นิพนธ์ต้นฉบับ

Physiological roles of Gamma-aminobutyric Acid (GABA) on isolated human fallopian tube smooth muscle activities.

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In this investigation, the physiological roles of GABA on isolated human fallopian tube smooth muscle activities were investigated. The longitudinal segments of fallopian tubes were obtained from reproductive age women during postpartum sterilization. The contractile activity was recorded in terms of force, rate, and form. The results revealed that GABA and related substances, muscimol and baclofen did not alter the response to either electrical transmural stimulation or exogenous norepinephrine. Furthermore, GABA and its agonists neither affected the frequency and amplitude of spontaneous contraction nor under a combination of adrenergic and cholinergic blocker conditions. In addition, acetylcholine produced an increase of forces and rates of contraction. However, forces of contraction produced by acetycholine were augmented by GABA and muscimol, but not baclofen. Pretreatment with bicuculline antagonized the enhancing effect of GABA as well as that of muscimol. These results suggested that GABA presumably is a facilitatory modulator of parasympathetic neurotransmission, not of sympathetic neurotransmission, and the effect is exerted by activation through GABA-A receptors.

Key words : *Gamma-aminobutyric acid, human fullopian tube*

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ได้ศึกษาบทบาททางสรีรวิทยาของกาบาต่อการทำงานของกล้ามเนื้อเรียบท่อนำไข่ของคน โดยใช้ส่วนตามยาวของท่อนำไข่จากสตรีที่ทำหมันหลังคลอดบุตร บันทึกการหดตัวของกล้ามเนื้อในรูปของความแรง อัตรา และรูปลักษณ์ ผลการทดลองพบว่ากาบาและสารเสริมฤทธิ์ เช่นมัสซิมอลและบาโคลเฟน ไม่มีผลเปลี่ยนแปลงการตอบสนองของกล้ามเนื้อต่อการกระตุ้นด้วยไฟฟ้าหรือการให้นอร์อิพิเนฟริน นอกจากนี้ กาบาและสารเสริมฤทธิ์ดังกล่าวไม่มีผลต่ออัตรา และความแรงของการหดตัวของกล้ามเนื้อที่เกิดขึ้นเอง และไม่มีผลต่อการหดตัวของกล้ามเนื้อที่เกิดจากการให้สารยับยั้งการทำงานของระบบแอดรีเนอจิก และโคลิเนอจิกร่วมกัน อะซิติลโคลีนเองสามารถเพิ่มความแรงและอัตราการหดตัวของกล้ามเนื้อได้ และความแรงที่เพิ่มนี้สามารถเสริมฤทธิ์ให้มากขึ้นได้โดยกาบาและมัสซิมอล แต่บาโคลเฟนไม่มีผล การเสริมฤทธิ์ของกาบาและมัสซิมอลนี้สามารถยับยั้งได้โดยไบคูคิวลิน จากผลการทดลองนี้อาจสรุปได้ว่ากาบาช่วยเสริมในการผ่านของกระแสประสาทพาราซิมพาเธติค แต่ไม่มีผลในประสาทซิมพาเธติค และผลการเสริมฤทธิ์นี้น่าจะออกฤทธิ์ผ่านทางตัวรับชนิดกาบา-เอ

The fallopian tube or oviduct, is dually innervated, having nerves from both the sympathetic and parasympathetic divisions of the autonomic nervous system. The presence of alpha-excitatory and beta-inhibitory adrenoceptors, have been shown by many investigators.[1-7] Estrogen enhances alpha-adrenoceptor activity.[6,8,9] The presence of cholinergic receptors has been demonstrated[1] which results in contraction of smooth muscle.[10]

The other possible neurotransmitter which has been reported to play a role on contractility of the fallopian tube is GABA (gamma-aminobutyric acid).[11-13] Most of the experiments have been done in rabbit[12] and rat[14] oviductal musculatures. In human fallopian tubes, Erdo et al.[15] reported only high densities of specific GABA binding sites which resemble those of GABA-A receptors. However, there is no documentation of GABA action on motility of human fallopian tubes.

The principal aim of this study was to elucidate whether GABA may play any physiological roles on human fallopian tube smooth muscle activities, and if so, is the GABA-A or GABA-B receptor is responsible for contractility of the tube.

Materials and Methods

1. Tissue Preparation and Measurement

The ampulla part of the human fallopian tubes were obtained from women in reproductive age who had undergone postpartum sterilization by tubular resection 2 days after delivery at the Department of Obstetrics and Gynecology, Chulalongkorn Hospital. The tissues were immediately immersed in 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, $NaHCO_3$ 2.2, and D-glucose 49.2. The volume was adjusted to 1,000 ml by the addition of distilled water. The tissues were carefully dissected free of surrounding blood vessels and connective tissues. The specimens were determined to be normal by gross examination. Longitudinal segments 1 cm long were cut. The muscle preparations were then suspended in 20 ml organ bath perfused with Krebs solution. The solution temperature was thermostatically maintained at 37°C and was continuously aerated with a mixture of 95 % O_2 and 5 % CO_2. The tissue was allowed to equilibrate in the bath for 1 hour and the spontaneous motility of the longitudinal muscles was recorded by use of an isometric transducer connected to a dynogragh (Beckman type RM). A resting tension of 1.0 g was applied to counterbalance the muscle tension.

2. Chemicals

The following drugs were used: GABA, muscimol, baclofen, bicuculline methiodide, norepinephrine bitartrate, phenoxybenzamine HCl, propanolol HCl, acetylcholine chloride, and atropine sulfate. All drugs were purchased from Sigma, dissolved in Krebs solution, and added directly to the muscle baths.

3. Experimental Procedures

3.1 Effects of electrical transmural stimulation (TS)

The tissues were stimulated transmurally for 180 s with biphasic electrical pulses of 1 ms duration and supramaximal voltage (90 V) through two platimum 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 responses.

3.2 Effects of GABA and GABA agonists on contractility under TS conditions.

10^{-3} M GABA, 10^{-5} M muscimol (a GABA-A agonist) and 10^{-6} M baclofen (a GABA-B agonist) were tested in the experiments. The effects of these drugs on contractile responses under TS conditions were recorded in terms of force, rate and form.

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

3.3 Effects of adrenergic and cholinergic-induced response.

After measurements of spontaneous activity, 10^{-3} M acetylcholine (ACh) or 10^{-5} M norepinephrine (NE) was added to the organ bath and the contractile activity was recorded.

3.4 Effects of GABA and agonists under adrenergic and cholinergic induced response condition

To examine whether GABA acts as co-regular or modulator of the response of tissue through autonomic neuro-transmitters, GABA or GABA agonists in the same doses as described in 3.2 were added into the tissues which had been pretreated with ACh or NE in the same doses as in 3.3

To confirm the effects of GABA , pretreatment with 10^{-5} M bicuculline was added to the medium after ACh or NE. Then GABA or agonists was applied and the effects examined.

4. Statistical analysis.

The results were presented as mean ± SEM. Student's paired t-test was used to evaluate the levels of significant difference between all of the mean values. The probability values that were less than 0.05 were considered to be significant.

Results

Most human fallopian tube preparations exhibited persistent spontaneous contractile activity after equilibration as shown in figure 1 A. The motility pattern consisted of rhythmical phasic contractions with varying amplitude.

1. Effects of electrical transmural stimulation (TS)

TS on the fallopian tubes produced a rapid phasic contraction and then inhibition in a frequency dependent manner in the inhibitory responses (figure 1 B). The percentage of inhibition was 67.75±8.88, 69.87±6.16, 74.04±8.42, 81.50±6.93, 88.28±7.02 and 90.14±6.20, for stimulation with 2,4,6,8,10 and 15 Hz of frequency, respectively. The maximum frequency used in this experiment was 20 Hz which produced nearly complete inhibition of contraction (94.38±4.07 %).

2. Effects of NE on contractile response.

As shown in figures 1 C and $2,10^{-5}$ M of NE significantly ($p < 0.05$) decreased the amplitude and basal tone of contraction. The percentage of NE-induced inhibition was 79.83±10.25. Comparison of the effects of NE and TS showed a similar pattern of inhibition. When TS was tested under NE - induced condition, the contraction was inhibited completely (figure 1 C).

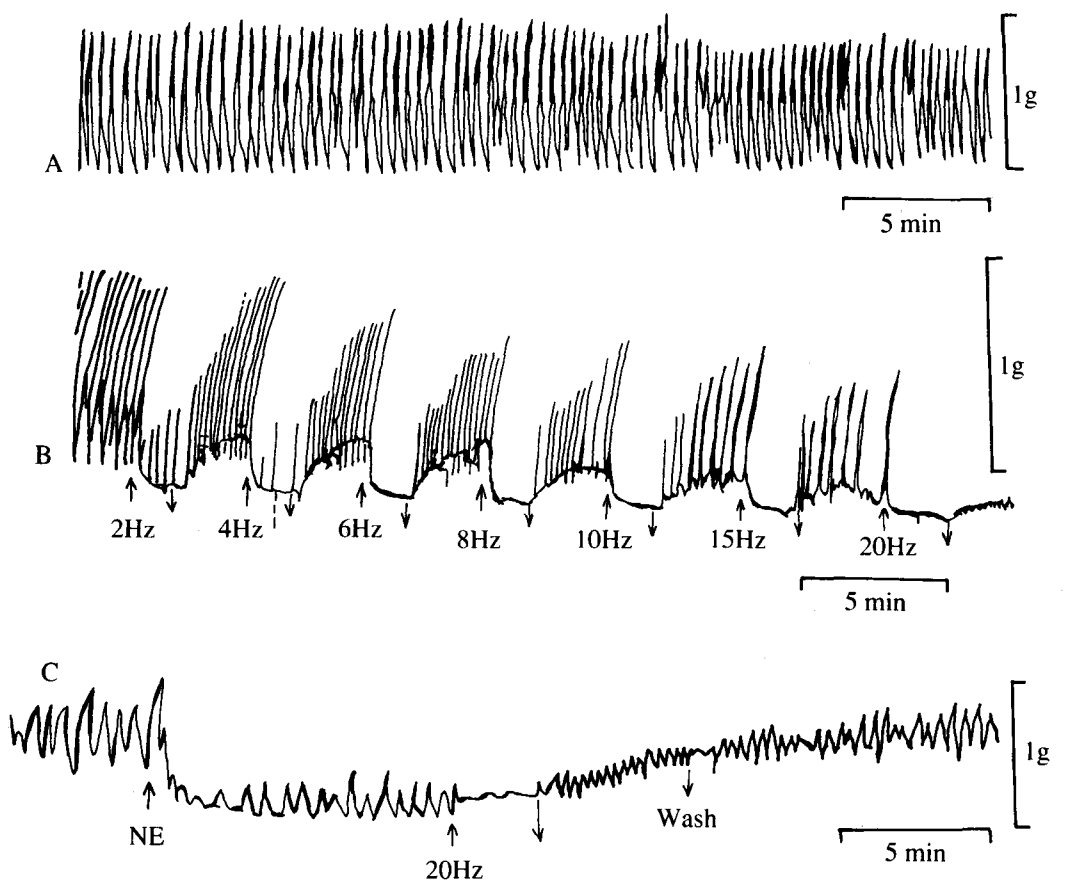

Figure 1. A. Tracing shows spontaneous contraction of the longitudinal preparation of the human fallopian tube under resting tension 1 g.

B. The effects of electrical transmural stimulation (TS) for 180 s with biphasic electrical pulses of 1 ms duration and supramaximal voltage (90 V). Varying frequencies from 2 to 20 Hz were investigated.

C. The effects of 10^{-5} M norepinephrine and TS on spontaneous contraction.
↑ = start of TS or drug application
↓ = end of TS

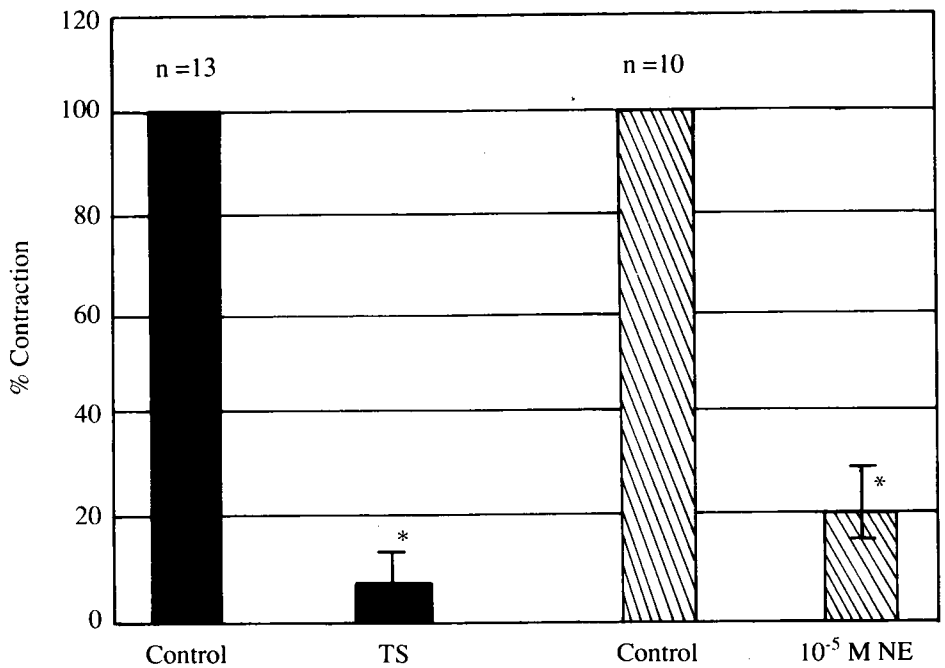

Figure 2. Comparison of the effect of 20 Hz frequency of electrical transmural stimulation (n=13) with the effect 10^{-5} M NE (n=10) on fallopian tube contractility. The solid bar is electrical transmural stimulation and the hatch bar is 10^{-5} M NE application. The response is expressed as per cent contraction, while the control is designated 100 per cent. The data represent the mean±SEM (* $p < 0.05$ compared with control).

3. Effects of GABA and GABA agonists on contractile response.

As shown in figure 3,10^{-3} M GABA has no significant effect on spontaneous contraction of fallopian tubes. Furthermore, GABA cannot reduce the inhibitory response induced by TS. Similarly, the GABA - A agonist (muscimol 10^{-5} M) and the GABA - B agonist (baclofen 10^{-5} M) produced non-significant effects neither on spontaneous contraction nor TS of the fallopian tube.

To establish whether GABA stimulates or inhibits the contraction of smooth muscles directly, the effect of GABA following blockade on receptors for NE and ACh was investigated. A combination of 10^{-5} M phenoxybenzamine (an alpha-adrenergic blocker), 10^{-6} M propanolol (a beta-adrenergic blocker) and 10^{-4} M atropine (a cholinergic blocker) together substantially reduced the contraction of the longitudinal muscle. This response could not be effected by GABA 10^{-3} M as demonstrated in figure 4.

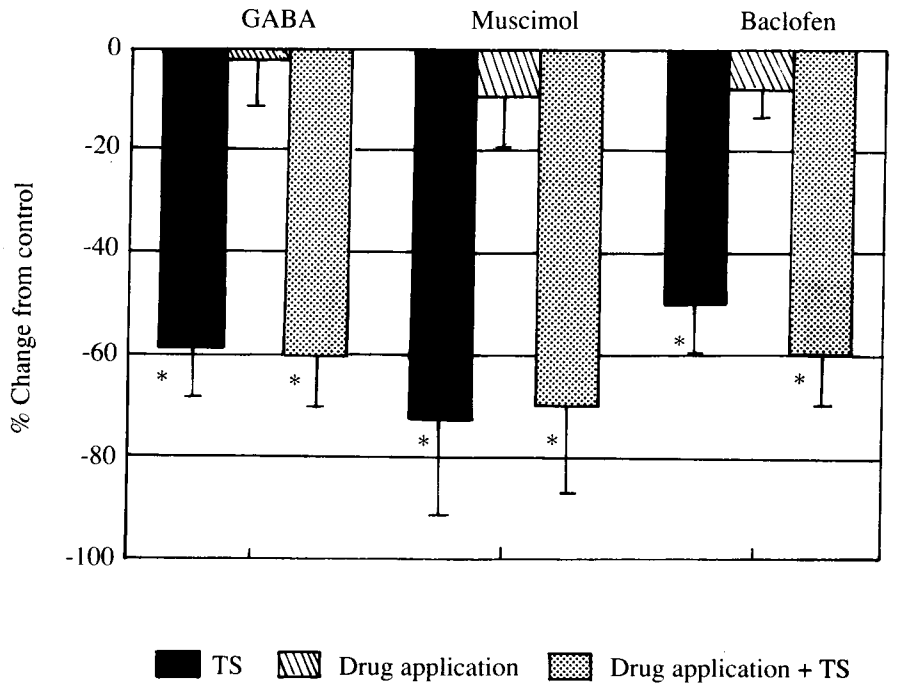

Figure 3. Effect of 20 Hz frequency of electrical transmural stimulation (TS) before and after 10^{-3} M GABA, 10^{-5} M muscimol and 10^{-6} M baclofen application. The data are expressed as per cent change from control (spontaneous contraction before transmural stimulation). Values are mean ± SEM, n=13 for GABA experiment; n=8 for muscimol experiment and n=14 for baclofen experiment. The solid bar is transmural stimulation, the hatch bar is drug application, and the dotted bar is a combination of drug application and transmural stimulation (* $p < 0.05$).

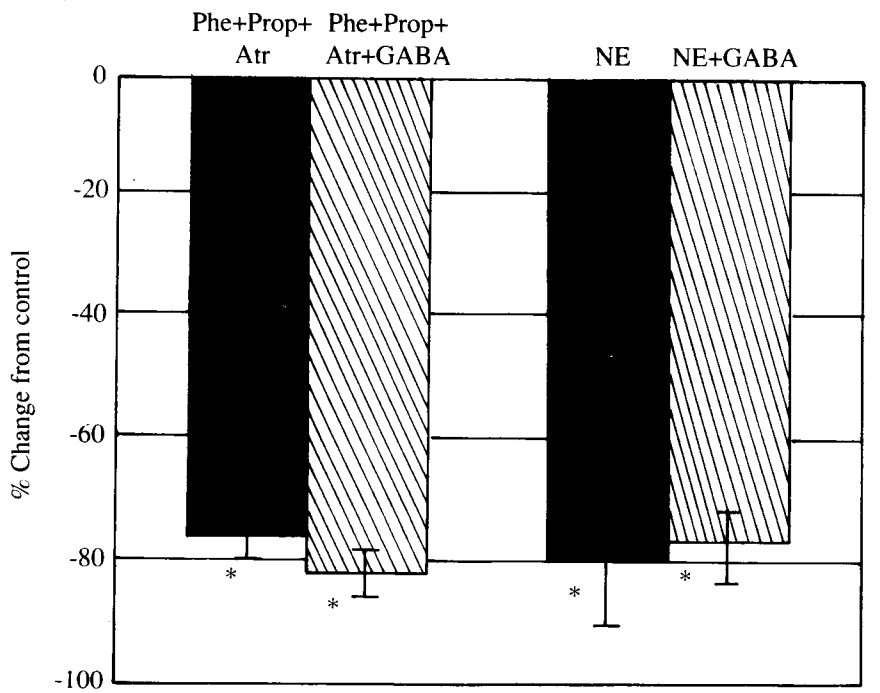

Figure 4. Comparison of the effect of 10^{-3} M GABA on the fallopian tube contractile response in the presence of a combination of 10^{-5} phenoxybenzamine (Phe), 10^{-6} M propranolol (Prop) and 10^{-4} M atropine (Atr), and in the presence of 10^{-5} M NE. The data are represented as per cent change from spontaneous contraction (control). The values are mean ± SEM (n=10 for each experiment). (* $p < 0.05$).

4. Effects of GABA and it agonists under adrenergic and cholinergic - induced response conditions.

While NE inhibited the contractility and had a relaxing effect on smooth muscle, GABA cannot alter these effects as shown in figure 4. ACh exerts a stimulatory action which was characterized by a marked increase of contraction frequencies, but not the amplitude, and by an elevation of the basal tone. When 10^{-3} M GABA was applied into the medium, the ACh contraction was significantly augmented ($p < 0.05$), as shown in figure 5. The increasing effect was mimiced by 10^{-5} M muscimol, but not baclofen 10^{-6} M (figure 5). When ACh contraction was disignated as 100 %, the augmentation by GABA and muscimol were 143.74±36.24% and 155.08±2.60% respectively but only 109.56±2.60% by baclofen. Pretreatment of 10^{-6} M bicuculline in the bathing medium prevented the enhancing effect of GABA and muscimol on fallopian tube contractions induced by ACh (figure 5).

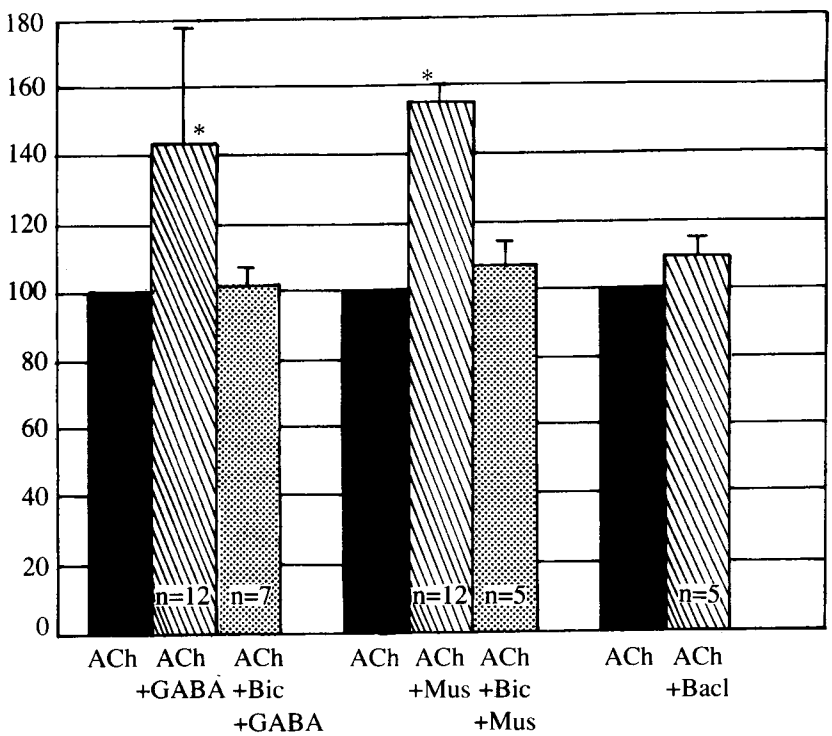

Figure 5. Effect of 10^{-3} M GABA, 10^{-5} M muscimol (Mus) and 10^{-6} M baclofen (Bacl) on ACh - induced contraction in the presence or absence of 10^{-5} M bicuculline (Bic), while ACh - induced contraction is designated as control. The data are represented as per cent change from ACh - induced contraction. The values are mean±SEM. The n indicates the number of specimens tested. (* $p < 0.05$).

Discussion

This study demonstrated that the spontaneous activity of human fallopian tubes was decreased by TS and the responses were frequency dependent (figure 1 B). In addition, NE application showed similarity in the qualitative aspects of the responses (figure 1 C). These results are in agreement with previous investigations.[3,16] From these studies it could be suggested that the response of tissue to TS resulted from release of NE from adrenergic nerves, and subsequent action on inhibitory beta-adrenergic receptors. The mechanism responsible for NE inhibition of spontaneous smooth muscle contracton may be hypothesized in the two following mechanisms. First, hyperpolarization of the membrane is considered. A previous study reported that catecholamines produced a hyperpolarization of the membrane and hence spontaneous activity was inhibited.[17] A second possible explanation is inhibition through the cAMP system. Phillippe and Bangalore[18] reported that beta-adrenergic receptor stimulation of adenylate cyclase, with subsequent cAMP production, resulted in smooth muscle relaxation.

To determine whether GABA modulates through sympathetic neural mechanisms or stimulates receptors on smooth muscle cells directly, a comparison of the effect of GABA, GABA under TS, GABA under NE - induced condition and GABA under the combination of adrenergic and cholinergic blockers was carried out. It was found that GABA and its agonists did not show the direct effect on spontaneous fallopian tube movement either at resting condition or under TS (figure 3), and also did not alter the effect of NE on contraction (figure 4). Furthermore, GABA has no effect on the tissues incubated

with a combination of phenoxybenzamine, propranolol and atropine (figure 4). Therefore, these results suggest that GABA may not relate to the alteration of smooth muscle function, and the action is probably not mediated through sympathetic nerves. However, it has been pre viously demonstrated that GABA inhibits the release of NE and the twitch response to TS in isolated vas deferen which was thought to be mediated via GABA-B receptors located on adrenergic nerve terminals.[19]

Our study indicates that ACh exerts a stimulatory action on tissue preparation (figure 5). Triner et al.[20] suggested two possible mechanisms of Ach. First an increase in intracellular ionized calcium which then mediated the inhibition of adenyl cyclase system and reduced cAMP in the tissue. resulting in the contraction of the muscles. Second, a change in sodium and potassium fluxes across the cell membrane leading to increasing activity ATP-ase which may become a potential competitor of adenyl cyclase and then reduce cAMP formation.

In this experiment, stimulatory actions of ACh were augmented by GABA and muscimol, but not baclofen, and their increase of ACh-induced contraction is suppresed by bicucullin (figure 5). 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 through GABA-A receptors. However, the present data is consistent with the data of previous studies which have identified a bicuculline-sensitive type of GABA receptor (GABA-A) in the fallopian tube of humans and rat.[15,21]

Erdo and Wolff[22] suggested that GABA-A receptors are coupled to chloride channels to regulate the chloride movement across the membrane, leading to hyperpolarization or depolarization, depending on the direction of the chloride gradient. For depolarization, GABA activates GABA-A receptors and an increase in Cl^- conductance, and if the resting chloride potential is less negative than resting membrane potential, the resulting movement of Cl^- out of cell will cause depolarization.[23] On the other hand, depolarization could cause the direct activation of the voltage-sensitive calcium channel and consequently facilitate calcium influx which appears to be the second messenger of the effect of GABA.[23] The reduction of membrane resting potential may be accompanied by the occurrance of K^+ and Cl^- outward cotransport, without a change in intracellular Na^+.[24,25] The potential may be near the threshold that action potential occurred. This effect is a complex neuroplastic response that leads to an increase in synaptogenetic capacity. Thus, the postsynaptic acceptance for ACh is elecvated above the normal level, resulting in facilitatory modulation of ACh-induced contraction. The relation-ship 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 GABA remain to be elucidated.

In conclusion, we have shown that GABA possesses a facilitatory modulator of parasympathetic neurotransmission, not of sympathetic neurotransmission, and the effect is exerted by activation through GABA-A receptors.

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