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Ventricular arrhythmia incidence in the rat is reduced by naloxone

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ABSTRACT

This study characterized the antiarrhythmic effects of the opioid receptor antagonist naloxone in rats subject to electrically induced and ischemic arrhythmias. Naloxone (2, 8 and 32 μmol/kg/min) was examined on heart rate, blood pressure, and the electrocardiogram (EKG) as well as for effectiveness against arrhythmias produced by occlusion of the left anterior descending coronary artery or electrical stimulation of the left ventricle. Naloxone reduced blood pressure at the highest dose tested while heart rate was dose-dependently reduced. Naloxone dose-dependently prolonged the P–R and QRS intervals and increased the RSh amplitude indicative of effects on cardiac sodium (Na) channels. Naloxone prolonged the Q–T interval suggesting a delay in repolarization. Naloxone effects were comparable to the comparator quinidine. Naloxone (32 μmol/kg/min) reduced ventricular fibrillation (VF) incidence to 38% (from 100% in controls). This same dose significantly increased the threshold for induction of ventricular fibrillation (VFt), prolonged the effective refractory period (ERP) and reduced the maximal following frequency (MFF). The patterns of ECG changes, reduction in ischemic arrhythmia (VF) incidence and changes in electrically induced arrhythmia parameters at high doses of naloxone suggest that it directly blocks cardiac Na and potassium (K) ion channels.

1. Introduction

Coronary heart disease commonly manifests as myocardial ischemia in the heart and results in arrhythmias. 'Sudden death' due to lethal ventricular fibrillation (VF) arrhythmias remains the main cause of death in the US. Death rates from VF have not changed dramatically since the inception of antiarrhythmic drug development programs [1]. There is a need for effective drugs to prevent arrhythmias despite the introduction of useful interventional cardiac techniques. It is apparent that there is no endogenous or exogenous chemical which *sine qua non* will solve the problem of prevention by drugs of arrhythmia-induced sudden death. Moreover, currently approved drugs and research efforts have been stopped due to serious drug safety issues encountered during drug development [2]. Thus there is a need to continue to explore new, and even old synthetic, chemical avenues in a search for useful drugs.

Naloxone is a clinically used opioid receptor antagonist [3] that is a synthetic congener of oxymorphone, an opioid analgesic [4]. Naloxone reduces ventricular arrhythmias produced in multiple experimental models [5–10]. It is postulated that naloxone exhibits antiarrhythmic activity by two mechanisms. Parratt and Sitsapesan [11] and Lee [9] suggest that during ischemia endogenous opioid peptides (EOP) are released from the myocardium and mediate arrhythmias by binding to their respective opioid receptors [12]. Thus, EOP may be intrinsically arrhythmogenic [13–15] and since naloxone blocks EOP receptors this is the mechanistic basis for antiarrhythmic activity.

Other studies [8,16,17] suggest that these effects of naloxone are not mediated by interactions with opioid receptors. Instead, naloxone has direct effects on the heart [18] due to blockade of cardiac ion channels [19,20] similar to the effects of naloxone observed on Na and K currents in guinea pig atria [21] and morphine in the squid giant axon [22,23]. Naloxone reduced the upstroke and prolonged the duration of the cardiac action potential (AP) [24]. Pugsley et al. [19,25] showed that cardiac Na and K channel block by arylacetamide κ opioid receptor agonists reduce cardiac arrhythmias [10,19,25].

The present study examined the antiarrhythmic actions of naloxone using ischemia and electrically induced arrhythmia rat models. These studies were integral to a wide ranging series of studies conducted as part of a drug discovery program aimed at finding a better antiarrhythmic drug.

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2. Materials

Male Sprague-Dawley rats from the U.B.C. Animal Care Centre (275–325 g) were used in these studies according to the IACUC guidelines established by the University and EU Directive 2010/63/EU for animal experiments.

3. Animal preparation

Adult rats were anesthetized (pentobarbital, 60 mg/kg, i.p.) and the trachea cannulated. Animals were artificially ventilated (100% oxygen) at a 10 ml/kg stroke volume and rate of 60 strokes/min. Body temperature was maintained (36–37 °C) using a heating lamp. The right jugular vein was cannulated for drug administration and blood pressure (BP) recorded from the left carotid artery. A lead II configuration was used to record the ECGs [25,26].

4. Naloxone dose–response curves

The effects of naloxone (solubilized in isotonic saline) on heart rate, blood pressure and EKG measures were examined in anesthetized, ventilated rats ($n = 8$/group). Naloxone doses were infused at rates of 2.0, 8.0 or 32.0 μmol/kg/min. Recordings were made 5 min after infusion (at a rate of 1 ml/h) or just prior to coronary occlusion. Heart rate was calculated from the EKG using a Model 7D Grass polygraph (Quincy, MA, USA) while ECG intervals (P–R, QRS and Q–T) were directly determined from the traces. No Q–T interval correction for heart rate was applied [27]. The RSh amplitude was measured as the difference between the peak of the R-wave and the trough of the S-wave [25,26].

4.1. Coronary artery occlusion

Surgical procedures are described by Pugsley et al. [10]. A left thoracotomy was produced and a polyethylene occluder placed around the left coronary artery in anesthetized, ventilated, cannulated rats. The chest was closed and the animal was allowed to recover (45 min) prior to drug administration. The serum K level (Ionetics Potassium Analyzer) was determined from a blood sample taken prior to occlusion since this ensures adequate ventilation of the animal and that physiologically relevant K levels exist since this electrolyte can influence arrhythmia incidence [10,28].

Animals ($n = 8$/group) were given an infusion of either isotonic saline (control) or naloxone (at either 2.0, 8.0, or 32.0 μmol/kg/min) using a random block design study protocol. The BP and ECG were recorded 5 min after infusion and a blood sample (0.25 mL) taken before occlusion. Quinidine ($n = 8$) was the positive control in this study in order to compare naloxone findings since it blocks Na and K channels in the rat and is antiarrhythmic [29]. Quinidine (2.0 μmol/kg) was given as a slow bolus dose (given over 5 min).

ECG, arrhythmias, BP, heart rate and mortality were monitored for 30 min after occlusion. Arrhythmias designated as ventricular premature beats (VPB), ventricular tachycardia (VT) and ventricular fibrillation (VF) were summed using an Arrhythmia Score (AS) [30] for each animal. Values were assigned based on the time of occurrence, incidence and duration of arrhythmia type: 0 (0–49 VPBs), 1 (50–499 VPBs), 2 (>499 VPBs ± 1 episode of spontaneously reverting VT or VF), 3 (>1 VT/VF episode or both with a duration <60 s), 4 (VT/VF alone or both with 60–120 s duration), 5 (VT/VF or both with duration >120 s), 6 (lethal VF starting <15 min post-occlusion), 7 (lethal VF starting 4–15 min), 8 (lethal VF starting 1–4 min post-occlusion) and 9 (lethal VF starting <1 min post-occlusion) [10,30]. The ECG had a positive S–T segment during the pre-dose interval so signs of drug-mediated changes could be discerned prior to occlusion and allowed for assessment of post-occlusion changes. Specific ECG changes occur that are associated with ischemia including a rapid increase in amplitude of the ECG signal defined by an increase in the R-wave.

In rats it is difficult to assess the morphology of the T-wave of the ECG thus difficult to assess changes in the elevation of the S–T wave as a percentage of the R-wave amplitude [31]. The S–T segment changes in rats are biphasic – fall after ligation to baseline [28] and increase to a maintained maximum [32]. Since S–T segment elevation changes with ischemia and varies with each animal the maximum and time to maximum S–T segment elevation were determined in all study groups.

After the post-occlusion observation period, a second blood sample was taken from surviving animals. Hearts were subjected to Langendorff perfusion with cardiogreen dye (1.0 mg/ml) to expose the occluded zone (or cardiac zone-at-risk). All aspects of these studies were performed according to the Lambeth Conventions [33,34].

4.2. Exclusion criteria used in occlusion studies

Exclusion criteria were applied in this study including: (1) the animal's BP remained above 25 mmHg; (2) animals exhibited normal sinus rhythm with discernable ECG intervals prior to occlusion; (3) prior to occlusion only 15 VPB were allowed; (4) serum K levels had to be 2.9–3.9 mM before ischemia; (5) ischemia included increases in the R-wave height and elevation of the S–T segment [28]; (6) the occluded (ischemic) zone was 25–50% of the weight of the left-ventricule. Failure to meet these criteria resulted in exclusion and replacement of the animal to balance group size [33,34].

5. Electrical stimulation

Arrhythmias can be produced by electrical stimulation of the left ventricle [35]. Thus, the influence of drugs on ventricular vulnerability (*i.e.*, arrhythmia induction) can be assessed as well as a probe of the drug effects on Na and K channels.

In anesthetized rats, left-ventricle electrical stimulation using two Teflon™-coated silver wire electrodes inserted through the chest wall was conducted [36]. A constant current square wave Grass stimulator (Grass, model SD9 or S88) was used to stimulate the left ventricle. The threshold current for capture (i_T – μA), threshold pulse-width (t_T – ms) for induction of extrasystoles, threshold current for induction of VF (VFt – μA), maximum following frequency (MFF – Hz) and effective refractory period (ERP-ms) were determined [19].

The variables i_T and t_t assess excitability [37] and measure Na channel availability. Ventricular fibrillation threshold (VFt) is the current required to develop VF [35]. ERP and MFF are measures of AP refractoriness [25]. ERP measured drug changes in the absolute refractory period while MFF probes the relative refractory period. The electrical stimulation variables were determined 5 min after each dose of infused saline or naloxone ($n = 8$/group). Naloxone was given cumulatively at 2.0, 8.0 and 32.0 μmol/kg/min. Quinidine was given as a single 2.0 μmol/kg slow bolus dose ($n = 8$).

6. Drugs

Naloxone hydrochloride dihydrate ((5α)-4,5-epoxy-3,14-dihydroxy-17-(2-propen-1-yl)morphinan-6-one) (PubChem CID: 5464092) and quinidine hydrochloride monohydrate (6′-methoxycinchonan-9-ol hydrochloride monohydrate) (PubChem CID: 16219921) were purchased from Sigma–Adrich Chemical Co. (St. Louis, MO). Naloxone was dissolved in 0.9% sodium chloride while quinidine was dissolved in 22% ethanol/78% distilled water.