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

M.J.A. Walker[a], E.S. Hayes[b], D.A. Saint[c], G. Adaikan[d], S. Abraham[e], A.L. Goldin[f], G.N. Beatch[g], B.A MacLeod[a], R.A. Wall[a], M.K. Pugsley[h,*]

[a] Department of Anesthesia, Pharmacology & Therapeutics, Faculty of Medicine, The University of British Columbia, 2176 Health Sciences Mall, Vancouver BC, V6T 1Z3, Canada
[b] BioCurate Pty Ltd, Parkville, VIC, Australia
[c] Department of Physiology, Faculty of Medicine, University of Adelaide, Adelaide, SA, 5005, Australia
[d] Department of Obstetrics & Gynecology, Faculty of Medicine, National University of Singapore, Singapore
[e] Department of Pharmacology, IIBR, Ness Ziona, Israel
[f] Department of Microbiology & Molecular Genetics, University of California, Irvine, CA, United States
[g] Clinical Science, Xenon Pharmaceuticals, Burnaby, BC, Canada
[h] Safety Pharmacology/Toxicology Consultant, Fairfield, CT, 06825, United States

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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 (VF_t), 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 (I_{Ksus}) outward potassium currents, albeit with reduced potency relative to sodium current blockade. Sodium 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.

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

* Corresponding author.
E-mail addresses: rsdaa@interchange.ubc.ca (M.J.A. Walker), e.hayes@biocurate.com (E.S. Hayes), david.saint@adelaide.edu.au (D.A. Saint), p_ganesan_adaikan@nuhs.edu.sg (G. Adaikan), abrahm_s@netvision.net.il (S. Abraham), agoldin@uci.edu (A.L. Goldin), gnbeatch@gmail.com (G.N. Beatch), mkpugsley@yahoo.com (M.K. Pugsley).

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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 Instruments, 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