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Review Article

New Drug Discovery of Cardiac Anti Arrhythmic Drugs: Insights in Animal Models

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Abstract

In the normal heart cardiac rhythm is controlled by microscopic and macroscopic structures. Abnormality of pacemaker or electrical conduction aberrations causes arrhythmic disorders. Cardiac Arrhythmias may be fortunate, typical, threatening and eventually fatal. Cardio-vascular arrhythmia happens commonly in clinical practice affecting patients drastically on digitalis, anesthesia, and acute myocardial infarction. Both traditional and genetic animal models of arrhythmias, their characteristics, and their significance are summarized as: In Vitro Models: Isolated guinea pig papillary muscles, Action potential and refractory period in isolated guinea pig papillary muscle, Lutgendorf technique & Acetylcholine or potassium induced arrhythmia. In Vivo Models-Chemically induced arrhythmia: Aconitine antagonism in rats, Digoxin induced arrhythmia in rats, Strophanthin/ouabain induced arrhythmia, Adrenaline induced arrhythmia & Calcium induced arrhythmia. Electrically induced arrhythmia: Ventricular fibrillation electrical threshold, programmed electrical stimulation induced arrhythmia & Sudden coronary death models in dogs. Exercise induced ventricular fibrillation. Mechanically induced arrhythmia: Reperfusion arrhythmia in rats, Reperfusion arrhythmia in dogs & two stage coronary ligation in dogs. Genetic models of arrhythmias. Conclusion: Experimental models of cardiac arrhythmias, traditional and genetic gives quick summary of the most popular animal models, their characteristics, and their significance for new drug discovery of antiarrhythmic agents.

Keywords: Cardiac Arrhythmias; In-vitro Animal Models; In-vivo Animal Models; Genetic Animal Models

Introduction

The most prevalent disease in the world right now is cardiovascular disease, which has serious personal and financial consequences. One of the most prevalent forms of heart illness and a leading cause of death is cardiac arrhythmia (approximately 17 million). The disease known as cardiac arrhythmia is characterized by disturbances in the heart's regular beat. Cardiac arrhythmias are linked to abnormal cardiac excitation wave initiation, abnormal car diac excitation wave propagation, or some combination of the two. Cardiac arrhythmias can be present in a variety of ways, and the exact mechanism underlying them is still unclear. The heart rate can also be used to categorize arrhythmias. A significant public health concern is common arrhythmias, notably atrial fibrillation (AF) and ventricular tachycardia/fibrillation (VT/VF). Traditional antiarrhythmic (AA) medications for AF are ineffective and carry the potential for fatal VT/VF. Implantable cardiac defibrillators seem to provide a novel but poor treatment for VT/VF. Due to safety issues or limited advantages in compared to current medication, only few

AA medicines have been successful in the recent few decades. In view of the most recent understanding of molecular and cellular pathways, the Vaughan Williams classification (one medicine for one molecular target) seems overly restrictive. Atrial-specific and/or multichannel blockers, upstream therapy, and anti-remodeling medicines are some of the new AA medications that are being developed.

We concentrate on the cellular mechanisms behind aberrant Na+ and Ca2+ handling in AF, congestive cardiac failure, and hereditary arrhythmias, as well as on cutting-edge methods for restoring ionic equilibrium. Rycals, dantrolene, and flecainide, medications that inhibit abnormal diastolic calcium release through the ryanodine receptor RyR2 and excessive Na+ entry (ranolazine), have extremely intriguing antiarrhythmic characteristics. These medications work by normalizing channel activity as opposed to blocking it. Ranolazine blocks anomalous persistent Na+ currents preferentially over normal peak currents, with little impact on normal channel function (cell excitability and conduction). The concept of "normalization" is equally applicable to RyR2 stabilizers, which solely stop aberrant opening and diastolic Ca2+ leakage in pathological tissues and have no impact on systolic function. The many ways that AA medicines work may expand the range of safe therapeutic choices for the management of arrhythmias in a few pathophysiological circumstances [1].

Any deviation or interruption from the regular heartbeat is referred to as an arrhythmia. The heart's fundamental rhythm is a strictly controlled phenomenon that is intended to ensure maximum effectiveness and peak performance. It is a dynamic phenomenon that varies in response to the body's metabolic requirements. The normal heart's various microscopic and macroscopic structures have a role in the cardiac rhythm. When any part of this pattern is broken or altered, an arrhythmia may develop. Failure of the pacemaker or electrical system to trigger and conduct impulses correctly is one of the arrhythmic disorders. Other arrhythmias take place when aberrant foci inside the heart disrupt the regular sequence of electrical stimulation and start the electrical sequence momentarily or repeatedly [2-4]. Arrhythmias can be advantageous, symptomatic, dangerous to life, or even lethal. Their effects depend on both how they present and whether there are any significant aberrant structural abnormalities of the heart. Cardiovascular arrhythmias are a common issue in clinical practice, affecting up to 25% of patients receiving digitalis, 50% of patients receiving an aesthesia, and more than 80% of patients with acute myocardial infarction. Ventricular extra-systole is one of the two ways that arrhythmia appears. A site other than the SA node is where the impulse comes from.

Early QRS complex with a broad aberrant shape is seen. Ventricular tachycardia is characterized by a rapid series of regular ventricular contractions occurring at a rate of 140–170 beats per minute. Around 100 beats per minute is the rate. Historically, antiarrhythmic drugs have been categorized into four separate types based on their mechanism of action. Based on their effects on phase-0 depolarization and repolarization, class I drugs—those that work by blocking the sodium channel—are split into three subgroups, IA, IB, and IC: Subclass IA medications typically delay repolarization while being a moderately potent sodium channel blocker (increase QRS). Drugs of subclass IB typically shorten repolarization, induce little to no change in action potential length, and have the lowest potency as sodium channel blockers. The most effective sodium channel blockers are those of subclass IC medicines, which have negligible repolarization effects (inc. PR, inc. QRS). Although moricizine is a Class I medication, it has also been variously classified as IA, IB, or in its own subgroup. By inhibiting beta-adrenergic receptors, class II medications have an indirect effect on electrophysiological parameters (increasing PR). Although the methods by which Class III medicines work are unclear (interference with potassium conductance is one putative mechanism), they act to prolong repolarization (increasing refractoriness), with little impact on the rate of depolarization (QT). Class IV medications, which mostly block L-type channels on the AV nodal calcium channel, are relatively selective (increase PR).

Cardiac Arrhythmias remains among the most challenging human disorders to diagnose and to treat. The complex pathophysiology of human cardiac arrhythmias has proven difficult to model. Direct correlations between the traditional arrhythmia's mechanisms, including abnormal excitability, conduction, or repolarization and underlying molecular or cellular biology are poorly defined, as the primary etiologies of many human arrhythmias remain unknown. Since the causes of several arrhythmic syndromes have been identified, genetic models reproducing the mechanisms of these arrhythmias have become feasible. Initial murine modelling has revealed that in many cases the pathophysiology of the respective human disease is more complex than had been suspected. Insights from human genetic studies and animal models strongly suggest that the primary molecular defects may contribute at many stages in the causal chain leading to cardiac arrhythmia. The comprehensive analysis of each arrhythmia will require knowledge not only of membrane effects of the primary defects, but also downstream intracellular signals, the developmental results of these perturbations, and the integration of compensatory responses and environmental factors. Precise modelling will require not only mutation of specific residues in known disease genes, but also the systematic study of each of many steps in cardiac arrhythmogenesis. Ultimately, such models will enable unbiased screening of for disease mechanisms and novel therapies.

Although no animal model can accurately resemble human disease condition and species differences also exist, close similarities with human suffering from or threatened by arrhythmias can be developed by selecting appropriate model and species. Though an animal is not the same as a human patient, cardiac arrhythmogenic mechanisms derived from animal experiments have tremendously helped us to diagnose and adapt therapeutic strategies. The following are the standard models useful for the screening of antiarrhythmic new drug discovery.

Methods (Animal Models) in Antiarrhythmic Drugs Screening

Cell culture technique

Studies on isolated ventricular Myocytes

Isolated ventricular myocytes can be used to assess ventricular arrhythmias, particularly torsade's de pointes. We can better understand the processes causing torsade's de pointes by analyzing action potential and patch clamp techniques in isolated ventricular myocytes. Decapitated guinea pigs (weighing 250–350 g) are slaughtered, and their hearts are extracted and retrogradely perfused with oxygenated calcium-free HEPES buffered saline at a rate of 10 ml/min for 5 minutes at 37°C. The same solution containing 300 U/ml type II collagenase and 0.5 to 1.0 U/ml type XIV protease is then applied once more to the area for 8 min. Finally, free HEPES buffered saline with 0.2 mM calcium chloride is applied to the area for an additional 5 min. The heart is broken down and divided into little pieces cut up, agitated until single cells are dissociated in a 20 ml HEPES buffered saline containing calcium chloride. The cells are then put back together in HEPES buffered saline and kept at room temperature. Conventional glass electrodes linked to the headstage of an Axoclamp 2A amplifier are used to record transmembrane potential. At a rate of 2 ml/min, HEPES-buffering saline is superfused into cells at 370C. Action potentials are evoked by sending quick current pulses (1 ms, 1.2 times threshold) across the recording electrode with an active bridge current. During the stabilisation phase, cells are stimulated at 1 Hz, at 1 and 3 Hz during the control phase, and at 10 min following superfusion with the test medications at cumulatively increasing drug concentration. For each circumstance, the digital averaging and measurement of four distinct action potentials are performed. Square bore and borosilicate microelectrodes are used for voltage clamp experiments. 25 mM KCl, 5 mM KATP, and 0.5 M K+ gluconate is added to borosilicate capillary tubes. The individual cells are voltage clamped using an EPC-7 clamp amplifier list. Cell perfusion is reduced by performing voltage clamp in whole cell recording mode and using a 1 ml syringe to maintain consistent negative pressure on the electrode. When cells are superfused with calcium-free HEPES buffered saline at a rate of 2 ml/min, outward K+ currents are monitored. Measurements of action potentials of currents in each cell under control conditions and during superfusion with two successively increasing doses of a specific drug are used to determine concentration response relations [5]. To measure significance within treatment variations, action potentials are evaluated using a three-way ANOVA. The significance of each treatment's meaning when compared to the control mean values is assessed using the Dunnett's t test.

In-vitro models

Isolated guinea pig papillary muscles

It is possible to identify and categorize potential anti-arrhythmic medications into classes I, II, III, and IV using a quick and precise non-microelectrode technique. Papillary muscle excitability developed tension (DT), and effective refractory period (ERP) in right ventricular guinea pigs are all measured. Excitability is reduced by blocking Na+ channels, refractory time is extended by blocking K+ channels, and cardiac muscle tension is reduced by blocking Ca2+ channels. Stunned guinea pigs (weighing 200–400 g), their carotid arteries cut. The heart is removed as soon as the thoracic cage is opened. A container containing physiological fluid that has been prewarmed and preoxygenated is inserted with the myocardium within. After being stripped of its pericardium, atria, and other structures, the heart is pinned to a dissection tray. The right ventricle opened, tendinous end of papillary muscle ligated with silk thread to ensure chordae tendineae are released from the ventricle, and other end clamped into tissue holder with platinum wire field electrodes. Transferring the preparation to a tissue bath with physiological salt solution kept at a constant temperature and pressure. The force transducer and the muscle are connected by the silk thread. At a stimulus frequency of 1 Hz and a stimulus duration of 1 MS, muscles are field stimulated to contract isometrically. A polygraph recorder captures the developing tension while a Grass constant voltage stimulator delivers pulses. By measuring created tension at various stimulation frequencies (0.3, 0.5, 0.8, 1.0, and 1.2 Hz), the force frequency curve is obtained. An agent's inotropic effect is measured as a percentage change in developed tension at 1 Hz between pre- and post-treatment [6-7]. Calculated changes in effective refractory period (post treatment minus pretreatment), strength duration curve degree of shift (geometrical area between pre and post treatment curves), and after treatment produced tension at 1 Hz percent changes. Depending on how the molecule affects developed tension, excitability, and effective refractory period, it is categorized as a class I, II, III, or IV antiarrhythmic agent.

Patch-Clamp Experiments in CHO Cells

Using the common whole-cell patch clamp method, cells expressing hKv1.5 or hKv4.3 with hKChIP2.2b were evaluated. 16 cells were taken out of the tissue culture flask physically and put into a perfusion chamber with a solution comprising (in mM): NaCl 140, KCl 4.7, CaCl2 2, MgCl2 1.1, HEPES 10, and pH adjusted to 7.4 with NaOH. To heat polish, the patch pipettes, borosilicate glass capillaries were removed. Pipettes had resistances of 2-3 MV following filling with NaCl 10, KCl 120, EGTA 1, HEPES 10, and MgCl2 (all in mM; pH 7.2 with KOH). Currents for hKv1.5 and hKv4.3 + hKChIP2.2b were recorded using voltage pulses of 500 ms duration applied at a frequency of 0.1 Hz from the holding potential of 280 mV to +40 mV. The EPC-9 patch clamp amplifier from HEKA Elektronik in Lambrecht, Germany, along with the software Pulse were used to record the data, which was then saved on a PC for subsequent study. The EPC-9's compensation circuit was used to account for the series resistance, which ranged from 4 to 9 MV and was compensated by 80%. The solutions used in the tests were heated to 36.6oC while the cells were continuously superfused [6-7].

Isolation of Porcine Atrial Myocytes.

All animal-related research was done in compliance with German animal protection laws and the US National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-13, revised 1996). It was also approved by the State of Hessen's Ethical Review Board. Pentobarbital was used to

induce anaesthesia in male German Landrace pigs weighing 15 to 30 kg. 17 The heart was quickly removed following a left thoracotomy, the pericardium was cut, and the lung was retracted. The heart was then quickly placed in an oxygenated, Ca2+-free Tyrode solution that contained (in mM): NaCl 143, KCl 5.4, MgCl2, 0.25, NaH-2PO4, HEPES 5, and glucose 10. The pH was then adjusted to 7.2 with NaOH. The hearts were then fixed to a Langendorff apparatus and infused with Tyrode solution at 37C under constant pressure through the left circumflex coronary artery (80 cm H2O). The left atrium received adequate circulation since all coronary arteries that descend to the ventricular walls were tied off. Atrial tissue was softened by perfusing the atrium with the same Tyrode solution for a further five minutes after the atrium was free of blood and contractions had stopped. This Tyrode solution now contained 0.015 mM CaCl2 and 0.03% collagenase (type CLS II, Biochrom KG, Berlin, Germany) (;20 min). After that, trituration was used to mechanically separate the left atrial tissue into minute pieces. A nylon mesh filter was used to filter the cells after they had been rinsed with a storage solution comprising (in mM): L-glutamic acid 50, KCl 40, taurine 20, KH2PO4 20, MgCl2 1, glucose 10, HEPES 10, and EGTA (pH 7.2 with KOH). In the storage solution, the separated cells were kept at room temperature [6-7].

Isolation of Guinea Pig Ventricular Myocytes

Using the same method as before, enzymatic digestion was used to isolate the ventricular myocytes. A concussion and cervical dislocation were used to quickly kill 400-g Dunkin Hardley Pirbright White guinea pigs. The thorax was opened, and the hearts were taken out and put into ice-cold isotonic saline right away. The same solutions used for isolating pig atrial myocytes were utilised to retrogradely perfuse the hearts at 37C through the aorta [2].

Action potential and refractory period in isolated guinea pig papillary muscle

The left ventricular guineas pig papillary muscle records an intracellular action potential after electrical activation. The stimulation frequency is changed to calculate the refractory time. A substance's effects on the length of the effective refractory period can be pro- or antiarrhythmic. The test compound's inotropic impact is also identified. To make the sacrifice, 250–300 g Marioth strain guinea pigs are stunned, the carotid artery is severed, the thoracic cage is opened, the heart is extracted, and the heart is then placed in a container of prewarmed, preoxygenated Ringer's solution. The two most powerful papillary muscles are cut, and the left ventricle is opened. Action potential is measured using a common microelectrode method. To determine the refractory period, rectangular pulses of 1 V and 1 ms length are applied to the papillary muscle at intervals of 500 ms. The second stimuli are then applied at decremental intervals until contraction stops [8]. Before and after the administration of the medication, contractile force and relative refractory period are measured. Values for ED and ED are established. Log 25ms probit analysis results in ED values that are scored.

Langendorff technique

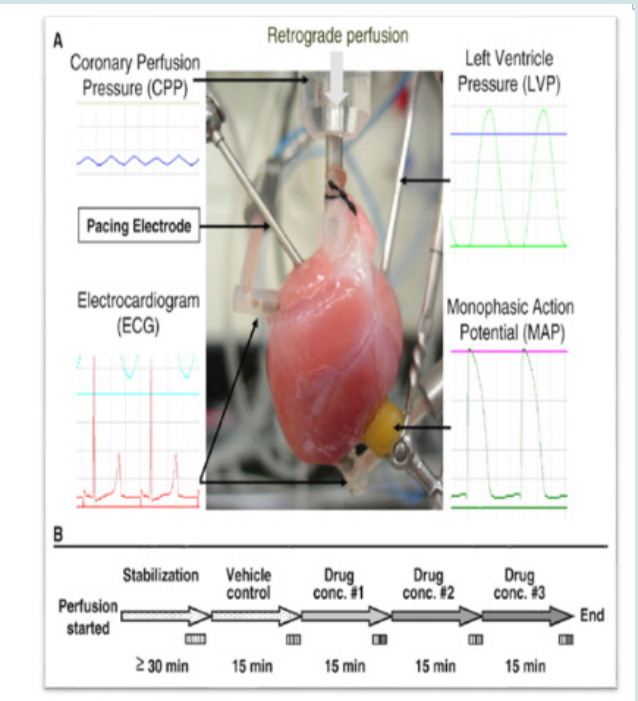

Figure 1: The isolated heart and experimental protocol. A. The front view of an isolated guinea pig heart mounted on the Langendorff apparatus and with all the electrodes and probes positioned. B. The standard protocol consisted of a minimum 30 min stabilization period once the heart perfusion was started, followed by 4 periods of each 15 min perfusions for the vehicle control and reference compound exposure at 3 ascending concentrations. CPP, LVP, ECG and MAP were monitored and recorded continuously throughout the experiment. The heart beats spontaneously under sinus automaticity rhythm except during the brief pacing as illustrated by the horizontal bars. (Guo L, Dong Z, Guthrie H. Validation of a guinea pig Langendorff heart model for assessing potential cardiovascular liability of drug candidates. Journal of Pharmacological and Toxicological Methods. 2009 Sep-Oct, 60(2):130-151. PMID: 19616638. DOI: 10.1016/j.vasch.2009.07.002) [35].

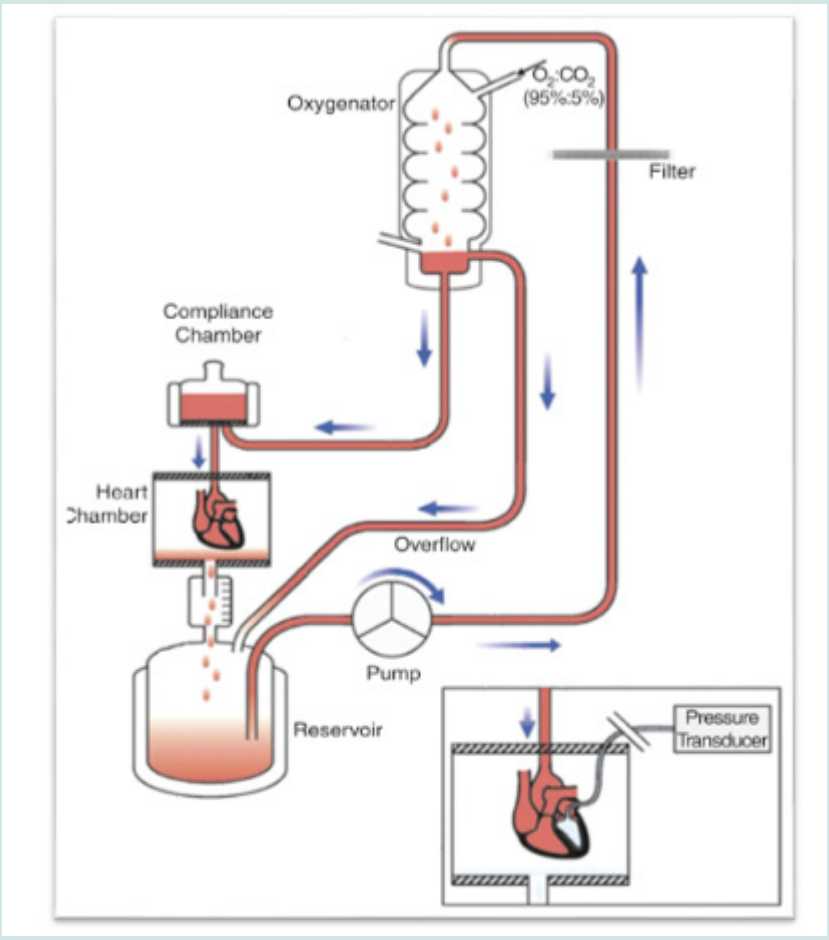

Figure 2: Isolated Langendorff heart perfusion model. (Guo L, Dong Z, Guthrie H. Validation of a guinea pig Langendorff heart model for assessing potential cardiovascular liability of drug candidates. Journal of Pharmacological and Toxicological Methods. 2009 Sep-Oct, 60(2):130-151. PMID: 19616638. DOI: 10.1016/j.vasch.2009.07.002) [35].

This technique's fundamental idea is that the heart is supplied with oxygenated saline solutions at constant pressure or flow in a retrograde route from the aorta. Like the in-situ heart during diastole, retrograde perfusion shuts the aortic valves. Through the coronary arteries branching off the coronary sinus and the expanded right atrium, the perfusate is displaced. By stunning, guinea pigs of either sex that weigh between 300 and 500 g are sacrificed. At 37, the heart was rapidly removed and placed in a dish with Ringer's solution. Lung and pericardial tissues that are related are taken out. Below the point of division, the aorta is located and severed. The aorta is punctured, the cannula is tied, and oxygenated Ringer's solution is then injected into the heart. The heart is moved to a double-walled perfusion device made of plexiglass that is kept at a temperature of 37 °C. At a constant pressure and temperature of 40 mm Hg and 37 °C, oxygenated Ringer's solution is perfused from a reservoir. The LAD coronary artery is bound with a ligament, and an occlusion is maintained for 10 minutes before reperfusion. Before or after occlusion, test chemicals are given via perfusion medium. Because pulsatile stimulation and arrhythmia induction involve epicardial ECG electrodes (rectangular pulses of 0.75 msec duration, usually of 10 V; frequency 400-1800 shocks per min). At the top of the heart is a little steel hook with a rope. A force transducer measures contractile force isometrically, and the results are recorded on a polygraph. A chronometer connected to the polygraph measures heart rate. The perfusion medium is infused with drugs. Both the control group and the test group's ventricular fibrillation or ventricular tachycardia frequency and duration are noted (Figures 1 & 2) [9].

Acetylcholine or potassium induced arrhythmia.

For the investigation, New Zealand white rabbits weighing between 0.5 and 3 kg are employed. Hearts are instantly extracted from the sacrificed animals. In Ringer's solution, additional tissue is separated from the atria. The bottom portion of the bath has an electrode to which the atria are connected and spent. Acetylcholine (3 x 10 g/ml) or potassium chloride (0.10 g) are used to induce fibrillation in the atria. Five minutes later, the trial is stimulated with rectangular pulses of 0.75 ms length, typically at 10 V. (Frequency 400-1800 shocks per min). A kymograph record is made mechanically. Up to 10 minutes are given to control arrhythmias to develop and persist. A test substance is introduced to the bath after allowing the bath to sit empty for 30 minutes, after which fibrillation is once more produced. Following the administration of the test substance, the preparation is washed and allowed to resume normal contraction if the atria does not stop fibrillating within 8 to 10 min-

utes. If fibrillation vanishes instantly or within five minutes of the test medicine being added to the organ bath, the test chemical is successful [10,11].

In-Vivo methods

In vivo models used to screen for new drug discovery of antiarrhythmic agents are depicted as:

Chemically induced Arrhythmia

A wide variety of substances, either alone or in combination, can cause arrhythmias. When sensitising drugs like intravenous adrenaline and anesthetics like ether, chloroform, or halothane are administered together, the result is an arrhythmia. Different animals react differently to these arrhythmogenic chemicals.

Aconitine Antagonism in Rats

Activating sodium channels over time, aconitine, a plant alkaloid from aconitine root, causes ventricular arrhythmias. Rats that have consumed aconitine can be used to test drugs that are thought to have anti-arrhythmic effects. Urethane (1.25g/kg) is administered intraperitoneally to anaesthetize male Ivanovas rats (300-400 g). Aconitine (5 ug/kg) is dissolved in 0.1 N HNO3 and administered 0.1 ml/min continuously into the rat's saphenous vein. Every 30 s, an ECG in lead II is recorded. 5 minutes before the infusion of aconitine, a test substance is administered intravenously or orally. The test group receives a larger dose of aconitine than the untreated group, which results in a measure of antiarrhythmic activity. About ventricular extrasystoles, tachycardia, fibrillation, and death, the antiarrhythmic action of the test substance is quantified by the amount of aconitine/100 g animal (infusion duration) [12,13].

Aconitine-Induced Arrhythmias in Whole Guinea Pigs

Guinea pigs weighing 300 to 400 g were anesthetized with sodium pentobarbital (50 mg/kg i.p.). Respiration was maintained with artificial ventilation (under room air, volume 1.5 ml/100 g, rate 55 strokes/min) through the cannula in the trachea to maintain pCO2, pO2, and pH within the normal range. Polyethylene tubing was inserted into the right jugular vein to administer aconitine and the test drugs. The left carotid artery was cannulated to monitor systemic blood pressure. The standard limb leads of the ECG were recorded. Blood pressure, heart rate, and ECG (lead I and II) were continuously monitored using a polygraph recorder (NEC San-ei Instruments Ltd., Tokyo, Japan). After 15 min of stabilization, vehicle or NCX inhibitors were administered to different groups for each dose of the drug or vehicle (i.e., 0–30 mg/kg) as i.v. bolus injection through the jugular vein. Five minutes later, 25 g/kg of aconitine was injected to induce ventricular arrhythmias in the whole-animal model (Lu and Clerck, 1993) (Fig. 1, protocol I). Each animal received only one dose (treatment) of either vehicle or any of the NCX inhibitors. The doses of SEA were 1 to 10 mg/kg and those of KBR were 1 to 30 mg/kg (Figure 3) [14].

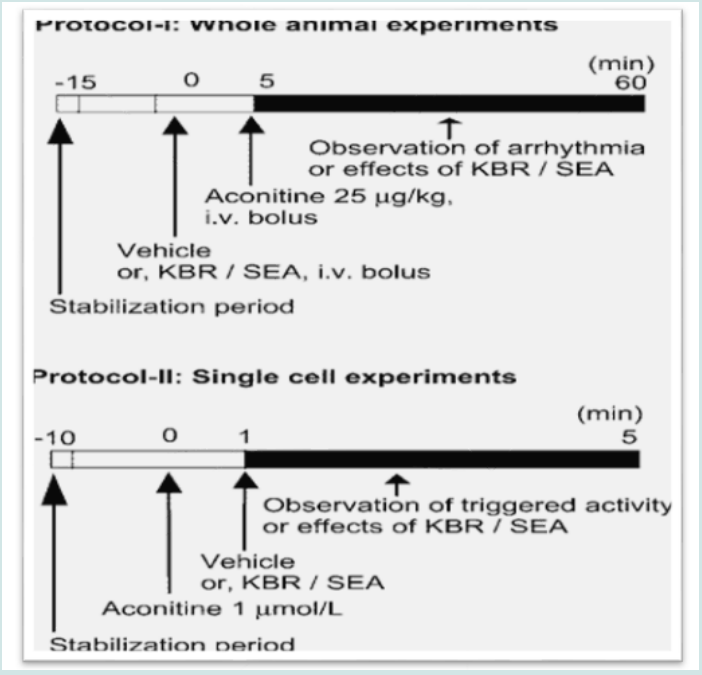

Figure 3: Diagram of the experimental protocols. Protocol I was used in the whole-animal experiments, and protocol II was used in single-cell experiments. In protocol I, after stabilization, vehicle or each dose of either KBR or SEA was pre-treated. Then aconitine (25 g/kg, i.v. bolus) was administered. The black column indicates the duration for observing arrhythmias. A total of 130 animals were entered in these series of experiments. In protocol I, 74 were used but six were ruled out according to exclusion criteria. The number of animals assigned to each dose of control (vehicle), KBR, and SEA were shown in Fig. 3. In protocol II, after stabilization, aconitine (1 M) was perfused first, then vehicle, or each dose of either KBR or SEA was post-treated. A total of 13 cells were entered in protocol II. (Amran MS, Hashimoto K, Homma N. Effects of sodium-calcium exchange inhibitors, KB-R7943 and SEA0400, on aconitine-induced arrhythmias in guinea pigs in vivo, in vitro, and in computer simulation studies. Journal of Pharmacology and Experimental Therapeutics. 2004 Jul 1;310(1):83-9. PMID: 15028781. DOI: 10.1124/jpet.104.066951) [14].

Digioxin induced arrythmia in guinea pigs.

Ventricular extrasystoles, fibrillation, and mortality are brought on by digoxin overdose. Antiarrhythmic medications make these symptoms last longer. Pentobarbitone sodium (35 mg/kg) is administered intraperitoneally to anaesthetize male Mariota guinea pigs (350-500 g). The animal is kept on artificial breathing (45 breaths/min) while having its trachea, jugular vein, and one carotid artery catheterized. Until cardiac arrest, digoxin is given through the jugular vein using a perfusion pump at a rate of 85 g/kg in 0.266 ml/min. Throughout the entire experiment, steel needle electrodes are used to capture the ECG. Through the carotid artery, blood pressure readings are taken. Before the infusion, the test medication is given intravenously or orally one hour before. Recorded is the amount of time before ventricular extrasystoles, fibrillation, and cardiac arrest occur. A statistical comparison is made between the total amount of digoxin administered (ug/kg) to cause ventricular fibrillation extrasystoles, ventricular fibrillation, and cardiac arrest following treatment with the test medication and controls receiving digoxin alone [15].

Strophanthin /Ouabain induced Arrhythmia.

Acute poisoning with cardiac glycoside (Strophanthin K). causes ventricular tachycardia and multifocal ventricular arrhythmia. Pentobarbitone sodium (30–40 mg/kg) is administered intraperitoneally to anaesthetize dogs (20 kg) of either sex. To administer the test compound and the arrhythmia-inducing drug (V. brachialis), two peripheral veins are cannulated (V. Cephalica antebrachialis). The ECC is recorded using lead II needle electrodes at various time intervals. Through the brachialis vein, strophanthin K is given at a rate of 3 g/kg/min. Strophanthin infusion is stopped 30–40 minutes later if ventricular tachycardia or multifocal ventricular arrhythmia develops. After the arrhythmias has stabilized for 10 minutes, the test substance is given. If the extrasystoles stop occurring right away after taking the medication, the test chemical is thought to have an antiarrhythmic action. If the test substance does not have a beneficial impact, larger doses are given at intervals of 15 minutes. The next dose is given after a steady arrhythmia recurs if the test chemical successfully reverses arrhythmias [16,17].

Adrenaline induced Arrhythmia.

High doses of adrenaline may cause arrhythmia to occur. Pentobarbitone sodium (30–40 mg/kg) is administered intraperitoneally to dogs (10–11 kg) to induce anaesthesia. A cannula is placed in the femoral vein, and 2-2.5 g/kg of adrenaline is then infused. Atrial and Lead II ECGs are both recorded. Three minutes after the infusion of adrenaline, the test medication is given [18]. A test substance is deemed to have an antiarrhythmic action if extrasystoles stop occurring right away after taking the medication.

Calcium induced Arrythmia.

Wistar albino rats (60-130 grams) are anesthetized with Nembutal (60 mg/kg) intraperitoneally. Ventricular flutter and fibrillation are induced by administration of 2 ml/kg 10% aqueous calcium chloride through the femoral vein. During the injection and for 2 minutes thereafter, the cardiac rhythm and behaviour are studied by means of a cardioscope connected to the animal with 2 percutaneous, precordial, clamp electrodes. The test drug is administered two minutes prior to calcium chloride infusion [19].

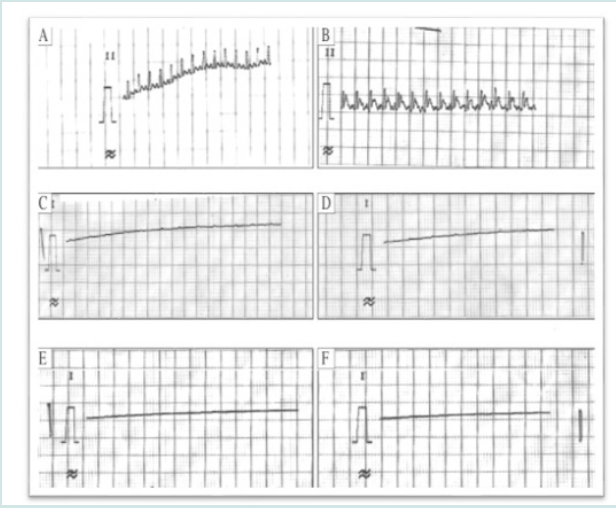

Figure 4: Representative electrocardiogram of Group II (10% CaCl2) at different time intervals. The ECGs were detected in Group II [Arrhythmia control group 1, 10% CaCl2 (50 mg/kg) (n = 6)]. with Dose 10% of CaCl2 (at different time intervals after intravenous administration). Arrhythmia, cardiodepression and mortality were observed at 20 min after 10% of CaCl2 administration. A: Normal reading (Control); B: Dose 10% of CaCl2 (at 0 min after administration) induced arrhythmic, C: Dose 10% of CaCl2 (at 5 min after administration) induced arrhythmic; D: Dose 10% of CaCl2 (at 10 min after administration) induced arrhythmic; E: Dose 10% of CaCl2 (at 15 min after administration) induced complete cardiodepression; F: Dose 10% of CaCl2 (at 20 min after administration) caused complete cardiodepression and Mortality. (Sharma AK, Kishore K, Sharma D, Srinivasan BP, Agarwal SS, Sharma A, Singh SK, Gaur S, Jatav VS. Cardioprotective activity of alcoholic extract of Tinospora cordifolia (Wild.) Miers in calcium chloride-induced cardiac arrhythmia in rats. Journal of Biomedical Research. 2011 Jul 1;25(4):280-6. PMID: 23554702. DOI: 10.1016/S1674-8301(11)60038-9) [1].

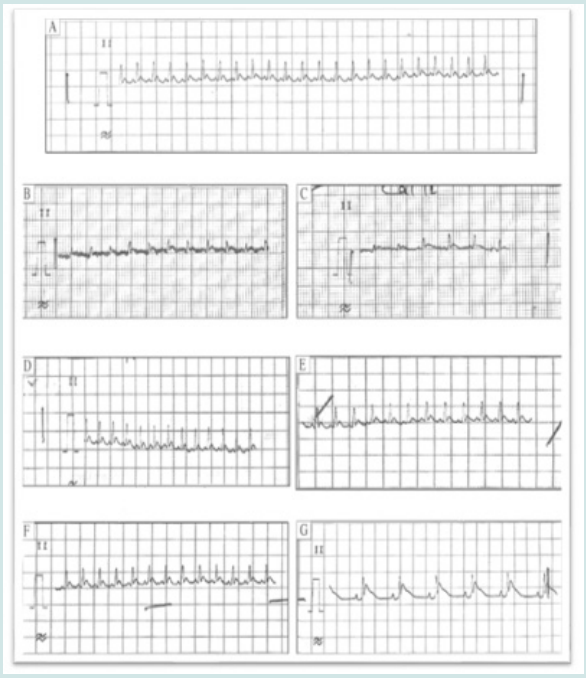

Figure. 5: Representative electrocardiogram of different groups (Group I to Group VIII, n = 6) A: Normal reading (Control); B: Group III: Arrhythmia control [5% CaCl2 (25 mg/kg)]; C: Group IV CaCl2 (5%)-induced arrhythmia+alcohol (95%, 0.5ml, iv); D: Group V: CaCl2 (5%)-induced arrhythmia+verapamil (5 mg/kg, iv); E: Group VI CaCl2 (5%)-induced arrhythmia+Tinospora cordifolia dried ethanolic extract dissolved in saline.(150 mg/kg, iv); F: Group VII CaCl2 (5%)-induced arrhythmia+Tinospora cordifolia extract dissolved in saline (250 mg/kg, iv); G: Group VIII CaCl2 (5%)-induced arrhythmia+Tinospora cordifolia dried ethanolic extract dissolved in saline (450 mg/kg, iv) (n = 6). (Sharma AK, Kishore K, Sharma D, Srinivasan BP, Agarwal SS, Sharma A, Singh SK, Gaur S, Jatav VS. Cardioprotective activity of alcoholic extract of Tinospora cordifolia (Wild.) Miers in calcium chloride-induced cardiac arrhythmia in rats. Journal of Biomedical Research. 2011 Jul 1;25(4):280-6. PMID: 23554702. DOI: 10.1016/S1674-8301(11)60038-9) [1].

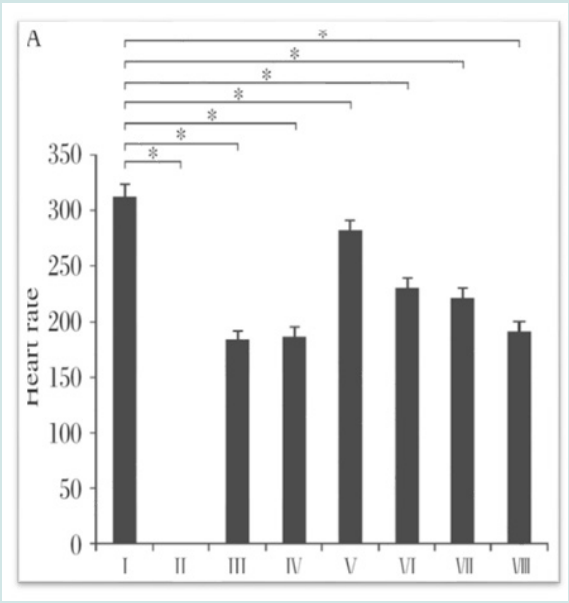

Figure 6: Comparison between the effects of Tinospora cordifolia extract and Verapamil on heart rate. *P < 0.05 for statistically significant vs control (Group I). (n = 6). Group I: Control; Group II: 10%CaCl2; Group III: 5%CaCl2; GroupIV: Alcohol 95%+5% CaCl2; Group V: Standard: CaCl2 (5%) +verapamil (5 mg/kg, iv); Group VI: CaCl2(5%) + Tinospora cordifolia (150 mg/kg, iv); GroupVII: CaCl2(5%) +Tinospora cordifolia (250 mg/kg, iv); GroupVIII: CaCl2(5%) +Tinospora cordifolia (450 mg/kg, iv). (Sharma AK, Kishore K, Sharma D, Srinivasan BP, Agarwal SS, Sharma A, Singh SK, Gaur S, Jatav VS. Cardioprotective activity of alcoholic extract of Tinospora cordifolia (Wild.) Miers in calcium chloride-induced cardiac arrhythmia in rats. Journal of Biomedical Research. 2011 Jul 1;25(4):280-6. PMID: 23554702. DOI: 10.1016/S1674-8301(11)60038-9) [1].

In this work, Tinospora cordifolia (T. cordifolia) alcohol extract was tested for its ability to prevent Cacl2-induced arrhythmia. Rats were given intravenous infusions of Cacl2 (25 mg/kg) to induce arrhythmia. Then, T. cordifolia extract (150, 250, and 450 mg/kg) and verapamil (5 mg/kg, iv) were administered to the animals. Electrocardiogram in Lead II was seen. We measured the levels of potassium, sodium, and calcium in the plasma. Verapamil decreased the heart rate by 9.70%, T. cordifolia at 150, 300, and 450 mg/kg by 26.30%, 29.16%, and 38.29%, respectively, while Cacl2 decreased the heart rate by 41.10% when compared to the control group. In rats given verapamil and T. cordifolia, the PQRST waves were restored to normal, and atrial and ventricular fibrillation were controlled. Cacl2 raised the levels of calcium and salt while lowering potassium in the blood. T. cordifolia boosted potassium levels while dose-dependently lowering calcium and salt levels. As a result, T. cordifolia can be employed in therapeutic settings to treat antiarrhythmic conditions such as atrial and ventricular fibrillation and flutter, as well as ventricular tachyarrhythmia (Figures 4-6) [1].

Electrically induced Arrhythmia

Flutter and fibrillation are caused by serial electrical stimulation, and some of the most common arrhythmias of clinical significance can be mimicked. In dogs under anaesthesia, either before or after the test substance is administered, the flutter threshold or the ventricular multiple response thresholds can be measured [20].

Ventricular Fibrillation electrical threshold

To compare antifibrillatory drugs, consider the highest frequency at which atria might respond to stimulation. Ventricular threshold has been measured using a variety of electrical stimulation approaches, including single pulse, train of pulse, continuous 50 Hz, and sequential pulse stimulation. Dogs (8–12 kg) are maintained on artificial respiration after being given sodium pentobarbital (35 mg/kg) intraperitoneally to anaesthetize them. The heart is hung in a pericardial cradle, the chest is opened, and measurements of blood pressure and temperature are taken. An Ag-AgCl stimulating electrode is implanted in a Teflon disc that is sutured to the anterior surface of the left ventricle, and the SA node is crushed [20]. Anodal constant current (3 ms square) is supplied through the driving electrode for 400 ms. A digital stimulator is used to programme electrical stimulation. Each ventricle's surface is covered with a recording electrode. A 0.2 to 1.8 s train of 50 Hz pulses is delivered 100 ms after every 18th basic driving stimulus to determine the ventricular fibrillation threshold (VFT). The current intensity of the pulse train necessary to induce sustained ventricular fibrillation is defined as the VFT. When ventricular fibrillation occurs, the heart is immediately defibrillated and allowed to recover to control condition for 15-20 min. The femoral vein is used for drug administration. VFT is measured before and after the test medication is administered, and the results are compared using the student's t-test [21].

Programmed Electrical stimulation induced Arrhythmia.

Dogs (8–12 kg) are put to sleep using artificial respiration after receiving 30 mg/kg of pentobarbital sodium intravenously. One inserts a cannula into the external jugular vein. The heart is made visible with a thoracotomy that is performed between the fourth and fifth ribs. The solitary LAD is the left anterior descending coronary artery. A ligature is then knotted around the artery and the needle after a 20-gauge hypodermic needle has been inserted on the LAD. The vessel suffers from significant stenosis because of the removal of the needle. The LAD is pumped for five minutes. The LAD is blocked for two hours with a silicon rubber snare to cause ischemic damage to the heart. The vessel is then repercussed for another two hours while critical stenosis is present. An epicardial bipolar electrode is sutured on the interventricular septum, close to the site of blockage, during the LAD reperfusion period. Subcutaneously implanted silver disc electrodes are used to monitor the ECG. After 6–9 days, the chest is reopened, and an electrode implanted in a non-infarcted area is used to execute programmed electrical stimulation. A further stimulus is supplied after 15 pacing stimuli with a 200 MS pacing stimulus that is set. The study makes use of animals that have ventricular fibrillation and persistent ventricular tachycardia. Before scheduled electrical stimulation begins, heart rate and ECG intervals are recorded. A test medication is then given 30 minutes later. Before and after the administration of test medications, the lowest current intensity of pulse necessary to cause sustained ventricular fibrillation is recorded, and the mean values of 10 experiments are compared using Student's t-test [22].

Sudden Coronary Death Model in Dogs

One of the main causes of death in affluent nations is sudden coronary death. The protection provided against sudden coronary mortality is examined using this canine model. Pentobabital sodium (30 mg/kg) is administered intravenously to anaesthetize male mongrel dogs (weighing 14 to 22 kg). The animals' tracheas are cannulated, and artificial breathing is used to keep them alive. To administer the test substance/saline, the jugular vein is cannulated. The left anterior descending coronary artery (LAD) is isolated after the chest cavity has been opened to reveal the heart, and a 20-gauge needle is then inserted on the LAD. The needle is then removed when a ligature has been tied across the artery and it, causing a crucial vascular stenosis. In the event of serious stenosis, the LAD is blocked for 2 hours with a silicon rubber snare and then reperfused for another 2 hours.

For pacing the left atrium, a bipolar epicardial electrode and a bipolar plunging stainless steel electrode are sutured to the left atrial appendage and interventricular septum, respectively. Two comparable stainless-steel electrodes, one at the distribution of the LAD distal to the blockage and the other in the distribution of the Left circumflex coronary artery (LCX)., are sutured on the left ventricular wall. Through the wall and into the LCX lumen, a silver-coated electrode is inserted, and it is then sutured to the surface of the heart right next to it. Subcutaneous implants are used to place silver disc electrodes for ECG monitoring. Animals are then allowed to heal after the surgical incision has been closed. Animals are given test medicine once they have recovered. A lead II ECG is recorded for 30 seconds every 15 minutes on a cardiocasette recorder using a direct anodal 15-A current from a 9-V nickel-cadmium battery that is coupled to a subcutaneously implanted disc electrode. Ani-

mals were slaughtered after experiencing steady anodal current for 24 hours or when ventricular fibrillation appeared. The thrombus mass in the LCX is taken out and weighed once the hearts have been removed. To investigate the site of the infarction, the heart is cut into sections and stained with tetrazolium triphenyl chloride (TTC stain). Using recordings from the cardiocassette, the start of ventricular ectopy and fatal arrhythmia is investigated. Evaluation of both sustained and non-sustained tachyarrhythmias [23].

Exercise induced Ventricular Fibrillation

Exercise-based tests that simulate coronary patients' conditions most precisely may involve coronary constriction. This model is appropriate for assessing the effects of antiarrhythmic medications on cardiovascular parameters in an exercise-plus-ischemia test. Sodium pentobarbitone (10 mg/kg, intravenously) is used to anaesthetize mongrel dogs (15–19 kg), after which the chest cavity is opened, and the hearts are exposed and supported by a pericardial cradle. A 20 MHz pulsed Doppler flow transducer and a hydraulic occluder are positioned around the left circumflex artery. The epicardial surface of the left and right ventricular electrograms are sutured with a pair of insulated silver coated wires, and a Gould Biotachometer is used to measure the heart rate. The left ventricle is then punctured with a solidstate pressure transducer that has been previously calibrated. Afterward, the left anterior descending coronary artery is blocked in two stages (partially occluded for 20 min and then tied off).

The animal's neck has leads from the cardiovascular equipment that have been tunnelled under the skin. Animals are given antibiotics and analgesics to lessen their discomfort. The animals are trained to lie quietly on a lab table without constraint for three to four weeks after the development of myocardial ischemia. They then walk on a motor-driven treadmill during this recovery time. On the motor-driven treadmill, vulnerability to ventricular fibrillation is then assessed. In the first three minutes of the protocol, the animals run at 6.4 km/hr (0% grade) as a warm-up. Every three minutes, the grade is raised by 0%, 4%, 8%, 12%, and 16%. The left circumflex coronary artery is blocked during the final minute of activity, the treadmill is halted, and the blockage is maintained for an extra minute (total occlusion time, 2 min). If the animal loses consciousness, an electrical defibrillator is utilised.

If ventricular fibrillation occurs, the blockage is freed. After receiving a pre-treatment with the test drug, the exercise plus ischemia test is repeated, and the results are compared to those of the control (saline) group. A Medtronic model 5325 programmable stimulator is used the next day to calculate the effective refractory period both at rest and during myocardial ischemia. A flowmeter is also used to study how the test substance affects coronary blood flow.[24]A Gould model 2800 S eight channel recorder is used to capture all hemodynamic information, including the rate of change of left ventricular pressure. The results are averaged, and analysis of variance is used to examine the reactive hyperemia response to each blockage during the refractory phase. The chi-square test with Yate's adjustment is used to analyse how the medication intervention affected the development of arrhythmias.

Mechanically induced Arrhythmia

Both ischemia and reperfusion have the potential to cause arrhythmias. Multiple phases of arrhythmia can be examined via coronary ligation or ischemia-induced infarction. Late arrhythmia is the focus of the two-step coronary ligation approach. In several species, the impact on reperfusion arrhythmia can be evaluated.

Reperfusion Arrhythmia in Rats

Ventricular arrhythmia and myocardial infarction are the outcomes of ligation of the left major coronary artery. During the ligation and subsequent reperfusion, an electrocardiogram is taken. In cardiac slices, p-nitro blue tetrazolium chloride staining is used to quantify the amount of infarcted tissue. Pentobarbitone sodium (60 mg/kg) is administered intraperitoneally to anaesthetize Sprague-Dawley(350-400gm) rats. The jugular vein is cannulated to provide test medications while the animal is kept on artificial breathing. Using a pressure transducer attached to a polygraph, blood pressure is taken from the carotid artery. The heart is visible when the chest is opened. In the case of infarct size investigations, the left coronary artery is found, ligated for 15 to 90 minutes, and then re-perfused for 30 minutes. Five minutes prior to the ligation, test medication is given. Throughout the entire experiment, peripheral blood pressure and ECG lead II are continually recorded. During the occlusion and reperfusion periods, the number of premature ventricular beats, ventricular tachycardia, and fibrillation are counted. [25-26]. The animal is sacrificed at the conclusion of the reperfusion phase, and the infarct size is measured using TTC (p-nitro blue tetrazolium trichloride) staining. To visualize the infarct tissue (blue/violet, stained healthy tissue, unstrained necrotic tissue) the heart is dissected, sliced into transverse sections (1 mm thick), and stained with TTC produced in Sorensen phosphate buffer containing 100 mM, L-maleate. Slices are imaged, and the baby area is calculated using planimetry using all of the slice projections. Comparing drug-treated animals- variation in hemodynamic parameters and infarct size to control values.

Reperfusion Arrhythmia in Dogs

Dogs with coronary artery ligation may experience elevated blood pressure, heart rate, heart contractility, left ventricular end diastolic pressure, and ventricular arrhythmias, particularly during the reperfusion period. The anesthetic thio-butobarbital sodium (30 mg/kg intraperitoneally) is administered to dogs (20-25 kg) to induce anesthesia. The dogs are then maintained on intravenous chloralose (20 mg/kg) and 250 mg/kg urethane intravenously, followed by the subcutaneous delivery of 2 mg/kg(morphine). The animal is then kept on an artificial breathing system: To administer the test substance, the saphenous vein is cannulated, and lead II of the ECG is constantly monitored. A pressure transducer is attached to a cannulated femoral artery to measure blood pressure. The left ventricular pressure curves are used to calculate heart rate and left ventricular end diastolic pressure. An increase in left ventricular pressure is used to evaluate myocardial contractility. Rats were

used in the experiment in a similar manner. 90 minutes are spent ligating the coronary artery. The test substance is given twenty minutes before ligation. The animal is repercussed for 30min. [27-28]. During the experiment, all the mentioned parameters are recorded. Comparing drug-treated animals to vehicle controls reveals changes in metrics (mortality, hemodynamics, and arrhythmia).

Two Stage Coronary Ligation in Dogs

Methohexitone sodium (10 mg/kg) is administered intravenously to anaesthetize dogs (8–11 kg) and keep them breathing artificially. The heart is visible when the chest is opened. Two phases of coronary ligation are carried out after the location of the left coronary artery. A 21- gauge needle and two ligatures are put around the artery. The needle is removed after the initial ligature has been tied around the artery. The second ligature is firmly secured around the artery 30 minutes later. After the second ligature has been knotted for 30 minutes, the chest is closed in layers and the dog is given time to recover. Arrhythmias start to appear after 24 and 48 hrs following ligation and disappear after 3-5 days. The atrial electrogram, lead II ECG, and mean blood pressure are all recorded. Following coronary artery ligation, test medications are administered as an infusion for 10 minutes [29]. Animals given drugs are compared to vehicle controls for changes in metrics (mortality), hemodynamics, and arrhythmia). Boyden and Hoffman's canine model, which causes tricuspid regurgitation and right atrial enlargement by banding the pulmonary artery, may also have a clinical analogue in people who have both tricuspid regurgitation and chronic obstructive pulmonary disease [30]. Instead of an anatomical barrier, a functional zone of blocking and area of sluggish conduction create the conditions for re-entry in these dogs. The first documented model of canine atrial flutter using sterile pericarditis also shows functional entrance [31]. Atrial flutter frequently occurs in patients after cardiac surgery, and this may be associated with postoperative sterile pericarditis, which is why this model was created.

Genetically Induced Arrhythmia

a) Genetic arrhythmia

It has been reported that a group of German shepherd dogs have inherited ventricular arrhythmias and are more prone to abrupt demise, which typically happens while they are sleeping or at rest following an active or exciting day. These canines can be used to evaluate possible antiarrhythmic medications. Although there is commonly noticeable T wave notching on the ECG, the QT interval is not prolonged. The ventricular tachycardias are quick polymorphic, follow lengthy R-R intervals, and are almost certainly brought on by early depolarizations of the Purkinje system, which activate the triggered activity. Both the transient outward current's density and its time constant of inactivation are decreased in the epicardial myocytes [30]. Additionally, there are deficiencies in cardiac sympathetic denervation. This dog model initially reminds one of the congenital long QT- syndrome, which is characterized by bradycardia-induced polymorphic ventricular tachycardia, rapid mortality, and genetic abnormalities in ion channels that control repolarization. The dogs do not, however, exhibit a prolonged QT interval. No IO deficiency has been reported in patients with long QT syndrome. [31-33]. However, patients have been reported to have polymorphous ventricular tachycardia (Torsade de pointes) with a normal QT interval, suggesting that this animal model may have a human counterpart.

Conclusion

Antiarrhythmic medications often work by altering either the conduction velocity, the length of the refractory period, or both. The features of the Na+ and Ca+2 channels and the passive electrical properties of heart tissue both play a role on conduction velocity. Action potential duration and refractory period duration vary significantly between species due to considerable changes in the K+ currents, which essentially control repolarization. It is evident that there are species-specific distinctions in the variables that affect how an arrhythmia develops, and it is also evident that no animal model can accurately represent an arrhythmic human being. Nevertheless, developing diagnostic and therapeutic approaches for both supraventricular and ventricular arrhythmias has clearly benefited from the knowledge gleaned from animal investigations.

We are confident that in the future, new knowledge will be gained from experiments carried out at various levels, including single cells, cell cultures, excised cardiac preparations, isolated whole hearts, whole hearts in conscious and anaesthetized animals, and systems expressing and testing the functions of molecules involved in electrical excitation. The development of new methods for diagnosis and treatment will result from the integration of these findings rather than from the use of a single model or experimental approach. In animals with "naturally" occurring cardiovascular illness, electrophysiological research should be promoted. Animal models have been central to the advances in our understanding of the mechanisms of human arrhythmia but have also highlighted issues fundamental to all forms of disease modelling. In any complex process, it is preferable to recapitulate as much of the causal pathway as possible, rather than empirically model individual components. The mechanistic insights that have been gained over last few decades emphasize the complexity of the pathogenesis of clinical cardiac arrhythmia. Models capable of integrating the effects of both genetic and epigenetic modifiers will be required to dissect the multi-step pathways involved, which include myocyte heterogeneity, channel processing and downstream signaling.

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