Chapter 2

Methods and Materials

1. Preparation of the Isolated Rabbit Jejunum

Experiments were carried out on 1 cm segments of jejunum from rabbit of either sex. The rabbit, weighing 1.5-2 kg, were killed by a sharp blow on the skull base. The abdomen was opened, jejunum was cut into 1 cm segments whose contents were gently removed by washing with Tyrode's solution of the following composition (in g/l) 8 g NaCl, 0.2 g KCl, 0.3 g $MgSO_4.7H_2O$, 0.2 g $CaCl_2$ 0.05 g NaH_2PO_4, 1 g $NaHCO_3$ and 1 g glucose. After freeing one segment was suspended in 20 ml organ chamber of continuously oxygenated Tyrode's solution with the temperature controlled at 37 ± 1°C by means of a circulating water pump. One end of the strip was attached to a hook of the glass rod, the other end to a force-displacement transducer (Grass FT. 03C or Statham UC-2) by a thread and Beckman preamplifier or Biosciences Type FC 117. The ileum was equilibrated for 60 minutes under a resting tension of 1 g, before being exposed to any drug. During the equilibration period, the Tyrode's solution was changed every 30 min. Isometric contractions were recorded with a Beckman R411 Dynograph recorder or Polygraph recorder (Washington 400 MD 2C).

2. Preparation of the Isolated Guinea-pig Ileum.

Guinea-pig of either sex, weighing 250-400 g, was killed by sharp blow on the skull base. The abdominal wall was opened. The ileum was

excised and 1 cm segments were removed from the middle and terminal regions. Their contents were gently removed by washing with Tyrode's solution.

The segments of ileum was suspended in a 20 ml organ chamber of continously oxygenated Tyrode's solution (37 ± 1°C) similar to that used for rabbit's jejunum.

3. Preparation of the Isolated Rabbit Aortic Strip

Rabbit was killed by a sharp blow on the skull base, the throat and chest were cut opened. The aorta was isolated to the point as near to the heart as possible, and dissected free for as long a distance as possible. It was transferred to a petri-dish containing Kreb's solution the composition of which was as follows (in g/l) 5.54 g NaCl, 0.35 g KCl 0.29 g $MgSO_4.7H_2O$, 0.28 g $CaCl_2$, 0.16 g KH_3PO_4, 2.1 g $NaHCO_3$ and 2.1 g glucose, at room temperature (28-30°C). The aorta was cut spirally so as to produce a continuous strip, about 4 mm wide 3 to 4 cm long. A thread was attached at each end and the preparation was mounted in 20 ml organ chamber containing Krebs' solution at 37°C ± 1°C aerated with a mixture of oxygen (95%) and carbondioxide (5%).

One end of the strip was attached to a hook of the glass rod in the chamber while the other end attached to a Force-Displacement transducer (Grass FT. 03C) Beckman preamplifier. The strip was equilibrated for 60 minutes under a resting tension of 3 g before being exposed to any drug. During the equilibration period the Krebs' solution was changed every 30 min. Isometric contraction were recorded on a Beckman R411 Dynograph recorder.

4. The Organ Bath

The organ bath used in isolated preparations (Fig. 4) were of double walled Harvard type. It was composed of two compartments, the inner chamber, capacity 20 ml, for tissue preparation being immersed in physiological fluid, and the outer jacket for flow-through circulation of 37°C prewarmed water so as to provide constant temperature to the inner compartment. The circulating water was supplied by a thermoregulating water pump (Churchill type). The bath also had an oxygen inlet to oxygenate the inner chamber through a sintered glass opening. Usually, a series of 2-4 chambers was used in each experiment, to provide replication of the results.

5. Drugs

Drugs used were alkaloids from *Uncaria salaccensis*.

- 19-Epi-3-Isoajmalicine (I-1); M.W. 352
- 3-Isoajmalicine (I-2); M.W. 352
- Uncarine B (O-1); M.W. 368
- Mitraphylline (O-2); M.W. 368

Other drugs :-

- Barium chloride ($BaCl_2.2H_2O$) M.W. 244.31
- Carbachol chloride (carbachol) M.W. 182.6
- Histamine dihydrochloride M.W. 301.1
- 5-Hydroxytryptamine creatinine sulphate (5-HT) M.W. 387.4
- Noradrenaline hydrochloride (NA) M.W. 205.7

All chemicals used in the experiments were of analytical grade. Deionized distilled water was used for the preparation of Tyrode's, Krebs'

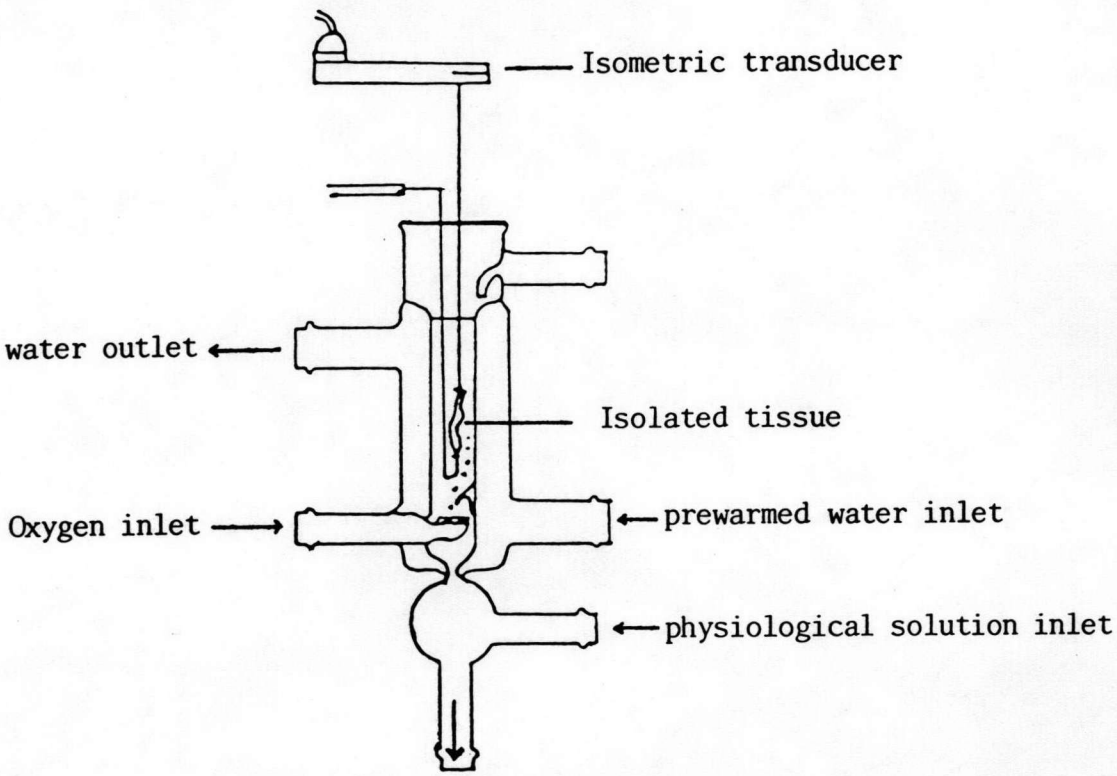

Figure 2. Organ Bath

and drug solutions.

6. Drug Administration

After the isolated tissues had been equilibrated for 45-60 min in the tissue chamber the drug was administered to the bath fluid in a cumulative regimen using either a microsyringe or an automatic micropipette. The volume of drugs used (up to 200 µl) did not cause any significant change in the ionic concentration of the saline solutions.

Tachyphylaxis was a frequent complication of dose-response measurements with 5-hydroxytryptamine on the quinea-pig ileum. It was found that tachyphylaxis could be avoided by the use of long dose cycles or by frequent changes of the bath fluid, usually six times between each dose of 5-hydroxytryptamine. The contact time of the agonist (5-HT) was 30 s and the interval between doses was 2 min (Brownlee and Johnson, 1963).

7. Data Acquisition

In each experiment a dose-response curve was constructed, usually employing five or six doses. Subsequently the dose-response curves were obtained in the presence of the antagonists. Subsequent series of doses were given after baseline tension was re-established usually within 45 min for isolated quinea-pig ileum, and 90 min for isolated rabbit aortic strip. Finally, the responses were obtained again at 1 h after washing out the antagonist. The concentration of the agonists and antagonists are expressed in molar of base in final bath concentrations.

Results were expressed as means and standard errors (S.E) of the means. Significance of the differences between "control" and "drug-treated" means were determined using "Student's *t* test". Values of P is less than 0.05 were taken to implicate statistical significance.

The affinity of a non-competitive antagonist for its receptor; pD_2, is the negative logarithm of the molar concentration of the antagonist that produces 50% reduction of the maximum response obtained with an agonist. pD_2 values were calculated by the equation (Broucke and Lemli, 1982) : $pD_2 = pD_x + \log \left(\frac{Eam}{Eabm} - 1 \right)$. pD_x is the negative logarithm of the molar concentration of the antagonist in the presence of which the maximum response of the preparation to the agonist is E_{abm}. E_{am} is the maximum control contraction to the agonist.

The affinity of a competitive antagonist for its receptor, pA_2, is the negative logarithm of the molar concentration of the antagonist which causes a 2-fold increase of the agonist concentration to obtain the same response. pA_2 values were calculated by the equation $pA_2 = -\log [B] + \log \left(\frac{[A]_2}{[A]_1} - 1 \right)$ (Tallarida *et al*, 1979). [B] represents the molar concentration of the antagonist in the presence of which 50% response of the preparation to the molar concentration of the agonist is $[A_2]$. $[A_1]$ is the molar concentration of the control agonist which produce 50% response.