

EXPERIMENTS

Experiment 1

OBJECT

To study the effect of K^+ , Ca^{++} , acetylcholine and adrenaline on perfused frog's heart .

Requirements

Recording drum, Sterling's heart lever, venous cannula and thread.

Chemicals

Calcium chloride, acetylcholine, KCl, $CaCl_2$, frog's ringer solution, etc.

Principle

Acetylcholine

It is parasympathomimetic drug. Muscarinic receptor is found in heart. In the heart acetylcholine causes activation of potassium ion channel, account for negative chronotropism (decrease heart rate) and negative inotropism (decrease force of contraction). Thus, the heart is inhibited. In perfused frog heart preparations stoppage of the heart is seen on upper side, while in isolated preparation it stops in diastolic condition.

Adrenaline

It is sympathomimetic having mixed agonists action. It produces increase in heart rate (positive chronotropic effect) and force of contraction (positive inotropic effect). Thus, adrenaline produces direct excitatory action on myocardial muscles mediated through β receptors present in the heart.

Calcium Chloride

In lower doses it increases heart rate and force of contraction but in high doses it inhibits the heart in systole characterized by straight line on upper side in isolated heart and on lower side in perfused frog heart.

Potassium chloride

It has also inhibitory effect on heart. In perfused frog heart preparation, it stops in systole while in isolated preparation, it stops in diastole.

Procedure

Take a pithed healthy frog in dissecting tray. Start dissection on ventral surface to expose the heart. While dissecting, abdominal aorta should be intact. Remove the pericardium carefully. Tie the branch of the aorta with a thread. The venous cannula is inserted into inferior vena cava by making an incision followed by constant washing with frog's ringer solution. Cut off a branch of the aorta and adjust the flow of frog's ringer solution. Pass a hook through apex of ventricle, connect it with thread to starlings heart lever. Observe the normal force and frequency of heart. Inject acetyl choline ($10\text{ }\mu\text{g}$) through the rubber tube. Start drum and record response for One minute. Stop drum and let the response to be normal again. Repeat the above procedure for KCl (2.5 mg), adrenaline ($10\text{ }\mu\text{g}$), $CaCl_2$ (2.5 mg) followed by 5 mg respectively. Record the graph, label it and then fix the graph with alcoholic reginous solution.

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Observations

<i>Drug</i>	<i>Pupil size</i>	<i>Light reflex</i>	<i>Corneal reflex</i>
Pilocarpine	Decrease	Present	Present
Physostigmine	Decrease	Present	Present
Ephedrine	Increase	Present	Present
Atropine	Increase	Absent	Present
Phenylephrine	Increase	Present	Present

Pilocarpine

<i>Time in minutes</i>	<i>Pupil size in mm</i>		<i>Corneal reflex</i>		<i>Light reflex</i>	
	<i>Control eye</i>	<i>Test eye</i>	<i>Control eye</i>	<i>Test eye</i>	<i>Control eye</i>	<i>Test eye</i>
0 minute (no drug)	-	-	-	-	-	-
10 minutes	-	-	-	-	-	-
20 minutes	-	-	-	-	-	-
30 minutes	-	-	-	-	-	-
40 minutes	-	-	-	-	-	-

Physostigmine

<i>Time in minutes</i>	<i>Pupil size in mm</i>		<i>Corneal reflex</i>		<i>Light reflex</i>	
	<i>Control eye</i>	<i>Test eye</i>	<i>Control eye</i>	<i>Test eye</i>	<i>Control eye</i>	<i>Test eye</i>
0 minute (no drug)	-	-	-	-	-	-
10 minutes	-	-	-	-	-	-
20 minutes	-	-	-	-	-	-
30 minutes	-	-	-	-	-	-
40 minutes	-	-	-	-	-	-

Ephedrine

<i>Time in minutes</i>	<i>Pupil size in mm</i>		<i>Corneal reflex</i>		<i>Light reflex</i>	
	<i>Control eye</i>	<i>Test eye</i>	<i>Control eye</i>	<i>Test eye</i>	<i>Control eye</i>	<i>Test eye</i>
0 minute (no drug)	-	-	-	-	-	-
10 minutes	-	-	-	-	-	-
20 minutes	-	-	-	-	-	-
30 minutes	-	-	-	-	-	-
40 minutes	-	-	-	-	-	-

Experiment 9

OBJECT

To study the effect of digitalis on frog's heart.

Requirements

Starlings heart lever, venous cannula, recording drum, kymograph paper, stand and thread.

Chemicals

Digoxin solution (100 µg/per ml): Weigh 10 mg of digoxin and dissolve in 10 ml distilled water. Take 1 ml of above stock solution and is diluted to 10 ml with distilled water.

1. Physiological salt solution.
 - (a) Frog's ringer solution.
 - (b) Frog's ringer solution without calcium chloride.
2. Calcium chloride solution 4%.

Principle

Digitalis being cardiotonic, discovered by William withering in 1776, has been therapeutically used in treatment of congestive cardiac failure (CCF). It inhibits a $\text{Na}^+ \text{K}^+$ ATP ase and there by increases influx of sodium ions. This causes increase in calcium ions intracellularly and hence an increase in force of contraction of heart. Due to increase in the end diastolic volume and decrease in the force of contraction of the heart of CCF patient, the size of the heart is increased. When digitalis is given to the CCF patient, there is increase in force of contraction of the heart. Due to decrease in the end diastolic volume, the size of the heart is also decreased. Digitalis increases the utilization of the energy production. Calcium ions play an important role in myocardial muscle contractility. When the heart is perfused with frog's ringer solution without calcium chloride, there is decrease in the force of contraction of the heart. But when this physiological solution is replaced with normal frog's ringer solution containing calcium chloride followed by gradual adding the dose of digoxin, there is increase in performance of the heart contractility. It is evident that digitalis and calcium ions both act synergistically.

Procedure

Take a pithed healthy frog in a dissecting tray. Start dissection on ventral surface to expose the heart. While dissecting, the abdominal aorta should be intact. Remove the pericardium carefully. Tie the branch of aorta with a thread. The venous cannula is inserted into inferior vena cava by making an incision followed by constant washing with frog's ringer solution. Cut off the branch of the aorta and adjust the flow of frog's ringer solution (15–20 drops per minute). Pass a hook through apex of ventricle, connect it with thread to starlings heart lever. Observe the normal extent of contraction of the cardiac muscles and the size of the heart.

Inject digoxin 0.1 ml through rubber tube. Start drum and record response for one minute. Stop drum and let the response to be normal again. Repeat the above procedure for 0.2 ml and 0.4 ml of digoxin replace the normal ringer solution with ringer solution without calcium chloride and record the response of the heart. Administer digoxin 0.1 ml and 0.2 ml along with calcium chloride of dose 0.1 ml and 0.2 ml respectively. Record and observe the response again. Immediate improvement of the cardiac performance is seen.

Experiment 13

OBJECT

To carry out test for pyrogen IP edition 1996 (main test).

Preparation of sample

Dissolve the substance being examined in or diluted with pyrogen free saline solution or other solution prescribed in the monograph. Warm the liquid being examined to approximately 38.5° C before injecting.

Dose

The volume of injection is not less than 0.5 ml per kg and not more 10 ml per kg of body weight.

Procedure

Take three selected rabbits of the previous experiments. Record the temperature of each animal at the interval of not more than 30 minutes, beginning at least 90 minutes before the injection of the solution being examined and continuing for three hours after the injection. Not more than 90 minutes immediately preceding the injection of the test dose, record the Initial temperature of each rabbit, which is the mean of two temperature recorded for that rabbit at an interval of 30 minutes in 40 minutes period. Rabbits showing a temperature variation greater than 0.2° C between two successive readings in the determination of “Initial temperature”, should not be used for the test. In any one group of the test animal, use only those animals whose “initial temperatures” do not vary by more than 1° C from each other and do not use any rabbit having a temperature higher than 39.8° C and lower than 38° C.

Inject the solution being examined slowly in to the marginal vein of the ear of each rabbit over a period not exceeding four minute unless otherwise prescribed in the monograph. Record the temperature of the each rabbit at half hourly intervals for three hours after the injection. The difference between “initial temperature” and the maximum temperature which is the highest temperature recorded for a rabbit is taken to be its response. When this difference is negative the result is considered as a zero response.

Interpretation

If the sum of responses of the group of three rabbits does not exceed 1.4° C and if the response of any individual rabbit is less than 0.6° C, the preparation being examined passes the test. If the response of any rabbit is 0.6° C or more, or if the sum of the responses of the three rabbits exceeds 1.4° C, continue the test using five other rabbits. If not more than three of the eight rabbits show individual responses of 0.6° C or more, and if the sum of responses of group of eight rabbits does not exceed 3.7° C, the preparation being examined passes the test.

Result

The sample of the test—Passed/failed the test.