CHAPTER 3

MATERIALS AND METHODS

Experimental animals

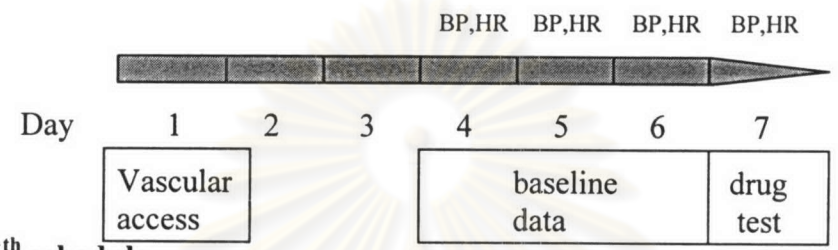
Ten healthy normal adult mongrel, mixed sex dogs (base on results of physical examination) weight from 15 to 20 kg were used in this study. The dogs were fed once daily with commercial diets (CP dog food®) and given water *ad lib*. Prior to beginning of the study, complete blood count, serum biochemical analysis, modified knott's test, fecal examination and complete urinalysis were evaluated to determine whether each dog was healthy. All dogs were vaccinated against common viral diseases and treated if there were ecto- and endoparasite infections.

Experimental protocol

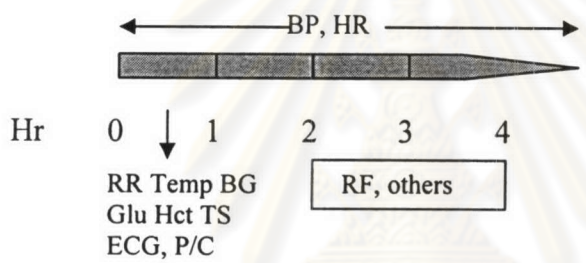
Before the experiment, the dogs were trained to get used to both investigator and experimental room. They were also trained to stand in a sling for recording of blood pressure. They were randomly divided into 2 groups. Group 1 (n=5), dogs received 0.2 mg/kg 0.2 % brimonidine ophthalmic solution *per os* and group 2 (n=5), dogs received 0.5 mg/kg of brimonidine ophthalmic solution *per os*. Each trial was conducted during an interval of 7 days. On day 1, vascular access was performed under general anesthesia for blood pressure measurement and sample collection. After two days of recovery period, thirty minutes baseline blood pressure was measured on days 4 and 5. On day 6, renal function, ECG, rectal temperature, blood glucose, blood gas, hematocrit, plasma total solid, and respiratory rate were determined. On day 7, all dogs received brimonidine following the assigned dose via single ingestion. Renal function was repeated four hours after brimonidine administration. Five hours after brimonidine administration, 0.1 mg/kg yohimbine HCl, specific antidote, was administered while renal function was reperformed. The blood pressure and heart rate were continuously recorded throughout the experimental period. Electrocardiogram,

rectal temperature and respiratory rate were recorded before and 4 hours hourly after brimonidine ingestion and after yohimbine administration. Blood glucose, blood pH, blood gas analysis, hematocrit and plasma total solid were measured before and third hour after brimonidine ingestion and after yohimbine hydrochloride administration (Fig. 3.1).

Trial schedule



Day 6th schedule



Day 7th schedule

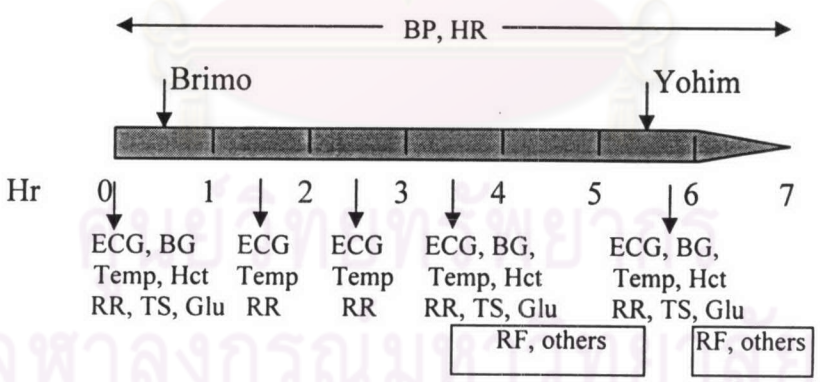


Figure 3.1- Experimental protocol time-line: Brimo = brimonidine, Yohim = yohimbine, BG = blood gas, ECG = electrocardiogram, RF = renal function, BP = continuous blood pressure monitoring, HR = heart rate, Temp = temperature, TS = total solid, Glu = glucose, Hct = Hematocrit, RR = respiratory rate, others = other parameters (including plasma osmolarity, plasma and urine electrolyte concentration)

Blood pressure and heart rate measurements

General anesthesia by halothane was performed in all experimental dogs. Left femoral artery was approached and polyvinyl catheter (inner diameter, 0.05-inch) was inserted. The catheter was used for blood pressure measurement and blood sampling. The free end of tube was tunneled under the skin to dorsal midline and exteriorized at thoracolumbar region. The exit catheter was connected with a pressure transducer (P23ID, Gould Electronics) that kept inside a dog jacket. Catheter was flushed before refilling with heparinized (1000U/ml) saline (0.9% NaCl) daily to prevent blood clot. The transducer was linked to the physiograph (Coulbourn instrument) during measurement of blood pressure. The signal was transformed by use of a specific software program and stored in a hard disk for subsequent analysis. Data were analyzed using a standard time series analysis technique. Signals were sampled (50 points/sec) and digitized to provide a mean of 3,000 sample points/min. Further analysis was performed, using a minute averaging of data to yield true blood pressure and heart rate.

Measurement of renal hemodynamics

Prior to renal clearance studies, food was withheld for 16 to 20 hours. Renal clearance procedures were performed with dogs resting in Pavlov slings without sedating or anesthetizing the dogs. Blood sample was obtained from femoral arterial catheter that was inserted on the first day for subsequent measurement of plasma concentrations of PAH, creatinine, osmolarlity and electrolytes. Following this collection of blood, an indwelling urinary catheter was placed. Then, creatinine/PAH solution (containing 0.65% of PAH, 1.25% of creatinine and 0.45% of NaCl in distilled water, pH 7.4) was administered subcutaneously as priming dose (6 ml/kg body weight) and maintainance dose (2 ml/kg) 35 minutes apart which were sufficient to maintain stable plasma concentrations of each compound during urine collections. After an equilibration period of 50 minutes (15 minutes after maintenance dose), the urinary bladder was emptied and rinsed with sterile water. Subsequently, 3 consecutive 20-minute (approx.) timed urine collections were made. The bladder was

standard deviation of MAP and HR in each hour of all dogs in either group. Osmolar clearance (Cosm) was calculated as a ratio between rate of osmolyte excretion (Uosm·V) and plasma osmolarity. The fractional excretion of the electrolytes was calculated by a ratio between clearance of electrolyte and GFR. Free water clearance (C_{H2O}) was calculated as a subtraction between Cosm and urine flow rate. The R-R interval was calculated as an inversion of heart rate and multiplied by 60 second.

Other measurements

The rectal temperature was measured for a minute by a mercury-thermometer. The respiratory rate was counted for a minute by the same investigators. The electrocardiography was performed by using ECG recording electrode linked Mortara instrument ELI 100 electrocardiograph.

Parameters

1. Mean arterial blood pressure (MAP).
2. Heart rate and R-R interval.
3. MAP/R-R interval.
4. Standard deviation of mean arterial pressure and heart rate (SDMAP and SDHR).
5. Lead II electrocardiogram (Q-T, P-R interval, duration of P wave and QRS complex, and amplitude of P and R waves).
6. GFR, ERPF, RBF and filtration fraction (FF).
7. Renal vascular resistance (RVR).
8. Fractional excretion (FE) of sodium, potassium, chloride, phosphorus and calcium.
9. Osmolar clearance ($U_{osm}\cdot V/P_{osm}$).
10. Free water clearance (C_{H2O}).
11. Urine output.
12. Arterial blood gas analyses (P_{CO2}, P_{O2}, pH, HCO_3 and O_2 saturation).
13. Hematocrit (Hct).
14. Plasma total solid.

15. Blood glucose concentration.
16. Rectal temperature (RT).
17. Respiratory rate (RR).

Data analyses

Data were expressed as means ± SE. Unless indicated, the results were compared by parametric method, one-way ANOVA with repeated measures design and post hoc analysis with Student-Newman-Keuls method were used to compare the data in all pairwise (i.e. renal function, fractional excretion of the electrolytes, C_{OSM}, C_{H2O}, urine output, Hct, TS, and blood glucose) and Dunnett's method was used to compare the data versus control values (i.e. MAP, HR, RR, EKG parameters, RR and rectal temperature). Non-parametric method was used in some data which normality failed, one way ANOVA with repeated measures on ranks was used. Differences between means were considered statistically significant at $P<0.05$. Sigma-stat software was used for statistical analysis.