Chapter II

Methods and Materials

1. In Vivo Preparation (Recording of systemic blood pressure)

1.1 Experimental animals:

Albino rats of both sexes, weighing 250-300 g, were used.

1.2 Anaesthesia:

The rats were anaesthetized by intraperitoneal injection of urethane (ethyl carbamate, dose 1.5 g/kg).

1.3 Tracheal Cannulation:

The trachea was cannulated with a polyethylene tube so as to facilitate respiration and the removal of excess secretions. Subcutaneous tissues overlying the trachea (namely lymph nodes, parotid glands, and submaxillary glands, together with connective tissues) were cleared by blunt dissection to expose the sternohyoid muscles. The sternohyoid muscles were split medially by blunt dissection exposing the trachea, which was then cleared from the adjacent tissues. Two cotton strings were passed under the trachea. An incision was made through the tracheal wall at a point about 1 cm posterior to the thyroid; later with experience, it was found that an incision made through the cartilage, instead of the muscle helped to avoid bleeding. Since the size of the trachea of this particular animal is very small, bleeding in the trachea is troublesome in the sense that only a small amount of bleeding can cause respiratory obstruction which leads to respiratory failure. A polyethylene cannula, diameter 2.5 mm,

length 5 cm, was inserted through the incision with the tip pointed toward the lung. The trachea was securely tied over the cannula. Care had to be taken so that respiratory obstruction usually caused by excessive mucous secretion, or in some cases from bleeding, was prevented. If such cases occured the tracheal cannula was cleared by suction with small polyethylene tube. The tube was gently inserted into the trachea so as to aspirate it, but not so deep as to injure the lung parenchyma.

1.4 Jugular Vein Cannulation:

The right external jugular vein was dissected from adjacent tissue, and cannulated for the subsequent injection of drugs.

A polyethylene catheter was used in this cannulation. The catheter tip was obliquely cut and smoothened to prevent puncturing the vascular wall. The Luer fitting of the catheter was connected to a three-way stop-cock and syringe. The syringe, stop-cock and catheter were filled with heparinized saline (100 iu/ml). Two cotton threads were passed under the exposed vein. One was used to tie off the vein cephalad to the heart. A small V-incision was made in the wall of the vein and the catheter was inserted through this incision. The catheter was advanced towards the heart so that the tip of the catheter was located in the superior vena cava. The vessel wall was tied on to the catheter.

1.5 Recording of Systemic Blood Pressure:

The left carotid artery was exposed by blunt dissection and isolated from the vago-sympathetic trunk. Three cotton threads were passed under the exposed carotid artery. The artery was ligated with one of the threads at a point as cephalad as possible. The artery

was occluded proximal to this tie, an incision made and a previously prepared catheter inserted and securely tied. A polyethylene catheter previously prepared in the same way as the venous catheter was inserted into the artery. To prevent accidental removal of the cannula, both arterial and venous cannulae were sewn on to the skin. Cure was taken so that air bubbles did not occur in either cannula.

Systemic arterial pressure was recorded on a Polygraph recorder (Washington 400 MD 2C) using strain gauge typed blood pressure transducer coupled to the oscillograph with a carrier preamplifier (Biosciences type FC 137).

2. In Vitro Preparation (Isolated atrial preparation)

Rat was killed by a sharp blow on the head. The abdominal and thoracic regions were immediately opened by midline incisions to expose the heart. The heart was quickly excised and placed in a petri-dish containing oxygenated Locke solution at room temperature (28° - 30° C).

2.1 Spontaneously-beating Preparation (Chronotropic Response):

Intact right and left atria were carefully dissected out in one piece, free from ventricular and connective tissue, and avoiding damage to the pace-maker region. The two atria were then separated, and the right atrium was subsequently suspended in 12 ml organ baths containing Locke solution (of composition, in millimolar/litre: NaCl 155.8; $CaCl_2$ 4.3; KCL 5.6; $NaHCO_3$ 1.8 and glucose 5) continuously bubbled with pure oxygen and maintained at 37° C by circulating thermoregulator. To assure spontaneous beating, care was taken so that the right atrium preparation was obtained with pace maker tissue. Each preparation was subjected to a resting tension of 1 g and left

to equilibrate in the bath until the rate and amplitude of spontaneous contractions were stable (usually 20-30 minutes after setting up). The preparations were later challenged with drugs. The spontaneous amplitude and rate of contractions, as well as the drug - evoked response of the tissue were recorded isometrically by mean of force-displacement transducer (Grass FT 03 C or Statham UC-2), Beckman preamplifier or Biosciences type FC 117 and Beckman Dynograph recorder (Type R) or Polygraph recorder (Washington 400 MD 2C). The rates of contraction of the atrial strips were measured off line on the Dynograph records at a paper speed of 10 mm sec^{-1}

2.2 Electrically-driven Preparation (Inotropic Response):

The left atrium of each rat was carefully dissected out free from ventricular, connective and right atrial tissues. The preparation was fixed on a pair of thin platinum wire electrodes, placed in a 12 ml organ bath containing Locke solution at 37°C and continuously aerated with pure oxygen. The atrial strip was driven electrically with square wave pulses of 6-8 msec duration at a frequency of 4.5 Hz and supramaximal voltage of 5-10 volts, delivered by Grass-stimulator (Mode SD 9D). The tissues were subjected to a resting tension of 1 g and allowed to equilibrate (usually for a period of 20-30 minutes after mounting) until the force of contractions were stable before they were exposed to the drugs.

2.3 Study on the Effects of Drugs on Relative Refractory Periods (Dromotropic Effects):

The tissue preparation was essentially the same as described in 2.2. Left atria were removed from guinea-pigs and mounted in contact with two small electrodes attached to a grass rod and placed

in Locke solution at 37°C. The atria were stimulated at 1 Hz, 1 msec at 2.5-5 volts continuously for 30-45 min. The stimulation frequency was then increased slowly but progressively until the atria could no longer follow the stimulation frequency. This procedure was repeated at intervals of 30 min until the maximum driving frequency was reproducible on three successive occasions. Each substance to be tested (I-1, I-2, O-1 and O-2) was then administered and the maximum driving frequency was established 30 min after administration of each dose. The concentration of each antagonist required to reduce maximum driving frequency by 50% was measured.

3. The Organ Bath

The organ bath used in isolated preparations (Fig. 2) were of double walled Harvard type. They were composed of two compartments, the inner chamber, capacity 20 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 was supplied by a thermoregulating water pump (Churchill type). The bath also had an oxygen inlet oxygenate the inner chamber through a sintered glass opening.

4. Drugs

Drugs used were: Alkaloids from *Uncaria salaccensis*

- 3-Isoajmalicine (I-1)
- 19-epi-3-Isoajmalicine (I-2)
- Uncarine B (O-1)
- Mitraphylline (O-2)

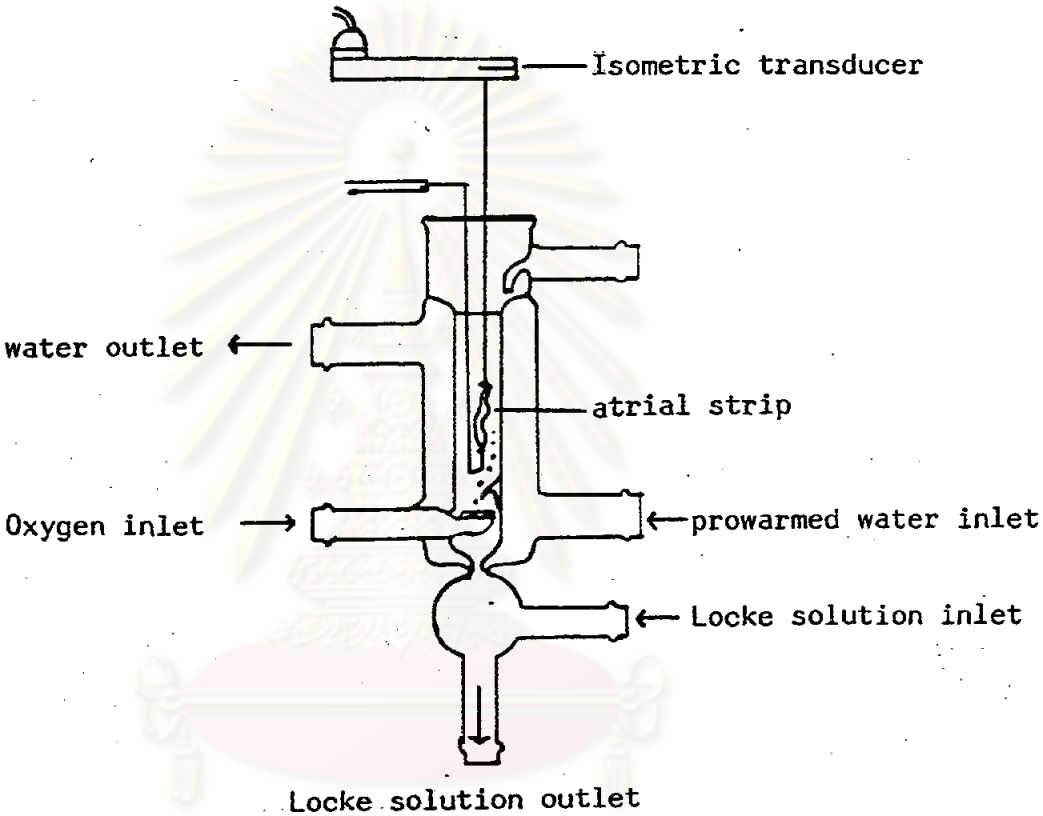

Figure 2. Organ Bath

Adrenaline, Atropine sulphate, 5-Hydroxytryptamine creatinine sulfate complex and Isoproterenol hydrochloride.

All drugs dissolved in distilled water were used.

5. Drug Administration

In vivo preparation, the drugs were injected into jugular vein via the cannulated catheter.

In isolated preparation, after tissues had been equilibrated for the minimum period of 20 min, the drug was administered to the bath fluid in a cumulative regimen using either a microsyringe or an automatic micropipette. Subsequent doses were given after a constant response to the previous dose had been established.

6. Analysis of Data

Results were expressed as means and standard errors (s.e) of the means. Significance of the differences between "control" and "drug-treated" means were determined using "student's t-test". Values of P of less than 0.05 were taken to implicate statistical significance.