CHAPTER II

MATERIALS AND METHODS

Animals

Males ablino rats weighing 250-300 gm. and fed ad libitum were used throughout this study.

1. In Vivo Preparation (Recording of Blood Pressure and Heart Rate)

1.1 Blood pressure

Rats were anesthetized with urethane 1.4 gm/kg. intraperitoneally. The trachea was cannulated with a polyethylene tube (diameter 2.5 mm., length 4 cm.) so as to facilitate respiration and removal of excess secretions. Systemic blood pressure was recorded on Beckman Dynograph recorder (Type RM) via pressure transducer (Statham P 23AA) connected to a cannula inserted into left femoral artery. Drugs were injected through a polyethylene tube inserted into the right femoral vein and internal carotid artery. In the latter case, three-way maden cannula was used, one side for administration of drugs, the others for inserting into the corotid artery in the opposite directions. In this manner, the blood supply of the carotid sinus was not affected during the test.

1.2 Groups of animals for experiment

1.2.1 Normal rats were divided into 9 groups to study:

: The effects of 1 mg/kg of intravenous piperine on blood pressure and respiration.

: The effects of 0.2 mg/kg atropine on the action of intravenous piperine.

: The effects of 5 mg/kg hexamethonium on the action of intravenous piperine.

: The effects of 0.7 mg/kg propranolol on the action of intravenous piperine

: The effects of 1 mg/kg phentolamine on the action of intravenous piperine.

: The combination effects of 0.7 mg/kg propranolol and 1 mg/kg phentolamine on the action of intravenous piperine.

: The effects of 1 mg/kg of intracarotidal piperine on blood pressure and respiration

: The effects of 2 % cocaine applied directly to carotid sinus on the action of intracarotidal piperine.

: The effects of 5 mg/kg hexamethonium on the action of intracarotidal piperine.

1.2.2 Bilateral Cervical Vagotomy rats.

Bilateral cervical vagotomy was performed at the beginning of the experiment before intravenous injection of 1 mg/kg piperine

1.2.3 Pithed rats.

1.2.3.1 Pithed Rats Preparation.

The rat was anesthetized with urethane 1.4 gm/kg intraperitoneally. The trachea was exposed and a piece of plastic tubing 4 cm. long and 2.5 mm. in diameter was inserted into the open trachea. Either the femoral artery or vein was isolated for canulation. The vagi were then cut and the jugular veins and carotid

arteries tied. With the rat on its back and the hind feet pinned to the operating board the animal was ready to be pithed pithing rod,2.2 mm. in diameter, 25 cm. long, with one end bluntly pointed, was made from an ordinary wire coat hanger. By holding the rat's head taut and in line with the vertebrae, with the thumb in the angle of the mandible and the forefinger around the top of the skull, the point of the pithing rod was inserted obliquely into and through the eye socket at an angle of approximately 45° to the long axis of the rat. After the skull was entered the rod was realigned with vertebral column and passed through the cranium and thence down the whole length of the spinal canal.

The tracheal cannula tubing was then promptly connected to the respirator system which included a side tube fitted with adjustable screw clamp. The respirator used was air-driven with adjustments for varying both rate and volume. Respiratory rates of 50-60 per min. were found to be optimum for the rat.

1.2.3.2 Pithed rats were divided into 5 groups to study:

: The effects of 1 mg/kg of intravenous piperine on blood pressure

: The effects of 0.7 mg/kg propranolol on the action of intravenous piperine.

: The effects of 1 mg/kg phentolamine on the action of intravenous piperine.

: The combination effects of 0.7 mg/kg propranolol and 1 mg/kg phentolamine on the action of intravenous piperine

: The effects of reserpine pretreatment (5 mg/kg i.p. for two consecutive days) on the action of intravenous piperine.

2. In Vitro Preparation

2.1 Groups of animals for experiment.

2.1.1 Normal rats were divided into 7 groups to study :

: The effects of piperine (3-48 ug/ml) on right atrial rate and left atrial force.

: The effects of propranolol, a beta blocking agent (0.03, 0.07, 0.15 ug/ml) on the action of piperine.

: The effects of 5-HT antagonist on the action of piperine

- cyprohepadine 0.02 ug/ml.
- methysergide 0.47 ug/ml.

: The effects of phentolamine, an alpha blocking agent (0.32 ug/ml) on the action of piperine.

: The effects of neuronal uptake inhibitor on the action of piperine.

- desipramine 0.27 ug/ml.
- cocaine 9.1 ug/ml.

2.1.2 Reserpine Pretreated Rats.

Rats were intraperitoneally injected with 5 mg/kg/day of reserpine for 2 consecutive days. In the third day, the animals were sacrificed and the hearts were isolated as described below.

2.2 Isolated Atrial Preparations.

Rats were sacrificed by blowing on the head. The chest was immediately opened and the heart was quickly removed. The heart was placed in a petri-dish containing Locke solution bubble with 100 % oxygen. The ventricle and connective tissues were carefully removed. The atrium was then separated into right and left atrium. Both isolated right and left atria were transferred into 25 ml. organ bath chambers

containing Locke solution aerated with 100 % oxygen and maintained at 37 °C by circulating thermoregulator. Isometric contraction was recorded using a force displacement transducer connected to a dynograph recorder with appropriate preampliflier. Tissue was equilibrated for 30-60 minutes under 1 gram preload before starting the experiments. During the equilibration period, the medium was replaced every 15 minutes interval with Locke solution until the rate and amplitude of contraction were stable.

The Locke solution contained : 155.8 mM NaCl, 5.6 mM KCl, 2.15 mM $CaCl_2$, 1.8 mM $NaHCO_3$ and 5.0 mM glucose.

The spontaneously beating right atria, and the left one were used in studying the rates and force of contraction respectively. The latter was driven electrically with square wave pulses delivered via platinum electrodes. The stimulus strength was 6 volts and the duration was 10 msec. The frequency of stimulation was kept constant at 240/min. The rates and contractile force were recorded with isometric force displacement transducer (Statham UC 3) connected to Beckman Dynograph recorder (Type RM) with Beckman preampliflier.

2.3 The Organ Bath Chambers

The organ bath chambers used in isolated preparations were of double walled type. They are composed of two compartments, the inner chamber, capacity 25 ml., for the mounted tissue preparations to be immersed in physiological solution and the outer jacket for flow through water circulation of 37 °C. so as to provide constant temperature to the inner compartment. The circulating water was supplied by a thermoregulating water pump (Churchill Type). The bath also has an oxygen inlet which gasses the inner chamber through a sintered gas opening.

3. Drugs

The drugs used are as follow.

- Piperine (1-piperoyl piperidine, Sigma Chemical Co.)
- Propranolol hydrochloride (Inderal; inj., I.C.I. Macclesfield)
- Reserpine (Serpasil; inj., Ciba-Geigy Limited)
- Methysergide hemimaleate (Sandoz)
- Cyproheptadine hydrochloride (MSD)
- Phenoxybenzamine hydrochloride (SKF lab)
- Phentolamine hydrochloride (Ciba)
- Desipramine hydrochloride
- Cocaine hydrochloride
- Atropine sulphate
- Hexamethonium bromide

All drugs were dissolved in normal saline except piperine used in in vitro and in vivo preparation dissolved in 95 % alcohol and in equal volume of dimethylsulfoxide (DMSO) and 95 % alcohol respectively.

4. Drug Administration

In vivo preparation, after the animals had been allowed to stabilize for at least 15 min, the experiment was begun. The drugs were injected into femoral vein via the cannulated catheter and flushed with normal saline.

In isolated preparation, after tissues had been equilibrated for the minimum period of 30 min, the drug was administered to the bath fluid using the microlitre syringe.

5. Statistical Analysis

Results were presented as mean $\pm$ standard error of the means.

Differences between means were compared by student's unpaired and paired t-test. They were considered significant when P values were less than 0.05.