Chapter III

Materials and Methods

Animal preparation

Experiments were carried out in twenty adults male mongrel dogs, weighting 8-19 kgs. The animals were fasted for 12 hours preceding the operation. On the day of the experiment, the dog was anesthetized with pentobarbital sodium (25 mg/kg bw.) intravenously. To maintain a state of light anesthesia, supplemental doses of pentobarbital (30-60 mg) were administered as required during the study. A tracheotomy tube was inserted. Two femoral vein were cannulated with polyethylene tube (PE 180). One for infusion of inulin and PAH, the other for slowly injection of Russell's viper venom. In order to study renal clearance, the priming solution containing p-aminohippurate (PAH) 1.2% and inulin 7.5% in isotonic saline were administered 0.5 ml/kg.bw. then the sustaining solution composed of 0.12% and 0.75% of PAH and inulin respectively, were infused at the rate of 1.8 ml/min.

One of femoral artery was cannulated with polyethylene tube (PE 200) which was connected to a pressure transducer (PE 23 AA) for measurement of arterial blood pressure and heart rate (Grass Model 7 Polygraph) and for collection of blood sample. Bilateral flank incision were made, both ureters were catheterized via retroperitoneal apporach with polyvinyl catheter (PV 190) for urine collection. Left

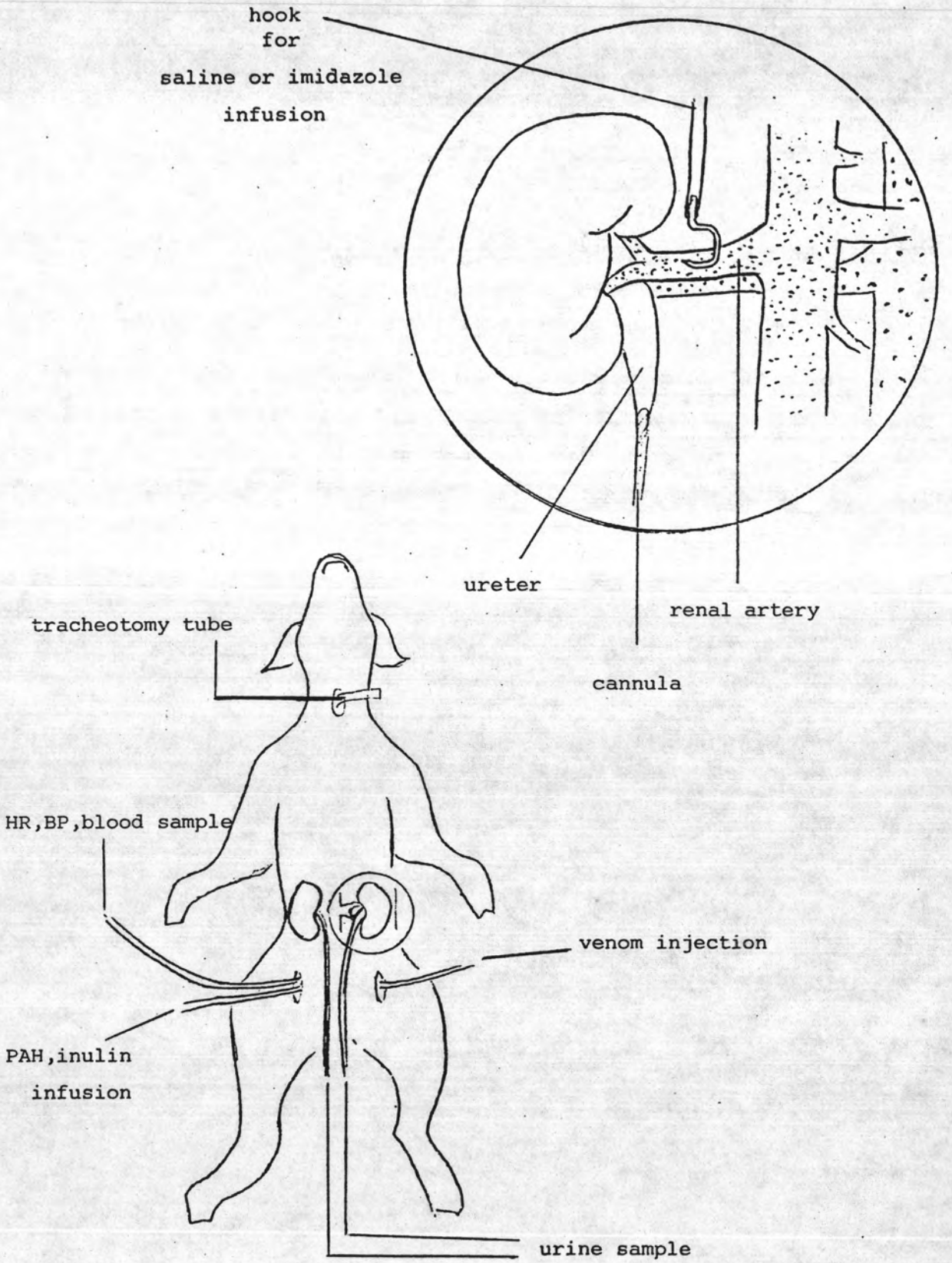


Fig.A: Scheme of experiment.

renal artery was hooked with the needle # 22 g connected to syringe pump model 341 A for infusion of normal saline alone in group I and normal saline then followed by imidazole in groups II, III and IV.

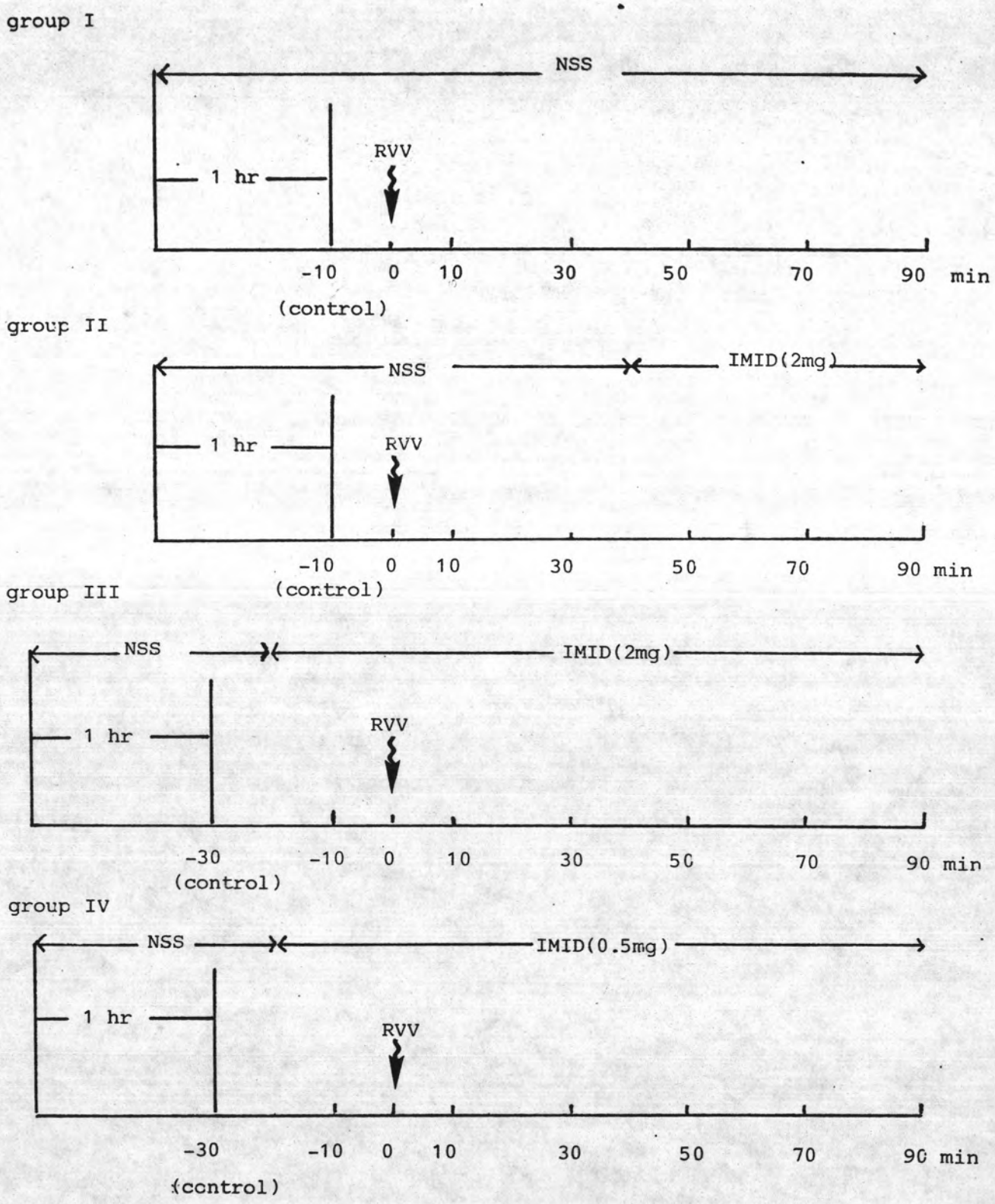
After an hour of infusion and the rate of urine flow was steady, duplicated sample for clearance study were obtained. Two urine samples were collected during 20 min interval. One arterial blood sample was drawn at the midpoint of the urine collection.

Experimental protocols.

To study the effects of Russell's viper venom on renal function during intrarenal infusion of thromboxane synthetase inhibitor (imidazole), twenty dogs were divided into four groups.

group I. Five dogs were used as control animal. After 1 hour of infusion of sustaining PAH and inulin solutions, the control sample of urine and arterial blood were obtained. Envenomation was performed immediatly by slow intravenous injection of Russell's viper venom 0.05 mg/kg for 10 minutes by peristaltic pump (Eyla model 3). The lyophilized Russell's viper venom (0.05 mg/kg bw) was dissolved in 20 ml of normal saline (NSS). Left renal artery was continuously infused with NSS at the rate of 0.3 ml/min throughout the experiment.

group II. Five dogs were treated in the same manner of gr.I but after 40 min of envenomation, 2 mg/kg/min of imidazole (IMID) was dissolved in 0.3 ml of NSS was continuously infused via left renal



RVV was given by intravenous injection.

NSS and IMID were infused via left renal artery.

Fig. B: Diagrammatic illustration of experimental protocols.

artery in place of NSS at the same rate of 0.3 ml/min.

group III. Five dogs were pretreated with the imidazole (IMID) at the rate of 2 mg/kg/min by intrarenal arterial infusion for 20 minutes before the envenomation and sustained throughout the experiment.

group IV. Five dogs were pretreated with 0.5 mg/kg/min of imidazole at the rate of 0.3 ml/min in the same manner of gr III.

Imidazole (MW = 68.1, crystalline grade I) was purchased from Sigma Chemical company. Russell's viper venom was donated by Thai Red Cross Society.

Determination of blood and urine samples

Determination of plasma and urine PAH concentrations were carried out by the method of Bratton and Marshall as modified by Smith (1962). Plasma and urinary concentrations were determined by the antrone method as described by Young & Raisz (1952).

The sodium and potassium concentrations in plasma and urine were determined by flame photometer (KLiNa flame operating; Beckman instrument), chloride by chloridometer (Buchler digital) , calcium by colorimetric method of Moorehead and Biggs (1974), inorganic phosphorus by method of Gomori (1941), osmolality by the freezing point osmometer (Advance osmometer model 3).

Packed cell volume was determined by the preparation of blood in an international microcapillary tube then centrifuged by Cray Adams micro hematocrit centrifuged, (Model 850 Ta) and measured by Hawksley micro hematocrit reader.

Calculation :

Using the Fick's principle, PAH clearance was used for determination of effective renal plasma flow (ERPF), inulin clearance was used for glomerular filtration rate (GFR).

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

mean arterial blood pressure (MAP) $= (P_S + 2P_D)/3$

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

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

filtration fraction (FF) $= \frac{GFR \times 100}{ERPF}$

renal vascular resistance (RVR) $= \frac{MAP \times 1333 \times 60}{ERBF \times 1000}$

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

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

urinary electrolytes excretion $= U_e V$

fractional electrolytes excretion (FE_e) $= \frac{U_e\ V/Pe}{GFR}$

Statistical analysis

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