MATERIALS AND METHODS

Experiments were performed on male and female rats (Wistar strain), weighing between 350-400 gm. They were obtained from the National Animal Center, Salaya Campus, Mahidol University. After arrival, the animals were allowed to adapt to the new environment for at least 7 days. All animals were housed in the animal room where the temperature was maintained approximately at 25° C with 12 : 12 hr dark-light cycle and received chow and water ad libitum.

Animal Preparation

All studies were started at approximately the same time of the day, about 9.00 a.m. Prior to each experiment, the animal was anesthetized with pentobarbital sodium (Nembutal sodium®, Abbott Laboratories, North Chicago, U.S.A.), 30 mg/kg of body weight intraperitoneally, and supplemental doses were given during the course of the experiment when necessary. The animal was then placed on a dissecting board in a supine position and the trachea was cannulated with a polyethylene cannula (internal diameter = 2 mm) to ensure a patent airway. Thereafter, the animal was allowed to breathe room air with a Harvard Rodent Ventilator (Model 683) throughout the experiment. The femoral vein was cannulated with a polyethylene cannula (PE 50, internal diameter = 0.58 mm) filled with heparinized saline (30 units/ml) for collection of blood samples and for infusion of solutions during the experiment.

For measurement of body temperature, the thermistor probe (YSI 423) was lubricated with petroleum jelly and was inserted upto at least

5 cm beyond the anal orifice. The rectal temperature was recorded continuously by a Tele-thermometer (YSI, Model 2100) during the experiment for use as an index of the changes of the core body temperature. Standard limb Lead II electrocardiographic needle electrodes were placed and the EKG was continuously monitored on a polygraph (Grass Instrument Co., Model 7, Quincy, Mass., U.S.A.).

Experimental Procedures

The animals were divided into 3 groups with 30 rats in group I and group II each and 45 rats in group III. The animals were given the chemicals as follows :

Group I Control group, the animals were given 0.9 % physiological saline solution (Maharaj Nakorn Chiang Mai Hospital, Thailand) 1 ml, intravenously.

Group II Lidocaine-treated group, the animals were given intravenous injection of 5 mg/kg body weight of Lidocaine hydrochloride (Xylocard®, Astra (Thailand) Co., Ltd.) dissolved in physiological saline solution and the volume of injection was adjusted to 1 ml. This dose was used according to the study of Olson, et al. (1984).

Group III Bretylium-treated group, the animals were given intravenous injection of 20 mg/kg body weight of Bretylium tosylate (Bretylol®, Dupont Merck, U.S.A.) dissolved in physiological saline solution and the volume of injection was adjusted as in Lidocaine-treated group. The dosage used is the maximum permissible dose for prophylactic effect against VF (Olson, et al., 1984).

All animals were given the chemicals mentioned above into the femoral vein as a single dose immediately before hypothermia procedure.

Hypothermia Procedure

Induced hypothermia was modified from the surface cooling technique of Horuichi and Koyamada (1964). Briefly, hypothermia was induced by immersing the animals in ice-water. The rectal temperature of the animals gradually declined at the rate of approximately 0.5°C/min and this surface cooling was continued until the rectal temperature reached 10°C or until ventricular fibrillation had spontaneously developed. The irregular EKG waves of more than 10 consecutive positive and negative deflections were defined as ventricular fibrillation. The incidence, the body temperature and the duration of ventricular fibrillation were recorded.

Collection of Blood Samples for Serum Potassium Analysis

Blood sample collection for serum potassium concentration determination was performed at normothermia (before induced hypothermia), at the core body temperatures of 25°C and 10°C, or when the ventricular fibrillation had immediately been defined. Then the experiment was stopped. At each blood sampling, approximately 0.7 ml of blood was withdrawn from femoral vein and the first 0.2 ml of blood was discarded before obtaining a sample for analysis. Immediately after each blood sampling, the animals received the same volume of physiological saline

solution for replacement of blood loss. All blood samples were collected into the glass test tubes and then were centrifuged by the centrifuge (H-103 N series, Kokusan Ensinki Co. Ltd., Japan) in order to separate serum from cellular constituents. Serum potassium concentrations were analyzed by Electrolyte Analyzer (Nova, Model Nucleus) using potassium ion selective electrodes.

Determination of the Relationship between Serum Glucose Concentration and Serum Potassium Concentration in Hypothermia

In order to determine the relationship between serum glucose concentration and serum potassium concentration as the body temperature declined, ten additional Wistar rats were subjected to the same hypothermia procedure and at each blood samplings the samples of 1 ml were collected and serum were separated for determination of potassium and glucose levels. The photometric glucose GOD - PAP method was used to determine serum glucose concentrations, using the special pack for multi-test analyser systems (Merck Mega, AU 510, Eris).

Statistical Methods

All of the data from the experiments were expressed as mean $\pm$ standard errors (mean $\pm$ SE). The numbers of animals that developed the ventricular fibrillation in each of the three groups, presented as percentage (%), were compared by a Hypothesis test for two proportions from independent groups. Statistic comparison between groups, as well as the changes of all parameters in each group, were performed using the unpaired Student's t test. The probability required for the significance was 95 % confidence ($p < 0.05$).