Chapter III

Material and methods

Tissue preparation and measurement

The human fallopian tubes were obtained from the Operation Section of the Department of Obstetric and Gynecology, Faculty of Medicine, Chulalongkorn University, at Chulalongkorn hospital. Segments of tissue were separated from surgical specimens during newly postpartum sterilization by tubular resection from women in reproductive age.

After operation, the tissues were immersed as quickly as possible in ice-cold oxygenated Krebs solution of the following composition (mM): NaCl 116, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHPO_4$ 1.2, $NaHCO_3$ 22, D-glucose 49.2, adjusted the volume to 1,000 ml by distilled water. Then, the tissues were transfered, and pinned on the paraffin waxed bottom of petri dish irrigated with Krebs solution which was continuously aerated with a mixture of 95 % O_2 and 5 % CO_2. The tissues were carefully dissected free of surrounding blood vessels and connective tissues. Each tube was examined grossly, only tubes with macroscopically normal appearance were used. Longitudinal segments, 1 cm long, were cut. The muscle preparation were then suspended in 20 ml organ bath perfused with Krebs solution. The solution temperature was thermostatically maintained at 37 $^{\circ}$ c and was continuously aerated with a mixture of 95 % O_2 and 5 % CO_2.

The segments were attached at one end to a glass retaining rod at the bottom of the organ bath, with the other end attached by cotton thread to an isometric transducer which was used to measure spontaneous motility of the tissues (Figure 1). A resting tension of 1.0 g was applied to counter balance the muscle tension.

Tissues were left to equilibrate for at least 60 min before drug application or electrical stimulation. Drug volumes used were never more than 100 uL, and were added directly to the solution after equilibration by a micropipette. Each tube was used only once to study the effects of each agent.

Estimation of the drug induced effects was achieved by measuring two parameters, i.e. frequencies (variation in number of contractions per minute) and amplitude (variation in height) of the contractions. The spontaneous activity before each addition of drugs or electrical transmural stimulation (TS) was designated as 'control' and the responses to the drugs or TS was expressed as the per centage change of this control according to the formula:

$$\frac{\text{Drugs (or TS) responses-control}}{\text{control}} \times 100$$

= % change of control spontaneous activity

A positive per cent change therefore indicates the excitation, while a negative is an inhibition.

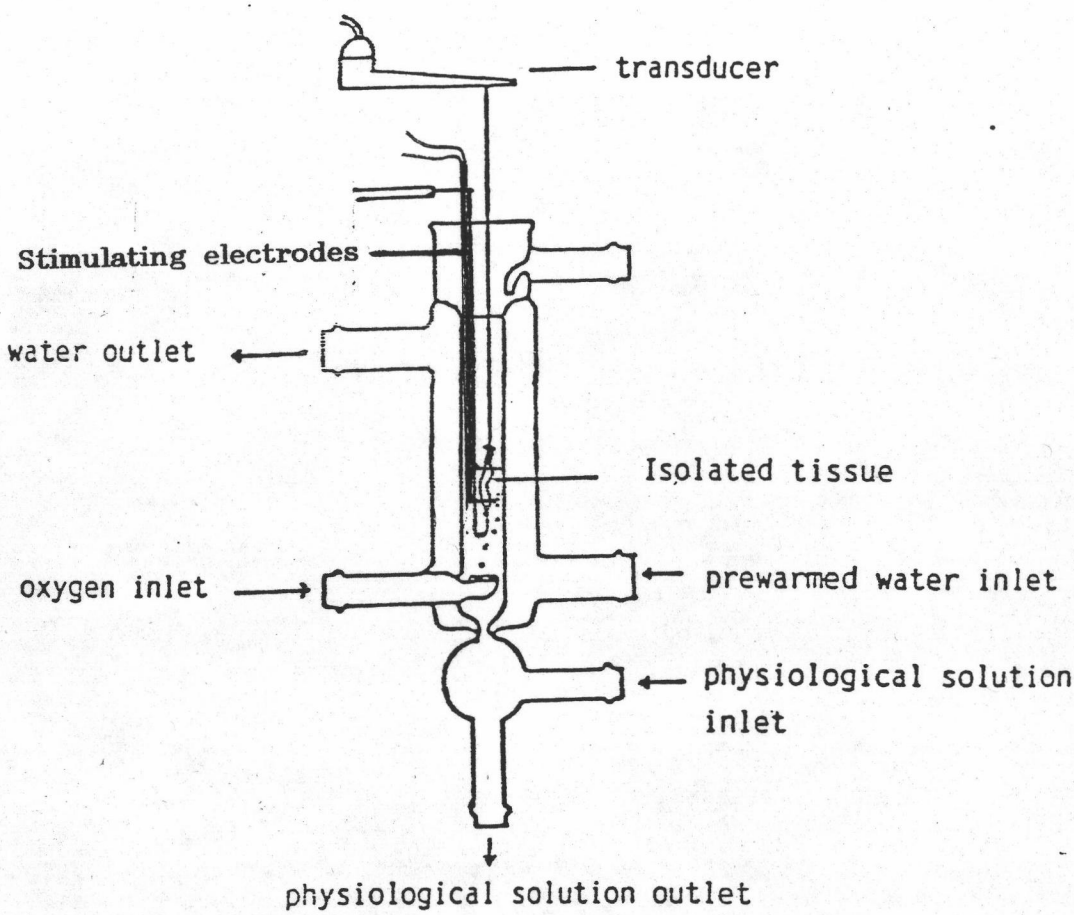

Figure 1 : Water jacketed organ bath

Chemical used

The following drugs were used: GABA, muscimol, baclofen, bicuculline methiodide, norepinephrine bitartrate, phenoxybenzamine HCl, propranolol HCl, acetylcholine chloride, atropine sulphate. All drugs were purchased from Sigma, and were dissolved in Krebs solution, added directly to the muscle baths. The final concentration of the drugs in organ bath was expressed in molar/millilitre (M/ml.).

Experimental protocols

Electrical Transmural Stimulation (TS)

A period of 1 hour was allowed to elapse for stabilization, the spontaneous motility of the longitudinal muscles was recorded under resting tension 1.0 g. Then, the tissues were stimulated transmurally for 180 s with biphasic electrical pulses of 1 ms duration and supramaximal voltage (90 V) through two platinum electrodes situated at the top and the bottom of the tissues. The effects of varying frequencies from 2 to 20 Hz were investigated to obtain the maximum response.

The tissues were allowed to return to a steady level of spontaneous activity before the next higher frequency stimulation in every experiment. The frequency which showed maximum response was chosen for all the subsequent experiments.

Effects of GABA and GABA agonists on contractility under TS condition.

To determine whether GABA regulates the motility of the fallopian tubes, the effects of GABA on the TS-induced response were examined in isolated preparations. After equilibration periods, measurement of spontaneous activity of the muscles was done, the tissues were transmurally stimulated using chosen frequency which produced maximal contraction. After allowed the tissues to recover, GABA concentration 10^{-3} M was applied to the medium. Compare the effect of transmural stimulation before and after GABA application condition and under TS condition.

In separate experiments the effects of, 10^{-5} M muscimol (a GABA-A agonist) and 10^{-6} M baclofen (a GABA-B agonist) were tested. The experiments under electrical TS were done in the same manner as those of GABA experiments described above. Pretreatment of the tissues with 10^{-5}M bicuculline (a GABA-A antagonist) 5 minutes before GABA and GABA agonist application was then tested subsequently and experiments were repeated under TS condition as described above.

To determine whether GABA or its agonists exert directly on the tissue it is neccesary to eliminate adrenergic and cholinergic-induced response. The adrenergic and cholinergic blockers were used in the experiments. Pretreatment with the combination of 10^{-5} M phenoxybenzamine (an alpha-adrenergic blocker), 10^{-6} M propranolol (a beta-adrenergic blocker) and 10^{-4} M atropine (a cholinergic blocker) was done before TS. After the tissues were allowed to recover, 10^{-3}M GABA (or GABA agonist) was applied and investigated.

Adrenergic and cholinergic-induced response of tissues

After measurements of spontaneous activity, 10^{-3} M ACh or 10^{-5} M NE was added to the organ bath and the contractile activity was measured for 5 min during exposure to the drugs. The drug was then washed out and the muscles were allowed to return to a steady level of spontaneous activity.

Effects of GABA and agonists under adrenergic and cholinergic-induced response condition.

To examine whether GABA acts as co-regulator or modulator of the response of tissues through autonomic neurotransmitters, effects of GABA during NE and ACh-induced response were tested.

After equilibration period, the spontaneous activity was measured 10^{-3} M ACh, or, in separate experiment, 10^{-5} M NE, was applied to the organ bath. Five minutes later, 10^{-3} M GABA was added and the effects investigated. To determine the type of receptor responsible for contractile responses, muscimol 10^{-5} M, or baclofen 10^{-6}M in separate experiment was applied instead of GABA.

To confirm the effect of drugs, pretreatment with 10^{-5} M bicuculline was established. Bicuculline was added to the medium after the 10^{-5} M NE or 10^{-3} M ACh was applied. Five minutes later, GABA 10^{-3} M or 10^{-5} M muscimol was applied and the effects examined.

Statistical analysis

The data were analyzed by means of Student's paired t-test and differences were accepted as significant at the 0.05 level of probability.