Chapter III

Materials and Methods

Animal preparation

Experiments were conducted on 20 male mongrel dogs, weighted 8-15 kgs. The animals were fasted for 12 hours preceding the operation. On the day of experiments, the dog was anesthesized with the intravenous injection of pentobarbital sodium (25-30 mg/kg bw.) initially, and received subsequent doses of 1-2 mg/kg bw. when necessary to maintain proper anesthesia throughout the experiment. A tracheal cannular was inserted by tracheostomy to secure free airways. The polyethylene catheter (PE 200) was introduced into left femoral artery for arterial blood pressure and heart rate recordings and for blood sample collection 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, paraaminohippurate (PAH), prazosin (0.7 g/kg), enalapril maleate (10 mg/kg), indomethacin (5 mg/kg) and Russell's viper venom (RVV) (0.1 mg/kg).

A flank incision was made, the polyvinyl catheter (PV 200) was inserted into the left ureter for urine collection. After surgical procedure, dogs were then giving the priming dose of 25 mg/kg.bw of inulin and 6 mg/kg.bw of PAH dissolved in 0.9% 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

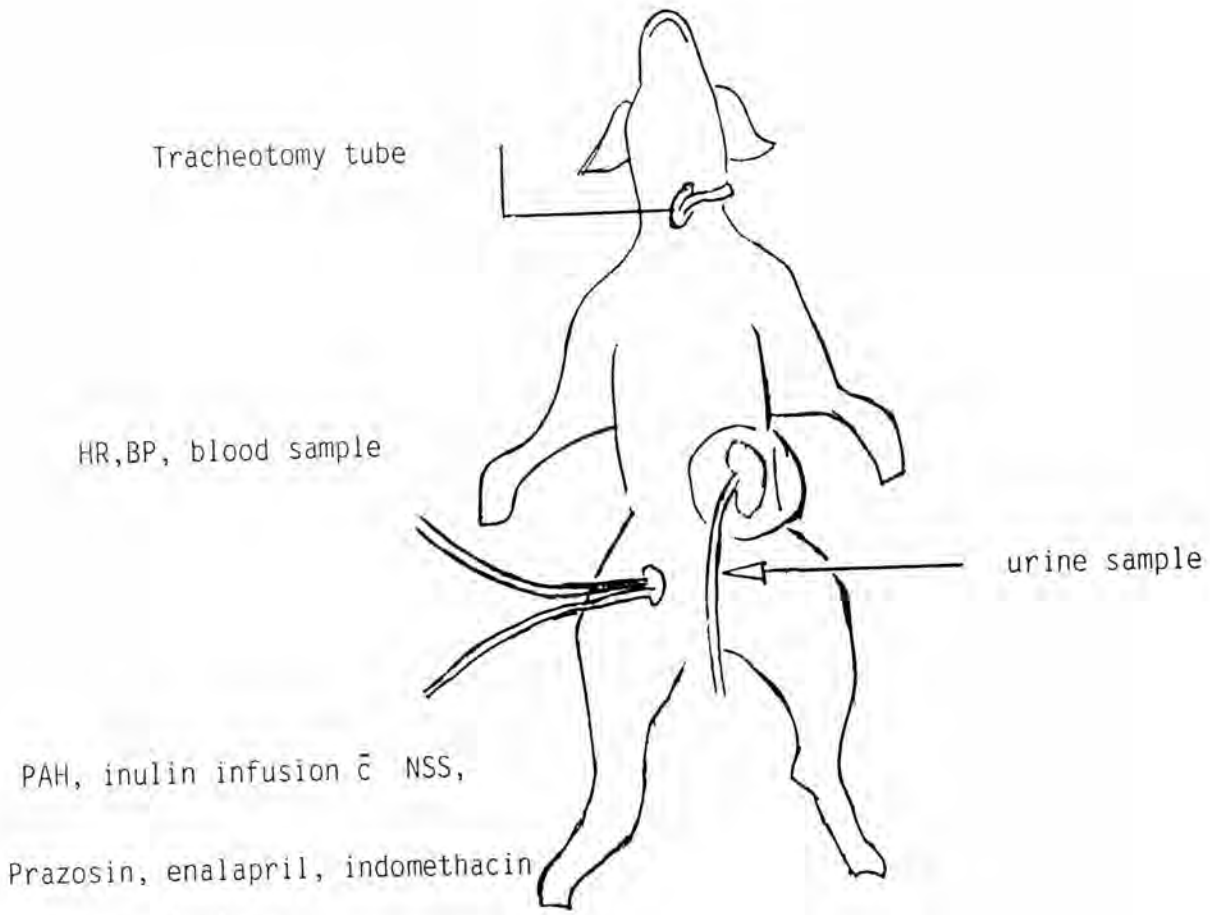


Figure A : Scheme of experiment

(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 and the rate of urine flow stabilized, urine samples for clearance study were obtained during 10 minutes collection. An arterial blood sample was drawn at the midpoint of the urine collection.

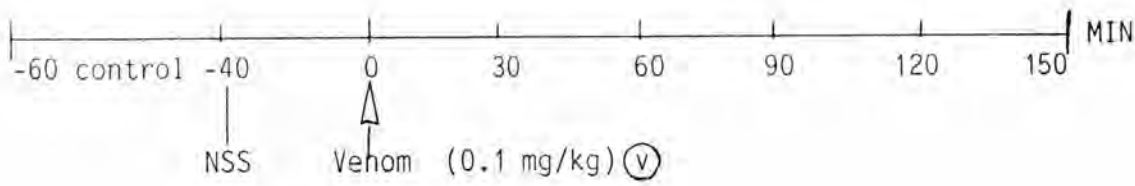
Experimental protocols

To study the plasma norepinephrine level and renal functions in dogs given Russell's viper venom and pretreated with prazosin, enalapril maleate and indomethacin, twenty dogs were divided into four groups.

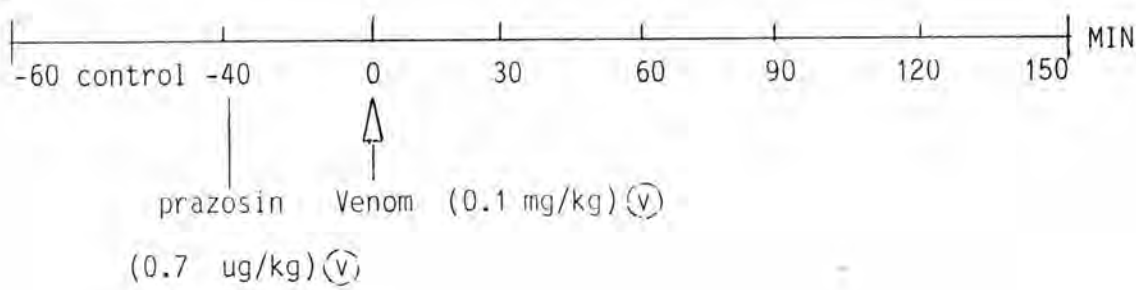
Group I , Five dogs were used as control animal. After one hour of infusion of sustaining PAH and inulin solutions, the control samples of urine and arterial blood were collected for 20 minutes (0-20 minutes). Intravenous infusion of 0.9 % NSS 20 ml was given to the animals of group I. Urine sample was collected for 40 minutes and arterial blood sample was drawn at the midpoint of this period. Envenomation was performed immediatly by intravenous injection of lyophilized Russell's viper venom 0.1 mg/kg.bw. dissolved in 20 ml. of normal saline solution (NSS) for 3 minutes. General circulation and renal functions were evaluated for 2 1/2 hours after venom injection.

-Intravenous infusion of 0.9% saline rate 0.57 ml/min----------

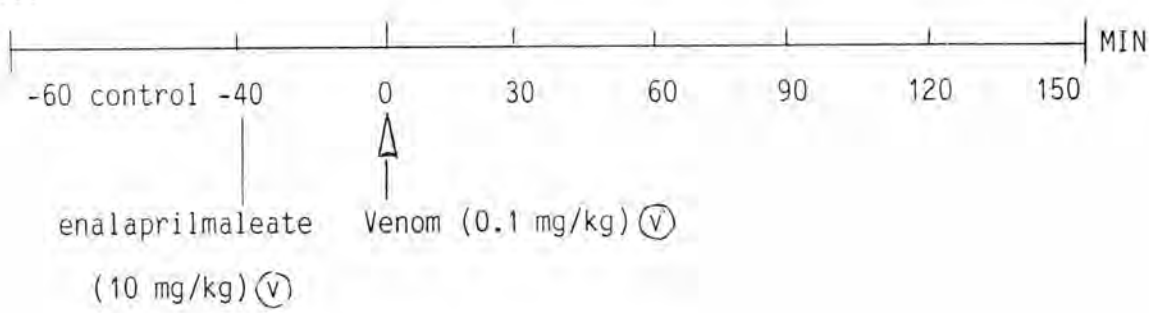
Group I



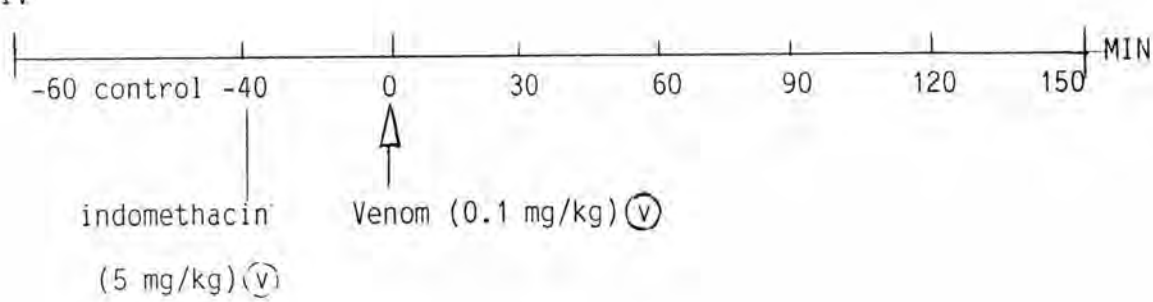
Group II



Group III



Group IV



Group II, III, IV Five dogs in each group were treated in the same manner of group I. After the control samples of urine and blood were collected for 20 minutes (0-20 minutes), 0.7 ug/kg.bw. of prazosin; 10 mg/kg of enalapril maleate and 5 mg/kg.bw. of indomethacin in 20 ml. of NSS were given intravenous to the animals of group II; group III and group IV respectively. Urine samples were collected for 40 minutes and arterial blood samples were collected at the mid point of this period of each group. Envenomation and procedure were perfomed as describe in group I until the end of experiment.

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).

Sodium and Potassium in plasma and urine sample were measured by flame photometer (Klina flame operating; Beckman instrument).

Packed cell volume was determined by microcapillary method (Howksley micro hematocrit reader).

Plasma norepinephrine was determined by extraction and fluorometric assay of catecholamines which were the combined techniques

and modification by Chang (1964), Ansell and Beeson (1968), Shellenberger and Gorden (1971), and Barchas (1972). This method was reported by Eleftheria Missirlis (1973) as follow:

Extraction of norepinephrine (NE)

1. Plasma 5 ml. was mixed with 5 ml. ethanol 75% and the mixture were centrifuge 3000 RPM 6 min. The clear supernatant was transferred to ml. centrifuge tube.

2. The standard solution of NE was prepared by serial dilution and the final concentration of 1 g/ml, 0.5 g/ml, 0.25 g/ml. and 0.125 g/ml were made.

3. The blanks used was 5 ml of ethanol 75%

4. The plasma, blank and standard solution of 5 ml. per tube were mixed with 200 mg. of alumina and pH was adjusted to 8 with sodium acetate in ethanol 75%, then shake 10 min, and centrifuge at 2000 RPM for 2 min. The NE was absorbed in the alumina which is precipitated. The supernatant was discarded.

5. The alumina was washed with 2 ml. cold deionized water and shake for 2 min, centrifuged at 2000 RPM for 2 min., then the supernatant was discarded.

6. NE was eluted from the alumina with 2.4 ml. 0.2 N acetic acid by shaking for 10 min then centrifuged at 3000 RPM for 2 min. The clear supernatant contains the NE.

7. The 2 ml. of supernatant were transferred to the test tube for the assay.

Fluorometric assay of NE

1. Each sample of 2 ml. in the test tube was added with 0.8

ml. of disodium ethylene diamine tetra acetate (EDTA) 0.1 M, pH 7, mixed well with vortex shaker then left for 2 min.

2. The iodine solution 0.4 ml. was added, mixed as the above then left in dark for 2 min.

3. The 0.8 ml. alkaline sulfite was added, mixed with vortex shaker and left for 2 min.

4. The 0.8 ml. glacial acetic acid was added mixed with vortex shaker and each tubes were placed in boiling bath for 2 min then allowed to cool for 10 min. It was ready for fluorescence measurement.

5. The mixture was transferred to a quartz cuvettes. The fluorescence was then measured for NE at wavelength 385 excitation and 485 emission.

$$\text{Calculation for NE (sample)} = \frac{\text{O.D. (sample) x weight (standard solution)}}{\text{O.D. (standard solution)}}$$

Abbreviation

NE	=	norepinephrine (ug%)
O.D.	=	optical density
Pd	=	Diastolic blood pressure (mm.Hg)
Ps	=	Systolic blood pressure (mm.Hg)

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) = $\frac{ERPF \times 100}{100 - PCV}$

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

urinary electrolytes excretion = UeV

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

mean arterial blood pressure (MAP) = Pd + 1/3 (Ps - Pd)

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

Statistical analysis

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