CHAPTER II

MATERIALS AND METHODS

A. Animals

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

B. Isolated Atrial Preparations

Rats were sacrificed by blowing on the head. The chest was quickly opened and the heart was removed. The excised heart was placed in a petri-dish containing Locke solution bubbled with 100% oxygen at room temperature (28-30°C). The ventricle and other extraneous tissues were carefully removed. The remaining atria were then separated into right and left sides. Both isolated right and left atria were then transferred and mounted in 25 ml organ bath chambers containing Locke solution aerated with 100% oxygen and maintained at 37°C by circulating thermoregulator. A preload of 1 gm was applied to the atria in every experiment performed.

The spontaneously beating right atria, and the left ones were used in studying the rate and force of contraction respectively. The latter was driven electrically with square wave pulses delivered via platinum electrodes. The stimulus strength was 5 volts and the duration was 5 msec. The frequency of stimulation was kept constant at 250/min. The rates and contractile force were recorded with isometric force displacement transducer (Grass FT 03 C) connected to Beckman Dynograph recorder (Type R) with Beckman preamplifier.

The atria were allowed to equilibrate until stable rate and contractile force were observed (usually 30-60 min after setting up) before the experiments began.

The composition of Locke solution is as follows : 155.8 mM NaCl, 5.6 mM KCl, 2.15 mM $CaCl_2$, 1.8 mM $NaHCO_3$ and 5.0 mM glucose.

C. The Organ Bath Chambers

The organ bath chambers used in the present investigation were of double walled Harvard 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℃ so as to provide constant temperature to the inner compartment. The circulating water was supplied by a thermoregulating water pump (Churchill Instrument Co. Ltd.). The bath also has an gas inlet which gasses the inner chamber through a sintered glass opening.

D. 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 above.

E. Drugs

The drugs used are as follow.

- Capsaicin (8-methyl-N-vanilly-6-nonenamide, Sigma Chemical Co.)
- Ouabain octahydrate (Sigma Chemical Co.)

- Propranolol hydrochloride (Inderal; inj., I.C.I. Macclesfield)
- Reserpine (Serpasil; inj., Ciba-Geigy Limited)
- Verapamil hydrochloride (Isoptin; inj., Knoll)
- Procainamide hydrochloride (Pronestyl; inj., Squibb)
- Methysergide hemimaleate (Sandoz)

Ouabain and Methysergide were dissolved in distilled water. Capsaicin was dissolved in absolute ethanol.

F. Statistical Analysis

Results were presented as means ± standard error of the means. Differences between means were compared by Student's unpaired t-test. They were considered significant when p values were less than 0.05.