Sympathomimetic and parasympathomimetic agents effects

Aims/Objectives

- To study the effect of sympathomimemtic and parasympathomimetic agents on guinea pig atria and ventricles.
- To study the frequency and strength of muscle contractions when using different drugs on guinea pig atria and ventricles.
- To be able to describe the effect of isoprenaline and propanolol AND acetylcholine and atropine on guinea pig atria and ventricular tissue.

1. 0 Introduction

The heart is enclosed in a double-walled sac called the pericardium. The superficial part of this sac is called the fibrous pericardium. This sac protects the heart, anchors its surrounding structures, and prevents overfilling of the heart with blood. It is located anterior to the vertebral column and posterior to the sternum. The size of the heart is about the size of a fist and has a mass of between 250 grams and 350 grams. The heart is composed of three layers, all of which are rich with blood vessels. The superficial layer, called the visceral layer, the middle layer, called the myocardium, and the third layer which is called the endocardium. The heart has four chambers, two superior atria and two inferior ventricles. The atria are the receiving chambers and the ventricles are the discharging chambers. The pathway of blood through the heart consists of a pulmonary circuit and a systemic circuit. Blood flows through the heart in one direction, from the atrias to the ventricles, and out if the great arteries, or the aorta for example. This is done by four valves which are the tricuspid atrioventicular valve, the mitral atrioventicular valve, the aortic semilunar valve, and the pulmonary semilunar valve.

The heart has a pacemaker activity, which is initiated through the sinoatrial (SA) node. The SA node is the pacemaker tissue located in the wall of the right atrium of the heart, near the entrance of the superior vena cava & thus the generator of the sinus rhythm. They do not contract, even though they possess contractile filaments. They send action potentials to the atrioventricular (AV) node, causing the ventricles to contract, pushing the blood through the aorta into the rest of the body.

The atrium of the guinea pig is able to survive in vitro & it is able to beat spontaneously as long as the SA node isn't damaged. In comparison to the atria, the ventricle has no pacemaker activity.

2. 0 Method

The heart was dissected in to atria and ventricles in the Ringer Locke solution to keep the heart tissue in optimal condition. Any fat around the heart tissue was trimmed and the atrioventricular (AV) junction was exposed. The AV junction appeared as a pale line. The heart was cut into two pieces, one of atria one of ventricles. Extreme care had to be taken whilst cutting the heart to make sure the sinoatrial (SA) node did not get damaged in the process because the SA node keeps the atria of the heart beating as it generates impulses.

Exp 1a:- Effects of isoprenaline

The atria of the heart was isolated and set up in the organ bath. The isolated atria was allowed to equilibriate in the organ bath for 15 minutes as some slight activity was witnessed. 0. 02ml Isoprenaline giving a final bath concentration of 4 x 10-6 M was added initially as 0. 02ml was the smallest

amount the Gilsen pipettes could measure accurately. This concentration was doubled for two more additions (0. 04ml : 8 x 10-6 M and 0. 08ml : 1. 6 x 10-5 M). The organ bath was washed out twice between adding doses and allowed to equilibriate for 10minutes before addition of the next dose. No response was witnessed on doubling the concentration so it was assumed that the atria had stopped functioning. Propranolol (5 x 10-6 M) was added to shock the atria into functioning again but no response was induced. Sample traces were provided showing what should have been observed.

Expt 1b:- Effects of Isoprenaline after Propranolol

Propranolol was added to the FBC of 5×10-6M. The preparation was left for 10 minutes so it could equilibrate. The EC50 dose of Isoprenaline was added and the response of the heart tissue was measured. No response was detected so sample traces were provided showing what should have been observed.

Expt 2a:- Effects of Acetylcholine To pace the heart 10µl of Isoprenaline was added. There was no response observed so 20µl of Isoprenaline was added. No response again so sample traces were provided showing what should have been observed.

Expt 2b:- Effects of Acetylcholine after Atropine

A dose of IC50 of acetylcholine was added in the presence of atropine.

No response was observed and sample traces were provided.

3. 0 Discussion

It can be predicted that as the concentration of isoprenaline is increased the force and frequency (known as inotropy and chronotropy respectively) of the

contractions would also increase due to the fact that isoprenaline is a sympathomimetic agent and a beta-adrenoceptor agonist. Theory dictates that an increase in the concentration of Isoprenaline would increase the force and frequency of the contractions. As the concentration of Isoprenaline is increased, the number of unoccupied receptors would reduce or disappear completely as the increasing concentration of isoprenaline would allow the occupation of more receptors resulting in a more forceful and frequent contractions. Figure 3. 1 clearly shows this as a higher concentrataion of isoprenaline is added more frequent and forceful contractions occurred. However when the isoprenaline was washed out the frequency and force of contractions returned to normal. In clinical situations Isoprenaline was used for bradycardia and heart block in an intravenous form but is no longer used as it was a non-selective beta-agonist. It would have worked for bradycardia (where the heart beats too slowly) by binding to receptors and increasing the force and contractions of the heart to somewhere near a normal rate. The increase of the heart rate would increase the amount of blood being pumped round the body. This is known as increasing cardiac output which is worked out by multiplying the heart rate by stroke volume.

Propranolol is a non-selective beta-blocker or antagonist. This means that it would be competing for the same receptors as isoprenaline but where isoprenaline has an effect propranolol would stop the effect from happening ie it would have affinity but not efficacy. Figure 3. 2 clearly shows this as when the propranolol is added the force and frequency of the contractions of the heart considerably lessened. When the EC50 dose of isoprenaline was added then the force and frequency of contractions increased again but not

to the initial same level as some receptors were still being occupied by the propranolol. Adding more isoprenaline would have restored the contractions to its previous level. Figure 3. 3 explains this in detail as it shows that maximum response is reached in both cases but in the presence of propranolol higher concentrations of isoprenaline are needed to overcome its antagonistic effects indicating isoprenaline has a greater affinity for beta receptors than propranolol. This indicates competitive antagonism where maximum response can be reached by increasing the concentration of the agonist whereas in non-competitive antagonism the maximum response cannot be achieved no matter how high you increase the concentration of the agonist. Clinically propranolol is used for hypertension, angina pectoris, supraventricular tachycardias. Due to its non-selective nature propranolol can also bind to β2 receptors causing bronchoconstriction and is therefore contraindicated in asthmatics.

Acetylcholine is a parasympathomimetic and binds to acetylcholine muscarinic receptors in this case the M2 receptors which are located on the heart. Due to it being a parasympathomimetic it has an inhibitory effect on the heart so the higher the concentration of acetylcholine used the weaker the frequency and force of the contraction. Figure 3. 5 shows this and the increasing concentrations of acetylcholine show a marked decrease in force and frequency with the FBC3 concentration nearly flat lining the heart tissue which means that a majority of the receptors were occupied by acetylcholine. Acetylcholine could be used for tachycardia (where the heart beats too fast) but due to its many side effects other parasympathomimetic agents are used.

Atropine is a muscarinic receptor antagonist and therefore competes with acetylcholine for the same receptors. Atropine inhibits the effect of acetylcholine so no reduction in force or frequency should be observed but rather an increase. This is confirmed in figure 3. 6 as the addition of atropine to the organ bath shows a marked increase in contractile force and frequency but when the IC50 dose of acetylcholine is added the force and frequency drop. This indicates competitive antagonism as figure 3. 7 shows that increasing the concentration of acetylcholine in the presence of atropine would overcome the antagonistic effects of atropine indicating a greater affinity of acetylcholine for muscarinic M2 receptors than atropine. Atropine is used in the treatment of bradycardia (which is a slow heart beat) by increasing the patient’s heart rate. It blocks the inhibitory action of the parasympathetic nervous system on the heart via the vagus nerve by acting as an antagonist on the muscarinic M2 receptors located in the heart thereby preventing the binding of acetylcholine to the receptors. As acetylcholine is a parasympathomimetic the binding of atropine to the receptors causes the sympathetic nervous system to come into effect which increases the heart rate due to it having an excitatory effect.

During the experimental procedure there was no data obtained from the guinea pig atria or the ventricles, which was most likely due to the courier being stuck in traffic. A fresher specimen could have resulted in some traces being obtained.

Name two classes of drug with examples, which have cholinergic side-effects. What effects are these likely to have on the heart? Explain the pharmacological basis of any gastrointestinal side-effects.

Two classes of drugs which have cholinergic side-effects are Tricyclic antidepressants and antispasmodics. There are two types of cholinergic side effects; peripheral and central. Due to the introduction of SSRI's (eg citalopram), TCA's (eg amitriptyline) are no longer used as much as first line antidepressants. This is due to TCA's having too many side effects. The peripheral cholinergic side-effects include dry mouth, sedation, constipation, nausea, vomiting, cardiovascular side effects and urinary retention. Central side effects are cerebral and include impaired concentration, confusion, attention deficit, and memory impairment. TCA's in high doses may cause cardiac dysrhythmias coupled with the prolongation of the QT interval which may cause sudden death. This is known as Torsades de Pointes. One of the most common gastrointestinal side effects of TCA's is constipation. They block the action of acetylcholine (which is a neurotransmitter) on the muscarinic M3 receptors located on the smooth muscle of the gastrointestinal tract. The inhibition of acetylcholine slows the muscular contractions that propel waste matter through the digestive tract and reduce the intestinal secretions which lubricate the passage for faeces causing constipation.

Antispasmodics are another class of drugs which have cholinergic side effects. Examples of antispasmodics include atropine, dicycloverine, and mebeverine. Constipation, transient bradycardia (followed by tachycardia, palpitation and arrhythmias), reduced bronchial secretions and dry mouth are the main side effects of antispasmodics. Antispasmodics affect the

gastrointestinal tract by reducing the motility of the GI tract. Atropine binds to the muscarinic M3 receptors located on the smooth muscle of the GI tract inhibiting acetylcholine which reduces the concentration of intracellular calcium which has an effect of reducing the number of contractions thereby constricting the smooth muscle which reduces the motility of the gut therefore causing constipation.