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Methods in Drug Evaluation



Prafulla Chandra Tiwari

Foreword
Durga Nand Tripathi

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Chapter

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Cardiovascular Drugs

ABSTRACT: In this Chapter we will briefly review the evaluation of cardiovascular drugs such as evaluation of antihypertensive drugs *viz*. ACE inhibitors, angiotensin receptor blockers, drugs for congestive heart failure (CHF) and myocardial infarction. This will provide a basic understanding of functioning of cardiovascular drugs.

We will also discuss α-sympatholytic activity, β1-sympatholytic activity, β2-sympatholytic activity along with bradykinin and potassium channel openers.

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INTRODUCTION

Diseases associated with heart such as hypertension, congestive heart failure (CHF) and myocardial infarction are together termed as cardiovascular disorders. Over the years as our functioning of heart increases, several drugs have been discovered for treatment of these diseases. Drugs such as angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers are used in treatment of hypertension. Alpha and beta sympatholytics, bradykinin and potassium channel openers are also used extensively in the management of cardiovascular disorders.

In this Chapter we will learn about evaluation of these agents.

β-SYMPATHOLYTIC ACTIVITY IN THE ISOLATED RAT SPLEEN

Contractions are induced in sympathetically innervated organs such as spleen smooth muscle by α-adrenergic drugs (such as adrenaline, noradrenaline) or electrical stimulation. These effects are antagonized by α-adrenergic blocking drugs such as phentolamine.

Procedure

We will use male Sprague-Dawley rats weighing 180–220 g for the study and these animals will be sacrificed in CO_2 anesthesia. Spleen of the rats will be removed and will be cut longitudinally into two halves. Each part of spleen will be placed in organ bath containing physiological salt solution. Bath solution will be bubbled with carbogen and will be maintained at 37°C. After incubating it for 30 minutes under a tension of 0.5 g, contractile response of the tissue will be recorded by administration of adrenaline (10–6 g/mL)

or nor adrenaline 10–6 g/mL). After obtaining 3 approximately identical spasms, the test compound will be administered followed by the addition of the spasmogen 5 minutes later. The contractile response will be allowed to plateau and recorded.

Standard Compound

Phentolamine

Evaluation

Contractile force will be recorded at its maximal level before and after drug administration. Percentage inhibition of adrenaline or noradrenaline induced contraction will be determined.

α-SYMPATHOLYTIC ACTIVITY IN THE ISOLATED RAT ANOCOCCYGEUS MUSCLE

Rat anococcygeus muscle was used as pharmacological tool by Gillespie (1972, 1980), Gibson and Gillespie (1973). Rat anococcygeus muscles have dense adrenergic innervation and shows contractile response when stimulated by noradrenaline, acetylcholine, and 5-hydroxytryptamine. Histamine has no contractile effect on these muscles.

Procedure

Anococcygeus muscles arise from the upper coccygeal vertebra close to one another in the midline of the pelvic cavity. These muscles pass caudally, lying first behind and to one side of the colon. These muscles are joined together to form a ventral bar in front of the colon few mm from the anus. Extrinsic nerves will pass in a branch of the perineal nerve on either side to enter the deep surface of each muscle just before the formation of the ventral bar. After sacrificing the animals, abdomen of rats will be cut open in the midline. Pelvis split, bladder and urethra of the animal will be removed. Lower part of the urethra will be cleared properly so as to avoid damage to the ventral bar of muscle, which is the only region lying ventral to the colon. Colon will be then cut at the pelvic brim, pelvic portion will be pulled forward and the delicate connective tissue behind it will be cleared until the anococcygeus muscles came into view. These muscles will be then isolated. Extrinsic nerves on either side run in the posterior scrotal branch of the perineal nerve and leave it to enter the deeper surface of the anococcygeal muscles as these muscles lie on the lateral surface of the colon. Ventral bar will be cut and each muscle will be mounted in a 100 mL bath containing Krebs solution at 36°C. Proper aeration to the solution will be ensured with 95% O_2+ 5% CO_2. Tension will be measured with isometric transducers and will be displayed on a polygraph. Field stimulation of the intramural nerves will be applied after drawing the

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muscles through a pair of electrodes similar to those described by Burn and Rand (1960); when the muscles are stimulated through their extrinsic nerves the nerves will be drawn through similar electrodes. Stimulation of either intramural or extrinsic nerves will be with 1ms pulses at 20 Hz, and at a supramaximal voltage. Dose response curves will be established with doses of 2×10^{-7} to 4×10^{-6} M noradrenaline, 4×10^{-7} to 4×10^{-5} M acetylcholine or with graded frequencies of electrical stimulation.

Effect of noradrenaline will be diminished by α-adrenergic antagonists, such as 10^{-6} M phentolamine. Dose response curves will show a parallel shift which is characteristic of competitive antagonism.

Evaluation

Contractions of the anococcygeus muscle induced by α-adrenergic agonist after addition of the test compound will be compared with the initial values and will be expressed as percentage thereof.

β1-SYMPATHOLYTIC ACTIVITY IN ISOLATED GUINEA PIG ATRIA

An increase in the frequency and force of contraction of spontaneously beating isolated right atria and contractions of electrically stimulated isolated left atria are potentiated by β-adrenergic agonists such as isoprenaline (isoproterenol). Isoprenaline-induced effects are inhibited by β-sympatholytic drugs. Isolated right (a) and left (b) guinea pig atria are used for evaluating β-receptor blocking activity. β1-blocking activity is assessed by this test because heart predominantly contains β1-adrenoreceptors.

Procedure

Guinea pigs of Pirbright stain of either sex weighing 250–300 g will be used for the study. Guinea pigs will be sacrificed by stunning and exsanguination and their heart will be removed; right or the left atrium will be cut-off and will be mounted in a 50 mL organ bath with a preload of 100 mg containing Krebs-Henseleit solution maintained at 32°C and aerated with 95% O_2/5% CO_2. Contractions will be recorded isotonically using a lever transducer.

Right atrium: Isoprenaline will be administered into the organ bath after an equilibration period of 30 minutes, to potentiate inotropy and frequency of the isolated right atrium. Cumulative doses of isoprenaline will be added starting from a concentration of 0.05 μg/mL consecutive doses will be administered at 3 minutes intervals. Organ bath will be thoroughly flushed for 1 minute after obtaining stable maximum plateau of the effect. Flushing of the organ bath will be repeated twice 5 and 20 minutes later. Whole procedure will be repeated with same concentrations of isoprenaline (control baseline values = 100%). Test compound will be then added to the organ bath and 5 minute later, again isoprenaline will be given at cumulative doses. If the test compound has β-receptor blocking activity (β-sympatholytic).

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1. Higher isoprenaline concentrations are necessary to induce the same potentiation of inotropy and frequency

or

2. At same concentration of isoprenaline, increase in inotropy and frequency will be reduced.

After the end of experiment, again a cycle without test drug will be performed.

Left atrium: Left atrium will be stimulated by a square wave stimulator with 2 impulses's at a voltage of 15 V and impulse duration of 1 ms. β-adrenergic agonist isoprenaline will be added at concentrations of 0.05–0.1 mg/mL. Organ bath will be then thoroughly flushed for 1 minute after an equilibration period of 30 minutes. Flushing will be repeated twice 5 and 20 minutes later. Whole procedure is repeated with the same cumulative isoprenaline concentrations. Test compound will be added into the organ bath when a stable plateau of contractions is achieved, and after 3 minutes isoprenaline will be again added at cumulative concentrations. If test compound will have β-receptor blocking activity (β-sympatholytic), then it will inhibit isoprenaline-induced effects. Refractory period will also be determined before and after administration of drug.

Evaluation

Percentage inhibition of (a) isoprenaline-induced or (b) electrically-induced and isoprenaline-potentiated increased inotropy and frequency by test drug will be calculated by comparing it to pre-drug activity (= 100%).

Percent change in refractory period will be calculated. IC50 values will be determined from the individual dose response curves.

ANGIOTENSIN CONVERTING ENZYME INHIBITION IN THE ISOLATED GUINEA PIG ILEUM

Active angiotensin II peptide is formed from inactive angiotensin I through action of angiotensin-converting enzyme (ACE) on angiotensin I. Bradykinin is degraded to inactive products by ACE. Therefore we can measure activity of ACE by two ways: (1) newly formed angiotensin II activity and (2) inhibition of the activity of bradykinin. Inhibition of ACE results in decreased activity of the precursor angiotensin I and potentiation of the bradykinin effect. Ileum of guinea pig contracts in response to both, angiotensin II and bradykinin, and can be used for quantitative determination of ACE inhibiting activity.

Procedure: Guinea pigs of either sex weighing 300 to 500 g will be used for the study. Guinea pigs will be sacrificed by stunning and exsanguination. After sacrificing the animals there abdomen will be cut open. A cord will be tied around the intestine just distal to the pylorus which is then severed above the cord. Intestine will be gradually removed, and the mesentery is being cut away as necessary. When colon is reached, intestine will be cut free. Below the cord, the intestine will be cut halfway through, so that a glass tube can be inserted. Tyrode's solution will be passed through the tube and the intestine

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until the effluent is clear. Mesentery will be cut away from the intestine which was joined to the colon. Intestine will be cut into 3 cm long pieces. Most distal piece of intestine is used as it is most sensitive part of intestine. It will be then fixed with a tissue clamp and brought into an organ bath containing properly aerated Tyrode's solution at 37°C. Other end will be fixed to an isometric force transducer and responses will be recorded on a polygraph.

Angiotensin I antagonism: Angiotensin I will be added at a concentration of 10 ng/mL bath solution to the organ bath after equilibrium time of 30 minutes. Angiotensin I dose will be repeated once or twice until identical responses are obtained and the contractile force will recorded. Now the potential ACE inhibitor will be added to the organ bath. Angiotensin I will be again added after incubation period of 5 minutes. Contraction will be diminished depending on the activity of the ACE inhibitor.

Bradykinin potentiation: Guinea pig ileum will be prepared as described earlier. Bradykinin will be added at a concentration of 15 ng/mL bath solution to the organ bath after equilibrium time of 30 minutes. Bradykinin additions will be repeated once or twice until identical responses are obtained and contractile force will be recorded. Now potential ACE inhibitor will be added to the organ bath. Bradykinin will be again added to the organ bath after incubation period of 5 minutes. Depending on the activity of the ACE inhibitor contraction due to Bradykinin will be potentiated.

Evaluation

Angiotensin I antagonism: Contractions after addition of ACE inhibitors will be expressed as percentage of contraction without the ACE inhibitor. IC50 values (concentrations inducing 50% inhibition) of ACE inhibitor will be calculated using various doses of ACE inhibitors. Ramipril, enalapril, and captopril will be used as standards.

Bradykinin potentiation: Increase in contractions after addition of the ACE inhibitors will be expressed as percentage of contraction without the ACE inhibitor. Ramipril will be used as standard.

CONTRACTILE AND RELAXING ACTIVITY ON ISOLATED BLOOD VESSELS INCLUDING EFFECTS OF POTASSIUM CHANNEL OPENERS

Contracted smooth muscles are relaxed by potassium channel openers such as cromakalim, nicorandil and pinacidil. Data from patch clamp technique and ion flux experiments as well as by antagonism against potassium channel blockers explain these effects. Potassium channel openers are used as antihypertensive and anti-asthmatic drugs. High diversity of potassium channels including ATP-sensitive, voltage-sensitive and Ca^{2+} activated channels complicate this study.

Procedure: Either of these animals; male Pirbright White guinea pigs weighing about 400 g, or Chinchilla rabbits weighing about 3.5 kg, or Sprague-

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Dawley rats weighing 250–400 g will be used in the study. Test will be performed on thoracic aorta or the arteria pulmonalis.

Materials and solutions:

Physiological salt solutions (PSS) [mM]

	PSS I	PSS II	PSS III
NaCl	122	112	72 (92)
KCl	5.0	5	40 (20)
$CaCl_2$	1.2	–	–
$MgSO_4$	0.56	0.56	0.56
KH_2PO_4	1.2	1.2	1.2
$NaHCO_3$	25	25	25
EDTA	–	0.2	–
Glucose	12	12	12

Contracting agents

K^{2+} Ca^{2+}	40 mM + 0.5 mM
K^{++} Ca^{2+}	20 mM + 0.5 mM
U 46619 (thromboxane A2 analogue)	1 μM
A 23187 (calcium ionophore)	5 μM
Noradrenaline	1 μM
Phenylephrine	0.1 μM
Acetylcholine	1 μM
Oxyhemoglobin	10 μM
Methylene blue	10 μM

Animals will be sacrificed by stunning and exsanguination. Each drug will be tested on at least 4 isolated organs. Heart and the pulmonary artery will be quickly removed and will be immersed in PSS I at room temperature. Pulmonary artery thus removed will be dissected into rings and endothelial cells will be removed gently by rubbing the intimal surface. Spirally cut strips of 15–20 mm length and 1–1.5 mm width will be suspended in an organ bath containing 20 mL oxygenated (95% O_2, 5% CO_2) PSS I at 37°C and a tension of 380 mg. Changes in length will be recorded isotonically using a lever.

Test compounds will tested on vessels with intact endothelial lining, thoracic aorta of animal will be isolated and dissected free from surrounding tissue. Rings of 3 mm width will be cut and suspended in the organ bath containing PSS I. Isometric force will be measured with a force transducer under a resting tension of 500 mg.

Functional integrity of the endothelium will be tested before drug administration. 1 μM acetylcholine in the organ bath will result in transient relaxation.

Contraction of each vessel strip or ring is induced by addition of one of the contracting agents into the organ bath after an equilibration period of 1 hours.

Contractions in potassium-depolarized vessels will be induced by using three different PSS solutions will be used (PSS I for 30 minutes, PSS II for 3 × 15 minutes and PSS III). Contractions will be induced in the presence of PSS III by adding 0.5 mM Ca^{2+} into the organ bath.

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Cumulative concentrations of the test compound will be added into the organ bath to obtain drug-response curves when a stable plateau of contraction is achieved. Consecutive concentrations will be added either at 1 hour-intervals or when the response of the previous dose has reached a steady state level.

Only one concentration will be tested to study the time course of relaxation and the duration of action.

To test that whether mechanism of action of vasorelaxing agent is related to the liberation of nitric oxide; methylene blue or oxyhemoglobin (10 μM) will be added to the organ bath 15–30 minutes prior to the cumulative administration of the test compound. NO induced relaxation is selectively inhibited by methylene blue or oxyhemoglobin.

Evaluation

Mean values of relaxation ± SEM will be calculated. Height of contraction before the first drug administration will be taken as 100%. IC50 values will be determined from the individual dose response curves. IC50 is defined as the dose of drug leading to a 50% relaxation of the contraction induced by KCl or other agonists.

BLOOD PRESSURE IN PITHED RATS

Shipley and Tilden in 1947 have proposed use of pithed rat for evaluation of pressor agents. Pithed rat preparation is frequently used to evaluate drug action on the cardiovascular system since this preparation is devoid of neurogenic reflex control which can modulate primary drug action.

Procedure

For pithing male Sprague-Dawley rats weighing 250–350 g will be prepared under halothane anesthesia. For blood pressure monitoring and blood sampling left carotid artery will be cannulated. Trachea and right jugular vein will be also be cannulated. For pithing a steel rod, 2.2 mm in diameter and about 11 cm in length will be inserted through the orbit and foramen magnum down the whole length of the spinal canal. Animals will be ventilated with a small animal ventilation pump through the tracheotomy tube. Oxygen-enriched air will be inspirated by providing a flow of oxygen across a T-piece attached to the air intake of the ventilation pump. Rats will be ventilated at a frequency of 60 cycles/min with a tidal volume of 2 mL/100 g body weight. 0.3 mL of blood sample will be withdrawn from the carotid cannula and will be analyzed immediately for pO_2, pCO_2, pH, and derived bicarbonate concentration using an automatic blood gas analyzer after 30 minutes of pithing. By altering respiratory stroke volume of the pump, values will be adjusted to: pCO_2 30–43 mm Hg, pH 7.36–7.50, pO_2 87–105 mm Hg. Continuous registration of blood pressure and cardiac frequency will be performed via the left carotid artery. To measure α1 and α2 antagonism, first dose response curves will be registered using doses of 0.1–30 μg/kg

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IV. phenylephrine (a selective α1 agonist), and 1–1,000 μg/kg IV BHT 920, (a selective α2 agonist). The test drug will be administered intravenously and the agonist dose response curves are repeated again 15 minutes later.

Evaluation

If the curve of blood pressure response to the agonists is shifted, dose-response curves will be plotted on a logarithmic probit scale and potency ratios will be calculated.

BLOOD PRESSURE IN CONSCIOUS HYPERTENSIVE RATS (TAIL CUFF METHOD)

Systolic blood pressure can be measured indirectly by tail cuff method. In this method, cuff is quickly inflated to well above suspected systolic blood pressure and then pulse will be obliterated. Now pressure in the cuff will be released slowly and, as the pressure falls below systolic blood pressure, pulse will reappear. This method is analogous to blood pressure determination by sphygmomanometer in humans and can be applied not only at the tail of rats but also in dogs and small primates. Antihypertensive drugs in spontaneously and experimentally hypertensive rats is evaluated by this method.

Procedure

Male Charles River/Sprague Dawley spontaneous hypertensive rats weighing 300–350 g or rats with experimentally induced hypertension will be used for the study.

Surgical procedure for inducing renal hypertension: Male Sprague-Dawley/ Charles River stain rats weighing 80–100 g will be anesthetized by injecting 0.8 mL 4% chloral hydrate solution intraperitoneally. Both kidneys will be exposed retroperitoneally. Silver clip (0.2 mm diameter, 4 mm length) will be placed onto both renal arteries for inducing hypertension. Kidneys will be reposed and wound will be closed by suture. Operated animals will attain renal hypertension within 5–6 weeks, with a systolic blood pressure (BPs) of 170–200 mm Hg (mean normal physiological BPs for rats is 100 mm Hg). Only animals with a BPs = 180 mm Hg will be used for the tests.

Test Procedure

Six animals will be used per dose. Control group will receive normal saline only. To reduce spontaneous variations in blood pressure, animals will be adjusted to the experimental cage by bringing them into the restraining cage enclosed in a 31–32°C measuring chamber 3–4 times before starting the experiment for a period of 30–60 minute. A tubular inflatable cuff will be placed around the base of the tail and a piezo-electric pulse detector will be positioned distal to the cuff to measure blood pressure. Cuff will be inflated to approximately 300 mm Hg. Pressure in the cuff will be released slowly; systolic pressure will be detected and subsequently recorded on a polygraph.

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Test substance will be administered once per day over a period of 5 days intraperitoneally or by gavage. For a new compound usual screening dose will be 25 mg/kg. Blood pressure and heart rate will be measured on:
Day 1: Predose and 2 hours post drug
Day 3: Predose and 2 hours post drug
Day 5: Predose, 2 hours post drug and 4 hours post drug.

Between measurements, animals will be taken back to their home cages. Standard compounds used in the study will be:

- Endralazine (3 mg/kg PO)
- Nifedipine (3 mg/kg PO)
- Urapidil (5 mg/kg PO).

Evaluation

Mean systolic blood pressure before and after drug administration and the duration of the treatment will be determined. Percent decrease in systolic blood pressure under drug treatment will be calculated.

DIRECT MEASUREMENT OF BLOOD PRESSURE IN CONSCIOUS RATS WITH INDWELLING CATHETER

This method was described by Weeks (1960). Through this method we can directly measure arterial pressure in conscious rats by eliminating the influence of anesthesia on cardiovascular regulation.

Procedure

Implantation of cannula: Male rats of Sprague-Dawley stain of weight about 300 g will be used. We will anesthetize rats with 45 mg/kg pentobarbital IP. Neck and the abdomen of animals will be shaved and cleaned with 70% alcohol. Viscera of the animals will be exposed through a midline abdominal incision. Just above the bifurcation a segment of the abdominal aorta will be exposed. Adjacent to this segment of the aorta a trocar will be passed through the psoas muscles through the muscles of the back and under the skin until it emerges from the skin of the neck. Cannula will be inserted into the trocar and trocar will be withdrawn from the body. Cannula will thus come out from the neck, being anchored by silk sutures to the neck skin and to the psoas muscle. Heparin solution will be filled in cannula and the end projecting out from the neck skin will be blocked with a tight fitting stainless steel needle. Other end of the cannula will be implanted into the aorta. Above the bifurcation aorta will be wiped with a cotton-tipped applicator stick, occluded above this segment and punctured with a bent 27 gauge hypodermic needle. PE 10 catheter tip will be inserted through the needle and advanced up to the aorta. Intestine will be replaced and wound will be sutured. Rats will be allowed to recover for 1 week.

Blood pressure measurement: Occluding stainless steel needle will be removed and cannula will be flushed with diluted heparin solution. Rats will

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be placed in a small cage to restrict its movements; even so it will be free to move. Statham P 23 Db pressure transducer will be connected to cannula and blood pressure will be recorded on a polygraph. Test drugs or standards will be administered either subcutaneously or orally. Recordings will be taken before and after administration of drug over a period of 1 hour.

Evaluation

Degree and duration of change in blood pressure will be measured. Five rats will be used for each dose and compound. The maximal changes of each group will be averaged and compared with the standard.

BEZOLD-JARISCH REFLEX

Circulatory collapse after injecting veratrine intravenously was first described in cats and is known as Bezold-Jarisch-reflex (Bezold and Hirt 1867; Jarisch and Richter 1939a, b, Jarisch 1940). This test was recommended as a suitable animal model of shock by Fleckenstein et al. (1950).

Bezold-Jarisch-reflex is triphasic blood pressure response in cats or dogs characterized by a short-lasting fall in blood pressure accompanied by bradycardia, followed by a short lasting increase and than a long-lasting decrease of blood pressure after intravenous injection of veratridine or other veratrum alkaloids.

Procedure

Male Sprague-Dawley rats weighing 250–380 g will be used for the study. Rats will be provided food and water ad libitum, except those used for intraduodenal drug administration; these rats will be kept for overnight fasting. Animals will be anesthetized by intraperitoneal injection of 1.5 g/kg urethane. Left jugular vein or duodenum, trachea and left femoral vein will be cannulated for drug administration (IV or ID), facilitation of respiration and injection of 2-methyl 5-HT, respectively. Heart rate of the animals will be derived from a limb lead II ECG monitored through subdermal platinum electrodes and will be recorded with amplifiers on a polygraph. DRC of 2-methyl 5-HT (5–100 μg/kg, IV) will be recorded for each rat to establish submaximal dose response (usually 10 or 20 μg/kg, IV) which will show a reproducible bradycardic response. Each animal will then receive single dose of test compound or standard and will be challenged by 2-methyl 5-HT at 5, 15, 30, 60, 120, 180, 240, 300, 360, 420, and 480 minutes post dosing. Separate group of rats receiving vehicle (saline for IV, deionized water for ID) will be similarly tested in each study.

Evaluation

Duration of action of the compounds will be assessed by determining the period of time for which the inhibitory effects remain significantly different from vehicle controls.

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SCREENING FOR β-BLOCKING EFFECTS IN ANESTHETIZED DOGS

Isoprenaline will be administered intravenously 2–3 times to the anesthetized animal after a steady-state period of 30–60 minutes, and hemodynamic parameters will be recorded (control values = 100%). At cumulative doses (final concentrations of 0.01, 0.05 and 0.15 mg/kg) test substance will be injected intravenously. 10 minutes 'drug effects' will be monitored for each dose, by measuring hemodynamic parameters. Effect of isoprenaline will be tested again (3 times). If test compound does not have an inhibitory influence on isoprenaline effects, a second test compound will be administered. All hemodynamic parameters will be continuously measured during the whole experiment.

ANGIOTENSIN II ANTAGONISM

Antagonists of angiotensin II are tested in rats after elimination of cardiovascular reflexes by vagotomy and ganglionic blockade.

Procedure

Male rats of Sprague-Dawley stain weighing about 300 g will be used for study. Pentobarbital sodium IV 60 mg/kg will be used to anesthetize the animals. Carotid artery of the rats will be cannulated and will be connected to a Statham transducer P 23 Db and blood pressure will be recorded on a polygraph. For application of test compounds and for infusion both jugular veins will be cannulated. 3 mm dorsal of larynx both vagal nerves will be cut. 10 mg/kg pentolinium tartrate is injected intravenously for ganglionic blockade. For evaluation of one test drug at least of 5 animals will be used.

Intrinsic agonistic activity: Test compound doses of 1, 2, 4 and 16 μg/kg will be injected through the cannulated jugular vein after the blood pressure has reached a constant value and blood pressure will be recorded.

Antagonistic activity: Doses of 0.5; 1.0; and 2.0 μg/kg angiotensin II will be injected at interval of 10 minutes to record dose-response curves. Continuous infusion of the potential angiotensin II blocker at a dose of 10 μg/kg/0.1 mL/minutes will be started after 10 minutes. Doses of 0.5; 1.0; and 2.0 μg/kg angiotensin II will be injected again 10 minutes after beginning of the infusion.

Duration of activity: Angiotensin II will be administered as continuous infusion at a dosage of 1 μg/kg/0.02 mL/minutes. 0.1 mg/kg of the angiotensin II antagonist will be administered when blood pressure has reached an elevated steady state level. Duration and intensity of fall in blood pressure will be recorded.

Evaluation

Intrinsic agonistic activity: Increase in blood pressure indicates that test compound has intrinsic agonistic activity.

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Antagonistic activity: Increases in blood pressure after graduated doses of angiotensin II during the infusion will be expressed as percentage of the increase before infusion. Results thus obtained will be compared with known angiotensin II antagonists.

MYOCARDIAL INFARCTION AFTER CORONARY LIGATION

Acute reduction of pump function and dilatation of left ventricular chamber occurs in rats after ligation of their coronary artery. This method is used for evaluation of drugs used in acute myocardial infarction.

Procedure

Male rats of Sprague-Dawley stain weighing 200–300 will be anesthetized using diethyl-ether. Chest of the animal will be cut open by left thoracotomy. Thread will be inserted near the middle of the lateral margin of the cutaneous wound and will be carried through a tunnel of the left pectoral muscle around the cranial half of the incision. Heart of the rat will be brought outside gently by applying pressure on the abdomen. Around the left coronary artery a ligature will be placed near its origin and will be tightened. Heart will be repositioned in the thoracic cavity within seconds, and ends of the musculocutaneous thread will be tightened to close the chest wall and enable the animal to breathe spontaneously. If this process is done with speed then mechanical respiration becomes unnecessary.

For evaluation of drugs used for treatment of acute myocardial infarction, rats will be treated 5 minutes after and 24 hours after occlusion by subcutaneous injection (standard 5 mg/kg propranolol).

Rats will be anesthetized with 60 mg/kg IP pentobarbital and the right carotid artery will be cannulated with a polyethylene catheter connected to a pressure transducer, two days after surgery. Catheter filled with fluid will then advance into the left ventricle through the aortic valve for measurement of left ventricular systolic and end diastolic pressure.

Heart will be arrested by injecting 2 mL of 2.5 M potassium chloride after making hemodynamic measurements. Chest of the rat will be cut open, and the heart will be isolated and rinsed in 300 mM KCl to maintain a complete diastole. Through the ascending aorta a double-lumen catheter will advanced into the left ventricle. Right and left atria will be tied off with a ligature, and the right ventricle will be opened. A cryostatic freeze medium will be filled in the left ventricular chamber through the smaller of the two catheter lumens and will be connected to a hydrostatic pressure reservoir maintained at a level corresponding to the end-diastolic pressure measured in vivo. To allow fluid in the two lumens to equilibrate outlet (larger lumen) will be then raised to the same level as the inlet. With hexane and dry-ice heart will be frozen rapidly. With a cryostat hearts is cut serially into 40-μm thick transverse sections perpendicularly to the longitudinal axis from apex to base. From each heart at a fixed distance, eight sections will be obtained and will be collected on gelatin-coated glass slides. Heart sections thus obtained will be air-dried and will be incubated at 25°C for 30 minutes with 490 μM

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Prafulla Chandra Tiwari M Pharm (Pharmacology) is working as Senior Research Fellow at Department of Pharmacology and Therapeutics, King George's Medical University, Lucknow, Uttar Pradesh, India. He has done his M Pharm (Pharmacology) from Rajasthan University of Health Sciences, Jaipur, India. He is working on signal transduction and immune-modulation in various inflammatory and autoimmune disorders. He has published many papers in reputed international journals.

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