CHAPTER II

MATERIALS AND METHODS

Preparation of the isolated aorta.

Male Wistar rats, weighting between 250 to 350 g, were sacrified by a blow on the head. The thorax was exposed and the thoracic aorta quickly excised. The vessel was then transfered to a glass dish containning Krebs Henseleit-solution with the following millimolar composition : NaCl 118.1 ; KCl 4.7 ; $MgSO_4$ 1:2 ; KH_2PO_4 1.2 ; $NaHCO_3$ 25 ; $CaCl_2$ 2.5 ; and glucose 11.1. The solution's temperature was kept at 37 °C and constantly gassed with the mixture of 95 % O_2 - 5 % CO_2. The vessel was carefully cleaned of adhering tissues, and spiral strip (2-3 mm. in width, 10 mm.in length) was prepared (Figure 3, 4). Each strip was tied on one end to a glass-hook and placed in 20 ml. jacketed organ bath. The other end was connected to a force-displacement transducer (Harvard) via a cotton string, with 1.0 g. of tension. The transducer was connected to a Harvard oscillograph recorder (Cat. No 50-9323) in order to monitor isotonic contractions. The physiological solution in the organ baths was prewarmed at 37 °C and aerated with the mixture of 95 % O_2 - 5 % CO_2. An one-hour equilibration period was allowed before conducting further experiments, during

which the Krebs-Henseleit solution was changed every 15 min.

The organ bath

The organ bath was composed of two compartments : the inner chamber, 20 ml in capacity, for tissues preparation, and the outer jacket for flow-through circulation of 37°C prewarmed water which provided constant temperature control to the inner compartment (Figure. 5) The circulating water was supplied by a thermoregulating water pump (Churchill type). The bath also had an oxygen inlet which provided oxygen mixture to the inner chamber through a sintered glass opening.

Drugs and chemicals

- Potassium Chloride (KCl) M.W. 74.56
- Histamine dihydrochloride M.W. 184.10
- 5-Hydroxytryptamine creatinine sulphate (5-HT) M.W. 387.40
- Norepinephrine hydrochloride M.W. 255.70
- Calcium Chloride ($CaCl_2$) M.W. 147.02
- Methysergide M.W. 469.54
- EGTA M.W. 148.0
- Verapamil hydrochloride M.W. 491.08
- Diltiazem M.W. 451.00
- Ancistrotectorine was naphthalene-isoquinolene alkaloid from Ancistrocladus tectorius (Lour.) Merr.

All chemicals used were of analytical grade. Distilled deionized water was used for the preparation of Krebs-Henseleit and drug solution. Ancistrotectorine was adjusted to form of chloride salt by adding HCl to pH 5-6.

Physiological Solution

Krebs-Henseleit solution was composed of the following ingredients :

- NaCl	118.10	mM.
- KCl	4.70	mM.
- $MgSO_4$	1.20	mM.
- KH_2PO	1.20	mM.
- $NaHCO_3$	25.00	mM.
- $CaCl_2$	2.50	mM.
- Glucose	11.10	mM.

Calcium-free solution was made by excluding calcium from the Krebs-Henseleit solution and adding the appropiate amount of EGTA (1.0 mM) to chelate the possible traces of calcium.

High potassium-depalarizing solution was made by substituting KCl for NaCl on an equimolar basis

A. Responses of the aorta to KCl, NE, 5-HT and histamine in Krebs-Henseleit solution. After equilibration period, the tissues were exposed to specified agonist in the cumulative administration

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regimen. The administration was made by using a microsyringe or an automatic micropipette. The contractions induced by each dose of an agonist were recorded until the steady pattern obtained before any other administration of further doses. Dose-response study was carried out with different concentration of the agonist and terminated when the maximum response revealed. One tissue was exposed to only one agonist throughout the experiment.

B. Effect of ancistrotectorine on the responses to aortic strips to KCl, NE, 5-HT and histamine in Krebs-Henseleit solution. After the dose-response study with a specific agonist had been established, the tissues were then washed in Krebs-Henseleit solution until the base-line resting tension was again obtained. Ancistrotectorine 1.19 X 10^{-5} M and 2.37 X 10^{-5} M were introduced into the medium and Left in contact with the tissues for 15 min before the introduction of the same agonist.

C. Responses in Ca^{2+} -free environment : In general, the procedures were the same as those in experiment A and B. Only the medium was changed by substituting Ca^{2+} -free solution containning 1.0mM EGTA for Krebs-Henseleit solution.

D. Response of the aorta to $CaCl_2$ in Ca^{2+} -free, high potassium-depolarizing solution. Aortic

strips which had been equilibrated in Krebs-Henseleit solution, were rinsed four times at 10-min intervals with 20-ml of Ca^{2+}-free physiological solution containning 1.0 mM EGTA. Later, they were equilibrated in Ca^{2+}-free solution for 30 min and then $CaCl_2$ was administered to the medium in an accumulative manner.

E. Effect of ancistrotectorine on the response of aortic strips to $CaCl_2$ in Ca^{2+}-free, high potassium-depolarizing solution. After equilibration in Ca^{2+}-free, high potassium depolarizing solution containning 1.0 mM EGTA for 30 min, the tissues were exposed to Ca^{2+}-free for 20 min then ancistrotectorine was introduced into the solution. The tissues were exposed to ancistrotectorine for 15 min before the introduction of $CaCl_2$

F. Effect of verapamil and diltiazem on aortic strips and the interaction of ancistrotectorine to these agents. After equilibration, the tissues were exposed to an agonist as in A, then the tissues were washed until the base-line resting tension was obtained, verapamil in the concentration of 1.0×10^{-7} M. or diltiazem in the concentration of 1.0×10^{-7} M was introduced into the solution, and Left in contact with the tissues for 15 min before the introduction of the agonist. Finally, these tissues were washed again with Krebs-Henseleit solution until the base-line

resting tension was obtained then a combination of verapamil (1.0×10^{-7} M) or diltiazem (1.0×10^{-7} M) and ancistrotectorine (1.19×10^{-5} M) was administered into the medium for 15 min before introducing the agonist. In all experiments, one tissue was exposed to only one agonist and 2 doses of ancistrotectorine (1.19×10^{-5} M, and 2.37×10^{-5} M)

G. Effect of methysergide and the interaction with ancistrotectorine. The tissues were exposed to 5-HT 5×10^{-8} M - 25×10^{-6} M until the maximum contractions were obtained after equilibration time, they were then washed with Krebs-Henseleit solution until the base-line resting tension was obtained again. methysergide 1×10^{-7} M was introduced 15 min before the administration of 5-HT as control, after that the tissues were washed again to obtain their base-line resting tension then the combination of methysergide 1×10^{-7} M and 1.19×10^{-5} M ancistrotectorine were introduced for 15 min before introducing of 5-HT. Exposing to methysergide 1×10^{-7} M and 2.37×10^{-5} M ancistrotectorine for 15 min was used in the later experiment.

Results were expressed as means standard errors of the means (S.E.M.). Statistical significance of the differences between control and drug-treated groups were determined by Student's paired t-test, with the level of significance (P-value) equals to 0.05.

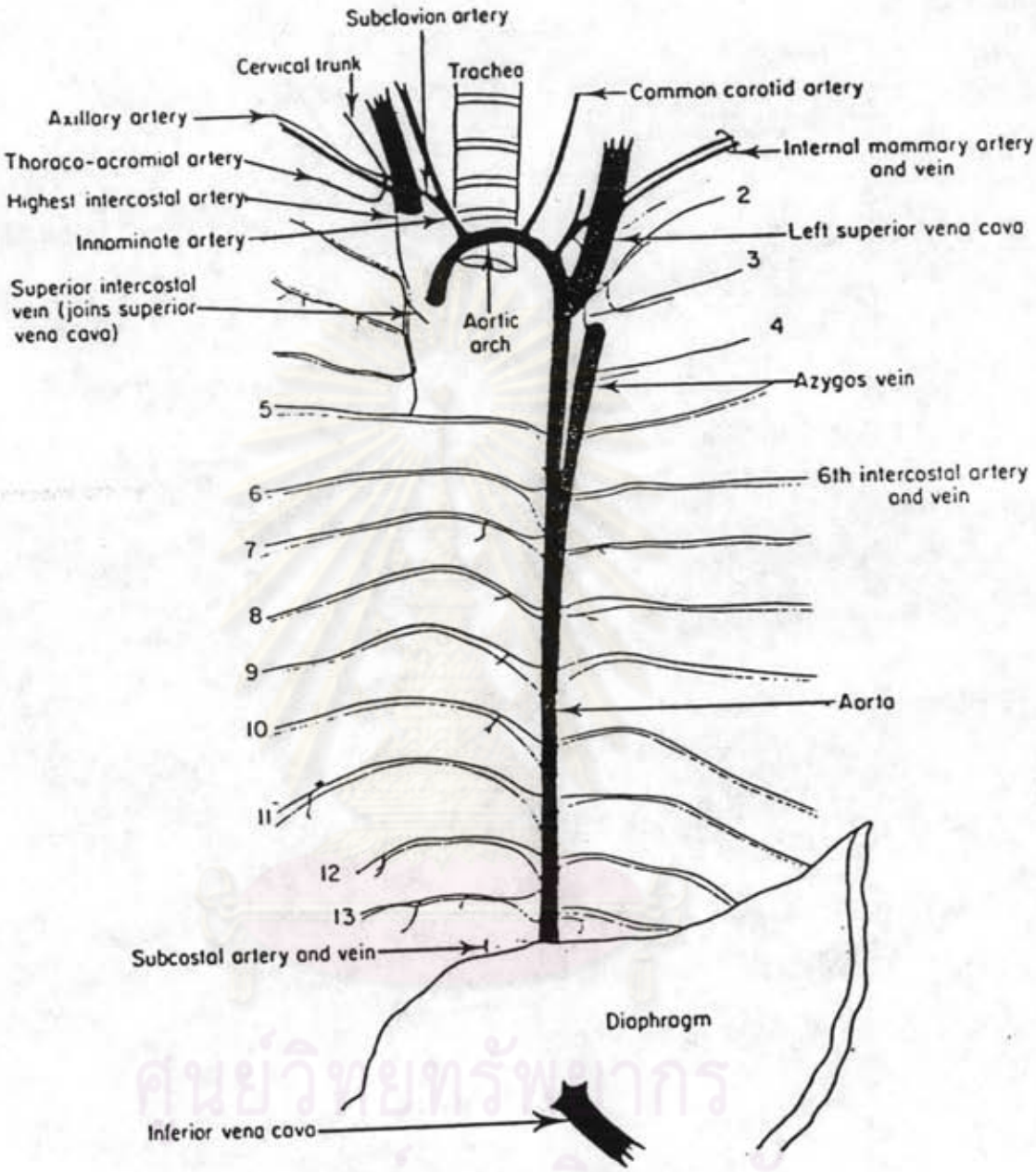

Figure. 3 The thoracic circulatory system of the rat

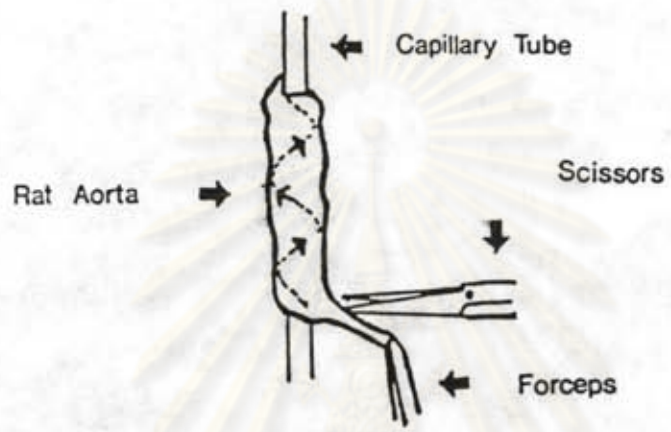

Figure. 4 The preparation of rat aortic strip

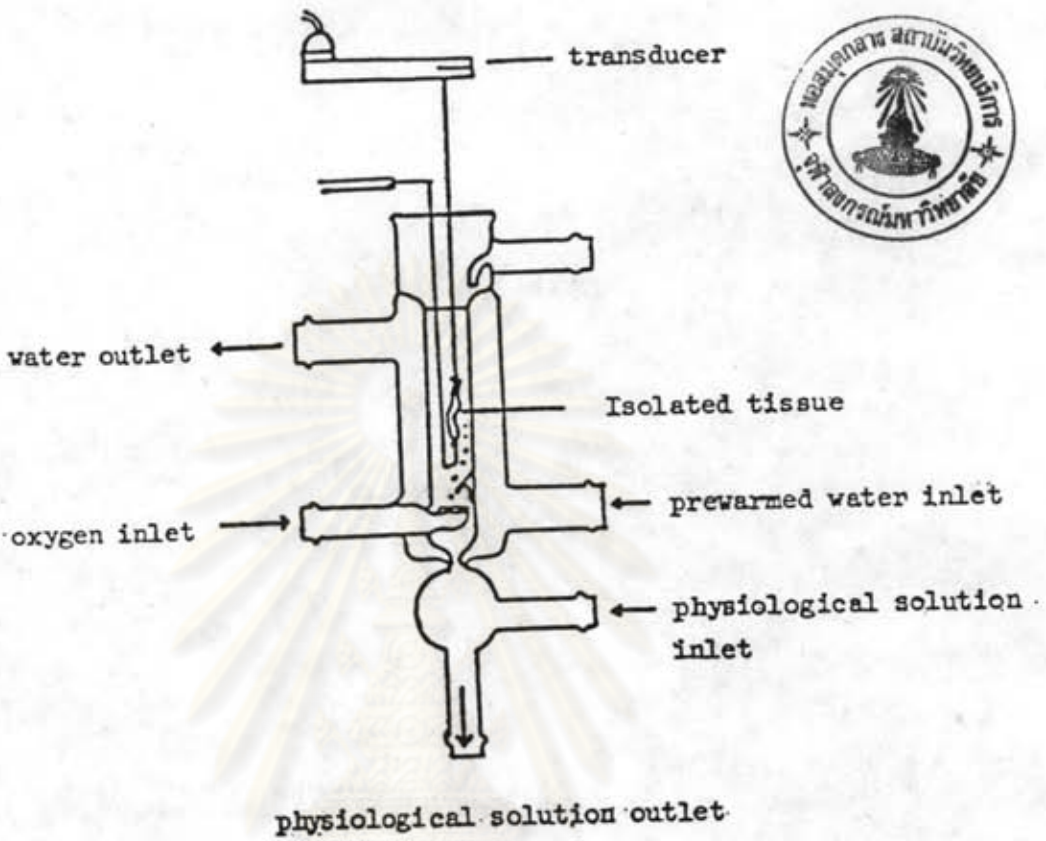

Figure. 5 The organ bath