Chapter III

Materials and Methods

Animals preparation

Twenty adult male mongrel dogs, weighing 10 - 15 kgs. were used in the experiments. Food and water were withheld for 12 hours preceding the investigation. On the day of the experiment, the dog was anesthetized with pentobarbital sodium 25 mg./kg.bw. intravenously. Supplemental doses of pentobarbital were administered as require during the study to maintain an even state of anesthesia. An endotracheal tube was inserted. One of femoral vein was cannulated with polyethelene tube (PE 180) for infusion. Before the clearance study, the priming solution containing p-aminohippurate (PAH) 8 mg./kg. bw. and inulin 50 mg./kg.bw. in isotonic saline (adjust to pH 7.4) was injected intravenously into femoral vein and followed immediately by sustaining solution at the rate of 1.5 ml/min. The rate of infusion was kept constant throughout the course of experiment by Peristaltic pump (Eyla model 3).

The composition of the sustaining solution contained PAH and inulin 2.5 mg and 5 mg in 1 ml of 0.9% NaCl respectively. One of carotid arteries was cannulated with polyethelene tube (PE 200) for blood collection and connected to the pressure transducer (PE 23 AA) for recording blood pressure and heart rate by polygraph (Grass model 7). Both of ureters were reached by bilateral flank incisions with a

retroperitoneal approach and tubulated with polyvinyl catheter (PV 190) for urine collection. After an hour of infusion and the rate of urine flow stabilized, urine samples were obtained during 10 minutes collection. Blood samples were obtained at the midpoint of the urine collection. Blood and urine samples were collected for determination of inulin clearance, PAH clearance, osmolality, sodium, potassium, chloride concentrations and also blood sample for measurement of packed cell volume.

Experiment procedures.

Four groups of five animals were used to study the effects of Russell's viper venom on cardiovascular and renal variables. Experiments were carried out as following :

Group I Control animals

Group II Splenectomized animals (10 days post splenectomy)

Group III Animals pretreated with indomethacin

Group IV Splenectomized animals pretreated with indomethacin

Animals in group III and group IV were fed indomethacin 100 mg daily for 3 days prior to experiment and 100 mg in the morning of the experiment.

On the day of the experiment, two periods of experiments were carried out.

The first period, cardiovascular and renal variables were performed as a control.

On the second period, the animal was given Russell's viper venom by intravenously into femoral vein at the doses 0.1 mg/kg.bw. The venom was prepared by dissolution the powder of lypophilized venom

1 mg in 1 ml of isotonic saline and then added up by isotonic saline to 20 ml (Tungthanathanich, 1983). Cardiovascular and renal functions was evaluated for 3 hours after venom injection.

Determination of cardiac output and plasma volume.

Both cardiac output and plasma volume were measured by dye dilution technique, using Evans blue (T - 1824). Cardiac output was measured by using technique as described by Chaiyabutr et al., (1980). A bolus of T - 1824 (0.5%) was injected into femoral vein. Then series of blood sample were collected from the carotid artery immediately, with 3 - 5 second after dye injection. Serial sample of arterial blood were collected by means of peristaltic pump and fraction collection. Each of sample approximately 1 ml/sec. was collected for a period of 10 - 14 second. Then the amount of dye in each blood sample was determined respectively by spectophotometry. In order to determine the plasma volume, a control sample of blood was collected before the dye injection and 15 minutes after dye injection and also determined by spectrophometry. Cardiac output was determined by dye dilution technique and was calculated as described by Hamilton et al., (1948). The plasma volume was calculated by the method of Kolmer (1951). Packed cell volume was determined by the preparation of blood in an internation microcapillary centrifuge and measured with an international microcapillary reader.

The method of determination blood and urine sample.

PAH was determined by the method of Bratton and Marshall as modified by Smith (1962). Determination of inulin was carried out

by the method of Schreiner as described by Smith (1962). Using the Fick's principle, PAH clearance was used for effective renal plasma flow (ERPF) and inulin clearance was used for glomerular filtration rate (GFR).

The compositions in the plasma and urine were measured as followed : sodium and potassium by flame photometer (Klina flame operating; Beckman instrument), chloride by chloridometer (Buchler digital chloridometry; Beckman instrument), osmolality by the freezing point osmometer (Advance osmometer model 3).

Abbreviations and derivations of variables used in text and figures.

MAP = mean arterial blood pressure (mm.Hg)

HR = heart rate (beat/min)

PCV = packed cell volume (%)

TPR = total peripheral resistance (dyne - sec/cm^5)

RVR = renal vascular resistance (dyne - sec/cm^5)

V = urine flow rate (µl/min/kg.bw.)

P_{in} = plasma concentration of inulin (mg/ml)

U_{in} = urinary concentration of inulin (mg/ml)

C_{in} = inulin clearance (ml/min/kg.bw.)

P_{PAH} = plasma concentration of PAH (µg/ml)

U_{PAH} = urinary concentration of PAH (µg/ml)

C_{PAH} = PAH clearance (ml/min/kg.bw.)

P_{Osm} = plasma osmolality (mOsm/Kg)

U_{Osm} = urinary osmolality (mOsm/Kg)

C_{Osm} = osmolar clearance (µl/min/kg.bw.)

C_{H_2O} = free water clearance (µl/min/kg.bw.)

P_e = plasma concentration of electrolytes (mEq/L)

U_e = urinary concentration of electrolytes (mEq/L)

Using the Fick's principle, PAH clearance was used for measuring effective renal plasma flow (ERPF) and inulin clearance was used for measuring glomerular filtration rate (GFR). The following calculation were performed :

glomerular filtration rate (GFR) $= \frac{U_{in}V}{P_{in}}$

effective renal plasma flow (ERPF) $= \frac{U_{PAH}V}{P_{PAH}}$

effective renal blood flow (ERBF) $= \frac{ERPF}{(100\text{-}PCV)} \times 100$

Filtration fraction (F.F.) $= \frac{GFR \times 100}{ERPF}$

Osmolar clearance (C_{Osm}) $= \frac{U_{Osm}V}{P_{Osm}}$

Free water clearance (C_{H_2O}) $= V - C_{Osm}$

Urinary electrolytes excretion (U_eV) $= U_eV$

Fractional electrolytes excretion $= \frac{U_eV/P_e}{GFR} \times 100$

$$\text{Renal fraction (R.F.)} = \frac{\text{ERBF} \times 100}{\text{cardiac output}}$$

$$\text{Total peripheral resistance (TPR)} = \frac{\text{MAP} \times 1333 \times 60}{\text{cardiac output}}$$

$$\text{Renal vascular resistance (RVR)} = \frac{\text{MAP} \times 1333 \times 60}{\text{ERBF}}$$

Statistical analysis

All data presented were normalized to individual body weight to allow comparison among the dogs. Data were reported as the mean value ± S.D. The paired t - test was used to estimate the statistical significance of difference between value obtained from the control period and from each period of the experiment. The unpaired t - test was used to estimate the statistical significance of difference between value obtained from the control group and each group of the experiment.