MATERIALS AND METHODS

Experimental animals

Adult Wistar rats of either sex, weighing 250-300 g, were supplied by the Animal house, Faculty of Science, Prince of Songkla University. They were fed with standard rat chow obtained commercially and tap water was given *ad libitum*. Rats were housed in groups of 6 (saline-treated, chronic morphine-treated and morphine-withdrawal rats) for each drug concentration (norepinephrine, epinephrine, isoproterenol and dopamine).

Morphine-dependent and morphine-withdrawal rats were prepared as described previously (Moises & Smith 1989). In brief, morphine tolerance and dependence were induced by giving repeated intraperitoneal (i.p.) injections of morphine sulfate every 8 h for 14 days. The dosage of morphine sulfate ranged from 10 mg kg^{-1}, 3 times a day (t.i.d.), on the first day, being doubled after every third day, to 100 mg kg^{-1}, t.i.d., on the last two days. Control animals were given i.p. injections of saline according to the same treatment.

Isolated rat atrial preparations

The rats were killed by a blow on the head and cutting the throat either 8 h (these animals constituted the morphine-dependent group) or 32 h (the morphine-withdrawal group) after a 14-day course of morphine treatment. The whole rat atria was isolated based on the method as described previously (Anonymous 1970). In brief, each isolated heart was removed as quickly as possible and placed in Krebs' solution of the following composition (mM) : NaCl 118.0, $NaHCO_3$ 25.0, KCl 4.7, $MgSO_4.7H_2O$ 0.57, KH_2PO_4 1.18, $CaCl_2$ 2.65 and glucose 11.0. All other tissue was cut away until nothing was left except the auricles. The isolated atria was mounted with 1 g preload tension in a 20 mL organ bath containing Krebs' solution aerated with 95% O_2 / 5% CO_2 and temperature was maintained at 35 °C. The pH of the equilibrated Krebs' solution was kept between 7.4 - 7.5. Then, the isolated atria was connected to a polygraph model 7D (Grass Instrument Co.,Quincy, Mass.,USA) and allowed to beat spontaneously. The atrial rate was recorded using a tachograph model 7 P44D (Grass Instrument Co.,Quincy, Mass.,USA) and a tension was directly read from the tracing obtained. Drugs were given in a single dose manner and the total volume of added drug was kept as small as possible and the maximal allowance was 1.0 mL.

Preparation of anesthetized rats

Both sexes of rats were anesthetized with sodium pentobarbital (35 mg kg^{-1}, Nembutal ^{R}, Abbott Laboratories, North Chicago, IL., USA) by i.p. injection. Each anesthetized rat was placed in a supine position, and its right jugular vein, left common carotid artery and trachea were

exposed surgically. A 25 cm length of polyethylene (PE-90) tubing (Intramedic^{R}, Clay Adams, NJ., USA) was inserted into the left common carotid artery for measurements of mean arterial blood pressure and heart rate via a pressure transducer model PT 300 and tachograph model 7P 44D (Grass Instrument Co., Quincy, Mass., USA), respectively coupled with a polygraph model 7D (Grass Instrument Co., Quincy, Mass., USA). A 15 cm length of PE-50 tubing was inserted into the right jugular vein for intravenous injections. The trachea was cannulated with a 3 cm length of PE-240 tubing to facilitate spontaneous respiration.

Drug administration

The isolated rat atrial preparation was equilibrated for at least 30 min with several changes of bathing medium. Each drug (norepinephrine, epinephrine, isoproterenol and dopamine) was added to the organ bath to give final bath concentration.

In each anesthetized rat, after a 30 min equilibration period, norepinephrine, epinephrine, isoproterenol and dopamine were injected at a dose range of 10^{-8} - $3x10^{-4}$ M which depended on each drug concentration used in the study. Each drug concentration was tested in 5 - 6 rats of the control group compared with chronic morphine-treated and morphine-withdrawal groups, respectively. Each injection was given at a fixed volume of 0.1 mL.

Drugs and chemicals

All chemicals used in this study were analytical grade reagents. *l*-Norepinephrine, *l*-epinephrine, *dl*-isoproterenol, and dopamine were purchased from Sigma Chemical Co., USA and were prepared fresh in Krebs' solution.

Data and statistical analysis

Increase of heart rate, mean arterial blood pressure or contractile force (tension) to all agonists were plotted against each drug concentration. The increase of mean responses between the control and experimental group of each drug concentration was compared by pairwised multi-comparisons of analysis of variance (ANOVA) followed by Duncan's multiple range test (*CLA* anova 2.0 software). The difference was assumed to be significant when $P < 0.05$. Each point in the curve represents the mean ± SEM of 5-6 measurements from different preparations.