Chapter V

Discussion

The present study demonstrates that the response of the tissues to electrical transmural stimulation is a decrease in the amplitude of spontaneous activity (figure 2 B). The responses appear to be frequency dependent, since the result showed the graded inhibition of human fallopian tube produced by increasing frequencies of electrical transmural stimulation. In addition, the results also show a similarity in the responses of the fallopian tube to NE and to transmural stimulation. The qualitatively aspects of the responses are very much alike.

The human fallopian tube has been previously reported an inhibitory respond to NE and electrical transmural stimulation. The inhibitory response to electrical transmural stimulation and NE were abolished by tetrodotoxin and guanethidine, while propranolol reversed the inhibitory response to NE and TS (Spilman and Harper, 1974; Molnar et al., 1976). These findings showed that the response of tissue to electrical transmural stimulation resulted from release of NE from adrenergic nerves, and subsequent action on inhibitory beta-adrenergic receptors. The results of present study are in agreement with these findings as shown in figure 2 B and figure 4.

The mechanism responsible for NE inhibition of spontaneous smooth muscle contraction may be hypothesized by the two following mechanisms. First, hyperpolarization of the membrane is considered. Previous study reported that catecholamines produced a hyperpolarization of the membrane

and hence spontoneous activity was inhibited (Magaribuchi and Osa, 1971). Second plausible explanation is the inhibition through the cAMP system. Since catecholamines play a significant role in the modulation of genital tract smooth muscle contractile activity. Phillippe and Bangalore (1989) reported that beta adrenergic receptor stimulation of adenylate cyclase, with subsequent cyclic adenosine 3',5'- monophosphate (cAMP) production, resulted in smooth muscle relaxation.

Beta adrenergic agents relax the rat uterus and hyperpolarize the muscle cell membrane were also reported by Kroeger and Marshall, (1973 and 1974). Their results suggested that beta adrenergic hyperpolarization is accompanied by an increase in potassium permeability of the cell membrane and by an increase in tissue cAMP. It was also stimulated the increasing of Ca^{2+} efflux (Kroeger et al., 1975; Scheid et al., 1979).

To determine whether GABA modulates through sympathetic neural mechanisms or stimulates receptors on smooth muscle cells directly, comparison the effect of GABA; GABA under electrical transmural stimulation; GABA under NE-induced condition and GABA under the combination of adrenergic and cholinergic blockers were investigated. It has shown in figure 6A, figure 7 and figure 8 in this experiment, GABA and its agonists have not shown the direct effect on spontaneous fallopian tube movements either at resting conditions or under electrical transmural stimulation. Figure 10 also demonstrates that these substances have not alter the effect of NE on fallopian tube contractility.

Since GABA has no effect on the tissues incubated with a combination of phenoxybezamine, propranolol and atropine (figure 8), therefore, the result suggests that GABA may not relate to the alteration of smooth muscle functions. On the other hand, GABA does not antagonize the effect of electrical transmural stimulation (figure 6), while TS releases NE form adrenergic nerve terminals. This suggests that the action of GABA on the isolated fallopian tube, is probably not mediate through sympathetic nerves. Although it has been demonstrated previously that GABA inhibits the release of NE and the twitch response to electrical stimulation in isolated vas deferens . These actions are thought to be mediated *via* GABA-B receptors located on adrenergic nerve terminals (Bowery et al., 1981).

The present study indicates the effects of ACh on smooth muscle contractility. ACh exerts a stimulatory action on tissue preparations characterized by a marked increase in contraction frequencies without any chages of amplitude (figure 12) and elevation of the basal tone. Triner et al. (1972) suggested the possible two mechanisms of the action of ACh on contractile response of smooth muscles. First increase in intracellular ionized calcium caused by ACh, which then may mediate the inhibition of adenyl cyclase system and reduce cAMP in the tissues, resulting the contraction of the muscles. The other possible mechanism is a change in sodium and potassium fluxes across the cell membrane leading to increasing activity of Na^+, K^+ - dependent ATP-ase which may become a potential competitor of adenyl cyclase for the same substrate. The Na^+, K^+-ATPase should have a higher affinity for ATP than adenyl cyclase. Thus, the cAMP formation could be slowed down by a relative lack of substrate.

In this experiment, GABA and its agonists have not shown the direct effect on spontaneous fallopian tube movement either at resting condition or under electrical transmural stimulation, or even under the combination of adrenergic and cholinergic blockers pretreatment. ACh exerts a stimulatory action on tissue preparations, characterized by a marked increase in contraction frequencies but not the amplitude. Forces of contraction produced by ACh-induced are augmented by GABA and muscimol, but not baclofen, and their increase of ACh-induced contraction is suppressed by bicuculline (figures 12 and 13). These actions together seem to indicate that the mechanism for GABA enhancement of ACh contraction may not be an increase in ACh release, but rather a postsynaptic modulatory effect on smooth muscle cells.

To establish type of GABA receptor responsible for this action, the result of present study demonstrates ACh-induced contraction is not affected by baclofen, whereas GABA and muscimol augment ACh action (figure 12). These results indicate that GABA may modulate cholinergic transmission through GABA-A receptors which may be on smooth muscles. The present data is consistent with the data of previous study, which have identified a bicuculline-sensitive type of GABA receptor (GABA-A) in the fallopian tube of the rat and the human (Erdo and Lapis, 1982; Erdo et al., 1984).

Erdo and Wolff (1990) reported that Fujiwara et al. (1975) were the pioneer which indicate that GABA receptors may be located on smooth muscle cells. It has been supported by autoradiographic studies with labelled muscimol.

Tetrodotoxin-sensitive contractile responses to GABAergic model compounds of the isolated rabbit oviduct (Erdo et al., 1984) and uterus (Riesz and Erdo, 1985) are consistent with the view that both GABA-B and GABA-A sites located on smooth muscle cells in these organs. Moreover, autoradiographic experiments indicated that a subpopulation of GABA-A sites in the guinea-pig urinary bladder may also be located on smooth muscle cells (Erdo et al., 1989c).

In general, GABA has been shown to produce hyperpolarization of postsynaptic membrane in the CNS through a bicuculline-sensitive receptors. However, depolarizing effects of GABA have also been observed in the superior cervical ganglia (Bowery and Brown, 1974). GABA also increases the release of catecholamines (CA) from the adrenal medulla (Sangiah et al., 1974), thereby suggesting that it may relate to depolarization of the chromaffin cells. In melanotrophs, it has also been demonstrated that GABA depolarized the cell membrane and caused a flurry of action potentials (Taraskevich and Douglas, 1982).

GABA-A receptors are couple to chloride channels to regulate the chloride movement across the membrane, leading to hyperpolarization or depolarization, depending on the direction of the chloride gradient (Erdo and Wolff, 1990.) Thus, the mechanism responsible for depolarizing effect of GABA could be hypothesized. The primary effect of GABA could be an increase in Cl^- conductance brought about be activation of GABA-A receptors, and if the resting chloride potential is less negative than resting membrane potential, the resulting movement of Cl^- out of the cell will cause depolarization (Virmani, Stojilkovic and Catt, 1990. quoted in Boakes et al.,

1984). On the other hand, depolarization could cause the direct activation of the voltage-sensitive calcium channel (VSCC). Entry of Ca^{2+} through VSCC thus appears to be the second messenger of the effect of GABA (Virmani et al., 1990).

GABA was found to exert morphogenetic effects on principal neurons of the superior cervical ganglion (Wolff et al., 1978, 1979). This effect is accompanied by a complex neuroplastic response that leads to an increase in synaptogenetic capacity, i.e., the postsynaptic acceptance for acetylcholine synapses is elevated above the normal in adult rats. The results of present experiment have shown that GABA plays a modulatory role in the regulation of ACh action on fallopian tube smooth muscles as a facilitatory modulator.

It has been reported that GABA directly affects the electrophysiological properties of endocrine cells isolated from the pars intermedia. The ionic and pharmacological characteristics of this action of GABA resemble those encountered at many GABAergic synapses in the CNS (Taraskevich and Douglas, 1982).

Taraskevich and Douglas (1982) also reported that GABA rapidly and reversibly depolarized the cells and profoundly reduced membrane resistance. The collapse of membrane resistance and potential in the pars intermedia cells in response to GABA seems to result from an increased in Cl^- conductance.

Kitayama et al. (1990) demonstrated that GABA caused an elevation of cytosolic free calcium ($[Ca^{2+}]_i$) *via* the GABA-A receptors in a

concentration-dependent manner, which was well correlated with an increase of of ^{45}Ca uptake, an increase of CA release and adepolarization of chromaffin cells. Since the GABA-induced rise of $[Ca^{2+}]_i$ was absolutely dependent on the presence of extracellular Ca^{2+}, at least on entry route for Ca^{2+} facilitated by GABA *via* a voltage-sensitive Ca^{2+} channel (VSCC) was suggested. In correlation with these results, GABA caused a larger increase of $[Ca^{2+}]_i$ as extracellular Cl^- was lowered. The maximal increase in $[Ca^{2+}]_i$ induced by GABA was about 300 nM, which is near the threshold that triggers the CA exocytosis from chromaffin cells.

Ca^{2+} influx through VSCC was required for GABA-induced luteinizing hormone (LH) release was also reported. Such entry of Ca^{2+} would result from activation of VSCC by depolarization due to the increased Cl^- conductance caused by GABA-A receptors activation (Anderson and Mitchell, 1986).

GABA evoked accumulation of extracellular potassium ion ($[K^+]_o$) have been recorded in many regions of both central and peripheral nervous system, always where depolarizing effects of GABA can be recorded (Morris and Liske, 1989). Although increase in K^+ and Cl^- conductance underly the hyperpolarizing actions of GABA, the exact mechanisms of both the depolarizing effects of GABA and the associated accumulation of $[K^+]_o$ are less well defined. It remains possible that a significant component of the depolarizing action of GABA may be secondary to a receptor-and transport-mediated increase in the extracellular level of K^+, and that this may importantly contribute to the well-recognized apparent "fading" of inhibitory effects.

Depolarizing effects of GABA are associated with an accumulation of $[K^+]_o$ (Morris and Liske, 1989). It is generally assumed that the depolarizing response is due to an increase in Cl^- conductance with outward movement of Cl^-. Ballanyi and Grafe (1985) reported the GABA-evoked depolarization in association with decrease in intracellular K^+ and Cl^- but not Na^+_i, and concluded that K^+ and Cl^- outward cotransport occured.

In summary, based on these results, the most probable mechanism for GABA enhancement of ACh contractions is that GABA may reduce the membrane resistance and potential. Such changes are resulting from an increase in Cl^- conductance. A shift of equilibrium potential for Cl^- should cause an activation of voltage-sensitive Ca^{2+} channel (VSCC) and consequently facilitates Ca^{2+} influx. Entry of Ca^{2+} through VSCC thus appears to be the second messenger of the effect of GABA. The reduction of membrane resting potential may be accompanied by the occuring of K^+ and Cl^- outward cotransport, but not change in Na^+_i. The potential may be near the threshold that action potential occured. This effcet is a complex neuroplastic response that leads to an increase in synaptogenetic capacity. Thus, the postsynaptic acceptance for ACh is elevated above the normal level, resulting in facilitatory modulation of ACh-induced contraction. The relationship between ACh and GABA indicates that both agents may be synergistically involved in the control of fallopian tube motility. However, the precise mechanisms of the modulatory effect of the modulatory effect of GABA remain to be elucidated.