Nitric Oxide Inhibits Smooth Muscle Responses Evoked by Cholinergic Nerve Stimulation in the Guinea Pig Gastric Fundus

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Abstract: In circular smooth muscle tissues of the guinea pig gastric fundus, transmural nerve stimulation (TNS) evoked an atropine-sensitive cholinergic excitatory junction potential (e.j.p.) and, after inhibiting the e.j.p. with atropine, an apamin-sensitive nonadrenergic noncholinergic (NANC) inhibitory junction potential (i.j.p.). The amplitude of e.j.p.s was similar when the frequency of TNS was low (<0.5 Hz), but it decreased successively (depression phenomenon) when the frequency was high (>1 Hz). The depression phenomenon was attenuated after inhibiting the production of nitric oxide (NO) with N\textsuperscript{\textalpha}-nitro-L-arginine (NOLA), but was not altered by inhibiting the i.j.p. with apamin. The e.j.p.s were increased in amplitude by the inhibition of cholinesterase activity, but they were decreased by NO produced from SNP with no alteration of their depression phenomenon. Isometric twitch contractions were depressed during high-frequency TNS. NOLA caused an increase in the amplitude of twitch contractions and the attenuation of their depression that changed the transient contraction produced by high-frequency TNS (1 Hz) to a tetanic one. SNP reduced the amplitude of twitch contractions, with no alteration of the membrane depolarization. The results suggest that NO produced during TNS has inhibitory actions on cholinergic transmission; the depression of e.j.p.s is mainly prejunctional events, and the depression of mechanical responses is mainly postjunctional events. [Japanese Journal of Physiology, 51, 693–702, 2001]

Key words: cholinergic transmission, junction potential, NO, NANC inhibition, depression.

Gastric smooth muscle receives projections from cholinergic excitatory and nonadrenergic noncholinergic (NANC) inhibitory nerves [1], and transmural electrical stimulation of these nerves elicits excitatory and inhibitory junction potentials (e.j.p. and i.j.p., respectively) in smooth muscles [2]. The cholinergic e.j.p. is inhibited by atropine, indicating that the potential is produced by an activation of muscarinic receptors [2]. In dog, the NANC i.j.p. is considered to be mediated by nitric oxide (NO) [3], since the potential is inhibited by N\textsuperscript{\textalpha}-nitro-L-arginine (NOLA), an inhibitor of NO synthase [4]. In the rat fundus, the i.j.p. is mimicked by S-nitrosocysteine, an NO donor [5], and could be attenuated, but it cannot be abolished by NOLA [6, 7], suggesting that NO and unidentified substances are involved in the i.j.p. formation. In the guinea pig stomach, nitroxidergic nerves are involved in the vagal projection to the stomach as an inhibitory mediator [8–10]. In this tissue, however, the NANC i.j.p. evoked by transmural nerve stimulation (TNS) is inhibited by apamin, but not by NOLA [11]. These controversial results suggest that the NANC i.j.p. is not produced by single substances, and the role of NO may differ between species and also between regions in the gastrointestinal tract [12]. A possible involvement of vasoactive intestinal polypeptide (VIP) [13,
s pituitary adenylyl cyclase activating peptide (PACAP) [15, 16], or carbon monoxide (CO) [17] is also reported in the inhibitory transmissions in gastrointestinal smooth muscles.

In smooth muscles isolated from the guinea pig gastric fundus, the facilitation phenomenon of e.j.p.s is observed in response to two TNSs with 2–5 s intervals, whereas the depression phenomenon is elicited in the e.j.p.s when a 2nd TNS is applied at short intervals (<1 s) [18]. TNS applied with short intervals will induce an overflow of transmitters from the junctional cleft and modulate the successive release of transmitters by activation of the prejunctional autoregulation mechanisms [19]. The possible involvement of co-transmitter substances in the prejunctional regulation of transmitter release is also considered [20]. However, the factors involved in these frequency-dependent alterations of the amplitude of e.j.p.s in gastric fundus remain unclear.

The present experiments were carried out to elucidate the factors involved in the depression of junction potentials, and mechanical responses appeared during trains of TNS with high frequencies in the guinea pig gastric fundus muscles. The results indicated that NO produced in response to TNS is one of the important factors for the prejunctional and postjunctional inhibitory modulations of cholinergic transmission mainly during high-frequency stimulation.

**MATERIALS AND METHODS**

Male albino guinea pigs, weighing 200–250 g, were anesthetized with fluoromethyl 2,2,2-trifluoro-1-(trifluoromethyl) ethyl ether (sevoflurane, Maruishi Pharm., Osaka, Japan), then decapitated. The animals were treated ethically according to the Guidelines for the Care and Use of Animals approved by the Physiological Society of Japan. The fundus region of the stomach was isolated, and the mucosal layer was removed by cutting with fine scissors. Smooth muscle tissues about 1.0 mm wide and 1.0 cm long were dissected along the circular muscle bundles (3–5 bundles), together with attached longitudinal muscle layers.

Isolated muscle strips were mounted on a silicone rubber plate fixed at the bottom of the recording chamber, with the mucosal layer upside. The recording chamber made from Lucite plate was 8.0×20.0 mm wide and 5.0 mm deep with a capacity of about 1 ml, and the muscle preparation was superfused with warmed Krebs solution at a constant flow rate of about 3 ml/min. A silver wire coated with enamel, except for the tip, was attached gently on the edge of the mounted tissue strip, and rectangular electrical pulses with brief durations (0.1–0.3 ms) were applied to the tissue through the wire for the transmural stimulation of intramural nerves (transmural nerve stimulation, TNS). Electric pulses were supplied by an electric stimulator (SEN-3301, Nihon Kohden, Tokyo, Japan). The effects of tetrodotoxin (3×10⁻⁷ M) on the electrical responses of smooth muscle membrane elicited by these stimuli were tested, and those abolished reversibly by tetrodotoxin were accepted as junction potentials. The TNS-evoked junction potentials were recorded from circular smooth muscle cells, using glass capillary microelectrodes (tip resistance, 50–80 MΩ), and were displayed on a cathode-ray oscilloscope (SS-7802, Iwatsu, Tokyo, Japan) and also stored in a personal computer for later analysis.

For recording the mechanical responses of smooth muscle tissues, both ends of the muscle strips were tied with fine silk thread and mounted vertically to a recording chamber with cylindrical shape (diameter 8.0 mm; height 1.5 mm; volume about 1.0 ml). The lower thread was fixed at the bottom, and the upper thread was connected to the arm of a mechatron-transducer (TB-612T, Nihon Kohden); the preparation was perfused with warmed Krebs solution at a constant flow rate of about 3 ml/min. A pair of rectangular silver plates 2.0 mm wide was fixed at the wall of the chamber, facing each other, and TNS was applied to the muscle through these plates. The resting tension of about 0.5 g was applied to each preparation, and isometric contractile forces produced by circular muscles were measured. The effects of tetrodotoxin on the mechanical responses elicited by TNS were tested, and those inhibited by tetrodotoxin were accepted as the responses elicited through the excitation of intramural nerves. The mechanical responses were displayed on a pen-writing recorder (National VP-6524A, Matsushita Electric Co., Tokyo, Japan).

The ionic composition of the Krebs solution was as follows (in mM): Na⁺ 137.4, K⁺ 5.9, Mg⁡²⁺ 1.2, Ca⁡²⁺ 2.5, HCO₃⁻ 15.5, H₂PO₄⁻ 1.2, Cl⁻ 134, glucose 11.5. The solution was aerated with O₂ containing 5% CO₂, and pH of the solution was 7.1–7.2.

Drugs used were acetylcholine chloride (ACh), atropine sulfate, apamine, neostigmine, NOLA, sodium nitroprusside (SNP), and tetrodotoxin (all purchased from Sigma Chem., USA). The chemicals were dissolved in distilled water as a stock solution and diluted further with Krebs solution to desired concentrations (the ratios of the dilution exceeded 1 : 1000). The dilution procedures did not alter the pH of the Krebs solution.

Values measured were expressed by the mean± standard deviation (SD). Differences between values
were tested by using an unpaired Student $t$-test, and probabilities of less than 5% ($p<0.05$) were considered significant.

RESULTS

Properties of junction potentials

In fundus smooth muscle of the guinea pig, TNS evoked an e.j.p. with the amplitude being 5–25 mV, and the potential was abolished by the application of atropine ($10^{-6}$ M) (Fig. 1). In the presence of atropine, TNS evoked an i.j.p. with the amplitude being 0.5–5 mV, and the potential was inhibited by apamin ($10^{-7}$ M). These results confirmed the properties of junction potentials reported previously in fundus smooth muscle of the guinea pig stomach [18].

TNS was applied in a train for 10 s at various frequencies (0.1–2 Hz), and the amplitude of e.j.p.s evoked by each stimulus was measured. The e.j.p.s with reproducible amplitude were evoked when stimuli were applied at low frequencies (<0.5 Hz). Increasing the frequency of stimuli over 0.5 Hz resulted in a generation of e.j.p.s with decreasing amplitude in a frequency-dependent manner (Fig. 1, A–E). The reduction in amplitude of e.j.p.s during the repetitive application of TNS, termed a depression phenomenon, was observed in all preparations examined. The amplitude of e.j.p.s evoked by single stimuli varied between preparations, and they were therefore normalized by having the value expressed as relative to the first of each train response. The depression phenomenon appeared when TNS was applied at frequencies higher than 0.5 Hz (see Fig. 1, C–E), and no measurable e.j.p. was evoked when the frequency exceeded 5 Hz ($n=5$, Fig. 2, C).

As TNS generates NANC i.j.p. together with the e.j.p. [18], the possibility exists that a TNS train enhances the amplitude of successive i.j.p.s, which may cause the depression phenomenon of the e.j.p.s, as a balance of two junction potentials. A possible involvement of i.j.p. components in the depression phenomenon was examined by observing the effects of repetitive stimulation on the amplitude of i.j.p.s in the presence of atropine and also the effects of inhibiting the i.j.p. with apamin on the e.j.p.s. In the presence of atropine ($10^{-6}$ M), TNS evoked i.j.p.s with the amplitude being 0.5–5 mV. Similar amplitudes of i.j.p. were evoked when a TNS train was applied at frequencies below 0.5 Hz. Higher frequency stimulations (≥0.5 Hz) produced a summation of the potential, resulting in a sustained hyperpolarization of the membrane during the train stimulation (Fig. 1, H–J). Because the amplitude of e.j.p. was a function of membrane potential and hyperpolarization increased it [18], the effects of inhibiting the i.j.p.s with apamin on the depression phenomenon of e.j.p.s were examined (Fig. 2). Apamin ($10^{-7}$ M) increased the amplitude of all e.j.p.s to a similar extent, and the first e.j.p. was increased to 127.0±14.0% ($n=12$) of control. However, the depression phenomenon of the e.j.p.s evoked by 1, 2, and 5 Hz frequencies was not altered. These results indicate that the depression phenomenon of e.j.p.s during a repetitive stimulation of nerves may not be causally related to the alteration of NANC i.j.p.s in the fundus smooth muscle.

The role of NO in the depression of e.j.p.s was observed by inhibiting the production of NO with NOLA [4]. In the presence of NOLA ($10^{-5}$–$10^{-4}$ M),
the amplitude of e.j.p. evoked by a single TNS remained unaltered \((10^{-5} \text{ M NOLA}, 101.0 \pm 18.0\%, n=8; 10^{-4} \text{ M NOLA}, 100.0 \pm 28.0\%, n=12; p>0.05\) for each). An expression of the amplitude of each e.j.p. by the normalized value indicated that the depression phenomenon was attenuated by NOLA. As a consequence, in the presence of NOLA the e.j.p.s with similar amplitude were evoked when TNS was applied at frequencies below 1 Hz (Fig. 3, A–D). The depression phenomenon of e.j.p.s elicited by high-frequency stimulation (\(>2 \text{ Hz}\)) was not fully antagonized by NOLA (Fig. 3, E). These results suggest that NO produced during TNS contributes partly to the depression phenomenon of e.j.p.s.

The effects of neostigmine on the e.j.p.s evoked by repetitive applications of TNS were observed in the gastric fundus muscles to evaluate the possible involvement of prejunctional cholinergic autoregulation [19, 20] in the depression phenomenon. In the presence of \(10^{-7} \text{ M neostigmine}, \) the amplitude of e.j.p.s evoked by single stimuli was increased to \(118 \pm 9\% (n=7)\) of control, with no significant change in the membrane potential (control, \(-51.1 \pm 3.5\text{ mV}, n=7\); in neostigmine, \(-51.6 \pm 5.9\text{ mV}, n=7; p>0.05\)). The depression phenomenon of e.j.p.s produced by 1 and 2 Hz stimulations was not altered by neostigmine (Fig. 4, A, B, E, and F).

The effects of a low concentration of atropine on the depression of e.j.p.s was also observed in smooth muscles of the gastric fundus to evaluate possible involvement of the prejunctional autoregulation mechanisms in the release of ACh. Atropine at a concentration of \(10^{-9} \text{ M}\) reduced the amplitude of e.j.p.s evoked by single stimuli to \(75 \pm 18\% (n=12)\) of control, with no alteration of the membrane potential (control, \(-50.7 \pm 3.8\text{ mV}, n=12\); in atropine, \(-51.3 \pm 4.5\text{ mV}, n=12; p>0.05\)). However, the depression phenomena observed by 1 and 2 Hz TNS were not altered by at-
ropine (Fig. 4, C, D, G, and H).

**Effects of sodium nitroprusside on electrical responses produced by TNS**

Attempts were made to evaluate the inhibitory actions of exogenously applied NO on the e.j.p. evoked in fundus smooth muscles of the guinea pig stomach. NO was supplied by applying SNP in the superfusate after inhibiting the production of endogenous NO with 10⁻⁵ M NOLA. A low concentration of SNP (10⁻⁸ M) did not alter the resting membrane potential (control, −49.8 ± 3.2 mV, n = 15; SNP 10⁻⁸ M, −54.7 ± 3.1 mV, n = 10, p > 0.05). High concentrations of SNP (>3×10⁻⁸ M) hyperpolarized the membrane in a concentration-dependent manner (SNP 3×10⁻⁸ M, −58.7 ± 3.4 mV, n = 10, p < 0.05; SNP 10⁻⁷ M, −61.7 ± 2.6 mV, n = 12, p < 0.05). In the presence of SNP (3×10⁻⁸ M), the amplitude of e.j.p.s evoked by TNS was reduced (Fig. 5, A and B). The inhibitory effects of SNP on the e.j.p.s appeared in a concentration-dependent manner, and in 10⁻⁷ M SNP the e.j.p.s were negligibly small (Fig. 5, C). The depression phenomena of e.j.p.s evoked by TNS trains with 1 and 2 Hz frequencies (Fig. 5, D and E) were also attenuated by 10⁻⁸ M SNP, with a significant reduction in the amplitude of e.j.p.s. Increasing concentrations of SNP to 3×10⁻⁸ and 10⁻⁷ M reduced further the amplitude of the first e.j.p.s of each train response and altered the depression phenomenon to a facilitation phenomenon of the e.j.p.s. Thus SNP could inhibit the e.j.p.s in the absence of any significant alteration of postjunctional membrane potentials. However, higher concentrations of SNP hyperpolarize the postjunctional membrane, indicating that the evaluation of the inhibitory actions of SNP on the e.j.p.s may be difficult from these types of experiments.

**Mechanical responses produced by TNS**

TNS with single stimuli elicited twitch contractions in fundus smooth muscles of the guinea pig stomach. The twitch contraction was abolished by atropine (10⁻⁶ M), indicating that it was elicited in response to acetylcholine (ACh) released from intramural nerves. In the presence of atropine, TNS elicited a relaxation of smooth muscles. These results confirmed the properties of the TNS-induced mechanical responses reported previously in the fundus muscles [21].

A TNS train with low frequencies (<0.05 Hz) evoked a similar amplitude of twitch contractions in response to each stimulus (Fig. 6, A). A successive reduction of the resting tension during TNS was often observed. Increasing the frequency of stimuli over 0.1 Hz resulted in a production of twitch contractions with successively decreasing amplitude (depression...
phenomenon), superimposed on the sustained elevation of resting tension (Fig. 6, B and C). At 1–2 Hz frequency stimulation, the amplitude of individual twitch contractions was reduced below detectable size, together with the sustained relaxation, and as a consequence muscle produced a transient contraction followed by a sustained relaxation during TNS (Fig. 6, E). The peak amplitude of the summed contraction produced by 1 Hz stimulation was $138.7 \pm 51.9\%$ ($n=14$) of twitch contractions. The amplitude of twitch contraction was measured as being relative to the first of each TNS train, allowing the comparison of contractions obtained from different preparations in the same scale (Fig. 7, A). A reproducible amplitude of twitch contractions was elicited when TNS was applied at low frequencies ($<0.05$ Hz). The depression phenomenon appeared in twitch contractions evoked at frequencies higher than 0.1 Hz.

The inhibition of NO production by NOLA ($10^{-5}$ M) resulted in a significant increase in the amplitude of twitch contractions evoked by single stimuli to $146.5 \pm 38.0\%$ of control ($n=16$), with no significant alteration of the resting tension (Fig. 6, F). In the presence of NOLA, the depression phenomena in twitch contractions produced by TNS with 0.1–0.5 Hz frequency were changed to facilitation phenomena (Fig. 6, G, H; Fig. 7, B). A marked alteration by NOLA also appeared on contractions produced by TNS with higher frequencies, and tetanic contraction was elicited during TNS with frequencies higher than 0.5 Hz (Fig. 6, I and J). The peak amplitude of tetanic contractions produced by 1 Hz TNS in the presence of NOLA was increased to $262.8 \pm 92.6\%$ ($n=14$, $p<0.05$) of the twitch contractions produced in the absence of NOLA. The effects of inhibiting the i.j.p. components by apamin on mechanical responses produced by TNS were observed in fundus smooth muscle of the guinea pig. TNS with low (0.1 Hz) and high (1 Hz) frequencies were applied to observe the effects of apamin on twitch and tetanic contractions, respectively. In the presence of $10^{-7}$ M apamin, amplitudes of twitch contractions produced by single stimuli were increased ($147.4 \pm 35.4\%$ of control, $n=3$, $p<0.05$). However, the depression phenomenon seen in twitch contractions elicited by 0.1 Hz TNS was not significantly altered (Fig. 8, A and B). Apamin augmented the peak amplitude of contractions produced by 1 Hz TNS ($185.7 \pm 38.5\%$ of control, $n=3$, $p<0.05$) and inhibited the relaxation produced during TNS (Fig. 8, D). These results suggest that the depression phenomenon of contractions produced by high-frequency stimulation may be mainly due to endogenous NO produced during TNS, and the contribution of apamin-sensitive i.j.p. component, if any, may be negligibly small.
Effects of sodium nitroprusside on mechanical responses produced by TNS or exogenously applied acetylcholine

The effects of SNP ($10^{-8}$M) on mechanical responses produced by TNS were also observed in the presence of $10^{-5}$M NOLA. SNP relaxed muscles and as a consequence abolished the resting tension and reduced the amplitude of twitch contractions produced by 0.1 Hz TNS to about 30% of control (Fig. 9, A and B). However, the amplitude of tetanic contractions produced by TNS with 1 Hz frequency was not markedly altered by SNP (Fig. 9, C and D). Thus the inhibitory actions of exogenously applied NO appear markedly on twitch contractions and weakly on tetanic contractions.

The effects of SNP on electrical and mechanical responses produced by exogenously applied ACh were also measured in fundus smooth muscle of the guinea pig. The concentration of ACh was chosen to be $10^{-7}$M, which was comparable to the estimated concentration of ACh acting on fundus smooth muscles during TNS (equal to $8 \times 10^{-8}$M [21]). An application of $10^{-7}$M ACh depolarized the membrane by $4.3 \pm 1.0$

**Fig. 6. Effects of NOLA on TNS-induced mechanical responses.** In smooth muscle of the guinea pig gastric fundus, a TNS train was applied for 1 min with different frequencies (A and F, 0.03 Hz; B and G, 0.1 Hz; C and H, 0.2 Hz; D and I, 0.5 Hz; E and J, 1 Hz), in the absence (A–E, control) and presence of $10^{-5}$M NOLA (F–J). All responses were recorded from the same tissue.

**Fig. 7. Changes in amplitude of twitch contractions produced by TNS.** In circular smooth muscle strips of the guinea pig gastric fundus, TNS was applied for 30 s with different frequencies, in the absence (A, control) and presence of $10^{-5}$M NOLA (B). The peak amplitudes of the TNS-induced contractions measured from the resting level were expressed by the relative value of contractions produced by the first TNS of each train (O, 0.5 Hz; ×, 0.3 Hz; △, 0.2 Hz; □, 0.1 Hz; ◊, 0.03 Hz). Mean values are shown (the SD of each value did not exceed ±20% of the mean value, n=6–12).

**Fig. 8. Effects of apamin on contractions produced by TNS.** In circular smooth muscles of the guinea pig gastric fundus, TNS-induced mechanical responses (A and B, 10 stimuli at 0.1 Hz frequency; C and D, 30 stimuli at 1 Hz frequency) were recorded in the absence (A and C, control) and presence of $10^{-7}$M apamin (B and D). All responses were recorded from the same tissue.
mV (n=6), and the value remained unaltered in the
presence of 10⁻⁸ M SNP (4.7±1.2 mV, n=6; p>0.05).
Increasing the concentration of ACh to 10⁻⁶ M depolarized the membrane by 15.7±4.9 mV (n=6), and
10⁻⁸ M SNP again did not alter the amplitude of depolarization (16.8±5.6 mV, n=6, p>0.05).

ACh produced a sustained contraction in fundus smooth muscles (data not shown), and in the presence of SNP, contractions produced by 10⁻⁷ M ACh were reduced by about 60%, and those produced by 10⁻⁶ M ACh were reduced by about 10% (Fig. 9, E). These results indicate that exogenous NO inhibits ACh-induced mechanical responses with no alteration of the electrical responses in fundus smooth muscles. However, the NO-induced inhibition appears only when the concentration of ACh is low and the inhibitory actions of NO on contractions produced by higher concentrations of ACh are weak.

**DISCUSSION**

The present experiments show that in fundus smooth muscle of the guinea pig, NO may be one of the factors to elicit the depression phenomenon of cholinergic transmission when they are evaluated from the effects of NOLA, an inhibitor of NO [4], on e.j.p's and contractions produced by TNS. In the stomach, nitro-ergic projections are confirmed by the immunohistochemical staining of the enzyme, NO synthase, in the vagal motor system [8, 9]. A functional involvement of NO in the adaptive relaxation as the inhibitory mediator is also reported in the guinea pig stomach [8–10, 12].

The depression phenomenon of e.j.p’s is one of the characteristic features in fundus smooth muscle of the guinea pig, and it appears when TNS is applied at high frequencies [18]. An involvement of many factors is considered for the depression phenomenon, such as (1) prejunctional autoinhibition by ACh, (2) desensitization of postjuncional muscarinic receptors to ACh during successive TNS, (3) release or production of inhibitory modulators during TNS, and (4) augmented i.j.p’s generated during a repetitive application of TNS preventing the successive e.j.p’s. The inhibition by neostigmine of acetylcholine esterase will increase the concentration of released ACh around the junctional clefts, which will allow ACh to overflow and stimulate prejunctional muscarinic receptors to activate the autoinhibition mechanism for the transmitter release [19, 20]. The present experiments indicate that the depression phenomenon is not modulated by neostigmine, suggesting that the prejunctional autoregulation mechanism is not causally related. A low concentration of atropine would modulate the muscarinic receptor–mediated prejunctional autoregulation mechanism [20]. An absence of any alteration of the e.j.p’s in the presence of atropine again suggests that the autoregulation mechanism may not be involved in the depression phenomenon. Exogenously applied ACh produces a sustained depolarization of the membrane in fundus smooth muscles [18], indicating that the progressive development of desensitization in the postjunctional muscarinic receptors is unlikely for the depression phenomenon. An absence of substantial contribution of i.j.p’s on the depression phenomenon of e.j.p’s is also confirmed in the experiments using apamin and atropine. Thus an involvement of unidentified factors is considered in the depression phenomenon of e.j.p’s.

The depression phenomenon is antagonized by NOLA, suggesting a possible involvement of endogenous NO. Inhibition by SNP of the e.j.p’s supports this possibility. Because the membrane depolarization produced by exogenously applied ACh is not altered by SNP, the inhibition by NO of the e.j.p. may not be a postjunctional event. The important difference in the
effects of endogenous and exogenous NO on the e.j.p. appears on the amplitude of the first e.j.p.s evoked by TNS trains; that is, the first e.j.p. is inhibited by exogenous NO, but not by endogenous NO. These data likely indicate that NO is a prejunctional inhibitory modulator of cholinergic transmission in the stomach, as suggested previously [11]. However, NO may not be the sole factor to elicit the depression of e.j.p.s, since NOLA antagonizes but cannot prevent all the depression phenomena of the e.j.p.s. In intestinal muscle tissues, many substances are released during TNS, such as VIP, substance P, or PACAP, together with ACh or NO [2, 12, 20], and the contribution of these substances in the depression phenomena of e.j.p.s cannot be ruled out.

In the circular smooth muscle of the guinea pig fundus, the decrease in amplitude of twitch contractions produced during repeated TNS is also termed the depression phenomenon. The depression phenomenon in the twitch contractions appeared when the frequency of TNS exceeded certain levels, as depression phenomenon did in the e.j.p.s. Both the electrical and mechanical events are blocked by atropine, indicating that they are mediated by ACh. The two phenomena appear in a frequency-dependent manner, but with different time scales. However, the TNS with frequencies, which produce the depression phenomenon in twitch contractions (>0.05 Hz), evokes e.j.p.s with similar amplitudes. It is speculated that the cellular mechanisms of the depression phenomena in the e.j.p.s may be different from those of the depression phenomena in twitch contractions.

NOLA antagonizes the depression phenomenon of twitch contractions and alters the transient contraction produced by high-frequency TNS to a tetanic contraction. These results suggest that the depression phenomenon observed in mechanical responses could be fully explained by assuming that NO produced during TNS inhibits successive contractions, and this contrasts the partial attenuation of the depression phenomenon of e.j.p.s by NOLA. The concentration of ACh working postjunctionally during TNS is estimated to be 8×10^{-8} M [21], and contractions produced by a comparable concentration (10^{-7} M) of ACh can be inhibited by SNP to an extent similar to the twitch contractions. These results could be explained if the NO-induced inhibition of twitch contraction is mainly postjunctional events. Thus the prejunctional and postjunctional events appear to be involved in the NO-mediated inhibition of cholinergic transmission in the gastric fundus. The depression phenomenon of e.j.p.s may be due to the successive reduction in the Ca^{2+} mobilization at functional nerve terminals [22, 23]. The attenuation by NOLA of the depression phenomenon of e.j.p.s may therefore be related to the facilitated supply of Ca^{2+} during TNS. The ACh-induced contraction is produced by an increase in Ca^{2+} concentrations in smooth muscle cells. SNP does not inhibit the ACh-induced membrane depolarization, suggesting that the attenuation by NOLA of the depression phenomenon of twitch contractions may be related to the removal of the inhibitory actions of NO on postjunctional events.

The inhibition by SNP of TNS-induced contraction is differentiated, and the amplitude of twitch contraction is reduced more than that of the tetanic contractions is. These differences may be related to the amount of ACh released during TNS, since similar differences are also observed in the inhibitory actions of SNP on contractions produced by exogenously applied ACh. Thus the inhibition by NO of muscle contraction appears when intracellular concentrations of Ca^{2+} ([Ca^{2+}]) are lower than certain levels. These results could be extrapolated to indicate that the production of NO is parallel to the amount of ACh released during TNS for functional antagonization of the cholinergic transmission.

It is concluded that in fundus smooth muscle of the guinea pig, NO may be involved in the inhibitory modulator of the cholinergic transmission when the effects are evaluated from junction potentials and contractions produced by TNS. The inhibitory actions of NO appear in pre- and postjunctional events.

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