Chapter III

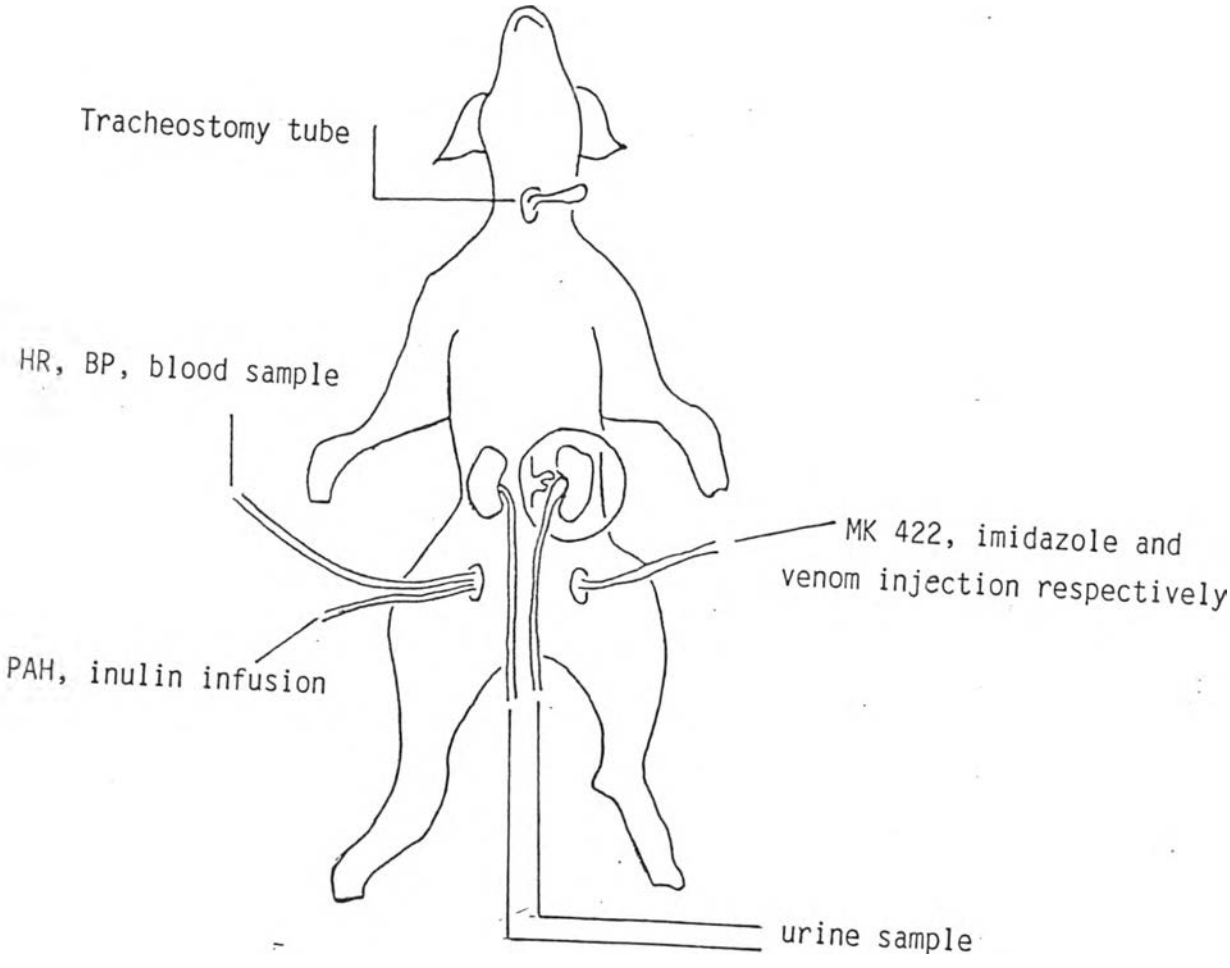
Materials and Methods

Animal preparation

Experiments were performed in 10 male mongrel dogs, weighing 8-16 kgs. The animals were fasted for 12 hours preceding the operation. On the day of the experiment, the dog was anesthetized with the intravenous injection of pentobarbital sodium (25-30 mg/kg bw.) initially, and recieved subsequent doses of 1-2 mg/kg bw. when nessary to maintain proper anesthesia throughout the experiment. A tracheal cannula was inserted by tracheostomy to secure free airways. The polyethylene catheter (PE 200) was introduced into left femoral artery for blood pressure, heart rate recordings and for collection of blood sample then it was connected to a pressure transducer (PE 23 AA) and polygraph recorder (Grass Model 7). Left femoral vein was cannulated with polyethylene tube (PE 180) for infusion of inulin, p-aminohippurate (PAH), enalaprile maleate (MK 422) (10 mg/kg), imidazole (5 mg/kg) and Russell's viper venom (RVV) (0.1 mg/kg).

Bilateral flank incision were made, both ureters were catheterized via retroperitoneal approach with polyvinyl catheter (PV 200) for urine collection. Left 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 prazosin in group II.

After surgical procedure, dogs were then given the priming dose of 25 mg/kg bw of inulin and 6 mg/kg bw of PAH dissolved in 0.9%



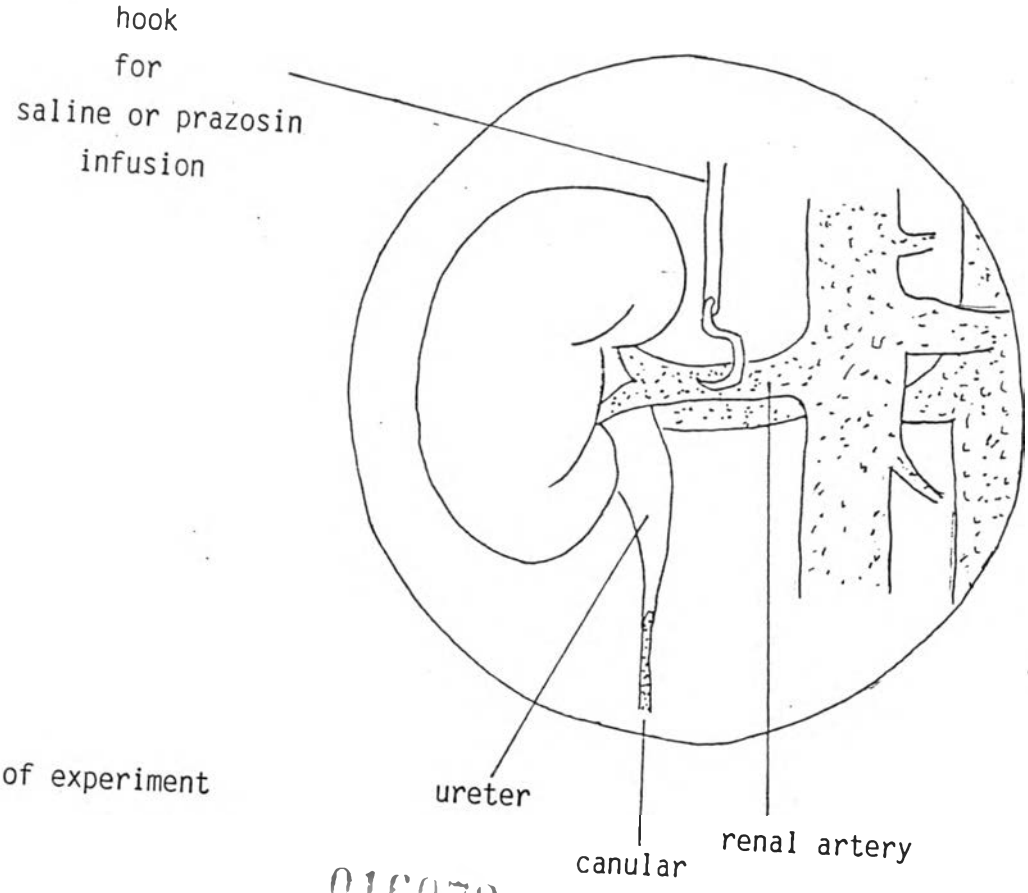


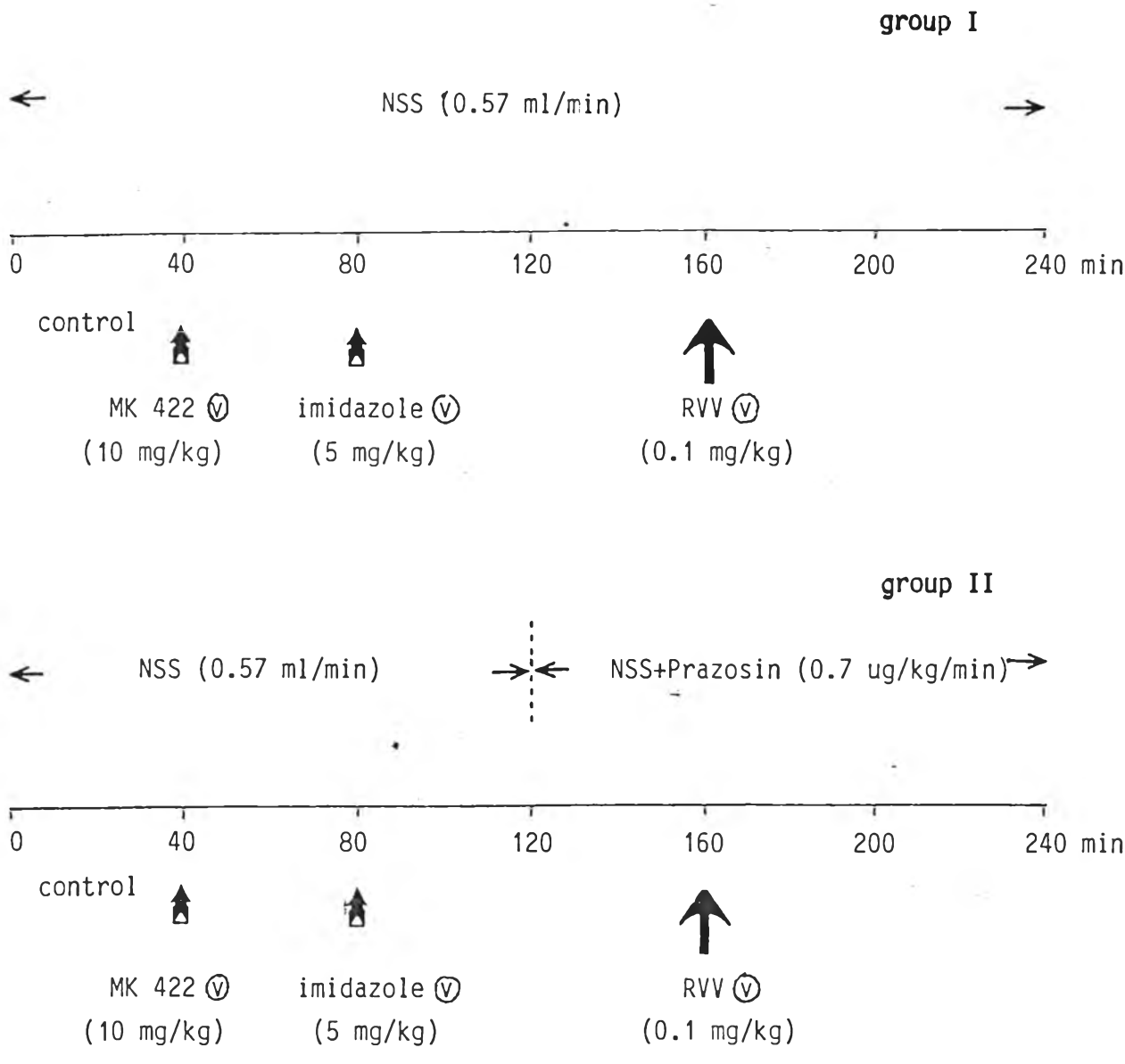
Fig A : Scheme of experiment

NSS intravenously and immediately afterward by the sustaining infusion of 500 mg% of inulin and 120 mg% of PAH at the rate of 1.4 ml/min with peristaltic pump (Eyla Model 3), that was sufficient to maintain the plasma inulin and PAH concentration at approximately 0.2 mg/ml and 0.02 mg/ml respectively. After an hour of infusion of priming doses of inulin and PAH and the rate of urine flow was steady, duplicated samples for clearance study were obtained. Two urine samples were collected 20 minutes 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 prazosin (α_1-adrenergic blocker), ten dogs were divided into two groups.

Group I Five dogs were used as control animal. After one hour of infusion of sustaining PAH and inulin solutions, the control samples of urine and arterial blood were collected for 40 minutes. Given (10 mg/kg) MK 422 and (5 mg/kg) imidazole were followed control period respectively. MK 422 sample of urine and arterial blood were collected for 40 min. then imidazole sample were collected in the same manner of MK 422 period. Envenomation was performed immediatly by intravenous injection of Russell's viper venom 0.1 mg/kg for 3 minutes. The lyophilized Russell's viper venom (0.1 mg/kg bw) was dissolved in 20 ml of normal saline (NSS). Left renal artery was continuously infused with NSS at the rate of 0.57 ml/min throughout the experiment.



MK 422, imidazole and RVV were given by intravenous injection.

NSS and prazosin were infusion via left renal artery.

Fig B : Diagrammatic illustration of experimental protocols.

Group II Five dogs were treated in the same manner of group I but after MK 422 and imidazole period respectively, 0.7 ug/kg/min of prazosin which dissolved in 20 ml of NSS was continuously infused via the left renal artery in place of NSS at the same rate of 0.57 ml/min.

Determination of blood and urine samples

Plasma and urine inulin concentrations were determined by the anthrone method as described by Davidson et al., (1963). Determination of plasma and urine PAH concentration were carried out by the method of Bratton and Marshall as modified by Smith (1962). Using the Fick's principle, inulin clearance was used for glomerular filtration rate (GFR) and PAH clearance was used for effective renal plasma flow (ERPF).

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 chloridometer; Beckman instrument), osmolality by the freezing point osmometer (Advance osmometer model 3).

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

Calculation :

$$\text{glomerular filtration rate (GFR)} = \frac{Uin\ V}{Pin}$$

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

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

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

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

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

urinary electrolytes excretion $= UeV$

fractional electrolytes excretion (FEe) $= \frac{UeV/Pe}{GFR} \times 100$

Mean arterial blood pressure (MABP) $= P_d+1/3\ (P_s - P_d)$

renal vescular resistance (RVR) $= \frac{MABP \times 1333 \times 60}{ERBF}$

Statistical analysis

Data were reported as the mean value ± S.D. The paired t-test was used to estimate the statistical significance of the difference between value obtained from the control period and from each period of the experiment.