CHAPTER III

Materials and Methods

Animals preparation

Adult male mongrel dogs, weighing 10-19 kgs. were used in the experiments. Food and water were witheld 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 required during the study to maintain an even state of anesthesia. An endotracheal tube was inserted. Both vagi were severed. One femoral vein was cannulated with polyethylene tube (PE 180) for infusion. Before the clearance study, the priming solution containing P-amino-hippurate (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 immediatly by sustaining solution at the rate of 1.5 ml/min. The rate of infusion was kept constant throughout the course of experiment by a peristaltic pump (Eyla Model 3). The composition of the sustaining solution contained 2.5 mg PAH and 5.0 mg inulin per ml of 0.9% saline. One of carotid arteries was cannulated with polyethylene 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 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, urine sample was obtained during 10 minutes and the blood

sample was collected at the midpoint of the urine collection.

Experimental procedures

Two groups of six dogs were used to study the effects of Russell's viper venom on cardiovascular and renal functions.

Group I Intravascular volume expansion.

The periods of experiments were carried out as followed:

Period 1. Cardiovascular and renal variables were performed as a control.

Period 2. The animal was given intravenously with Dextran 70 solution (6% in saline solution) 30 ml/kg.bw. at the rate of 60 ml/min. Cardiovascular and renal functions were performed after one hour period of equilibration for a second control. (0 minute)

Period 3. Following intravascular volume expansion the animal was given Russell's viper venom intravenously at the doses 0.1 mg/kg.bw. The venom was prepared by dissolution the powder of lyophilized venom 1 mg in 1 ml. of isotonic saline and diluted to 20 ml with isotonic saline (Tungthanathanich, 1983; Tongvongchai, 1984). Cardiovascular and renal functions were measured at 15, 30, 60 and 120 minutes after venom injection.

Group II Intestinal and splenic blood vessels occlusion.

On the day of the experiment the periods of the experiment were carried out as followed:

Period 1. Cardiovascular and renal variables were measured as control.

Period 2. In this period, the occlusion of the intestinal and splenic blood vessels were performed by ligation. After the occlusion, an infusion of Dextran 70 solution were performed to restore the blood pressure. One hour later, cardiovascular and renal functions were performed as a second control (0 minute).

Period 3. The animal was given Russell's viper venom intravenous injection at the doses 0.05 mg/kg.bw. Cardiovascular and renal function were measured at 15, 30, 60 and 120 minutes after envenomation.

Determination of cardiac output and plasma volume.

Cardiac output and plasma volume were measured by dye dilution technique, using Evans blue (T-1824). Cardiac output was measured by the technique described by Chaiyabutr et al., (1980) and calculated as described by Hamilton et al., (1948). A bolus of dye T-1824 (0.5%) was injected into femoral vein. Then series of blood sample were collected from the carotid artery immediately with 3-5 seconds after dye injection. Serial samples 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 seconds. Then the amount of dye in each blood sample was determined by spectrophotometry. In order to determine the plasma volume, blood samples were collected before the dye injection and 15 minutes after dye injection and also determined by spectrophotometry. The plasma volume was calculated by the method of Kolmer (1951). Packed cell volume was determined by microhematocrit centrifuge.

The method for the determination of blood and urine specimens.

PAH was determined by the method of Bratton and Marshall, modified by Smith (1962), inulin by the method of Schreiner as described by Smith (1962).

The compositions in the plasma and urine were determined as followed; sodium and potassium by flamephotometer (Corning Model 435), chloride by silver titration method (Cl/CO_2 Analyzer; Beckman instrument), Osmolality by freezing point osmometer (Advanced 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 (ul/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 ug/ml)

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

P_{Osm} = plasma osmolality (mOsm/kg.)

U_{Osm} = Urinary osmolality (mOsm/kg.)

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

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

P_e = plasma concentration of electrolytes (mEq/L)

U_e = urinary concentration of electrolytes (mEq/L)

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The following calculation were performed :

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

Renal plasma flow (RPF) $= \frac{U_{PAH}V}{P_{PAH}}$

Renal blood flow (RBF) $= \frac{RPF}{(100-PCV)} \times 100$

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

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

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

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

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

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Statistical analysis

All data presented were normalized to individual body weight to allow comparison among the dogs. Data were reported as the mean value $\pm$ 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.

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