CHAPTER II

MATERIALS AND METHODS

1. Animals

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

2. Groups of animals for experiment

2.1 Normal rats were divided into 4 groups to study :

2.1.1 The effects of 5-HT (0.2 - 7.75 µg/ml) on right atrial rate and left atrial force.

2.1.2 The effects of 5-HT antagonist on the action of 5-HT

- cyproheptadine 0.02 µg/ml
- methysergide 0.47 µg/ml

2.1.3 The effects of propranolol, a beta-blocking agent (0.15 µg/ml) on the action of 5-HT.

2.1.4 The effects of 5-HT antagonists and propranolol on the action of 5-HT.

2.2 Reserpine pretreated rats.

Reserpine was injected intraperitoneal in a dose of 5 mg/kg 2 days before the experiment, and divided into 2 groups to study effects of 5-HT antagonists.

3. Preparation of isolated rat atria

3.1 Spontaneously-Beating Preparation (Chronotropic Response)

Rats were killed by blowing on the head. The abdominal and thoracic regions were immediately opened by midline incissions to expose the heart. The heart was quickly excised and placed in a petri-dish containing oxygenated Locke solution (of composition, in millimolar/litre : NaCl 155.8; $CaCl_2$ 2.15; KCl 5.6; $NaHCO_3$ 1.8 and glucose 5) at room temperature (28° - 30°C). The ventricular and connective tissues were carefully removed. The left and right atria were then separated, and the right atrium was transferred into the 25 ml organ baths containing Locke solution continuously bubbled with pure oxygen and maintained at 37°C by circulating thermoregulator. Each preparation was applied a tension of 1 gm.. The rate and contractile force was recorded with isometric force transducer connected to a recorder (Beckman Dynograph recorder type R). The atrium was allowed to equilibrate until the rate and amplitude of spontaneous contraction were stable, and then the experiments began.

3.2 Electrical stimulation of the atria (Inotropic Response)

The isolated left atria was fixed on a platinum wire electrode, placed in 25 ml organ bath containing Locke solution at 37°C and continuously aerated with pure oxygen. The stimulus strength was 5 V. and the duration was 5 msec. The frequency of stimulation was kept constant at 250/min. The tissue was applied a tension of 1 gm. and allowed to equilibrate until the force of contractions were stable before they were exposed to the drug.

4. The organ bath

The organ bath used in isolated preparations were of double walled type. They were composed of two compartments, the inner chamber, capacity 25 ml, for tissue preparation immersed in physiological fluid and the outer jacket for flow-through circulation of 37°C prowarmed water so as to provide constant temperature to the inner compartment. The circulating water supplied by a thermoregulating water pump (Churchill type). The bath also had on oxygen inlet oxygenate the inner chamber through a sintered glass opening.

5. Drugs

Drugs used were :

- 5-Hydroxytryptamine creatinin sulphate (Sigma)
- Cyproheptadine hydrochloride (Merck Sharp & Dohm)
- Methysergide hemimaleate (Sandoz)
- Propranolol hydrochloride (inderal inj, I.C.I.)
- Reserpine (Serpasil inj, Ciba Geigy)

Cyproheptadine hydrochloride was dissolved in methanol all other drugs were dissolved in distilled water.

6. Drug Administration

After tissues had been equilibrated for the minimum period of 15 min, the drug was administered to the bath fluid by using a microsyringe, responses were recorded within 15 min of the administration of the drug. After repeated washing the preparation was allowed to recover for at least 15 min before the blocker was tested, and then the preparation was discarded.

7. Analysis of Data

Results were expressed as means and standard error of the mean (S.E.M.). Significance of the differences between "5-HT" and "blocker plus 5-HT" means were determined using "student's t-test". Values of P of less than 0.05 were taken to implication statistical significance.