CHAPTER III

MATERIALS AND METHODS

Animal preparations

Studies were conducted on 20 male mongrel dogs weighing 8-16 kilograms and devided into four groups. The animals were fasted for 12 hours prior to study. On the day of the experiment, they were anesthetized with intravenous injection of sodium pentobarbital 25-30 mg/kg.bw and supplemented dose of 1-2 mg/kg.bw to maintain a state of anesthesia throughout the experiment.

Tracheostomy was performed and the tracheal tube was inserted into the trachea for prevention air way obstruction under anesthetic condition. The femoral artery was cannulated with polyethylene tube (PE200) for the recording of arterial blood pressure and heart rate (Polygraph Model 79, Grass Instruments Co.) and for blood sample collections. The femoral vein was cannulated with polyethylene tube (PE180) for intravenous infusion of isotonic normal saline 10 ml/kg.bw to replenish fluid loss during surgical procedure and intravenous fluid infusion for renal clearance studies. A left flank incision was made and the left ureter was cannulated via a retroperitoneal approach with a polyvinyl catheter (PV190) for urine collection. A hook shape 21 gauge needle attached to polyvinyl catheter (PV80) was placed at the base of the left renal artery, antrograde to renal artery flow, for intrarenal artery injection of Sch 28080 10 μmol/kg.bw dissolved with DMSO 400 μl and intrarenal artery infusion of isotonic normal soline at a rate of 0.9 ml/min, with a constant syringe pump (Harvard apparatus intravenous syringe pump).

After the surgical procedure, the renal clearance studies were performed. The priming solution containing insulin 5 gm% and p-aminohippuric acid (PAH) 1.2 gm% in isotonic normal saline was administered 0.5 ml/kg.bw then the sustaining solution containing insulin 500 mg% and PAH 120 mg%, was infused with constant peristaltic infusion pump. (Eyla model 3) at the rate of 1.4 ml/min throughout the experiment.

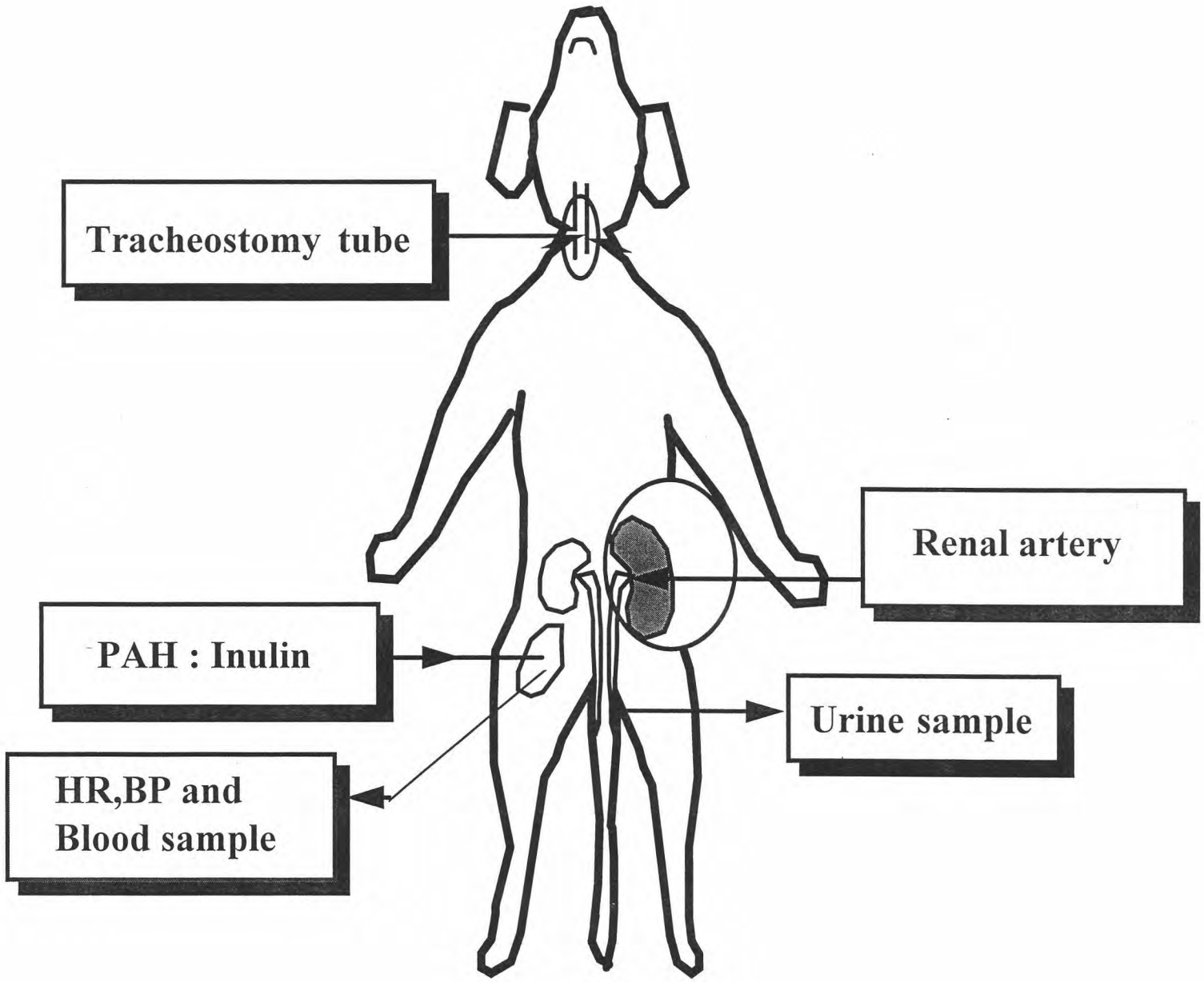


Figure A Scheme of experiment

Experimental design

Twenty dogs were devided into four groups.

Group I Five dogs were used as control animals. Before the experimental studies, animals were allowed free access to tap water and normal diet.

Group II Five dogs were induced to be hypokalemia by using oral furosemide administration in a dose of 120 mg/day for 14-20 days and stop drug administration 24 hours before experiment.

Group III Five dogs were bilaterally adrenalectomized (ADX). They were anesthetized with intravenous injection of sodium pentobarbital 25 mg/kg.bw. Bilaterally adrenalectomy of each animal was performed through flank Incision.

Group IV Five dogs were bilaterally adrenalectomized and pretreated to create furosemide induce hypokalemia(HypoK+ADX) in the same manner as group II.

Each adrenalectomized dogs were supplemented with daily intramuscular injection of prednisolone acetate 2.5 mg/kg.bw.

After equilibration time, at least 60 minutes after the solution infusion and the rate of urine flow was steady, duplicated samples for renal clearance studies were begun for control period. Arterial blood sample was collected with a midpoint of urine collection.

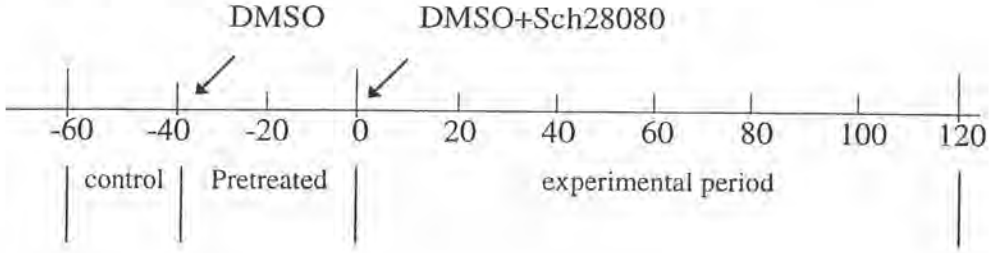
After completion of control measurements, all animals were pretreated with intrarenal arterial injection of 400 µl dimethyl sulfoxide (DMSO).

After two pretreated period of 20 minutes, Sch28080 (10 µmol/kg.bw) in 400µl of DMSO was administrated by intrarenal artery injection.

Blood pressure and heart rate were recorded. Blood and urine samples were collected over periods of 20 minutes for 9 periods.

In all groups, blood and urine samples were used for measurements of hematocrit (Hct), pH, pCO_2, electrolyte concentrations and urinary tritable acid.

Inulin clearance and p-aminohippuric acid clearance were measured for recording renal hemodynamics.



Determination of blood and urine samples

The glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were calculated from inulin and PAH clearance by using the Fick's principle. Plasma and urinary inulin concentration were determined by the anthrone method which modified the method of Young and Raisz (1952). Determination of plasma and urine PAH concentrations were carried out by the method of Bratton and Marshall as modified by Smith (1962). Effective renal blood flow (ERBF) was calculated from ERPF and hematocrit value (Hct).

Filtration fraction (FF) was calculated from GFR and ERPF. Renal vascular resistance (RVR) was calculated from MAP and ERBF.

The compositions in the plasma and urine were determined as followed : Sodium and potassium concentrations by flame photometer (Clinical Frame Photometer 410C : Corning Ltd.), chloride concentration by chloridometer (Chloride Analyzer 925 ; Corning Ltd.) and osmolality by the freezing point osmometer (Advanced Digimatric Osmometer model 3D3)

The pH measurements in plasma and urine were determined by the electrometric techniques with glass electrode pH meter (Hanna Instrument) 8520). Arterial blood gas was determined by blood gas analyzer (Blood gas analyzer 238 ; Corning Ltd.).

Pack cell volume was determined by microcapillaries centrifuge (Adams micro hematocrit centrifuge, model 850 Ta), and determined by international microcapillary reader (Hawkley micro-hematocrit reader).

The urinary titratable acidity and ammonium were determined by titrating a fresh specimen of urine with 0.05 N NaOH, using 1% phenolphthalein as an indicator

which modified the method as discribed by Conners (1975). In brief, the titration of weak acid with strong base were performed as following. 2.5 ml of urine sample was transfered to the flask containing 1 gm of potassium oxalate powder and 1 drop of 1% phenolphthlein indicator solution.

The direct titration of titrable acid (TA) was carried out by adding the standard 0.05 N NaOH, from a buret to the solution of sample. The end point was marked by the first permanent pink color (pH 8.2).

The volume of titrant required to reach the end point was read from the calibrated scale of the buret and calculated the normality of titrable acid and urinary titratable acid excretion.

To the above titrated mixture, added 2 ml of 37% formaline solution and $CaCO_3$ and mix well. Titrate again with 0.05 N NaOH to the first permanent pink color. The volume of titrate required to reach the end point was read and calculated the normality of ammonium and the urinary ammonium excretion.

$$\text{Normality of TA or } NH_4^+ = \frac{\text{0.5 N NaOH x Volume of NaOH was used}}{\text{Volume of urine (2.5 ml)}} \times 1{,}000$$

$$= \mu\text{Eq/ml of urine}$$

$$\text{Urinary TA or } NH_4^+ \text{ excretion} = \text{Normality of TA or } NH_4^+ \times \text{Urine flow rate}$$

$$= \mu\text{Eq/min/kg.bw}$$

$$\text{Urinary flow rate (V)} = \frac{\text{Volume of urine}}{\text{Time collection} \times \text{kg.bw}}$$

$$= \text{ml/min/kg.bw}$$

Calculation

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

Pulse pressure (PP) = Ps-Pd

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

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

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

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

Filtered load = GFR × Pe

Renal vascular resistance (RVR) = $\frac{MAP}{ERPF}$

Urinary electrolyte excretion = Ue × V

Fractional electrolytes excretion (FEe) = $\frac{(Ue \times V/P) \times 100}{GFR}$

Fractional water excretion = $\frac{V \times 100}{GFR}$

Urinary acid excretion(UAE) = $(U_{TA}V + U_{NH_3}V)$

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

Free water clearance (C H_2O) = V - Cosm

Free water reabsorption = Cosm-V

Statistical Analysis

All the data were presented as the means±SEM. statistical significance of thedifferences betaween period in the same group was determine by student's pair t-test P values less than 0.05 was considered significant.