Chapter III

Materials and Methods

Animals Preparation

Experiments were carried out in twenty-nine adult male mogrel dogs, weighting 10-18 kgs. The animals were fasted for 12 hours preceding the operation. On the day of the experiment, the dog was anesthetized with pentobarbital sodium (30 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 tracheal tube was inserted to secure free airway. Two femoral veins were cannulated with polyethylene tubes (PE 180). One for infusion of the clearance solution and calcium chloride solution, the other for infusion of calcium channel blocker (Verapamil). In order to study renal clearance, the priming solution containing p-amino hippurate (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 1.8-2.0 ml/min . The rate of infusion was kept constant throughout the course of experiment by peristaltic pump (Eyla model 3). A jugular vein was cannulated with polyethelene tube (PE 180) for infusion selective alpha-1 adrenergic blocker (Prazosin). One of femoral artery was cannulated with polyethelene tube (PE 200) for blood collection and connected to the pressure transducer (PE 23 AA) for

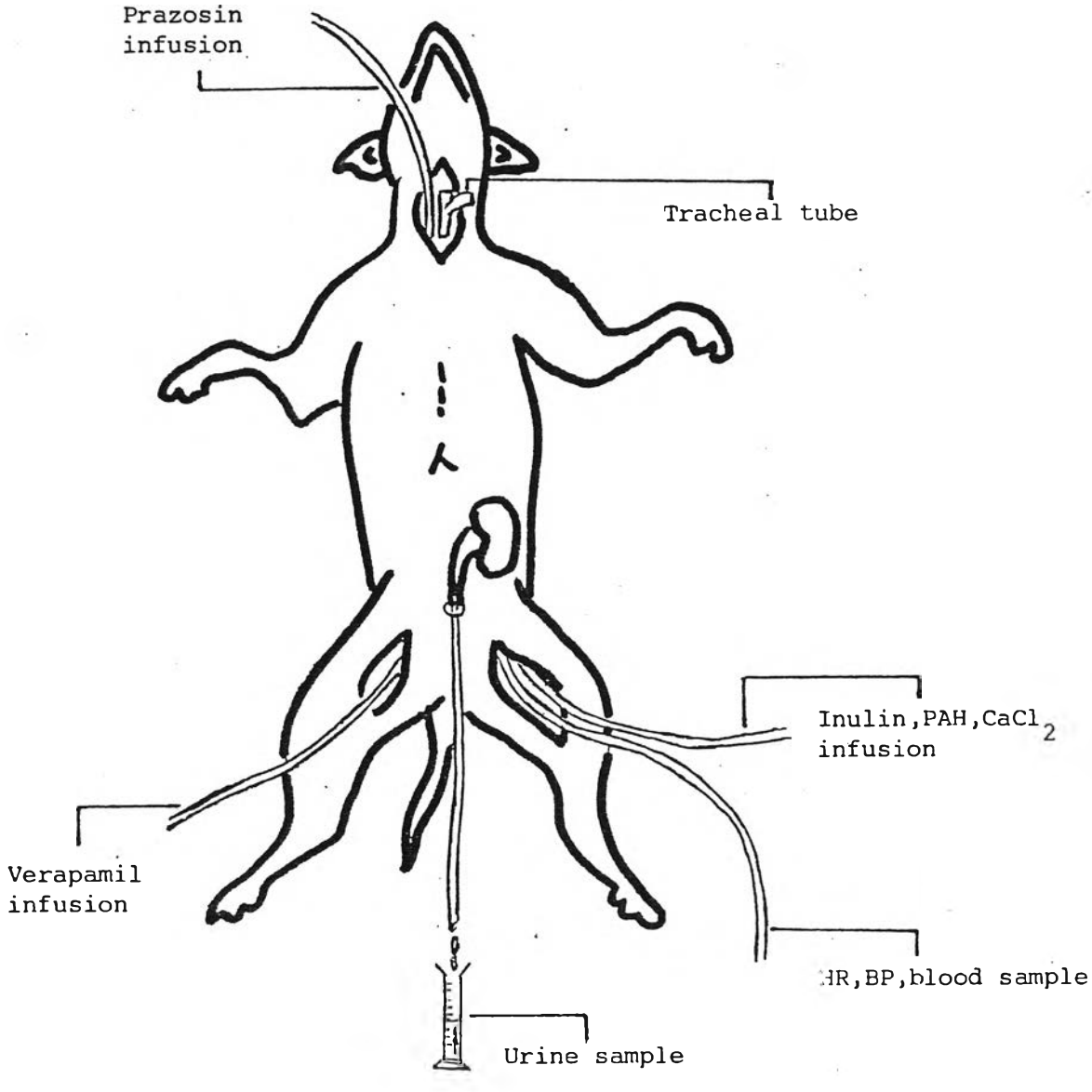


Fig.A : Scheme of experiment

recording of arterial blood pressure and heart rate (Grass Model 7 Polygraph). Left ureter reached by paracostal incision with a retroperitoneal approach and tubulated with polyvinyl catheter (PV 190) for urine collection .

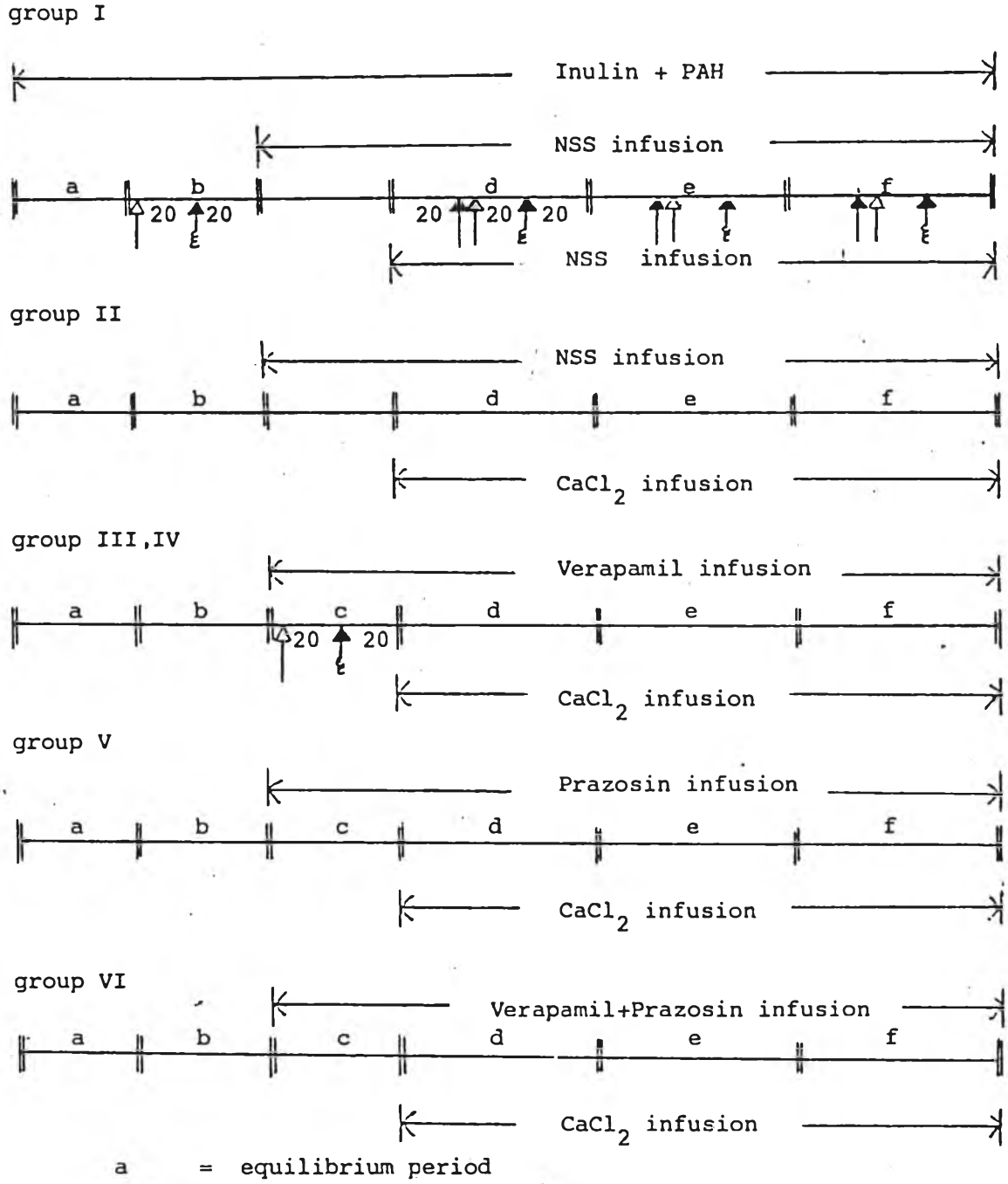
After an hour of infusion of inulin and PAH solution, and the rate of urine flow stabilized, urine samples were obtained during 20 minutes collection. Blood samples were obtained at the midpoint of the urine collection. Blood and urine samples were collected for measurement of inulin clearance, PAH clearance, osmolality, sodium, potassium, chloride, calcium, inorganic phosphorus concentration and also blood sample for measurement of packed cell volume.

Experimental protocols

As shown in Fig.B . Twenty-nine dogs were devided into six groups.

Group I : Four dogs were used as control animals. After 1 hour of infusion of sustaining inulin and PAH solution, the control sample of urine and arterial blood were obtained. Isotonic saline solution (NSS) as a control solution was performed by intravenous infusion at a rate of 1 ml/min via right femoral vein throughout the experiment.

Group II : Five dogs were used in this group. After 1 hour



a = equilibrium period

b = control period

c = before $CaCl_2$ infusion period

d,e,f = after $CaCl_2$ infusion period at 1^{st},2^{nd} and 3^{rd} hr.

20 = 20 minutes interval of urine collection

△ = the time that collect series blood samples for CO

▲ = the time that collect blood sample

ξ = the time that collect blood sample and collect for PV

Fig.B : Diagramatic illustation of experimental protocols.

of infusion of sustaining inulin and PAH solution, the control sample of urine and arterial blood were obtained. The 400 mEq/L calcium chloride solution was performed immediatedly by intravenous infusion with 2.5 ml/kg as the priming dose and followed by sustaining; which mixed in the sustaining clearance solution, in the dose of 0.025 ml/kg/min while NSS was still continuously infusion via right femoral vein throughout the experiment.

Group III : Five dogs were treated in the same manner of gr II. But 40 minutes before infusion of calcium chloride solution, the animals were pretreated with a low dose of calcium channel blocker (Verapamil), which was infused intravenously with the priming dose of 0.2 mg/kg and followed immediatedly by sustaining in the dose of 6 µg/kg in the rate of 1 ml/min in replacing of NSS via right femoral vein throughout the experiment.

Group IV : Five dogs were treated in the same manner of gr.III, but animals were pretreated with a high dose of Verapamil which was infused intravenously with the priming dose of 0.4 mg/kg and followed immediatedly by sustaining in the dose of 12 µg/kg in the rate of 1 ml/min via right femoral vein throughout the experiment.

Group V : Five dogs were treated in the same manner of gr.III, but animals were pretreated with the selective alpha-1 adrenergic blocker (Prazosin) which was infused intravenously with the priming dose of 1.15 mg/kg and followed immediatedly by sustaining in

the dose of 20 µg/kg in the rate of 1 ml/min via jugular vein throughout the experiment.

Group VI : Five dogs were treated in the same manner of gr.III, but animals were pretreated with the combination both of the high dose of Verapamil and Prazosin. The sustaining dose of the combined drugs were infused intravenously at the rate of 1 ml/min throughout the experiment.

Determination of cardiac output and plasma volume

Both cardiac output and plasma volume were measured by dye dilution technique, using Evan's 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 femoral artery immediatedly, with 3-5 second after dye injection. Serial samples of arterial blood collected by mean 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 samples 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 spectophotometry. Cardiac output was determined by dye dilution technique and was calculated as described by Hamilton et al. (1948). Plasma volume was calculated from the following equation.

$$PV = \frac{Id}{cd}$$

Where Id is total concentration of dye T-1824 bolus injection.

cd is the concentration of dye in the plasma at 15 minutes after dye injection.

Blood volume was estimated from plasma volume and packed cell volume as follow :

$$\text{Blood volume} = \frac{\text{Plasma volume} \times 100}{100 - \text{PCV}}$$

Determination of blood and urine samples

Plasma and urine inulin concentration were determined by the antrone method as described by Young and Raisz (1952). Determination of plasma and urine PAH concentration were carried out by the method of Marshall as modified by Smith (1962). Using the Frick's principle, PAH clearance was used for determination of effective renal plasma flow (ERPF) and inulin clearance was used for glomerular filtration rate (GFR).

The sodium and potassium concentration in plasma and urine were determined by flame photometer (Klina flame operating, BecKman instrument), chloride by chloride/carbon dioxide analyzer (BecKman instrument), calcium by colorimetric method of Moorehead and Biggs (1974), inorganic phosphorus by the method of Gomeri (1942), osmolality

by the freezing point osmometer (Advance osmometer model 3). Packed cell volume was determined by the preparation of blood in an international microcapillary centrifuge (Adam micro hematocrit centrifuge, model 850 Ta) and measured with an international microcapillary reader (Hawkley micro hematocrit).

Calculation :

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

Pulse pressure (PP) = $Ps-Pd$

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

Effective renal plasma flow (EFRP) = $\frac{U_{PAH} \times V}{P_{PAH}}$

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

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

Renal fraction (RF) = $\frac{ERBF \times 100}{CO}$

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

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

Urinary electrolyte excretion $= U_e \times V$

Fractional electrolyte excretion (FE_e) $= \frac{U_e V/P_e \times 100}{GFR}$

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

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

Statistical Analysis

Data were expressed as the mean value±S.E., the paired t-test was used to estimate the statistical significance between values obtained from control period and from each experiment period. The unpaired t-test was used to estimate the statistical significance of the differance between values obtained from the control group and each group of the experiment.