Materials and Methods

Animal preparation :

Twenty adult male mongrel dogs, weighing from 10-15 kg., were used in the experiment. On the day of the experiment, the dog was anesthetized with pentobarbital sodium 25 mg/kg.bw. intravenously and were subsequently given small maintenance doses as necessary. The tracheal tube was inserted by tracheostomy. A femoral vein cutdown was performed and a polyethylene catheter (PE 180) placed into the inferior vena-cava to infuse the solution. Before the clearance study was performed, the priming solution containing p-aminohippurate (PAH) 8 mg/kg.bw. and inulin 50 mg/kg.bw. in isotonic saline (adjust to pH 7.4) of 1 ml/kg.bw. was injected intravenously into femoral vein and followed immediately by sustaining solution at the rate of 1.5 ml/min. by using Peristaltic Pump (Eyla model 3). The composition of the sustaining solution contained PAH and inulin 2.5 mg. and 5 mg. in 1 ml. of 0.9% NaCl respectively. Polyethylene tube (PE 200) was inserted in the common carotid artery for blood collecting and connected to the pressure transducer (PE 23 AA) for recording blood pressure and heart rate by polygraph (Grass model 7). Both of ureters were reached by bilateral flank incisions with a retroperitoneal approach and tubulated with polyvinyl catheter (PV 190) for urine collection. After an hour of infusion, urine samples were obtained during 10 minutes collection. Blood samples were taken at the midpoint of the urine collection. Blood and urine sample were collected for determination of inulin clearance, PAH clearance, plasma concentrations of osmolality,

sodium, potassium and chloride. Blood samples were measured packed cell volume by international microcapillary centrifuge. After the control period, the one ureter was canulated and attached with pressure transducer (PE 23 AA) and the ureteral pressure was recorded on the Grass recorder. Before the beginning of experiment, the pelvic pressure was allowed rising to the stabilized level.

Experimental procedures :

Four groups of five animals each were used to study the effects of indomethacin on cardiovascular and renal variables.

Group I Administration of indomethacin after hypertonic solution (3% NaCl) injection.

Group II Administration of indomethacin after hypotonic solution injection (2.5% Dextrose in water)

Group III Administration of indomethacin after furosemide injection.

Group IV Administration of indomethacin after anti-diuretic hormone injection (Arginine Vasopressin)

1	CONTROL	U.OBSTRUCTION	3% NaCl	INDOMETHACIN
2	CONTROL	U.OBSTRUCTION	2.5% D/W	INDOMETHACIN
3	CONTROL	U.OBSTRUCTION	FUROSEMIDE	INDOMETHACIN
4	CONTROL	U.OBSTRUCTION	ADH	INDOMETHACIN

On the day of the experiment, four periods of experiments were carried out in each group as following:

The first period : Cardiovascular and renal variables were performed as a control.

The second period: The ureter was obstructed by attachment Polyvinyl catheter to a pressure transducer and let the pelvic pressure elevated to stabilized levels (30 min.). Cardiovascular and the contralateral kidney variables were performed.

The third period : The experiments were carried out in each group as following;

Group I : The animals were given 3 per cent NaCl intravenously into femoral vein at the dose 2.2 ml/kg.bw. to elevate plasma osmolality to 10 per cent (Fenner, 1982)

Group II :The animals were given 2.5 per cent Dextrose in water intravenously into femoral vein at the dose 18 ml/kg.bw. to decline plasma osmolality to 10 per cent (Fenner, 1982)

Group III: The animals were given furosemide intravenously into femoral vein at the dose 5 mg/kg.bw.

Group IV : The animals were given arginine vasopressin 1 unit by intramuscular injection.

After an administration of the solution in this period, pelvic pressure was observed.

General hemodynamics and contralateral renal functions were obtained.

The fourth period: A solution contained 5 mg/kg.bw. of indomethacin was injected into the femoral vein. (Indomethacin was dissolved in water alkalinized with equimolar $NaCO_3$ in 0.9% NaCl 10 ml.). Circulatory hemodynamics, pelvic pressure and contralateral renal functions were evaluated for 45 minutes after indomethacin.

Protocols :

Time (min)	Period	Ipsilateral kidney*	Contralateral kidney*	General circulation
0-60	1	Control	Control	Control
60-90	2	Ureteral obstruction Record pelvic pressure.	Record renal functions.	Record general circulations.
90-120	3	group 1 Hypertonic solution injection i.v. group 2 Hypotonic solution injection i.v. group 3 Furosemide injection i.v. group 4 ADH injection i.m.		
		Record pelvic pressure.	Record renal functions.	Record general circulations.
120-165	4	Indomethacin injection i.v.		
		Record pelvic pressure.	Record renal functions.	Record general circulations.

Determination of cardiac output and plasma volume.

Both cardiac output and plasma volume were measured by dye dilution technique, using Evans 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 the carotid artery immediately, with 3-5 seconds after dye injection. Serial samples of arterial blood were collected by means of peristaltic pump and fraction collection. Each of sample approximately 1 ml/sec. was collected for a period of 10-14 seconds. Then the amount of dye in each blood sample was determined respectively by spectophotometry. In order to determine the plasma volume, a control sample of blood was collected before dye injection and 15 minutes after dye injection and also determined by pectrophotometry. Cardiac output was determined by dye dilution technique and calculated as described by Hamilton et al.,(1948). The plasma volume was calculated by the method of Kolmer (1951). Packed cell volume was determined by the preparation of blood in an international microcapillary centrifuge (Clay-Adams) and measured with an international microcapillary reader.

The method of determination blood and urine samples.

PAH was determined by the method of Bratton and Marshall as modified by Smith (1962). Determination of inulin was carried out by the method of Schreiner as described by Smith (1962). Using the Fick's principle, PAH clearance was used for determination of effective renal plasma flow (ERPF) and inulin clearance for glomerular filtration rate

(GFR). The compositions of plasma and urine were measured as followed;

- Sodium and potassium by flame photometer (Klina flame operating; Beckman instrument)
- Chloride by chloridometer (Buchler digital chloridometry; Beckman instrument)
- Osmolality by the freezing point osmometer (Advance osmometer model 3)

Abbreviations and derivations of variables used in text and figures.

MAP = mean arterial blood pressure
HR = heart rate (beat/min)
PCV = packed cell volume (%)
TPR = total peripheral resistance (dyne-sec/cm^5)
RVR = renal vascular resistance (dyne-sec/cm^5)
V = urine flow rate (µl/min/kg.bw.)
P_{in} = plasma concentration of inulin (mg/ml)
U_{in} = urinary concentration of inulin (mg/ml)
C_{in} = inulin clearance (ml/min/kg.bw.)
P_{PAH} = plasma concentration of PAH (µg/ml)
U_{PAH} = urinary concentration of PAH (µg/ml)
C_{PAH} = PAH clearance (ml/min/kg.bw.)
P_{Osm} = plasma osmolality (mOsm/kg)
U_{Osm} = urinary osmolality (mOsm/kg)
C_{Osm} = osmolar clearance (µl/min/kg.bw.)
C_{H_2O} = free water clearance (µl/min/kg.bw.)
P_e = plasma concentration of electrolytes (mEq/L.)
U_e = urinary concentration of electrolytes (mEq/L.)
PP = pelvic pressure (mm.Hg)

The following calculation were performed :

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

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.) $= \frac{GFR \times 100}{ERPF}$

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

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

Urinary electrolytes excretion (U_eV) $= U_eV$

Fractional electrolytes excretion $= \frac{U_eV/P_e}{GFR} \times 100$

Renal fraction (R.F.) $= \frac{ERBF \times 100}{\text{cardiac output}}$

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

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

Statistical analysis

All data presented were normalized to individual body weight to allow comparison among the dogs. Data were reported as the mean ± S.D. The paired t-test was used to estimate the statistical significance of difference between value obtained from the control period and from each period of the experiment. The unpaired t-test was used to estimate the statistical significance of difference between value obtained from the control group and each group of the experiment.

Scheme of the experiment: