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# An examination of the cardiac actions of PD117,302, a κ-opioid receptor agonist

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ABSTRACT

These studies examined the opioid and non-opioid *in vivo* and *in vitro* actions of PD117,302 ((( ± )-trans-N-methyl-N-[2-(L-pyrrolidinyl)-cyclohexyl]benzo[b]thiophene-4-acetamide), a kappa (κ)-opioid receptor agonist. PD117,302 selectively labeled the κ-opioid receptor in guinea pig cerebellar membranes and in mice the $ED_{50}$ for analgesia was 2.3 μmol/kg. A non opioid cardiovascular assessment of PD117,302 showed that it dose-dependently increased left-ventricular peak systolic pressure in rat isolated perfused hearts but reduced heart rate and blood pressure in anaesthetized rats. Over the concentration range 0.3–30 μM *in vitro*, and dose-range 0.25–4 μmol/kg *in vivo*, PD117,302 dose-dependently prolonged the P-R interval, QRS width and Q-T interval of the rat heart ECG. Naloxone (either 1 μM or 8 μmol/kg) did not antagonize the observed ECG effects of PD117,302. Cardiac electrical stimulation studies in anesthetized rats showed that threshold currents for capture and fibrillation were increased and effective refractory period (ERP) prolonged. In rats subject to coronary artery occlusion PD117,302 reduced arrhythmia incidence. Intracellular cardiac action potential studies qualified the ECG changes produced by PD117,302 such that there was a dose-dependent reduction in the maximum rate of depolarization of phase 0 ($dV/dt_{max}$) and prolongation of the action potential duration (APD). In isolated cardiac myocytes PD117,302 dose-dependently (1–100 μM) reduced peak $Na^+$ current and produced a hyperpolarizing shift in the inactivation curve. Transient outward and sustained outward $K^+$ currents were blocked by PD117,302. Thus, the ECG changes and antiarrhythmic effects observed *in vivo* result from direct blockade of multiple cardiac ion channels.



## 1. Introduction

At doses producing analgesia, kappa (κ) receptor agonists such as ethylketocyclazocine and bremazocine produce diuresis and sedation (Dortch-Carnes and Potter, 2005). These benzomorphan compounds do not produce the untoward effects of emesis, respiratory depression or constipation associated with mu (μ) agonists such as morphine (Stein et al., 2000). The elucidation of κ opioid receptor pharmacology resulted from the synthesis of the arylacetamide class of compounds (Zhu et al., 1992). Prior to their development only a limited understanding of these receptors and their function was known because of the lack of selectivity of the benzomorphan compounds for the κ receptor.

Extensive structure–activity relationship studies were conducted and resulted in the synthesis of compounds with a high μ:κ ratio and with optimal affinity for the κ receptor (Clark et al., 1988). PD117,302 (( ± )-trans-N-methyl-N-[2-(L-pyrrolidinyl)-cyclohexyl]benzo[b]thiophene-4-acetamide), a thiophene arylacetamide derivative of U-50,488H, was one such drug which showed high affinity (nM) for the κ receptor and a lower affinity (μM) for the μ-receptor (Clark et al., 1988; Meecham et al., 1989). The pharmacological profile of PD117,302 suggested that it produced naloxone-reversible antinociception, locomotor impairment and diuresis, but lacked respiratory depression-related safety effects (Leighton et al., 1987; Clark et al., 1988; Hunter et al., 1990). Tortella et al. (1990) showed that PD117,302 is an efficacious anticonvulsant in rat seizure models with activity against N-Methyl-D-Aspartate (NMDA; $ED_{50}$=0.27 mg/kg, iv) and maximal electrical

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shock (MES; $ED_{50}$=16.3 mg/kg, subcutaneously) induced convulsions. Clinically, κ agonist drugs such as spiradoline (U-62,066E) produced analgesia; however, marked adverse effects including diuresis, sedation, dysphoria and hallucinations (Wadenberg, 2003) halted development. Despite such findings, synthesis and development of novel κ agonist drugs has continued (Kivell et al., 2014; Le Naour et al., 2014; Riley et al., 2014).

All opioids, but in particular the κ agonists, exhibit many complex pharmacological actions on the cardiovascular system (Pugsley, 2002). While the CNS effects are clearly opioid receptor mediated (Rech et al., 2012) the actions of these compounds, and the role of these receptors in peripheral tissues is not understood. The fact that some opioid compounds, when injected intravenously, mediate a reduction in cardiac contractility and venous pressure may not be dependent upon opioid receptor activation but rather may result from a direct effect of the opioid on cardiac and smooth muscle of the vasculature (Cheng et al., 2007). The presence of opioid receptors and their importance in the function of heart muscle remains undefined (Tanaka et al., 2014). Endogenous enkephalins occur in the atria, although the localization of endogenous opioid peptides (EOPs) in the heart has not been defined they are found in cultured adult rat ventricular cardiac muscle cells (Springhorn and Claycomb, 1992) but also other non-neuronal tissues (Denning et al., 2008). Opioid receptors are distributed in the atria and ventricles and studies suggest that blockade of the κ-opioid receptor alone is antiarrhythmic (Pugsley, 2002). It was postulated from early studies that EOPs were endogenous, potentially arrhythmogenic, components released from myocytes and related cells into the local cellular milieu during the development of myocardial ischemia (Wong et al., 1990; Tanaka et al., 2014).

Studies with a opioid receptor agonist and antagonist drugs in neuronal tissue suggest that there is, in addition to these receptor mediated actions, a distinct 'non-opioid' receptor mediated action of these drugs on ion channels mediating electrical excitability in both neuronal and cardiac tissue (Su et al., 2002; Xiao et al., 2003). These 'non-opioid' properties involve interactions with both cardiac sodium and potassium channels and reduce the incidence of ischemic cardiac arrhythmias (Pugsley et al., 1993, 1995). A number of structurally similar arylacetamide κ agonist drugs including (−)PD129,290, (+)PD129,289, U-62,066E and U-50,488H all dose-dependently decreased blood pressure (BP) and heart rate (HR) and at high doses these compounds prolonged the PR, QRS and Q-T intervals of the ECG. Neither Mr2266 nor naloxone reduced these cardiovascular actions (Pugsley et al., 1992a, 1992b, 1993, 1995, 1998). Consistent with neuronal studies, the primary basis for the cardiac effects is due to inhibition of voltage-gated sodium currents with electrophysiological properties similar to local anesthetics (Joshi and Gebhart, 2003).

In this study we assessed the opioid-related binding in guinea pig cerebellar membranes and analgesic properties in mice of PD117,302. A comprehensive cardiovascular examination was conducted to characterize whether this κ-opioid receptor agonist exhibited cardiac ion channel effects using *in vitro* and *in vivo* models. An electrophysiological profile of PD117,302 was conducted using epicardial intracellular potentials and isolated rat cardiac myocytes to assess drug effects on ionic currents and channels that contribute to the cardiac action potential and the ECG.

## 2. Materials and methods

### 2.1. Animals

Male Dunkin–Hartley guinea pigs (300–500 g) were used for all binding studies conducted at the University of British Columbia, Canada (U.B.C. Animal Care Centre). Male BALB/c mice weighing between 30 and 40 g were used for all analgesia studies conducted at U.B.C. Male Sprague–Dawley rats weighing between 200 and 300 g were used for all whole animal and isolated heart studies conducted at U.B.C while male Wistar rats (200–300 g) were used for isolated cell studies conducted at the Australian National University, Australia (A.N.U.). All studies conducted at U.B.C. and A.N.U were performed according to the guidelines established by the institutions respective Animal Care and Use Committee.

### 2.2. Opioid receptor radioligand binding assays

Guinea pig cerebellar membranes were prepared according to the method described by Maguire et al. (1992). Briefly, cerebellar tissue was homogenized with a polytron homogenizer in 20 volumes of 50 mM Tris–HCl (pH 7.5) buffer and centrifuged at 30,000 G for 15 min. Membrane homogenates were re-homogenized, centrifuged two additional times and resuspended in 50 mM Tris–HCl to a final tissue concentration of 20 mg/ml wet weight.

Membrane aliquots (500 μl) were incubated in duplicate (to a total assay volume of 1 ml) with either 0.5 nM of the μ-selective radioligand [$^3$H]-[D-Ala$^2$, Mephe$^4$, Gly-ol$^5$] enkephalin (DAMGO) or 0.5 nM of the κ selective ligand [$^3$H]U-69,593 and 9 increasing concentrations of unlabeled PD117,302. Non-specific binding was determined in the presence of 10 μM morphine or the unlabeled ligand U-69,593. 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 radioactivity bound to the filters was quantitated by liquid scintillation spectrometry at room temperature (25 °C)

#### 2.2.1. Binding data analysis

The data obtained from the competitive binding assays was analyzed by LIGAND (NIH, Bethesda, MD). This program uses a weighted, non-linear, least squares regression analysis to calculate the binding affinities and receptor densities. Data is expressed as an $IC_{50}$ value or the concentration of unlabeled ligand required to inhibit 50% of specific radioligand binding.

### 2.3. Analgesia studies in mICE

Individual mice ($n$=4/dose group) were randomly and blindly selected to be given an intravenous tail vein injection of either saline or PD117,302. (1.0, 1.4, 2.8 or 5.5 μmol/kg). A tail flick assay was conducted to investigate the central antinociceptive effects of the compound (Vecchietti et al., 1991).

### 2.4. Langendorff isolated hearts

Rat hearts ($n$=5/group) were mounted on a modified Langendorff apparatus (Curtis et al., 1986) and perfused with Krebs–Henseleit solution (pH 7.4), bubbled with 5% $CO_2$ in $O_2$ and maintained at a temperature of 34 °C. A compliant saline-filled balloon was inserted into the left ventricle. The developed pressure in the heart was recorded together with the maximum rate of intraventricular pressure development ($+dP/dt_{max}$). ECGs were recorded (band width 0.1–40 Hz) from silver-ball electrodes placed on the epicardial surfaces of the right atrium and left-ventricle.

### 2.5. Animal preparation in vivo

Adult rats ($n$=4–5/group) were anaesthetized with pentobarbitone (60 mg/kg, intraperitoneally) and the trachea