Jadavpur University

Master Degree Thesis

Parametric study of ECG in Wistar rats

KUHU BHADURI

Thesis Title

Parametric study of ECG in Wistar rats

Thesis submitted in partial fulfillment of the requirement

For the degree of Master of Pharmacy

Under the guidance of

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Submitted by

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2016

Decleration of originality and compliance of academic ethics

The auther, hereby ,declares that this thesis contains original research work, as part of his Master of Pharmacy program .This indicates that the work was done entirely by the author and the elements in the thesis are not a copy of or similar to any other thesis submitted /published elsewhere.

All works were perfomed under the supervision of Prof. SanmoyKarmakar at the Department of Pharmaceutical Technology , Jadavpur University.

All information in this document have been obtained and presented in accordance with academic rules and ethical conduct.

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Certification

This is to certify that Miss. KuhuBhaduri has carried out all research studies under my supervision at the Department of Pharmaceutical Technology, Jadavpur University, Kolkata, for the thesis titled **" Parametric study of ECG in Wistar rats** ''.

The ideas put into effect were original and are not a copy of or similar to any other thesis submitted/published elsewhere.

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KuhuBhaduri

May,2016

To my beloved parents

CONTENT

INTRODUCTION

Drug-induced prolongation of cardiac repolarization and proarrhythmias have become a primary focus of regulatory agencies and pharmaceutical companies. The first drug to be withdrawn from the market due to proarrhythmias (for the treatment of angina) in 1988, followed 5 years later by terodiline, which was initially used as an antinational agent , and later redeveloped for the treatment of unstable bladder disease. This was followed by many more withdrawals for a range of drugs from different pharmacological classes including gastemizole, cisapride, grepafloxacin,levacetylmethadol,lidoflazine,sertindole,sparfloxacin, terfenadine, and thioridazine (B.Darpo,2007). Drug-induced QT interval prolongation and the appearance of torsade de pointes (TdP) is recognized as a potential risk during treatment with a broad range of drugs including repolarisation –delaying antiarrhythmic, various antihistamines ,antipsychotics, antimicrobial agents etc.

Heart is a muscular organ that pumps blood to our body. Heart is at the center of your circulatory system. This system consists of a network of blood vessels, such as arteries, veins, and capillaries. These blood vessels carry blood to and from all areas of our body.

Blood carries the oxygen and nutrients that organs need to work well. Blood also carries carbon dioxide (a waste product) to lungs so we can breathe it out.

Heart is vital for our health and nearly everything that goes on in our body. Without the heart's pumping action blood can't move throughout our body.

An electrical system controls our heart and uses electrical signals to contract the heart's walls. When the walls contract, blood is pumped into our circulatory system. The electrical impulse begins from the Sinoatrial node (SA node) and then moves to the Auriculo ventricular node (AV node),followed by move to the purkinjefibres through the bundle of HIS.Ultimately it will results in the contraction of the atria and ventricles.

The SA node sends electrical impulse at a certain rate,the heart rate may still change depending on physical demands, stress, hormonal and other factors.This change in the heart rate ultimately results in the changes in normal rhythm of heart beat,which might precipitate a number of cardiovascular disorders, including arrhythmia,torsades de points ,Ventricular fibrillation .Which may represent in the Electrocardiogram (ECG).

Acetylcholine causes bradycardia, even transitory discontinuation of heart beats by sinus effect decrease of atrioventricularconduction,decrease of the strength of atrium contractions. Cardiac slowing is explained at least partly by cellular hyperpolarization resulting from opening of potassium channels linked to the G proteins and potassium exit out of the cell including an increase in polarization .The decrease of the force of contraction comes from a decrease of ca2+ entry in the cell probable by inhibition of adenylcyclase(Monahan 1990).

Adrenaline in your bloodstream achieves its effects on heart rate by stimulating the adrenergic receptors on cells throughout heart tissue once stimulated, these receptors pass the fight or flight message to a specialized type of protein called a G-protein. In turn , G-proteins stimulate other subtances inside cells that trigger a

cascading alert effect. The overall result of this process is an increase in heart rate, as well as an increase in the force of each individual heart contraction.

Intravenous injection of very low doses of acetylcholine in animals or human beings causes immediate and fugacious fall of the arterial pressure resulting from cardiac slowing and vasodilation.

It is the first globally harmonized regulatory guidance for assessment of cardiac safety during clinical drug development concurrently with ICHE14, a second ICH guideline also reached step 4 status for implementation by the three ICH regions: The Non –Clinical Evaluation of the potential for Delayed Ventricular Polarization (QT Interval prolongation) by human Pharmaceuticals (ICH S7B) (Grisanti et at .,2005).

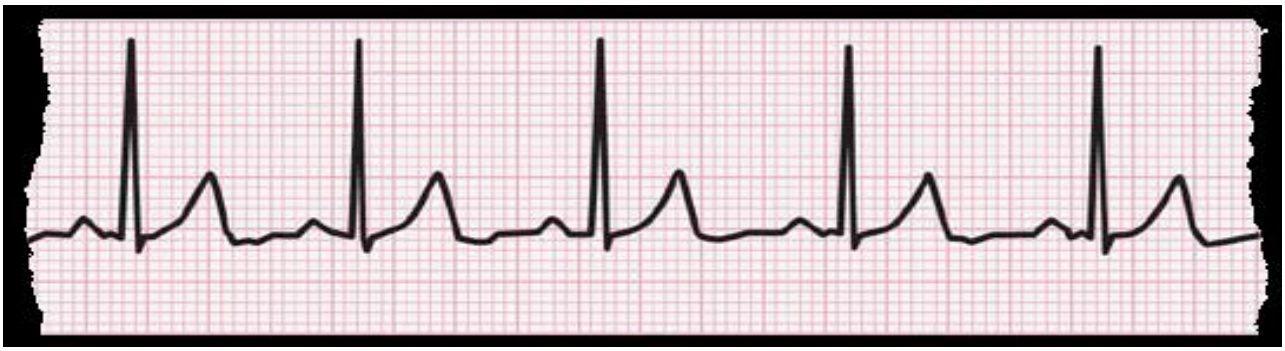
REVIEW OF LITERATURE

Arrhythmia is an abnormal heart rhythm. It may feel like fluttering . It can cause the heart rate to be too slow or too fast. Some arrhythmias don't cause any symptoms. There are two basic kinds of arrhythmias.

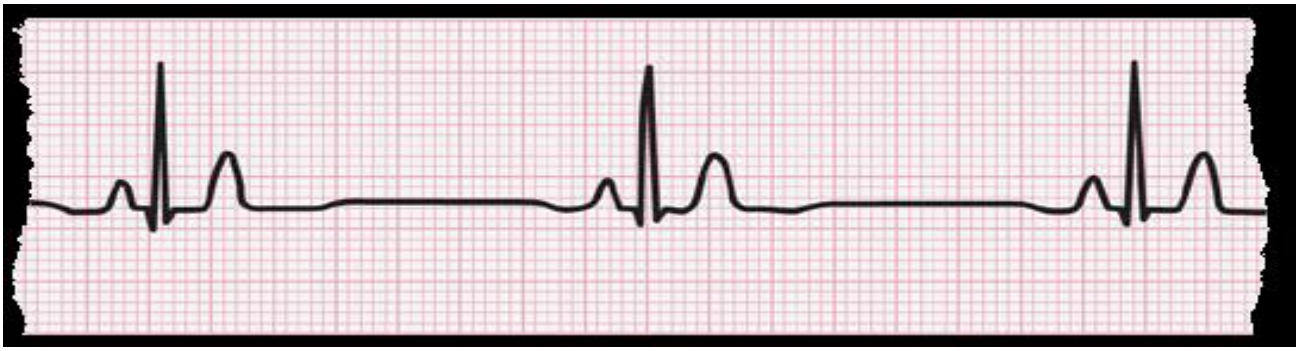
Bradycardiais when the heart rate is too slow — less than 60 beats perminute.

Tachycardia is when the heart rate is toofast — more than 100 beats per minute.

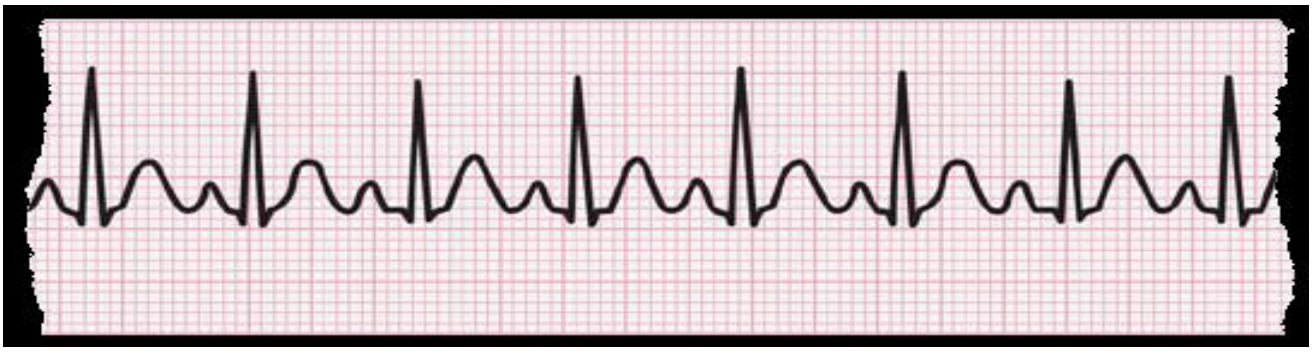
Tachycardia can reduce the heart's ability to pump, causing shortness of breath, chest pain, lightheadedness or loss of consciousness. If severe, it can also cause heart attack or death.



ECG strip showing a normal heartbeat



ECG strip showing **bradycardia**



ECG strip showing **tachycardia**

Symptoms includes

- Palpitations (a feeling of skipped heart beats, fluttering)
- Pounding in chest.
- Dizziness or feeling light-headed.
- Fainting.
- Shortness of breath.
- Chest discomfort.
- Weakness or fatigue (feeling very tired).

Arrhythmias diagnosed by

Electrocardiogram (ECG or EKG) is often used to diagnose arrhythmias. It creates a graphic record of the heart's electrical impulses.

Treatment may includes

- Lifestyle changes
- Medicine to prevent and control arrhythmias
- Medicine to treat related conditions such as high blood pressure, coronary artery disease, etc
- Anticoagulants to reduce the risk of blood clots and stroke
- A pacemaker to help your heart beat more regularly
  Cardiac defibrillation and implanted cardioverter defibrillators (ICDs)
- Cardiac ablation
- Surgery

Demographic pattern of arrhythmia world wide

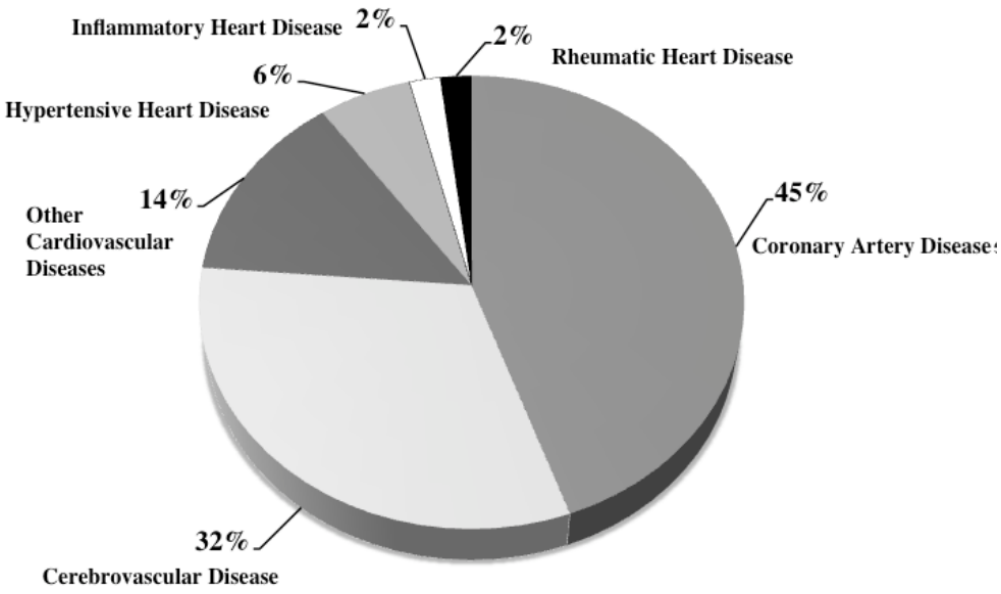


Fig 1.Distribution of Cardiovascular Diseases Accounting for Deaths Worldwide in2004

Cardiovascular Diseases in India

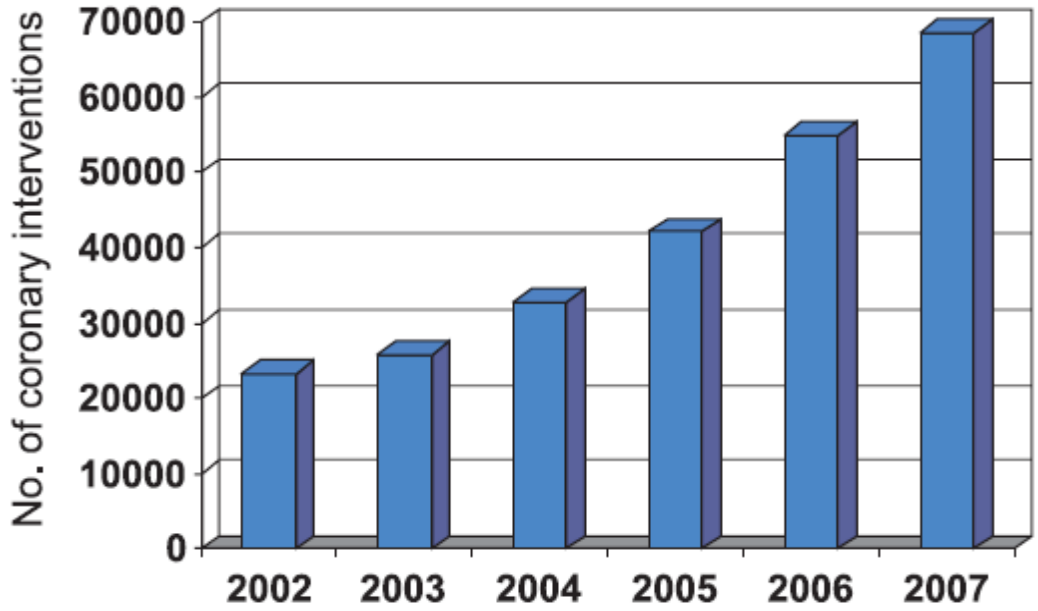


Figure 2. Total coronary interventions in India(Mariana Mirabel et,al 2007)

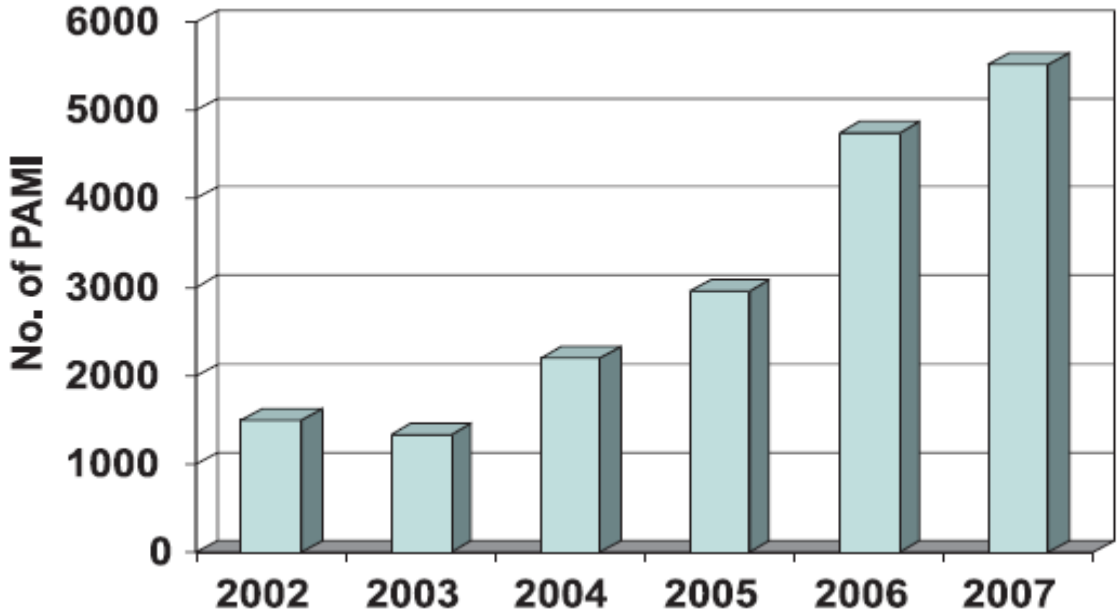


Figure 3. Data on intervention in acute MI (India) Cardiovascular Diseases – PatientLoad. (MareikeLankeit ,2007)

ECG

Electrocardiogram is a graphical representation of the bio-electrical currents generated by the myocardial cells. The conductivity of the body allows the detection of these currents on its skin. By placing a pair of electrodes on the body, an ECG voltage potential between them can be measured and recorded. This graphical representation (ECG) can be either printed on a paper or displayed on a monitor. The device capable of recording and printing the ECG on paper is called electrocardiograph. Einthoven was the inventor of the first device capable of printing the ECG on paper. Einthoven named the waves he observed on the ECG using five capital letters from the alphabet: P, Q, R, S, and T.

Fig 4. Electrocardiograph of human as taken from lead II (Venkatesh, 2010)

Significance Of the ECG curve

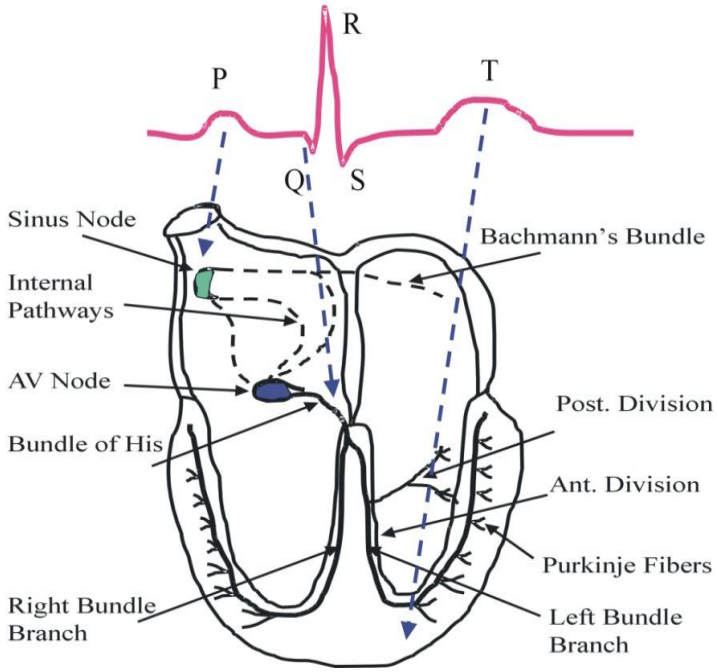


Fig 5. Components of Heart

- The ECG using five capital letters from the alphabet: P, Q, R, S, and T.
- P wave: represents the depolarization impulse across the atria.
- Q, R and Swaves: all these three waves represent the ventricular depolarization (the downward stroke followed by and upward stroke is called Q wave, the upward stroke is called R wave and any downward stroke preceded by an upward stroke is called S wave)
- T wave: represents the repolarization of the ventricles.

QT interval

In cardiology, the QT interval is a measure of the time between the startof the Q wave and the end of the T wave in the heart's electrical cycle.The QT interval represents electrical depolarization and repolarization of the ventricles. A lengthened QT interval is a marker for the potential of ventricular tachyarrhythmias like torsade's de pointes and a risk factor for sudden death.

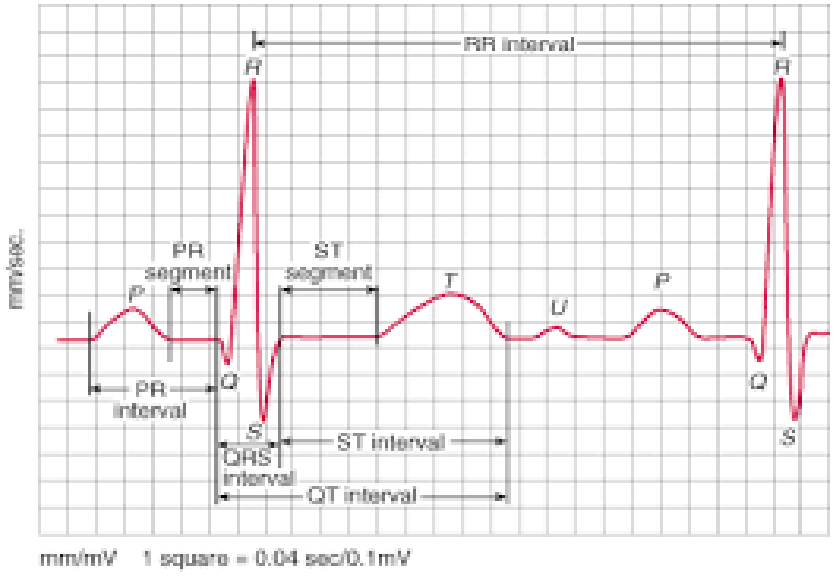


Fig 6.Normal ECG trace (sinus rhythm), with waves, segments, and intervals (Hart MA,1989)

Action potential duration

Across the ventricular cell membrane there is a steady potential difference of almost the same sizeas the equilibrium potential for K+ (- 94 mV) , that is - *90* mV. This negative potential isreferred to as the resting membrane potential(RMP) , because it represents the potential differenceacross the cell membrane (inside

negative) at res t between successive action potentials . Any process that reduce the absolute size of the RMP (ie, depolarize the membrane) tends to activate(open) fastNa+- channels. These channels contain fast opening and fast closing gates (inactivationgates) . Electrochemical forces favour the abrupt influx of Na+ from neighboring regions . Hereby ,thepotential is further diminished and more and more Na+- channels are activated or opened. Thethreshold potential for release of an action potential is a rise of 25 mV from - 90 mV . The cardiacaction potential is an all - or - none response,which can be divided into following phases.

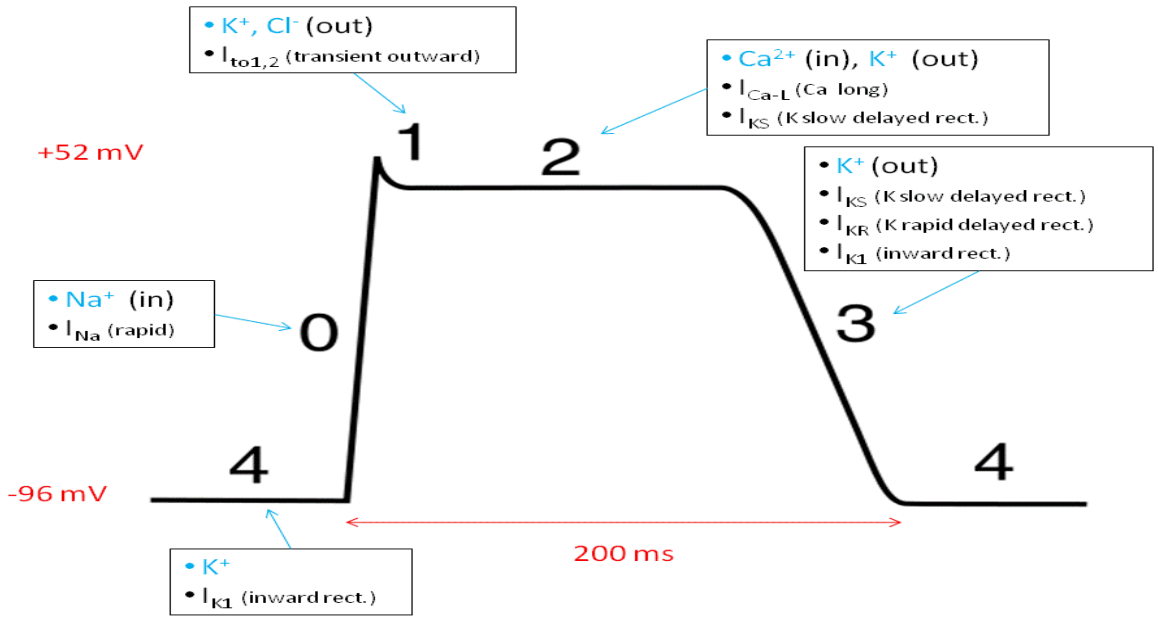


Fig 7.action potential duration (Hatzopoulos, 2002)

Roleof potassium currents in cardiac arrhythmias and QT prolongation

ProlongQT intervals induced by hypokalemia in rats might better be explained by the delayed action potential duration in cardiac tissue .Further, according to Yang and Roden (1996) , hypokalemia enhances the blockade of the current passing through delayed rectifier k^+ (IKr), thereby exaggerating the effect of the drug.

We would better like to emphasise that hypokalemia was observed to augment Clarithromycin induced QTc Prolongation (probably by delaying the action potential duration), which was subsequently rectified/reduced following K^+ supplementation.

Abnormal excitability of myocardial cells may give rise to ectopic beats and initiate re-entry around an anatomical or functional obstacle. As Kþ currents control the repolarization process of the cardiac action potential (AP), the Kþ channel function determines membrane potential and refractoriness of the myocardium. Both gain and loss of the Kþ channel function can lead to arrhythmia. The former because abbreviation of the active potential duration (APD) shortens refractoriness and wave length, and thereby facilitates re-entry and the latter because excessive prolongation of APD may lead to torsades de pointes (TdP) arrhythmia and sudden cardiac death. The pro-arrhythmic consequences of malfunctioning Kþ channels in ventricular and atrial tissue are discussed in the light of three pathophysiologically relevant aspects: genetic background, drug action, and disease-induced remodelling. In the ventricles, loss-of-function mutations in the genes encoding for Kþ channels and many drugs (mainly hERG channel blockers) are related to hereditary and acquired long-QT syndrome,

respectively, that put individuals at high risk for developing TdP. arrhythmias and

life-threatening ventricular fibrillation. Similarly, down-regulation of Kþ channels in heart failure also increases the risk for sudden cardiac death. Mutations and polymorphisms in genes encoding for atrial Kþ channels can be associated with gain-of-function and shortened, or with loss-of-function and prolonged APs. The block of atrial Kþ channels becomes a particular therapeutic challenge when trying to ameliorate atrial fibrillation (AF). This arrhythmia has a strong tendency to cause electrical remodelling, which affects many Kþ channels. Atrial-selective drugs for the treatment of AF without affecting the ventricles could target structures such as IKur or constitutively active IK,ACh channels.

Results of QT prolongation

Torsades de points

At the cellular level, the repolarisation phase of the myocytes is drivenredominantly by outward movement of potassium ions. A variety of different K^+ channel subtypes are present in the heart. Two important K^+ currents participating in ventricular repolarisation are the subtypes of the delayed rectifier current, I_{Kr} (“rapid”) and I_{Ks} (“slow”). Blockade of either of these outward potassium currents may prolong the action potential. I_{Kr} is the most susceptible to pharmacological influence. It is now understood that virtually without exception, the blockade of I_{Kr} current by these drugs is at least in part responsible for their

pro-arrhythmic effect. Blockade of the I_{Kr} current manifests clinically as a prolonged QT interval and the emergence of other T or U wave abnormalities on the surface ECG. The prolongation of repolarisation may result in subsequent activation of an inward depolarisation current, known as an early after-depolarisation, which may promote triggered activity. When accompanied by the presence of a notably increased dispersion of repolarisation, this may induce re-entry and provoke TdP, which is then sustained by further re-entry or spiral wave activity . Such phenomena are more readily induced in the His-Purkinje network and also from a subset of myocardial cells from the mid ventricular myocardium, known as M cells. Compared to subendocardial or subepicardial cells, M cells show much more pronounced action potential prolongation in response to I_{Kr} blockade. This property results in a pronounced dispersion of repolarisation (that is, heterogeneous recovery of excitability), creating a zone of functional refractoriness in the mid myocardial layer, which is probably the basis of the re-entry that is sustaining the TdP.

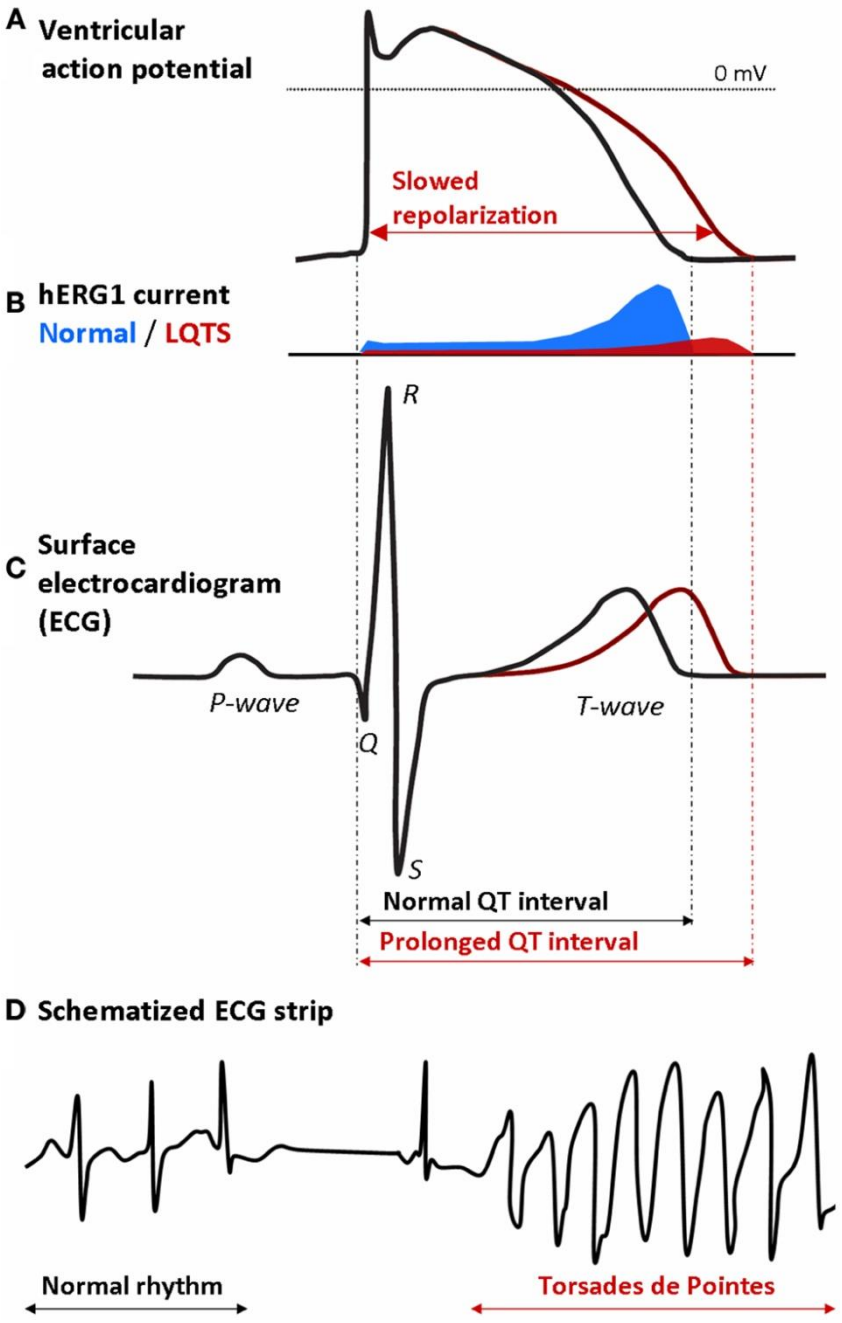


Fig 8.An electrographic rhythm strip demonstrates torsades de points (GowdaR.M et,al,2001)

Difference in ECG pattern of human and rodents

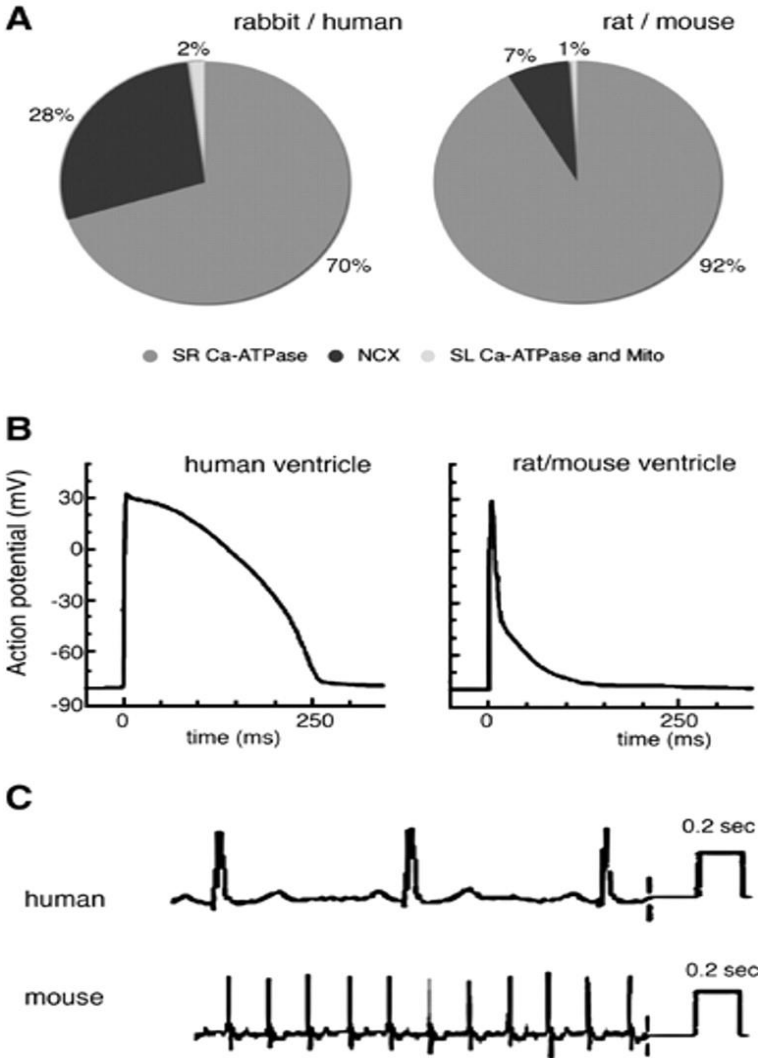


Fig 9.Difference in ECG pattern of human and rodents (Rat)(Hayes E et,al,1994)

DRUGS OF OUR CONCERN

Acetylcholine

An injection of acetylcholine causes bradycardia, even transitory discontinuation of heart beats by sinus effect decrease of atrioventricularconduction,decrease of the strength of atrium contractions.Cardiac slowing is explained at least partly by cellular hyperpolarization resulting from opening of potassium channels linked to the G proteins and potassium exit out of the cell including an increase in polarization .The decrease of the force of contraction comes from a decrease of ca2+ entry in the cell ,probable by inhibition of adenylcyclase.

Intravenous injection of very low doses of acetylcholine in animals or human beings causes immediate and fugacious fall of the arterial pressure resulting from cardiac slowing and vasodilation.

Adrenaline

Adrenaline in bloodstream achieves its effects on heart rate by stimulating the adrenergic receptors on cells throughout heart tissue once stimulated, these receptors pass the fight or flight message to a specialized type of protein called a G-protein.In turn ,G-proteins stimulate other subtances inside cells that trigger a cascading alert effect.The overall result of this process is an increase in heart rate,as well as an increase in the force of each individual heart contraction.

Ketamine

ketamine is a medication mainly used for starting and maintaining anesthesia.It induces a trance-like state while providing pain relief ,sedation and memory loss.

Others uses include for Chronic pain and for sedation in intensive care. Effects typically begin within five minutes when given by injection with the main effects lasting up to 25 minutes.

Xylazine

Xylazing HCL injection (Xylazine),a nonnarcotic compound ,is a sedative and analgesic as well as muscle relaxant .Its sedative and analgesic as well as muscle relaxant .Its sedative and analgesic activity is related to central nervous system depression .Its muscle relaxant effect is based on inhibition of the intraneuural transmission of impulses in the central nervous system .The principal pharmacological activities develop within 10 to 15 minutes after intramuscular injection .

Abstract

Literature survey reveals that there is perhaps no comprehensive report on Rat ECG. As a result rat ECG can not be reliably utilized for prediction of human effect.
The major reason behind this is due to the following factors:
a) Rat ECG pattern is different from that of human as rats is believed to lack iKr expression.
b) Heart rate varies widely unlike that of human being.

Cardiac arrhythmia is a dreaded disease that results from unprecedented increase or decrease in the cardiac rate.The disturbance in the typical sinus rhythm could be traced in electrocardiogram tracings .While a slight change in the QT interval within the ECG raises slight concerns.

Since Acetylcholine can open the potassium channels linked to the G proteins and helps outflow of potassium from the cells and produces hyperpolarization. So acetylcholine can causes bradycardia and even transitory discontinuation of heart beats by sinus effect.We also observed body weight can influence ECG pattern (eg, P,PQ,QRS,QT,PR,RRvalues).

Adrenaline can increase heart rate by stimulating the adrenergic receptors on cells throughout heart tissue once stimulated, these receptors pass the message to G-protein. In turn,G-proteins stimulate other subtances inside cell that trigger a cascading alert effect. Thereby, heart rate and force of contraction increases.

Considerable evidence has accumulated that a variety of drugs may prolong the heart rate of the surface electrocardiogram (ECG),

AIM& OBJECTIVES

Accordingly, we have taken up this study to investigate EGC pattern in rat particularly ECG segmental duration with varying heart rate. Therefore, we have employed Acetylcholine and Adrenaline to manipulate heart rate in rats and tried to document significant duration of different segments of ECG.

The aim of the present work is, ‘‘Parametric study of ECG in Wistar rats’’.
Since Acetylcholine can open the potassium channels linked to the G proteins and helps outflow of potassium from the cells and produces hyperpolarization. So acetylcholine can causes bradycardia and even transitory discontinuation of heart beats by sinus effect.We also observed body weight can influence ECG pattern (eg, P,PQ,QRS,QT,PR,RRvalues).

Adrenaline can increase heart rate by stimulating the adrenergic receptors on cells throughout heart tissue once stimulated, these receptors pass the message to G-protein. In turn,G-proteins stimulate other subtances inside cell that trigger a cascading alert effect. Thereby, heart rate and force of contraction increases.

MATERIALS &METHODOLOGY

Wister rats, BiopacMP36 system, Biopac electrode lead II, Acetylcholine Chloride was purchased from SRL, Adrenaline Bitartrate injection was purchased from Vulcan Laboratories PVT. Limited. Ketamine hydrochloride injection was purchased from Vulcan Laboratories and Xylazine injection from Indian Immunologicals**.**

Animal husbandry and maintenance

Adult male Wister rats with body weighs of 106-136 gms were used for the study. Animals were grouped and housed in cages not more than six animals per cage under suitable laboratory condition (temperature 25±2ºC, 50±20% relative humidity) with dark and light cycle (12/12) for minimum of seven days before beginning the experiment to adjust the new environment and to overcome the stress possibility incurred transport . Only healthy animals were assigned to the study .During this period they had free access to standard dry pellet diet (Nutrilab Rodent, Provimi) and water adlibitum. The study conducted was in accordance with the Institutional Ethical Committee .

METHODOLOGY

Atfirst Ketamine hydrochloride and Xylazine in combination form were administeredintraperitoneally (IP).

↓

Wait for few minutes to attain the anaesthetic condition

↓

In lead IIsystem,thepositive,negative,andground electrodes were placed on lateral position of Rat

↓

ECG patterns were recorded until baseline stabilization

↓

According to their body weight Various amount of Adrenaline was injected one by one rat intraperitoneally (IP)

↓

After that ECG pattern (P,PQ,QRS,QT,PR,RR values) was recorded for each rat.

Figure 1: Modified Lead II electrode placement in the rat model.

Test protocol

The Animals will be divided into two groups (n=5).

- group I rats were receive various concentration(0.5-3.5) microgram/mg of Acetylcholine , for seven days.
- group II rats were receive various concentration (0.2-0.8)mg/kg body weight of Adrenaline according to their body weight for seven days .
- Heart rate was calculated using the following equation:

$QTc = QT/(RR/150)^{1/2}$

FINAL IMPRESSION

Parametric study of ECG in wistar rat for the analysis of heart rate against the acetylcholine and Adrenaline administration according to dose as per body weight is shown in the above tables, the normal heart rate of rat 350bpm and after acetylcholine injection heart rate was below 300bpm (eg. 233,229,241,218bpm).

After Adrenaline injection heart rate was above 350bpm (eg. 400,382,370,355bpm).

So it is concluded from the above experiment that Acetylcholine and Adrenaline changes the heart rate gradually on time .

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