Chapter IV

Results

Representative tracings of the spontaneous contractions of the human fallopian tubes are shown in figure 2-A. Regular spontaneous activity of human fallopian tube was generally seen at the end of the equilibration period (45-60 min). The motility pattern consisted of rhythmical phasic contraction with varying amplitude and the frequency usually decrease gradually during the experiments.

Effects of electrical transmural stimulation

As shown in figure 2-B, the response of longitudinal preparation of tissues to varying frequencies of electrical transmural stimulation (TS) was demonstrated.

Electrical transmural stimulation (TS) with 1 ms duration, varied the frequency from 2 to 20 Hz and intensity of 90 V for 180 s on the fallopian tube produced a rapid phasic contraction and then inhibition. The inhibitory responses were frequency dependent manner. (Figure 2-B and figure 3). The per centage of inhibition is 67.75 $\pm$ 8.88 , 69.87 $\pm$ 6.16, 74.04 $\pm$ 8.42, 81.50 $\pm$ 6.93, 88.28 $\pm$ 7.02 and 90.14 $\pm$ 6.20, for stimulation with 2, 4, 6, 8, 10 and 15 Hz of frequency respectively. The maximal frequency used in this experiment is 20 Hz produced nearly complete inhibition of contraction (94.38 $\pm$ 4.07 %). (Values are shown in mean $\pm$ 2SE, n = 13).

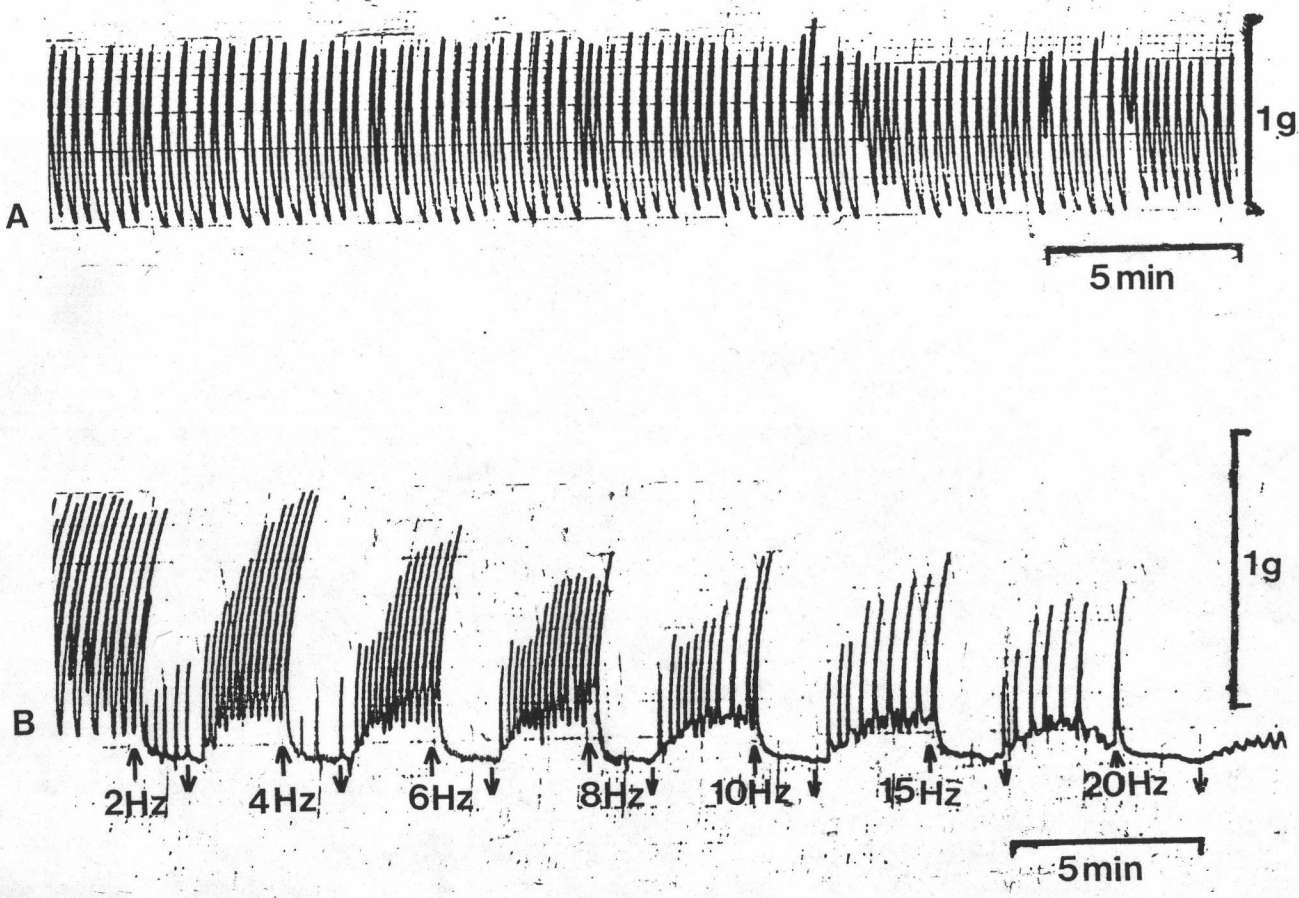

Figure 2 : Tracing of spontaneous contraction of the longitudinal preparation of the human fallopian tubes under resting tension 1.0 g (A) and the effects of electrical transmural stimulation (TS) for 180 s with biphasic electrical pulses of 1 ms duration and supramaximal voltage (90 V). The varying frequencies from 2 to 20 Hz were investigated (B).

↑= start of transmural stimulation;

↓= end of transmural stimulation.

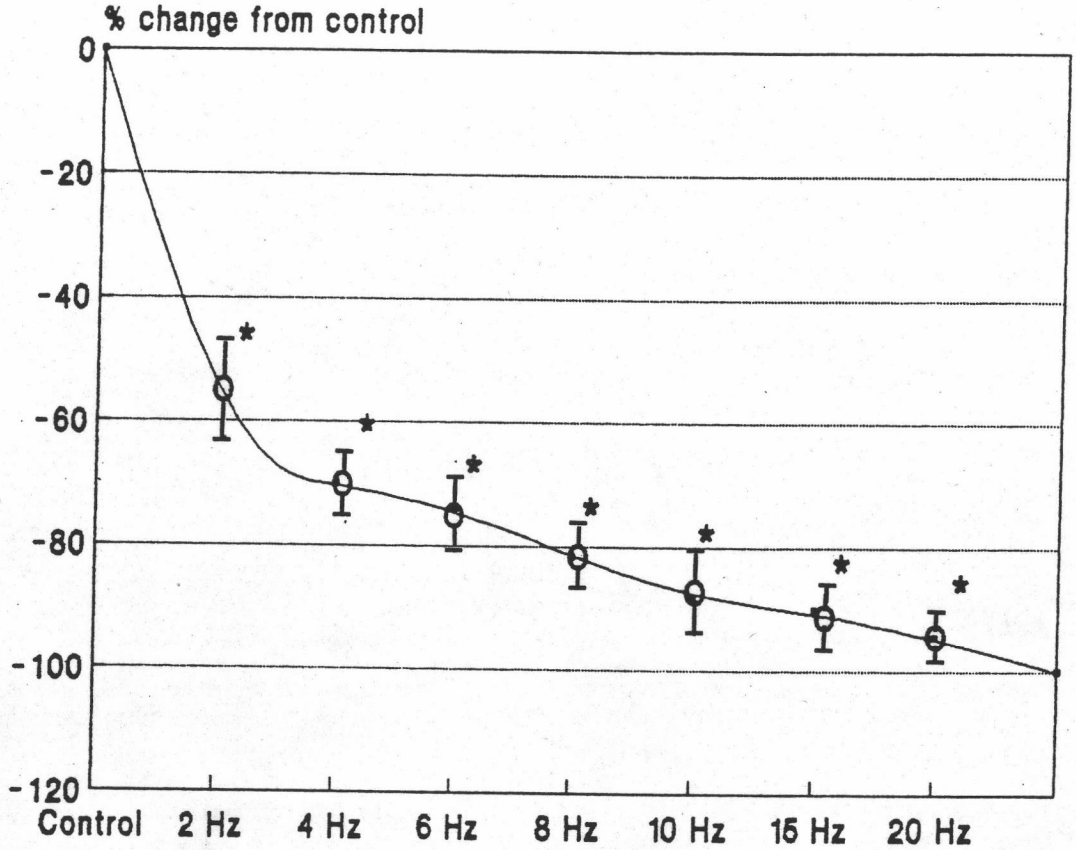

Figure 3 : Response of the longitudinal muscle of human fallopian tubes to electrical transmural stimulation of varying frequencies. The response is taken as the per cent change from spontaneous activity. The negative per cent change indicates an inhibition of contraction. The data represent the mean $\pm$ 2 SE (n=13). Significant levels : * $p < 0.05$, compare with control.

The characteristic effect of transmural stimulation was the amplitude of spontanous activity decreased and appeared to be frequency-dependent accompany with lowering of the basal tone which implied that the relaxation is occured.

The effect of NE on contractile response.

Application of 10^{-5} M NE to the medium produced an inhibition of contraction and relaxation of the muscle. This NE-induced inhibition shows a similar to that produced by electrical transmural stimulation. (Figure 4-A). Comparison the effect of NE and electrical transmural stimulation to the contractile response of the fallopian tube was also shown in figure 4-B and figure 5. Qualitatively these effects are very similar, NE inhibits a contractile pattern resembling that produces by TS. The percentage of NE-induced inhibition is 79.83 $\pm$ 10.25 (mean $\pm$ 2SE, n = 10). The characteristic effect of NE is decrease in the amplitude and decrease the basal tone. When the electrical transmural stimulation is tested under NE-induced condition, the contraction is inhibited completely (figure 4-B).

The present data shows a consistent with the previous data by Molnar et al. (1976) that transmural stimulation of the isolated human fallopian tube produced the inhibitory response resulting from activation of adrenergic nerves, subsequently NE release and then activation of beta adrenergic receptors.

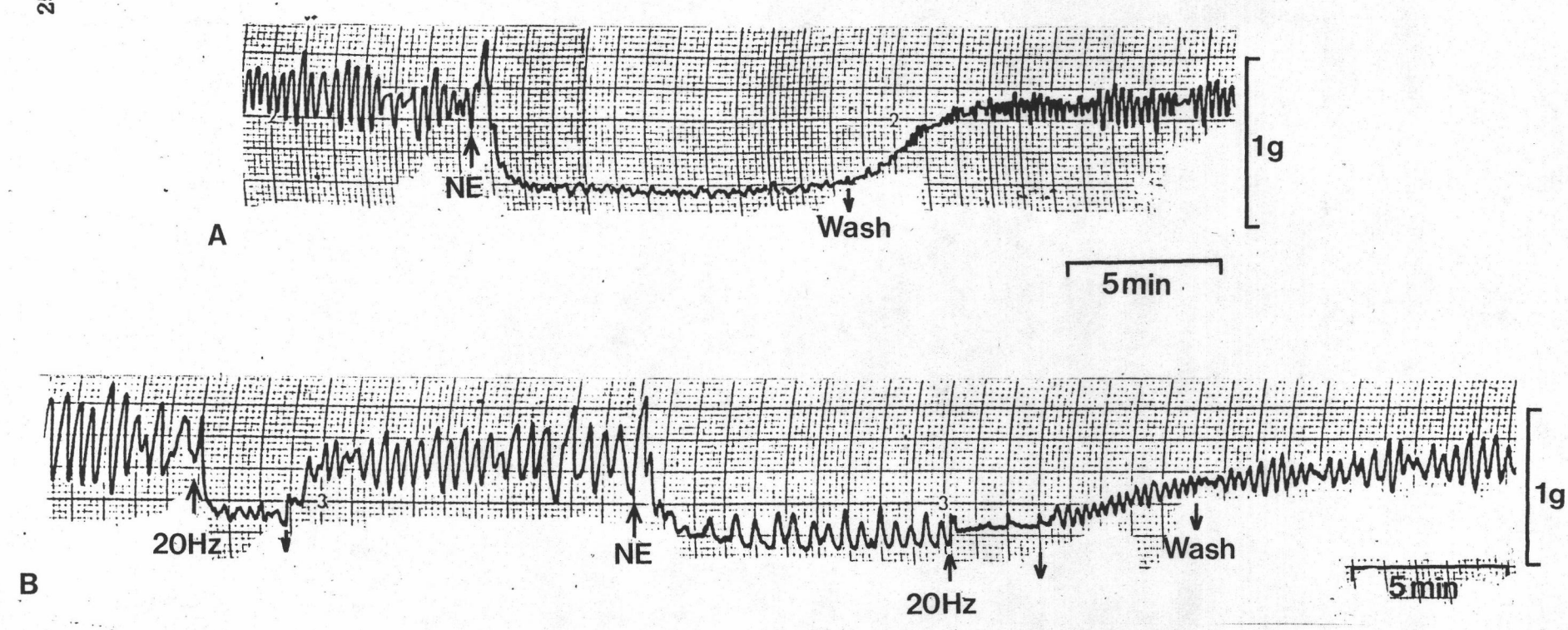

Figure 4 : (A) : The effect of 10^{-5} M norepinephrine on the fallopian tube contraction. (B) : Comparison of electrical transmural stimulation (TS) and the effect of 10^{-5} M norepinephrine on spontaneous contraction.

↑ = start of transmural stimulation or drug application;

↓ = end of transmural stimulation

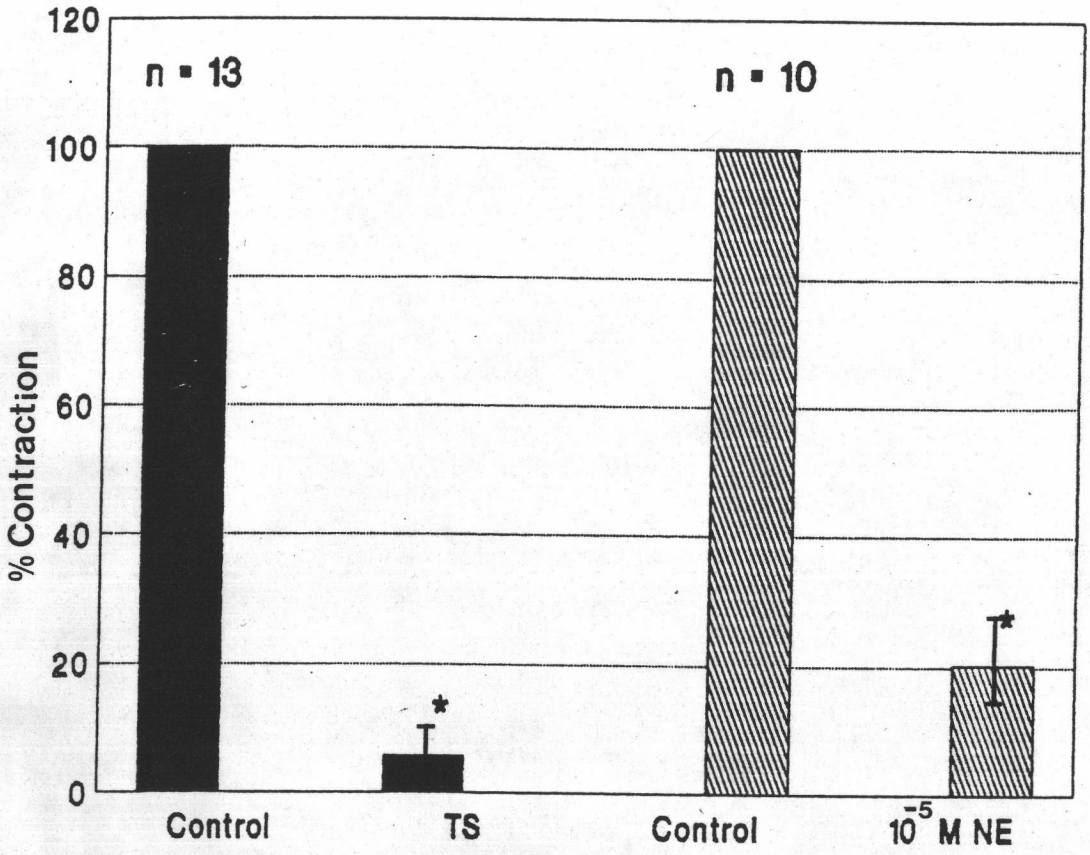

Figure 5 : Comparison the effect of 20 Hz of frequency of electrical transmural stimulation (n=13) with the effect of 10^{-5} M NE (n=10) on fallopian tube contractility. Solid bar is electrical transmural stimulation and hatch bar is 10^{-5} M NE application. The response is expressed as per cent contraction,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

Effects of GABA and GABA agonists on contractile response

To establish whether GABA stimulates or inhibits the contraction of fallopian tubes, if so, which receptors are responsible for the activity. The solution of 10^{-3}M GABA and its agonists, 10^{-5} M muscimol, a GABA-A agonist and 10^{-6} M baclofen, a GABA-B agonist were tested.

As shown in figure 6-A, GABA has no effect on spontaneous contraction of fallopian tube. Application of 10^{-3} M GABA to the medium resulting the little elevation of the basal tone but not the amplitude and frequency (n = 13).

To determine whether GABA affect the inhibitory action of electrical transmural stimulation, the tissues were stimulated with 20 Hz of frequency for 180 s before and after application of 10^{-3} M GABA to the medium. The result showed that GABA cannot reduce the inhibitory response induced by electrical transmural stimulation. The inhibitory response of the tissue after application of GABA showed a contractile pattern resembling that produced by electrical transmural stimulation before GABA application (figure 6-A) .

Quantitative analysis of the amplitude of contraction as per cent change from control in figure 9 showed that the inhibitory response resulting from electrical transmural stimulation before and affter GABA application are not significantly difference. The per centage of inhibition is 58.66 $\pm$ 9.50 and 60.23 $\pm$ 9.68 respectively. The per cent change of the amplitude resulting from GABA application to the tissue is about -1.98 $\pm$ 9.98 (negative value means inhibition),which is not significantly difference from

control. These results indicate that GABA has no effect on either spontaneous contraction or inhibition of NE release from adrenergic nerves resulting from electrical transmural stimulation.

The effects of 10^{-5}M muscimol, a GABA-A agonist, on contractile activity is shown in figure 6-B. Muscimol has no effect on the baseline tone of resting tension, the amplitude and also the frequency of contraction.

Comparison the effect of muscimol on inhibitory response induced by electrical transmural stimulation, the electrical transmural stimulation before and after 10^{-5}M muscimol were tested. The result showed that muscimol cannot alter the inhibitory response to transmural stimulation.

Quantitative analysis of the amplitude of contraction as per cent change from control is shown that the inhibitory responses resulting from electrical transmural stimulation before and after muscimol application are not significantly difference (figure 9). The per centage of inhibition is 72.60 $\pm$ 18.72 and 70.00 $\pm$ 17.70 before and after muscimol application, respectively (the values represent in mean $\pm$ 2SE, n = 8). The insignificant per cent change of amplitude due to muscimol application to the tissue compare with control is -9.17 $\pm$ 13.94 ($p > 0.05$).

The effect of baclofen, a GABA-B agonist on contractile response of the fallopian tube was shown in figure 7. Baclofen, 10^{-5} M was used in this study neither affected the frequency nor amplitude of spontaneous fallopian tube contraction. While the electrical transmural stimulation (90 V, 20 Hz for 180 s) produced the inhibition of contraction, 10^{-5} M baclofen could not

antagonize this effect. The contractile patern produced by electrical stimulation before baclofen application is resemble that produced by electrical stimulation after baclofen application.

Quantitative analysis of the amplitude of contraction resulting from electrical stimulation before and after baclofen application compare with the control is demonstrated. The per cent changes from control are -49.76 ± 13.26 and $- 59.64 \pm 12.63$, respectively (figure 9) (the values represent in mean $\pm$ 2SE, $n = 14$). These two values are significantly difference from control. While the effect of 10^{-5} M baclofen on per cent change from control of spontaneous contraction, is -7.78 ± 4.35, insignificantly difference from control.

To establish whether GABA stimulates or inhibits the contraction of smooth muscle directly, the effect of GABA following blockade of receptors for noradrenaline and acetylcholine, was investigated. 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) together substantially reduced the contraction of the longitudinal muscle. This responses could not be effected by GABA 10^{-3} M ($n = 10$) as demonstrated in figure 8.

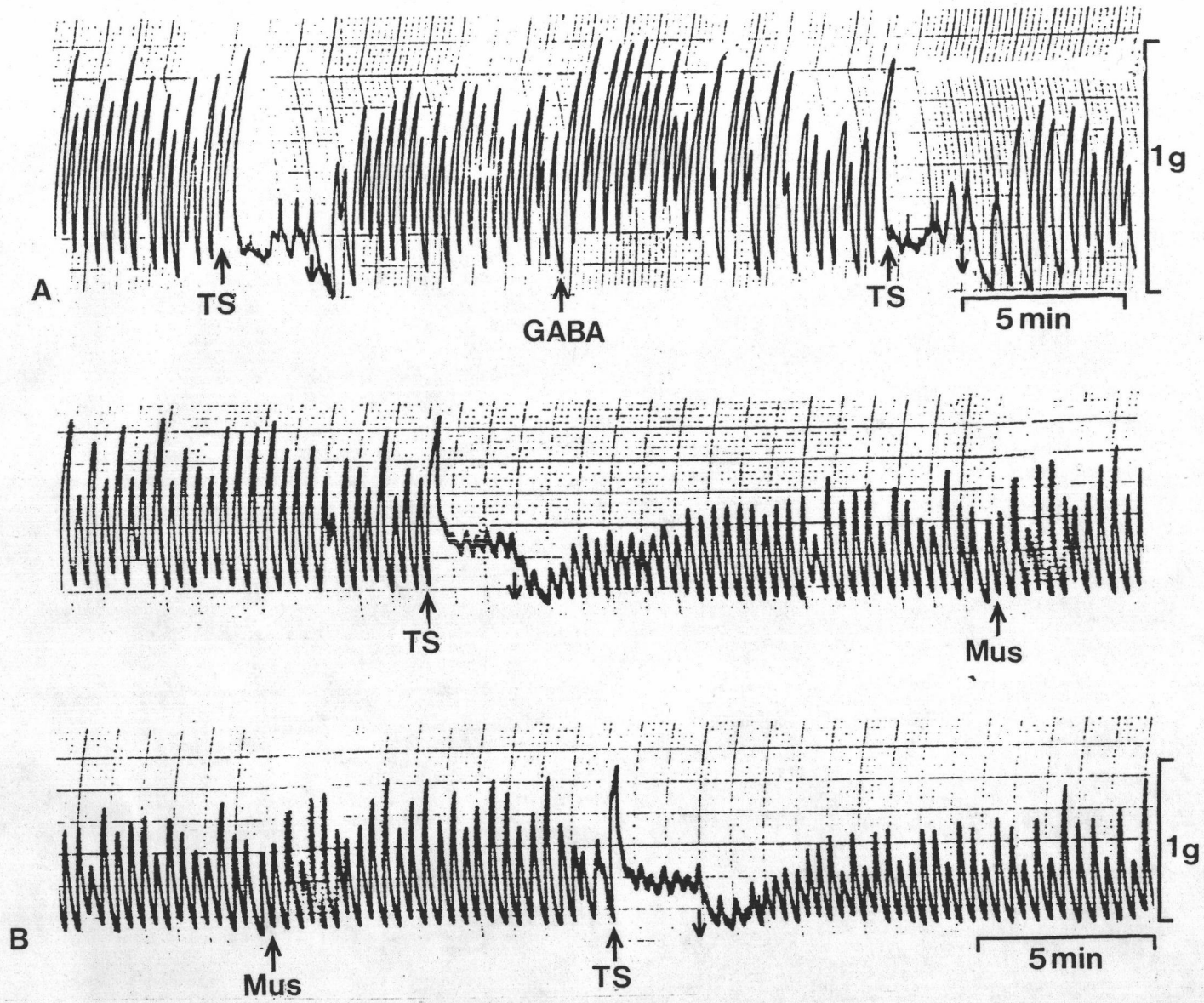

Figure 6 : Effect of electrical transmural stimulation (20 Hz of frequency) on human fallopian tube contractions. (A) Effect of 10^{-3} M GABA on electrical transmural stimulation; (B) : Effect of 10^{-5} M muscimol (a GABA-A agonist) on electrical transmural stimulation.

↑ = start of transmural stimulation

↓ = end of transmural stimulation

Mus=muscimol (n=13 and n=8 respective)

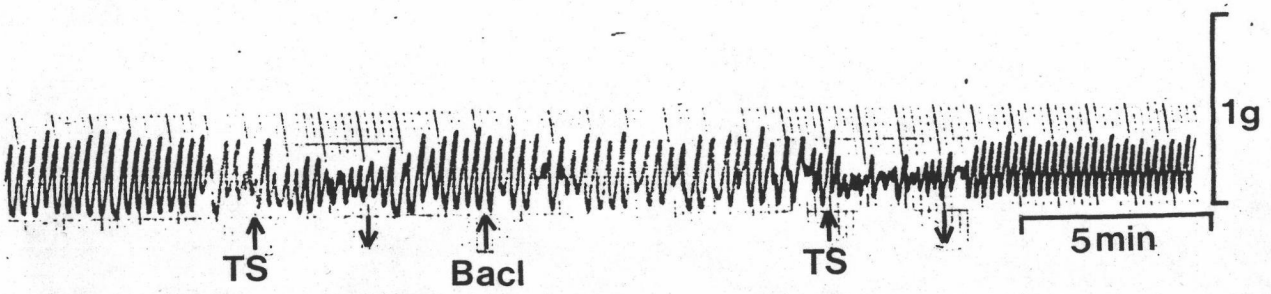

Figure 7 : Effect of 10^{-6} M baclofen (a GABA-B agonist) on electrical transmural stimulation (20 Hz of frequency).

↑ = start of transmural stimulation or drug application

↓ = end of transmural stimulation

Bacl=baclofen. (n=14)

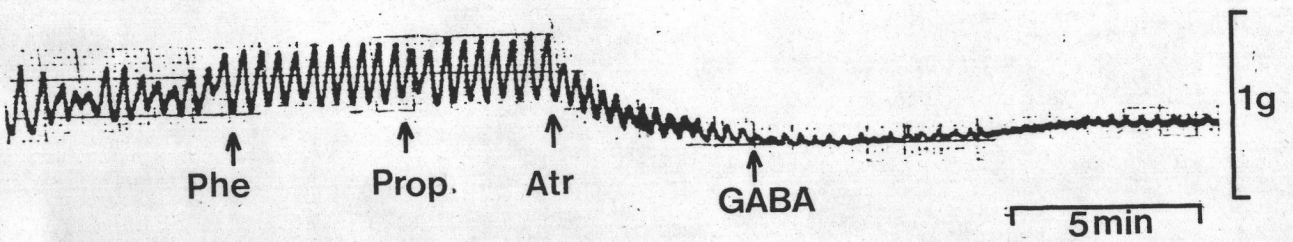

Figure 8 : Effect of 10^{-3} M GABA on human fallopian tube smooth muscle contractile response in the presence of alpha-adrenegic blocker (10^{-5} M phenoxybenzamine); beta-adrenergic blocker (10^{-6} M propranolol) and cholinergic blocker (10^{-4} M atropine)

↑ = drug application; Phe: phenoxybenzamine

Prop: propranolol; Atr: atropine (n=10)

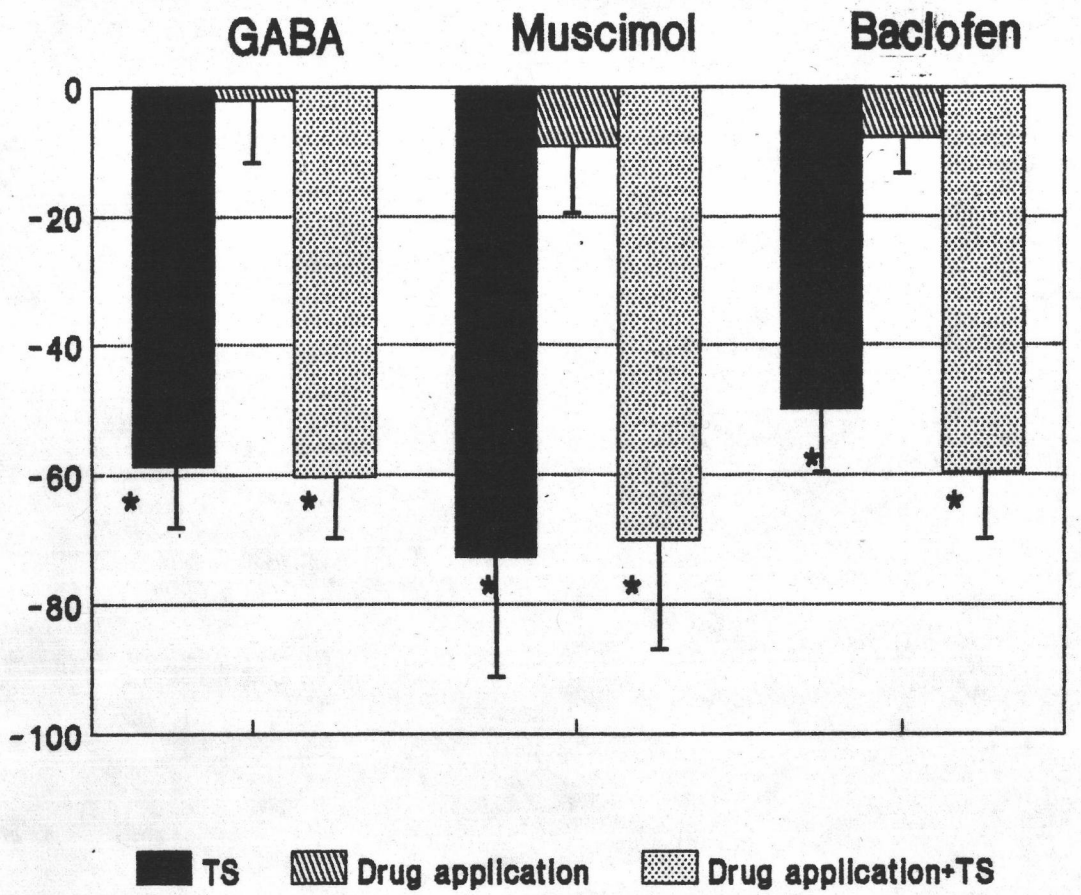

Figure 9 : Effect of 20 Hz of frequency of electrical transmural stimulation (TS) before and after 10^{-3} M GABA, 10^{-5} M muscimol and 10^{-6} M baclofen application. Data are expressed as per cent change from control (spontaneous contraction before transmural stimulation). Values are mean $\pm$ 2 SE.; n=13 for GABA experiment; n=8 for muscimol experiment and n=14 for baclofen experiment. Solid bar is transmural stimulation; hatch bar is drug application and dotted bar is combination of drug application and transmural stimulation (* $p < 0.05$, significantly difference from control).

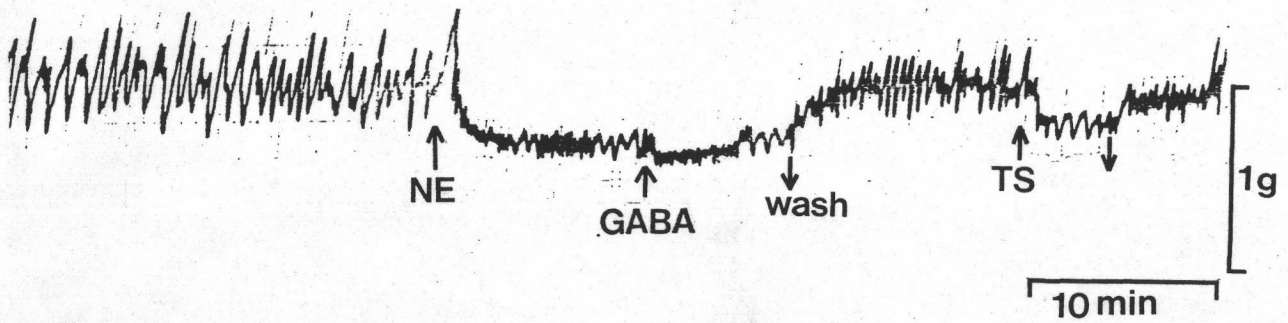

Figure 10 : Effect of 10^{-3} M GABA on fallopian tube contractile response under 10^{-5} M NE-induced inhibitory condition (n=10).

↑ = drug application or electrical transmural stimulation;

↓ = drug wash out or end of stimulation

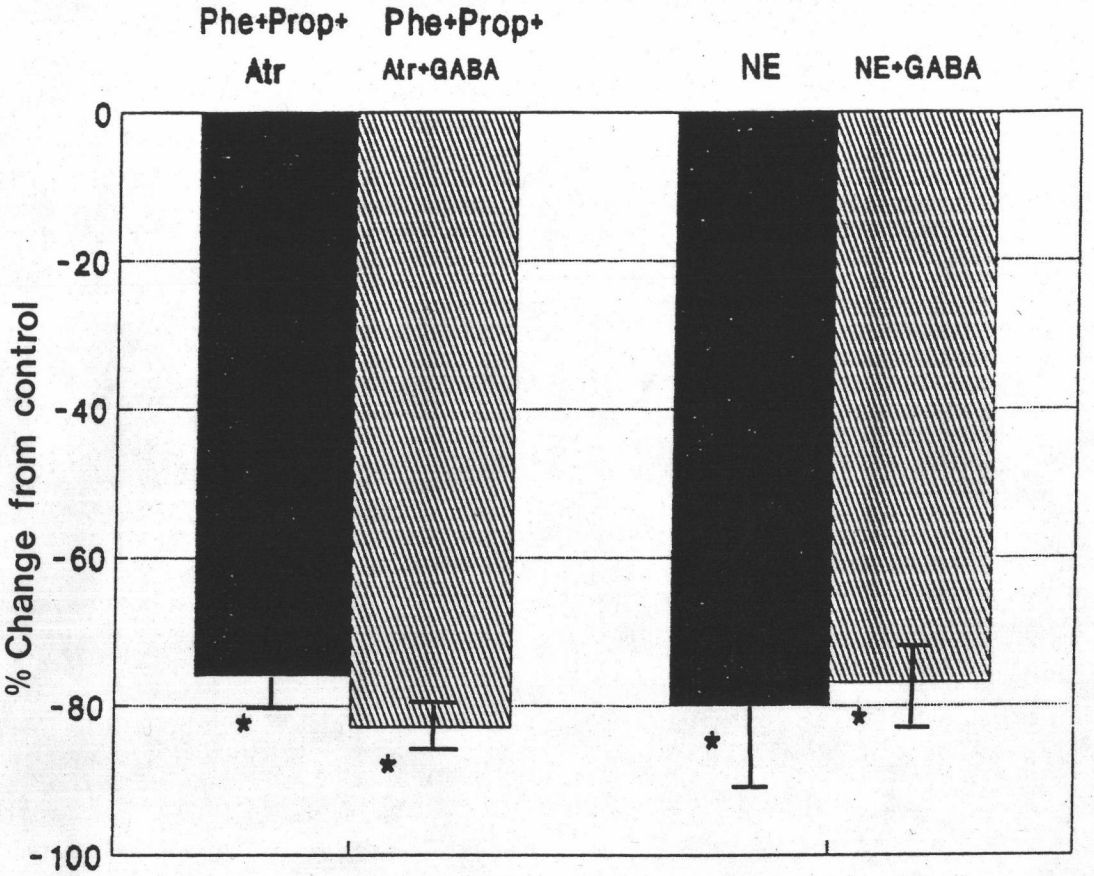

Figure 11 : Comparison the effect of 10^{-3} M GABA on the fallopian tube contractile response in the presence of combination of 10^{-5} M phenoxybenzamine, 10^{-6} M propranolol and 10^{-4} M atropine, and in the presence of 10^{-5} M NE. Data are represented as per cent change from apontaneous contraction (control). Values are mean $\pm$ 2 SE (n=10 for each experiment). (* $p < 0.05$, significantly difference from control). Phe = phenoxybenzamine; Prop = propranolol; Atr = atropine; NE = norepinephrine.

Effects of GABA and its agonists under adrenergic and cholinergic- induced response conditions

To establish whether GABA acts as co-regulator or modulator of the tissues response through autonomic neurotransmitters, NE from sympathetic nerves and ACh from cholinergic nerves. Effects of GABA on smooth muscle tissue under NE and ACh-induced response condition were tested.

While NE inhibited the contractility and had relaxing effect on smooth muscle, GABA cannot alter these effects as shown in figure 10. Quantitative analysis comparison of the effect of GABA under autonomic nerve blockade and under NE-induced condition is established. Whether GABA had a direct effect on smooth muscle or indirect through sympathetic nerves. As shown in figure 11, GABA can alter neither the effect of combination of autonomic neurotransmitter blockers nor NE. These results imply that GABA has no either direct effect on smooth muscle or through the modulation of sympathetic nerves by enhancing or inhibiting its neurotransmitter effect.

Acetylcholine exerts a stimulatory action on tissue preparations. This effect could be characterized by a marked increase of contraction frequencies but not the amplitude and by elevation of the basal tone. (Figure 12-A, B, C and figure 13-A, B).

To determine whether the site of action of GABA is on cholinergic nerves or on smooth muscle, the effect of GABA on the response to ACh was examined. When 10^{-3} M GABA was applied into the medium, the ACh contraction elicited by 10^{-3} M dose of ACh is augmented by 10^{-3} M GABA (figure 12-A). The increasing was mimiced by 10^{-5} M concentration of a GABA-A agonist, muscimol as shown in figure 12-B. Baclofen, a GABA-B agonist in concentration 10^{-6} M has no effect on the contractile response to 10^{-3} M ACh. (Figure 12-C).

In contrast to the effects on electrical transmural stimulation-induced and NE-induced inhibition, the contractile response to ACh 10^{-3} M is augmented by 10^{-3} M GABA and 10^{-5} M muscimol being 143.74 $\pm$ 36.24 % and 155.08 $\pm$ 2.60 % respectively when ACh contraction was designated as 100 % (values are represented in mean $\pm$ 2SE, n = 12 for each experiment). The contractile response to ACh-induced is augmented by 10^{-6} M baclofen being only 109.56 $\pm$ 2.80 %, (n = 5), without significantly difference from ACh-induced contraction. (Figure 14). Pretreatment of 10^{-6} M bicuculline in the bathing medium prevented the enhancing effect of 10^{-3} M GABA and 10^{-5} M muscimol on fallopian tube contractions induced by 10^{-3} M ACh (figure 13-A,B and figure 14, n = 7 and n = 5, respectively). Since GABA , muscimol and baclofen have been tested and showed no effect on either baseline tone of resting tension, or even amplitude and frequency of spontaneons contractions. However, GABA and muscimol increased the contraction induced by ACh. In addition, these effects are inhibited by bicuculline. These results indicate that GABA may act on fallopian tube contractility through parasympathetic neurotransmitter modulation.

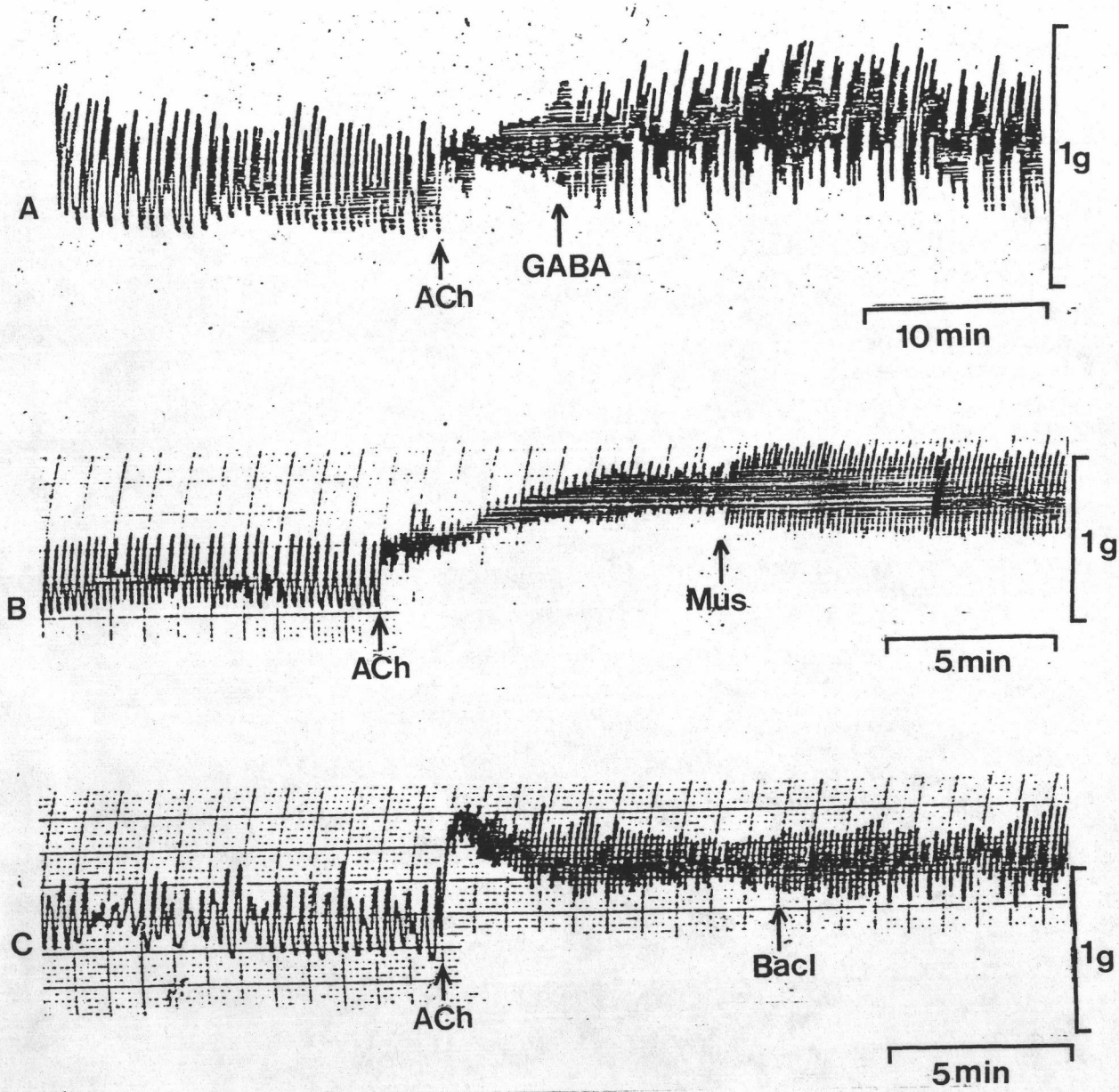

Figure 12 : Effect of 10^{-3} M GABA (A), 10^{-5} M muscimol (B) and 10^{-6} M baclofen (C) on 10^{-3} M ACh-induced contraction of the human fallopian tube (n=12, n=12 and n=5 respectively).

↑ = drug application, ACh = acetylcholine,

Mus = muscimol, Bacl = Baclofen.

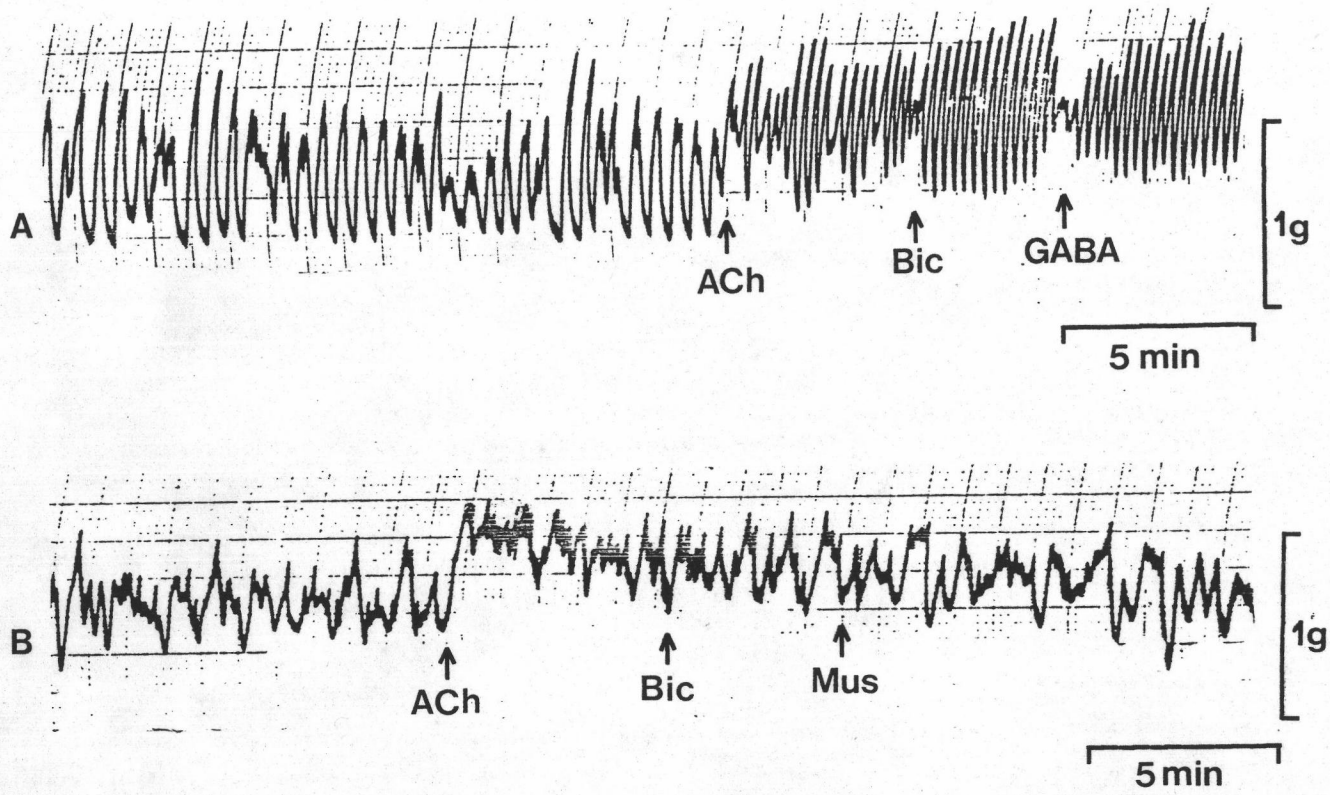

Figure 13 : Effect of 10^{-3} M GABA (panel A) and 10^{-5} M. muscimol (panel B) on ACh-induced contraction of the fallopian tube in the presence of 10^{-5} M bicuculline (n=7 and n=5 respectively).

↑ = drug application, Bic = bicuculline,

ACh = acetylcholine , Mus = muscimol.

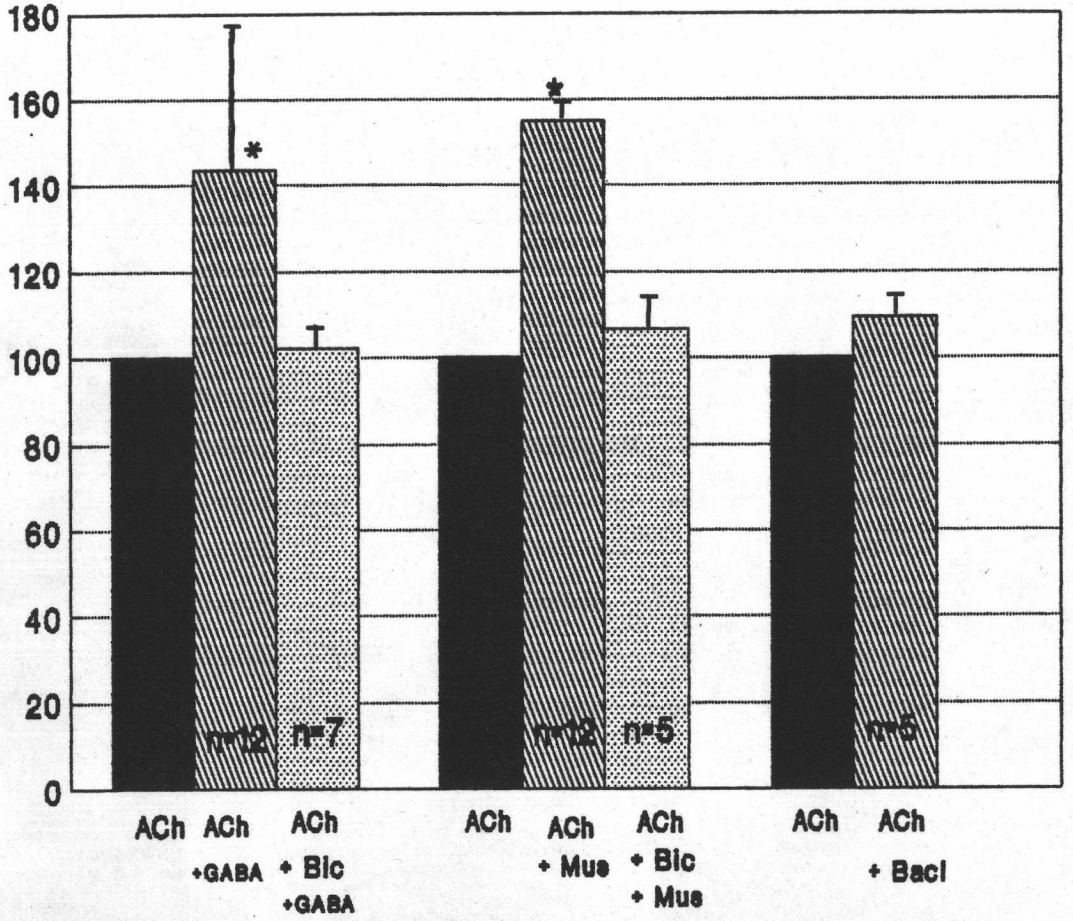

Figure 14 : Effect of 10^{-3} M GABA, 10^{-5} M muscimol (Mus) and 10^{-6} M baclofen (Bacl) on ACh-induced contraction in the presence or absence of 10^{-5} M bicuculline (Bic), while ACh-induced contraction is designated as control. Data are represented as per cent change from ACh-induced contraction. Values are mean $\pm$ 2 SE. The n indicates the number of specimen tested. * $p < 0.05$ is significantly difference from control.