CHAPTER 3

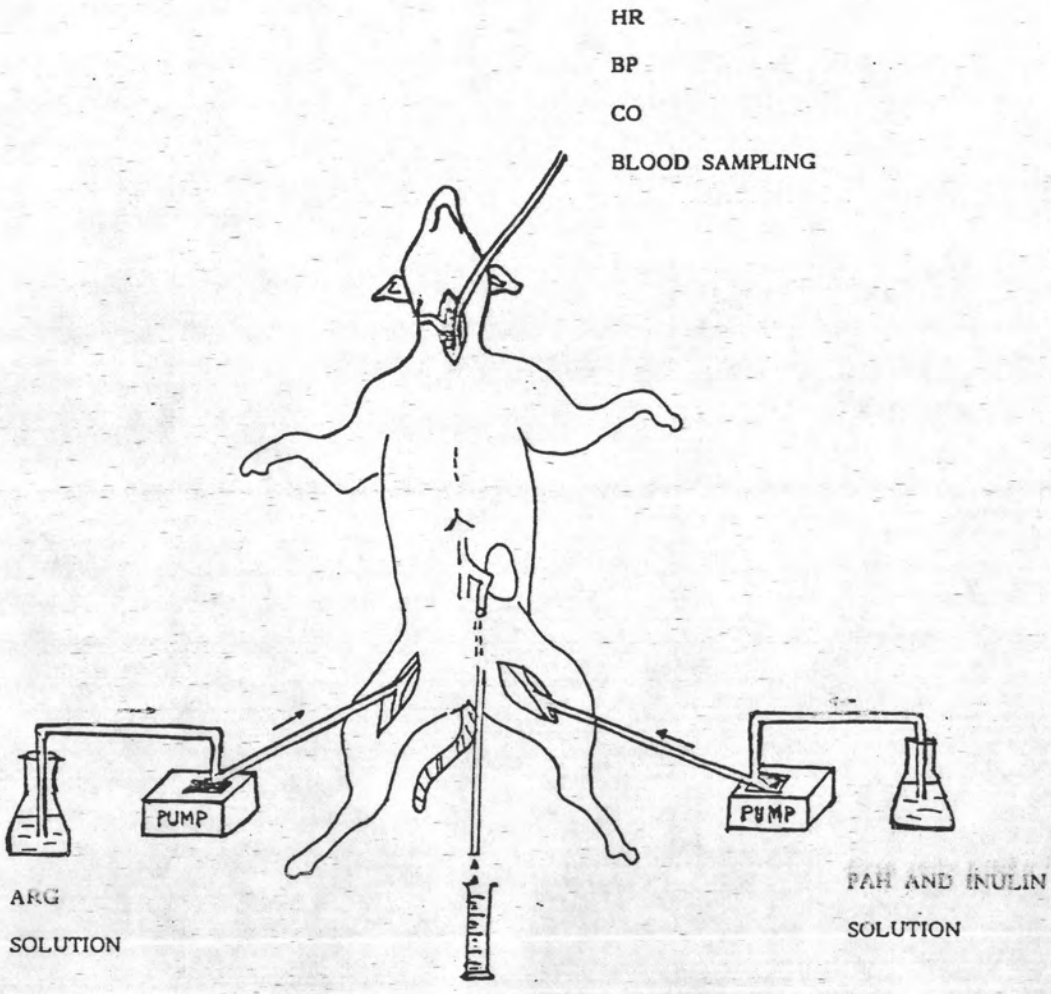
Materials and Methods

All experiments were performed in 10 adult male mongrel dogs weighing from 9.5 to 14.5 kg.

Animal preparation

After fasting for 12 hours, the dog was anesthetized with pentobarbital sodium (25 mg/kg). To maintain the state of light anesthesia, supplemented doses of pentobarbital (30-60 mg) were administered as required during the study. After tracheostomy tube insertion, both femoral veins were cannulated with polyethylene tubes (PE 200) : one for the infusion of inulin and para-aminohippurate (PAH) and the other for the infusion of ARG. The left carotid artery was also cannulated with polyethylene tube (PE 240) which was connected to a pressure transducer (Stratum PE 23 AA) for blood sampling and measurement of heart rate, arterial blood pressure (Grass Model 7 Polygraph), and cardiac output. Following left flank incision, left ureter was catheterized via retroperitoneal approach with polyvinyl catheter (PV 190) (Figure A).

FIGURE A SCHEMA OF THE EXPERIMENT



Experimental protocols

The animals were divided into 2 groups

In each group the experiment was divided into 8 periods. Each periodwas 30 min. The parameters in each period were shown in Table 1.

Group 1

An isotonic saline solution was injected at a bolus dose (10 ml/kg) into one femoral vein and continuously infused at a rate 1.5 ml/min throughout the experiment. A priming solution containing inulin 7.5 g/dl and PAH 1.2 g /dl in isotonic saline was administered at a dose 0.5 ml /kg into the other femoral vein and the sustaining solution containing inulin 0.75 g/dl and PAH 0.12 g/dl wasinfusedat a rate 0.5 ml/min throughout the experiment. After one hour of infusion when inulin and PAH concentrations in plasma reached steady levels and steady urine flow was obtained, the control period started. Urine was collected at 30 min interval and a sample of blood was drawn at the mid point of each urine collection for determination of inulin and PAH clearances, osmolarity, sodium, potassium, and hematocrit. The heart rate and the arterial blood pressure were recorded just before blood sampling and CO was measured approximately 5 min after blood sampling. Following the control period, 45 ml of isotonic saline solution containing ARG at a dose 2.5 mmol/kg was infused at a rate 1.5 ml/min within

Table 1 The parameters measured in both groups

Period	Parameter
Control	GFR, RPF, CO, MAP, HR, Hct
1st ARG	Na, K, Osmolarity in urine and plasma
A*	GFR, RPF, MAP, HR, HCT
B*	Na, K, Osmolarity in urine and plasma
IND	The same as control and the first ARG
2nd ARG	
C**	The same as A and B
D**	

Each period is 30 min

* Period after the first ARG infusion

** Period after the second ARG infusion

30 min. Inulin and PAH clearances representing glomerular fitration rate (GFR) and renal plasma flow (RPF) were determined during ARG infusion, 30 min and 60 min after infusion. CO was measured only during ARG infusion. Then indomethacin (IND) at a dose 10 mg/kg dissolved in isotonic saline was injected into the femoral vein within 1 min. CO and renal clearances were studied within 30 min interval after IND injection. ARG at the same dose was again infused for 30 min. CO and renal clearances were studied during the infusion. Sixty minutes after ARG infusion, only renal clearances were studied within each 30 min interval.

Group 2

In 5 dogs ARG at dose 5 mmol/kg was used instead of ARG 2.5 mmol/kg. This was followed by indomethacin (10 mg /kg) and again by ARG (5 mmol/kg) CO, inulin and PAH clearance were determined in the same order as in group 1.

Analytical methods

Inulin and PAH concentrations were determined by methods as described by Davidson et al (1963) and Smith (1962) respectively. CO was measured by dye-dilution technique using Evans blue dye as previously described (Chaiyabutr, Faulkner and Peaker, 1980). Osmolarity was measured by cryoscopic osmometer (OSMOMAT 030, GONOTEC).

Sodium and potassium were measured by flame photometer (KLINA FLAME, BECKMAN).

Calculation

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

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

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

Glomerular filtration rate (GFR) = $\frac{Uin \times 100}{Pin}$

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

Renal vascular resistance (RVR) = $\frac{\text{Mean arterial pressure}}{\text{Renal blood flow}}$

Total peripheral resistance (TPR) = $\frac{\text{Mean arterial pressure}}{\text{Cardiac output}}$

Fractional excretion of electrolyte = $\frac{Ue \times V / Pe \times 100}{GFR}$

Osmolar clearance (Cosm) $= \frac{Uosm \times V}{Posm}$

Free water clearance ($C H_2O$) $= V - Cosm$

Statistical analysis

All values were presented as mean ± SEM. Values within the same group were analysed by paired t-test. A p value <0.05 was statistically significant.