CHAPTER II

MATERIALS AND METHODS

1. Experimental Animals

Albino rats of wistar strain of both sexes with body weights varying between 200-300 grams were used.

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

2.1 Anaesthesia:

The animals were anaesthetized with sodium pentobarbital 40 mg/kg intraperitoneally. Supplementary doses of sodium pentobarbital were given during experiment as required to maintain anaesthesia.

2.2 Tracheostomy and Cannulation:

The trachea was cannulated with a polyethylene tube so as to facilitate respiration and removal of excess secretions. Subcutaneous tissues overlying the trachea were removed by blunt dissection to expose the sternohyoid muscles. The sternohyoid muscles were separated medially by blunt dissection to expose trachea. Two linen ligatures were passed under the trachea. An incision was made through the tracheal wall for insertion of a polyethylene cannula diameter 2.5 mm, length 4 cm. The trachea was securely tied over the cannula.

2.3 Jugular Vein Cannulation:

The right jugular vein was cleared from adjacent tissue and cannulated for the subsequent infusion of drugs.

A polyethylene catheter filled with normal saline was used in this cannulation. The tip of catheter was obliquely cut and smoothened to prevent trauma on vascular wall. The Luer fitting of the catheter was connected to a three-way stop cock. Two linen ligatures were passed under the exposed vein. One was used to tied off the vein tightly as far towards the head end of the animal as possible. A small V-incision was made on the vessel wall 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.

2.4 Recording of Blood Pressure and Heart Rate:

The left carotid artery was exposed by blunt dissection and isolated from the vago-sympathetic trunk. Two linen ligatures were passed under the exposed carotid artery. The artery was ligated tightly with one of the ligature as far towards the head end of the animal as possible. The artery was occluded proximal to this tie. A V-incision was made and a previously prepared catheter inserted and securly tied. A polyethylene catheter was filled with heparinized saline (100 iu/ml) before inserted

into the artery. To prevent accidental removal of the cannula, both arterial and venous cannulae were sewn on to the skin.

Systolic, diastolic blood pressures and heart rate were recorded on Beckman Dynograph recorder (type RM) using Statham P 23AA pressure transducer coupled to Strain gauge coupler (type 9872) with Beckman preamplifier.

Mean blood pressure derived by calculation from

$$\text{Mean blood pressure} = P_D + \frac{1}{3}(P_S - P_D)$$

P_D = diastolic blood pressure

P_S = systolic blood pressure

3. In Vitro Preparation (Isolated atrial preparation)

The rats were killed by a sharp blow on the head. The abdominal and thoracic regions were immediately opened by midline incision 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).

3.1 Spontaneously-Beating Preparation (Chronotropic Response):

Intact both atria were carefully dissected out in one piece, free from ventricular and connective tissues, and avoiding damage to the pace-maker region. The two atria were then separated, and the right atrium was subsequently suspended in 20 ml organ baths containing Locke solution of following composition (in millimolar/litre):

NaCl 155.8, $CaCl_2$ 4.3, KCl 5.6, $NaHCO_3$ 1.8 and glucose 5 in distilled deionized water. The temperature of the organ bath was maintained at 37°C by circulating thermoregulator and Locke solution medium was continuously aerated with pure oxygen. 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. The left atrium was equilibrated 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 Statham force-displacement transducer (type UC2) coupled to Strain gauge coupler (type 9803), Beckman preamplifier and Beckman Dynograph recorder (type RM).

3.2 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 20 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 msec duration at a frequency of 4.5 Hz and supramaximal voltage of 8 volts,

delivered by Stimulator. The tissues were subjected to a resting tension of 1 g and allowed to equilibrate (usually for a period of 20-30 min after mounting) until the force of contractions were stable before they were exposed to the drugs.

3.3 The Organ Bath:

The double walled Harward type organ bath was used in isolated preparations (Fig. 2). It 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 pro-warmed water so as to provided 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 to oxygenate the inner chamber.

4. Drugs

Drugs used were: 3α-dihydrocadambine, indole glycosidic alkaloid from Anthocephalus chinensis A. Rich. leaves (ALKALOID)

Acetylcholine chloride, Atropine sulphate, Isoproterenol hydrochloride, Propranolol hydrochloride, Histamine dihydrochloride, Mepyramine maleate, Cimetidine, Hexamethonium bromide, Tyramine hydrochloride.

All drugs were dissolved in normal saline except ALKALOID used in in vitro preparation dissolved in Locke solution and doses are expressed as the salt.

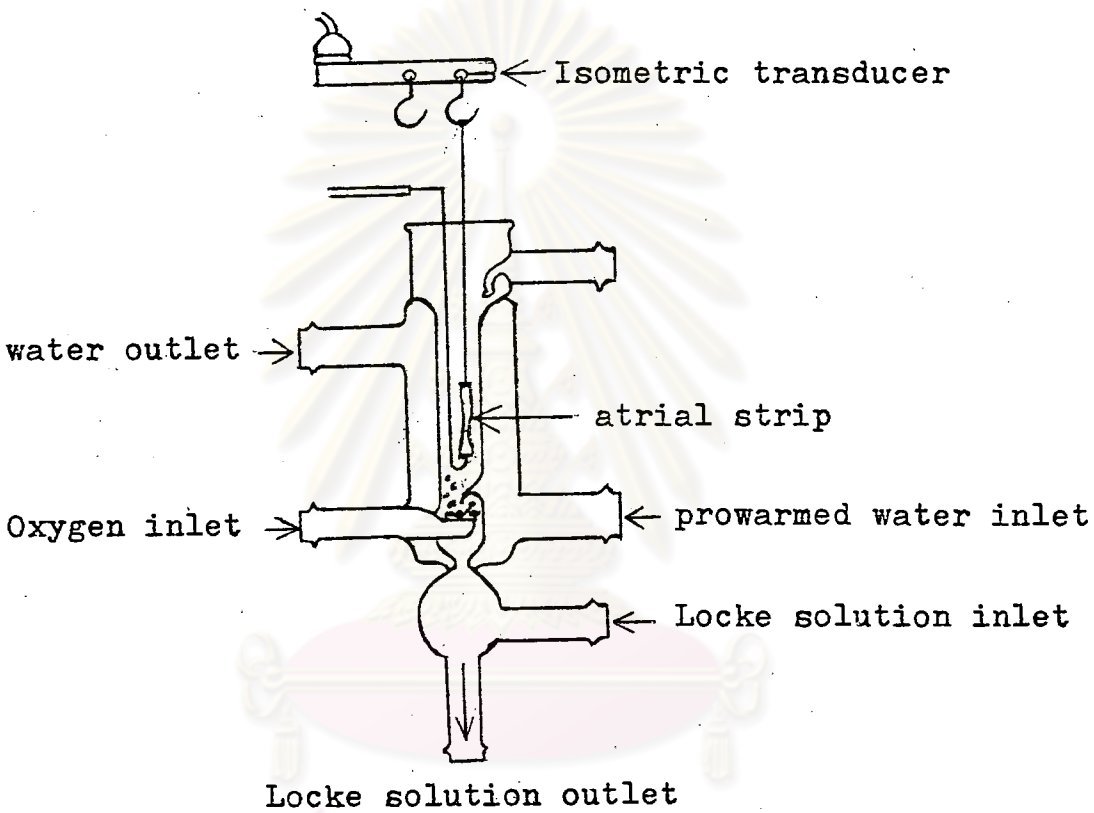

Figure 2. Organ Bath

5. 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 infused at the rate about 1 ml/min into jugular vein via the cannulated catheter and flushed with normal saline.

In isolated preparation, after tissues had been equilibrated for the minimum period of 20 min, the drug was administered to the bath fluid using an automatic micropipette.

6. Analysis of Data

Experimental data were expressed as means ± S.E.M. Statistical significance was tested according to Student's t-test for paired or unpaired variates.