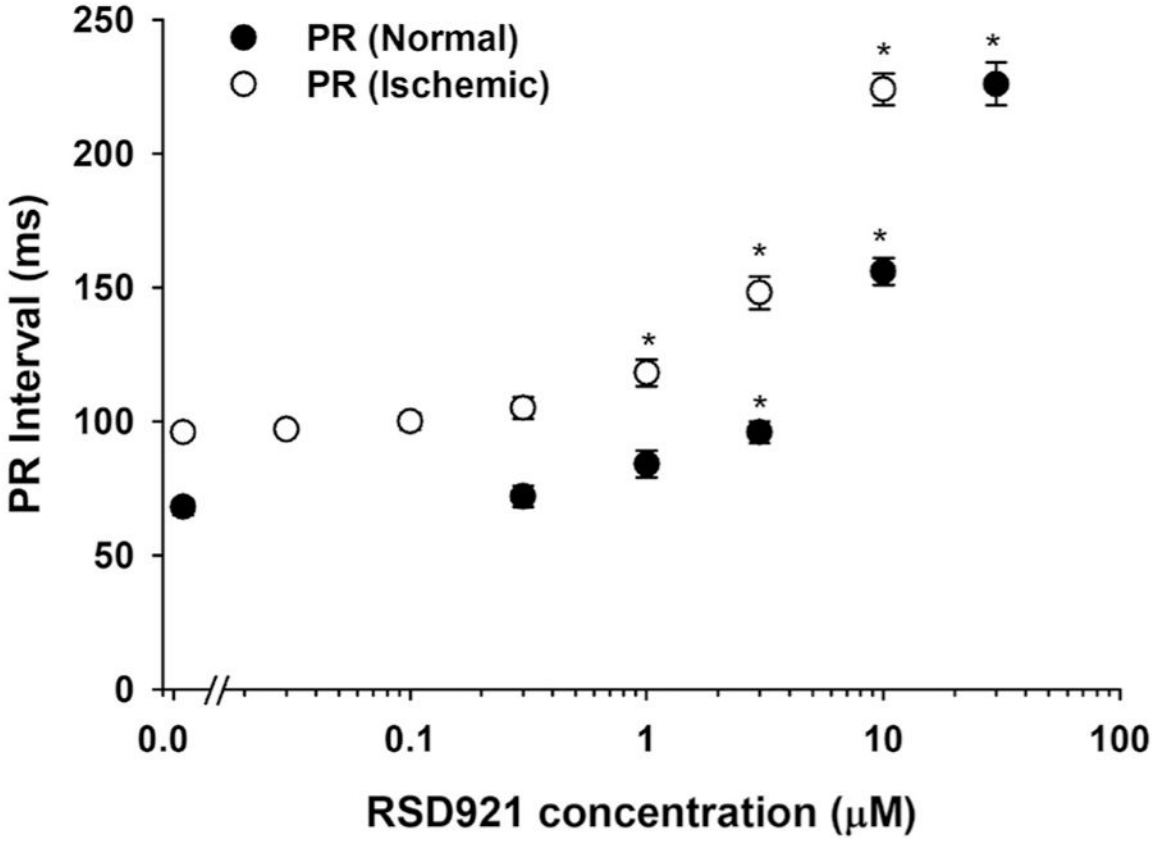

Fig. 2.

Concentration-response effects of RSD921 on the PR interval of the ECG in rat Langendorff hearts. Data shows the percent change from control after 5 min exposure to different concentrations of drug in a normal (pH7.4; K^+ 3.4 mM) or ischemic (pH6.4; K^+ 10.1 mM) PIPES buffer at 35 °C. Values are mean ± s.d. for n = 6. Using a nonlinear curve fit for the data, the correlation coefficient for the PR interval was $p < 0.001$.

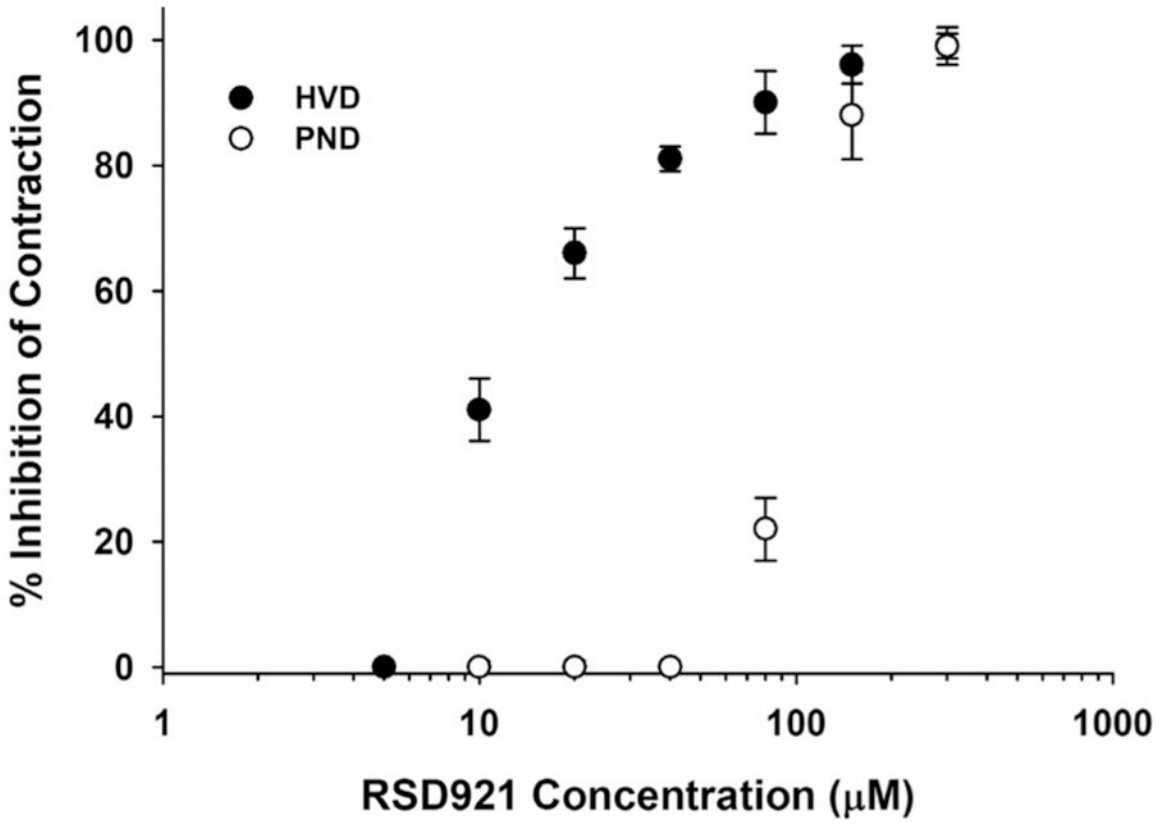

Fig. 3.

Concentration-response effects of RSD921 on the rat phrenic nerve/diaphragm and hypogastric nerve/vas deferens neuromuscular preparations. Stimulation (at a frequency of 20 Hz for the hypogastric and 0.2 Hz for the phrenic nerve) was delivered using electrodes placed on the each nerve above the level of the Krebs-Henseleit bathing solution. The effect of RSD921 on nerve-induced contractions was determined by cumulative additions at 3 min intervals. EC_{50} values were calculated for each preparation. Using a nonlinear curve fit for the data, the correlation coefficient for the rat phrenic nerve/diaphragm curve was $p < 0.05$ and for the hypogastric nerve/vas deferens was $p < 0.001$.

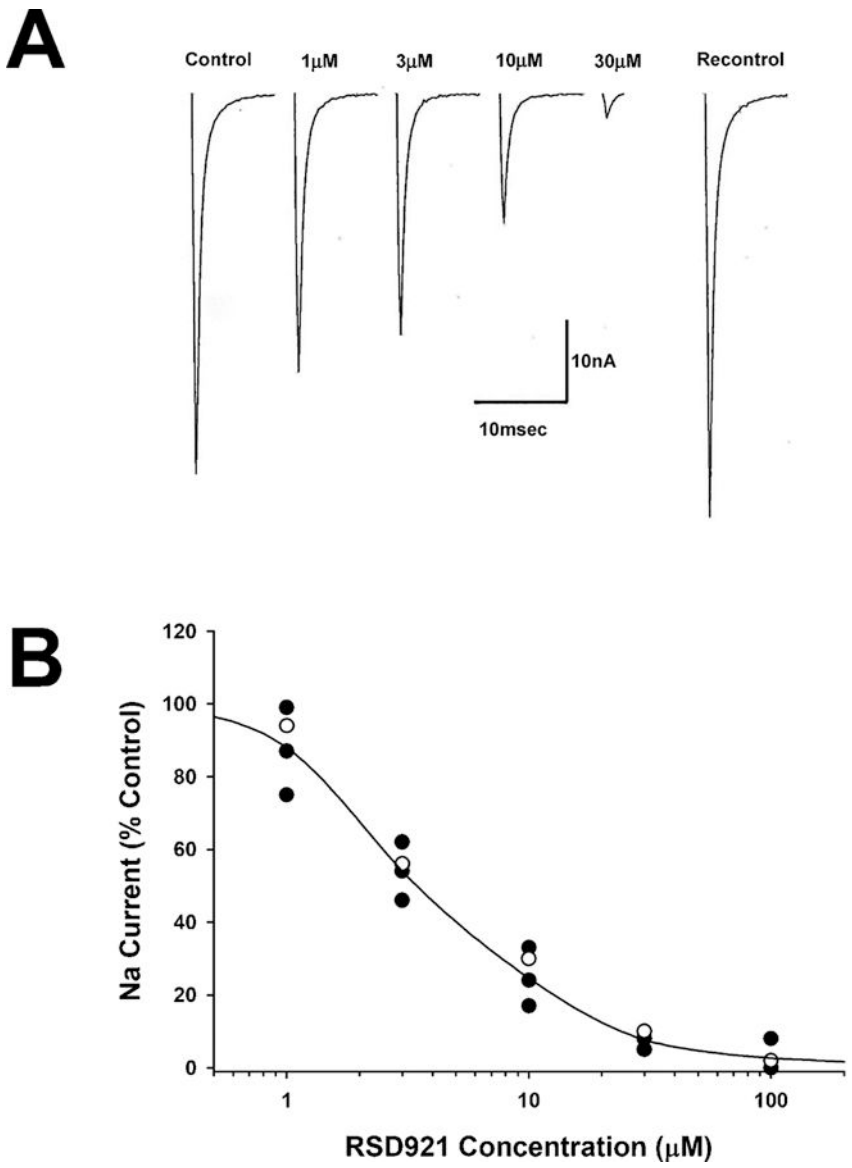

Fig. 4.

The effects of RSD921 on sodium currents in isolated rat myocytes. Panel A shows sodium current traces evoked by a voltage-step from a pre-pulse potential of −150 mV to a potential of 0 mV. The voltage step was delivered at 3 s intervals and RSD921 was added to the bath solution at the concentrations indicated. The recontrol (washout) current amplitude is comparable to the control. Panel B shows the concentration-response curve for blockade of the sodium current by RSD921 (n = 3). The half-maximal sodium current block (IC_{50}) from this data, using the best fits of the equation $I_{Na} = 1/[1+(KA/[A])$ was 3.8 ± 0.5μM with a slope (Hill coefficient, nH) of 1.3 ± 0.2. In the presence of naloxone (5μM) the IC_{50} was unchanged (open symbols). The curves described by the solid lines were best fit by the Hill equation as described in the Methods.

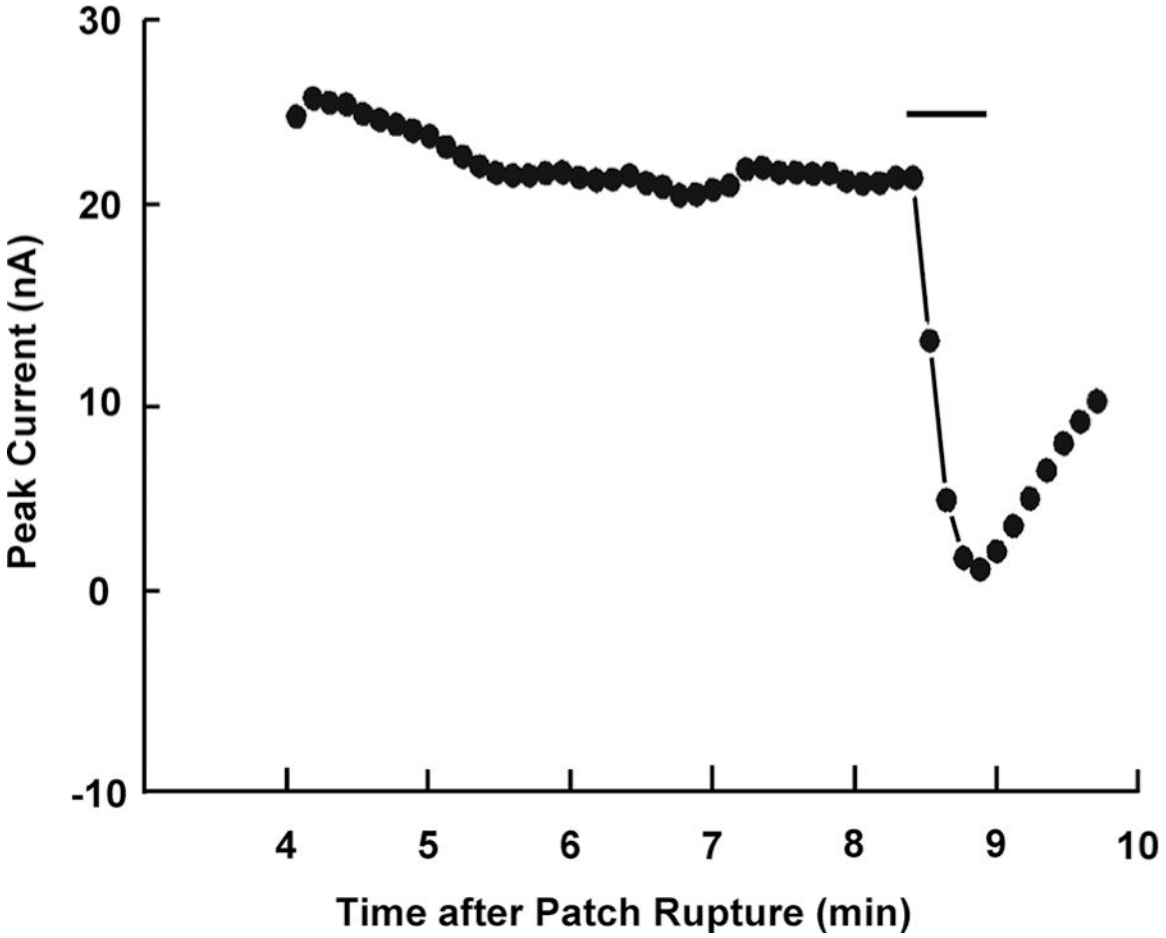

Fig. 5.

The ion channel blocking effects of RSD921 were examined to determine if block occurs with application of drug outside or inside the myocyte. Whole cell recording was achieved with RSD921 (130μM) inside the patch pipette. Sodium currents were evoked by a voltage step to 0 mV from a pre-pulse potential of −150 mV for 4 min after patch rupture of the myocyte. These voltage steps were given at 6 s intervals and currents generated were plotted as a function of time. RSD921 (130μM) was added to the bath solution as indicated by the bar).

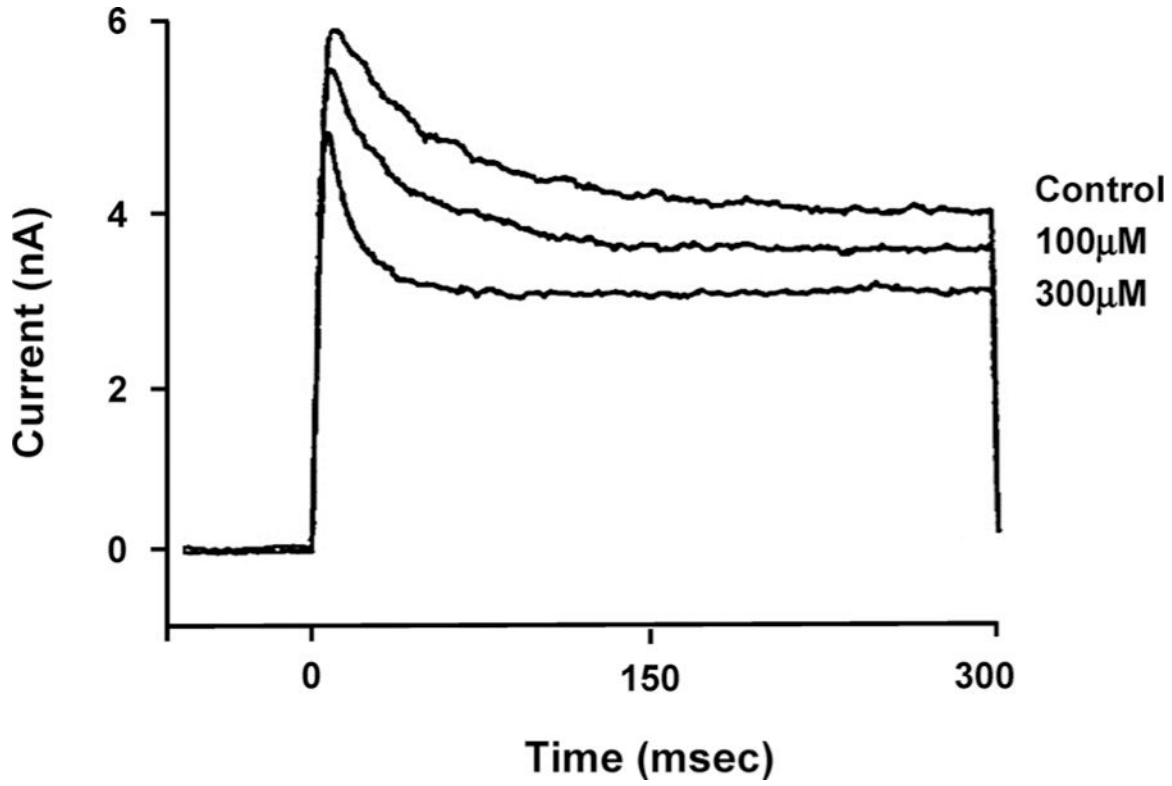

Fig. 6.

The effects of RSD921 (100 and 300μM) were examined on depolarization-induced transient outward (I_{to}) and sustained outward plateau (I_{Ksus}) potassium currents. Currents were elicited by depolarisation to +50 mV from a pre-pulse potential of −150 mV for 300 msec. RSD921 was perfused into the bath in the presence of 50μM tetrodotoxin (TTX) and currents subsequently evoked.

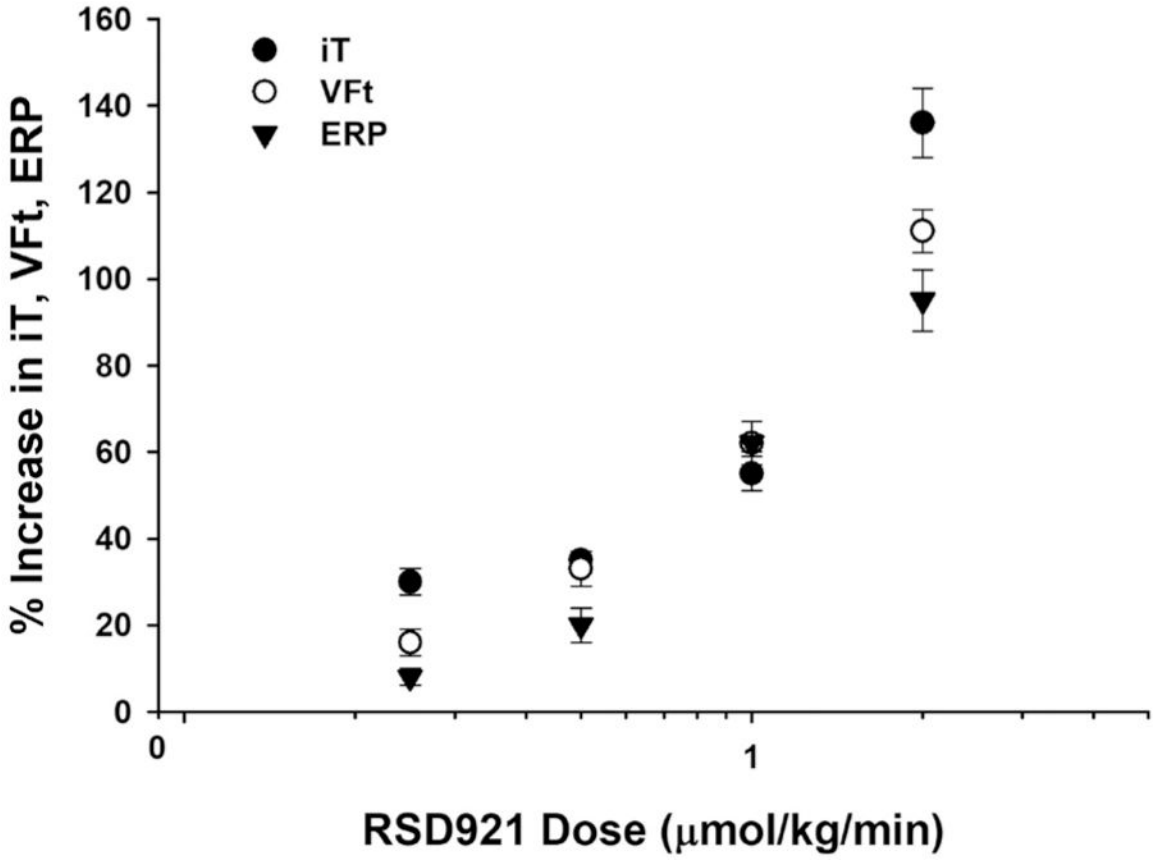

Fig. 7.

Effects of RSD921 on electrical stimulation variables and induction of arrhythmias in anaesthetized rats. RSD921 dose-dependently increased the threshold current for induction of extrasystoles (i_t) and ventricular fibrillation (VF_t) and prolonged the effective refractory period (ERP). Values (mean ± s.d. for n = 5 animals) were measured after completion of each dose. *Indicates a significant difference from pre-treatment at $p < 0.05$. Using a nonlinear curve fit for the data, the correlation coefficient for VF_t was $p < 0.01$.

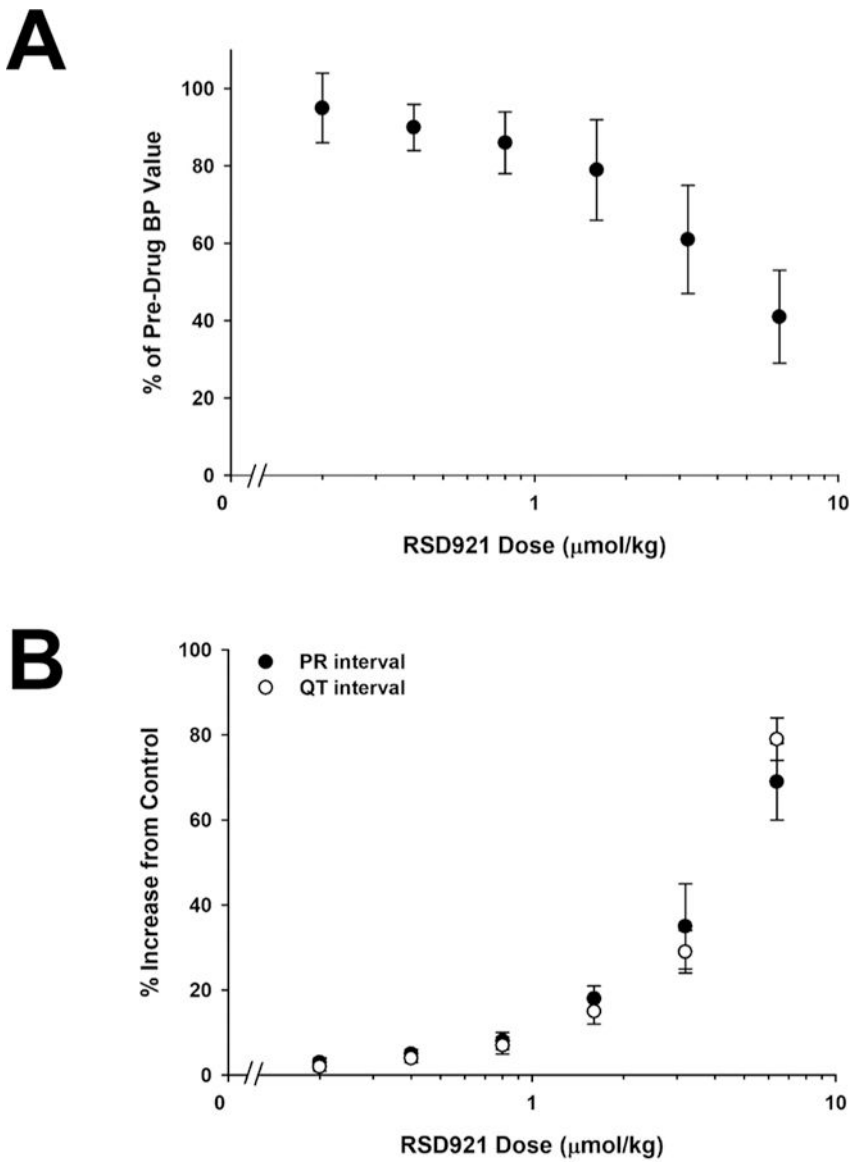

Fig. 8.

Effects of RSD921 on the blood pressure (panel A) (BP) and ECG (panel B) measures (PR and QT intervals) in male and female baboons (n = 4). Values (mean ± s.d. for n = 4 animals) shown were measured 2 min. after completion of each bolus dose. *Indicates a significant difference from pre-treatment at $p < 0.05$. Using a nonlinear curve fit for the data, the correlation coefficient for the BP and QT interval was $p < 0.01$ and the PR interval was $p < 0.001$. Pre-treatment mean control PR interval was 126 ± 3msec and QT interval was 262 ± 18msec.

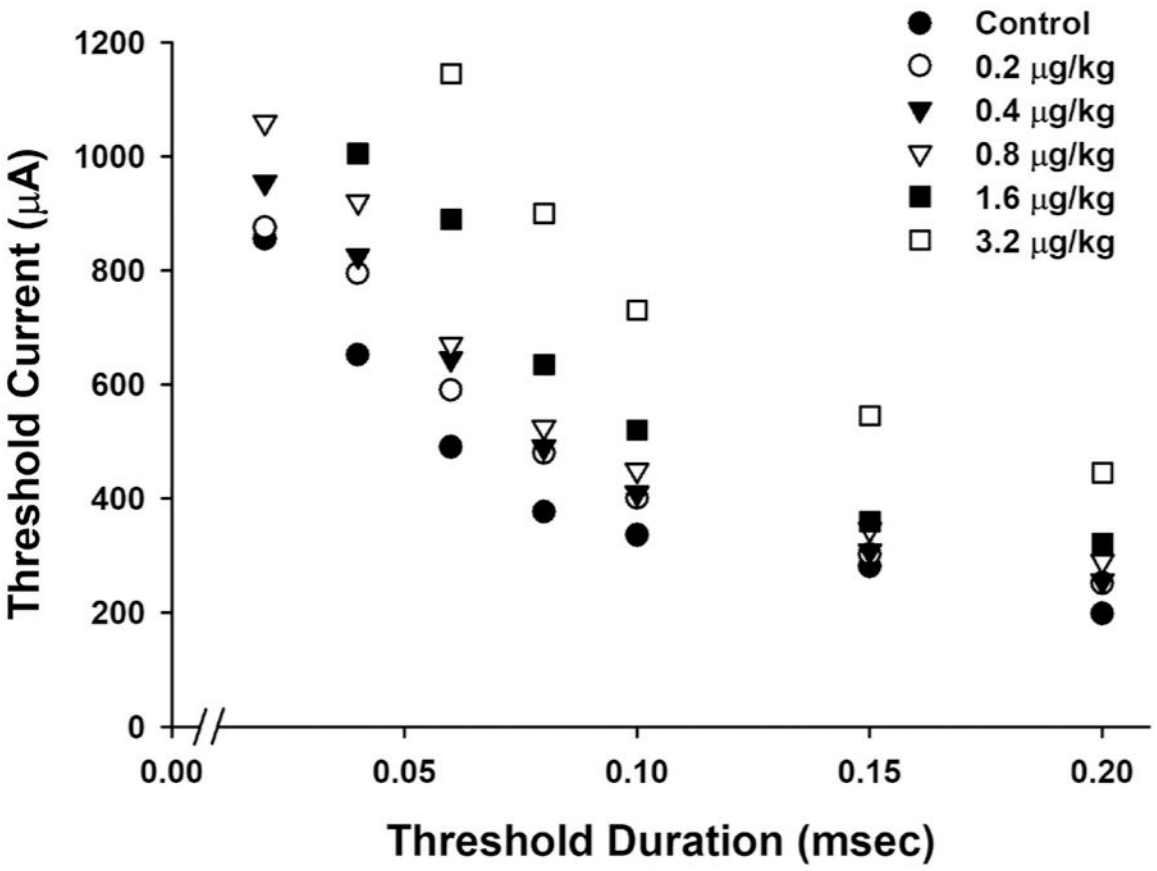

Fig. 9.

Effect of RSD921 (0.2–6.4 μmole/kg), given as a bolus dose, on monophasic action potential threshold current (i_t) *versus* duration (t_t) in the right ventricle of anaesthetized baboons. Data shown is for electrophysiological variables determined 3 min (i_t, t_t and i_t *vs* t_t) after dose administration. Using a nonlinear curve fit for the data, the correlation coefficient for all i_t *vs* t_t curves was $p < 0.05$. For the sake of clarity, error bars are omitted in the figure.

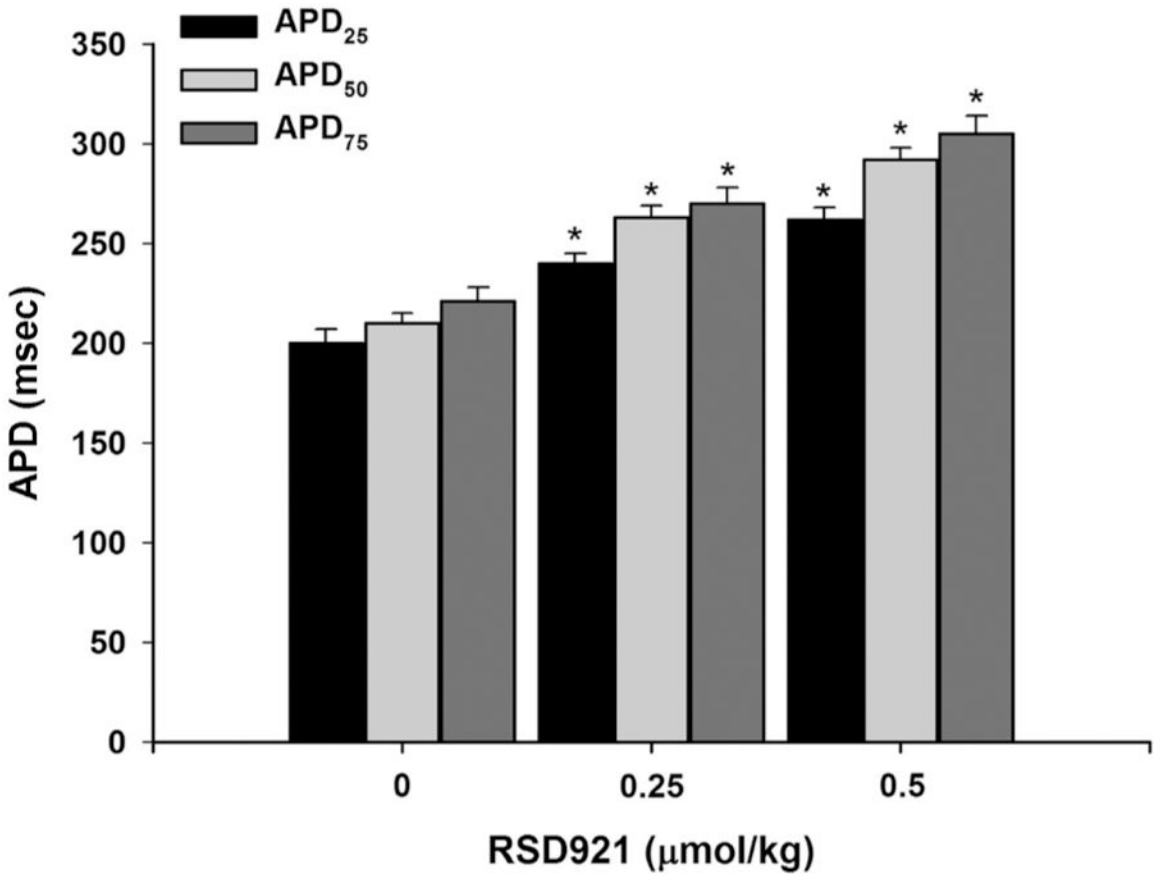

Fig. 10.

Effect of RSD921 (0.2–6.4 μmole/kg), given as a bolus dose, on the cardiac APD in the right ventricle of anaesthetized baboons. Data shown is for changes in APD (25, 50 and 75%) determined 10 min after dose administration. *Indicates a significant difference from pre-treatment (0 μmol/kg) at $p < 0.05$.

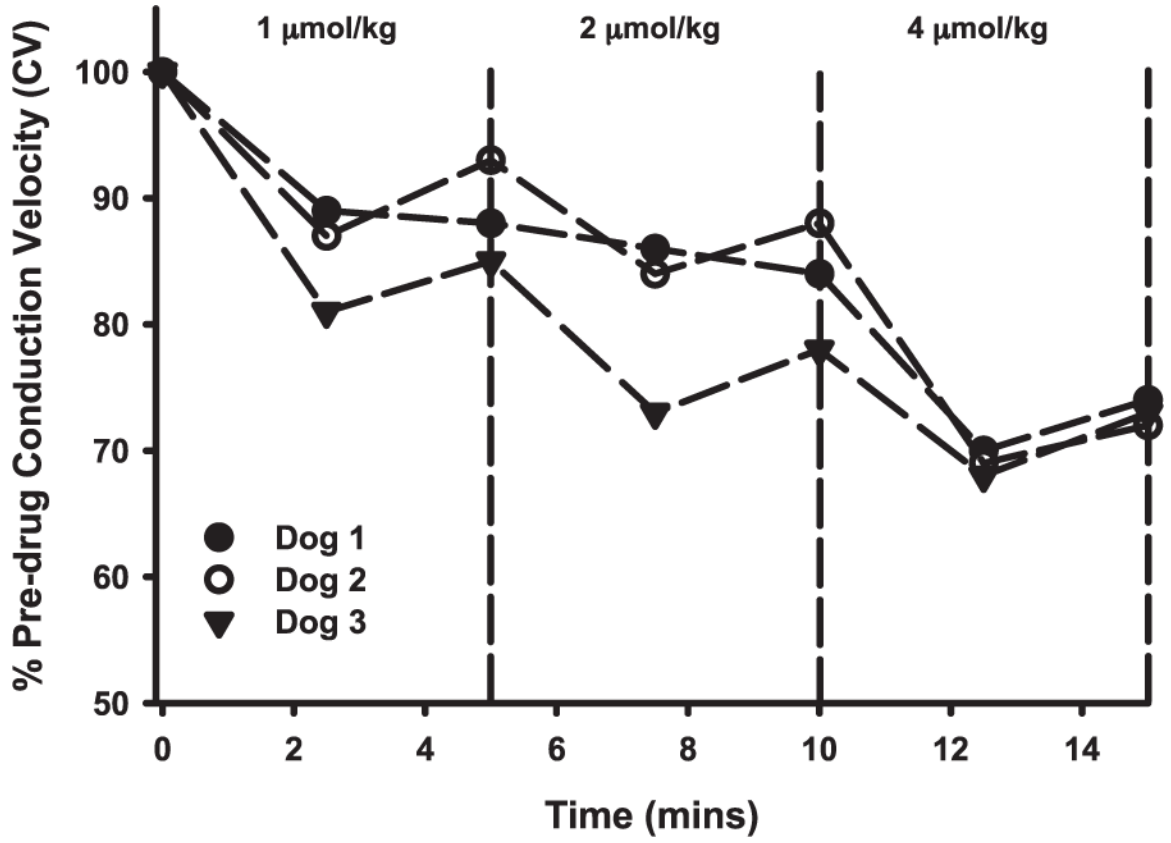

Fig. 11.

Dose response effects of RSD921 on conduction velocity (CV) in anaesthetized dogs (n = 3). RSD921 (1, 2 and 4 µmol/kg) was given every 5 min producing a dose-related decrease in CV. Individual CV values are plotted for each dog.

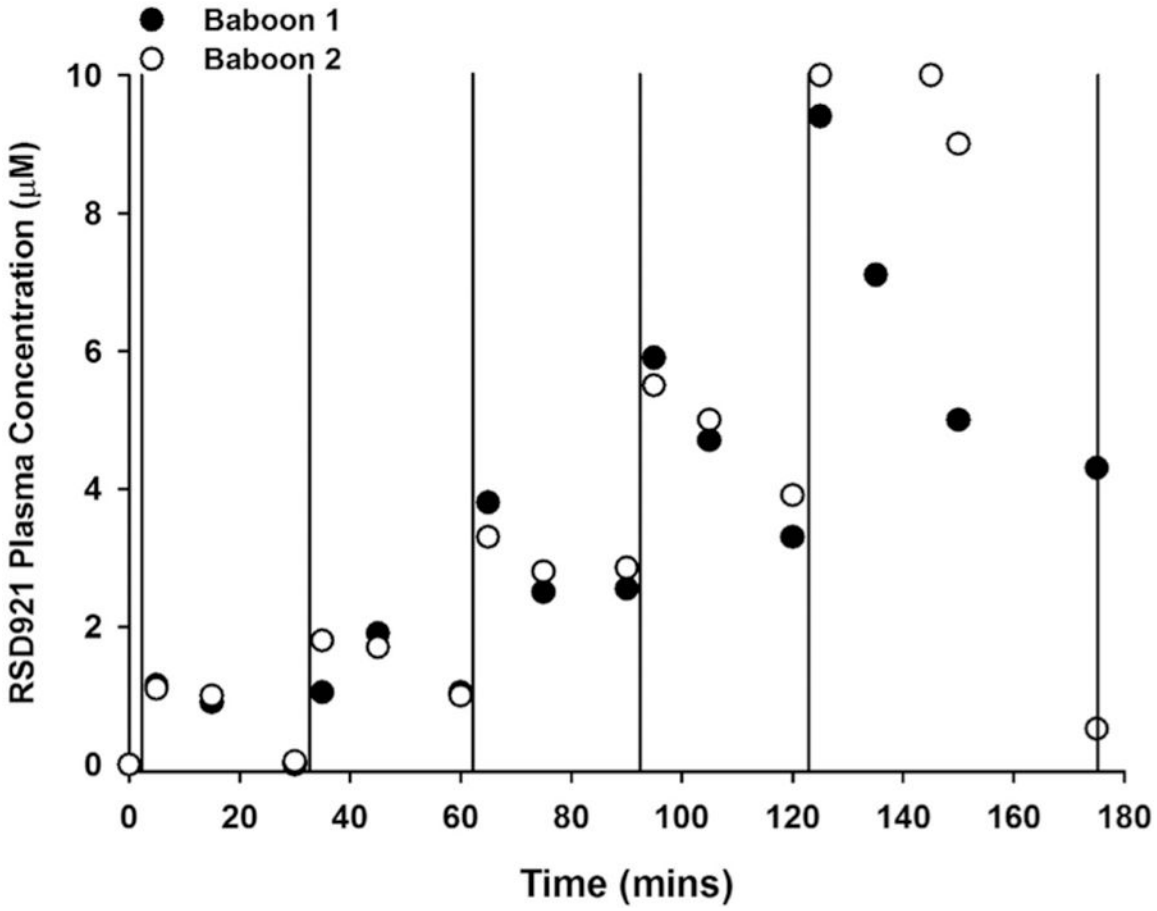

Fig. 12.

Plasma concentrations associated with cumulative i.v. bolus doses (0.2–3.2 μmol/kg) of RSD921 in anaesthetized male baboons (n = 2). Individual plasma values are plotted for each baboon.

Table 1

The cardiovascular and ECG effects of RSD921 in anesthetized rats.

Dose (μmol/kg)	BP (mmHg)	HR (beats/min)	P-R (ms)	QRS (ms)	RSh (mV)	Q-T (ms)
0	152 ± 6	420 ± 15	60 ± 2	30 ± 1	0.42 ± 0.04	48 ± 2
0.5	141 ± 4	407 ± 10	63 ± 3	31 ± 2	0.46 ± 0.05	50 ± 3
1.0	130 ± 2*	385 ± 13*	68 ± 2*	35 ± 1*	0.57 ± 0.04*	53 ± 2
2.0	112 ± 5*	347 ± 16*	75 ± 5*	36 ± 1*	0.62 ± 0.05*	56 ± 4*
4.0	92 ± 7*	297 ± 11*	87 ± 4*	37 ± 2*	0.71 ± 0.06*	57 ± 5*
8.0	44 ± 6*	221 ± 17*	112 ± 3*	39 ± 2*	0.88 ± 0.04*	59 ± 3*

The effects of a single slow bolus intravenous doses of RSD921. Values are mean ± s.d. (n = 5) for the variable indicated. BP = mean arterial blood pressure; HR = heart rate, and the P–R, QRS and Q–T are ECG intervals while RSh describes a change in the amplitude of the RS wave.

* Indicates $P < 0.05$ for comparison with control (saline).

Table 2

Antiarrhythmic profile of RSD921 in anaesthetized rats.

Dose (μmole/kg)	PVC	Log_{10} PVC	VT	VF	Mortality	AS
0	94	2.0	9/10	8/10	8/10	6.0 ± 0.7
0.1	62	1.8	7/7	5/7	3/7	4.6 ± 0.8
0.25	30.4	1.5	3/5	1/5	1/5	2.4 ± 1.3
0.5	123.2	2.1	3/6	0/6	0/6	1.7 ± 0.8
1	34.6	1.5	2/5	0/5	0/5	1.8 ± 1.0
2	20.0	1.3	3/11	0/11	10/11	ND

Ventricular tachycardia (VT), ventricular fibrillation (VF) and mortality are listed as a ratio of the total number of rats. Premature ventricular contraction (PVC) incidence is log_{10} normalized. Arrhythmia scores (AS) are listed as group mean ± s.d. ND = Not determined as all the animals dosed in this group upon occlusion had insufficient cardiac output to survive the 30 min observation period.

Table 3

The duration of regional anesthesia produced by RSD921 in the sciatic nerve of mice.

Drug Concentrations (%)	Lidocaine (min)	RSD921 (min)
0.063	0.7 ± 0.3	4 ± 0.6
0.125	1.3 ± 0.6	13 ± 4
0.25	5 ± 0	40 ± 3
0.5	8 ± 1	52 ± 3
1.0	23 ± 3	81 ± 8

Time (min) refers to time to recovery from anesthesia. Values are expressed as mean ± s.d.