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The Possible Role of Nitric Oxide on Enteric Nerves-Mediated Relaxation of the Gastric Smooth Muscle of the Guinea Pig

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= Abstract = **The influence of the enteric nerves stimulation on the contractility of gastric circular muscle was studied in guinea pig stomachs. The enteric nerves were activated by electric field stimulation(EFS; 90 V, 1 ms, 32 Hz square pulses for 1 s). EFS produced initial transient contraction followed by relaxation and slow recovery. The initial contraction was sensitively blocked by treatment with atropine. The following relaxation still occurred in nonadrenergic noncholinergic(NANC) state. EFS-induced relaxation was reduced by L^G-nitro-L-arginine(L-NNA), a nitric oxide(NO) synthase inhibitor. The relaxation was restored from the suppressed state after the application of L-arginine(L-arg), a substrate of nitric oxide synthase. With conventional intracellular recording, slow wave and inhibitory junction potential(IJP) were recorded. L-NNA had no effect on IJP, while apamin blocked it. In conclusion, it is suggested that NO may play a major role in EFS-induced relaxation. The exact mechanism of this relaxation is unknown, but the relaxation does not result from the IJP induced by the activation of apamin-sensitive potassium channels.**

Key Words: *EFS, L^G-Nitro-L-arginine, Nitric oxide, L-Arg, Nonadrenergic noncholinergic, Inhibitory junction potential*

INTRODUCTION

It has been recognized that the stimulation of a certain class of nerves within the gut wall elicits relaxation or contraction. These phenomena have been commonly referred to as NANC(nonadrenergic noncholinergic) because neither adrenergic nor cholinergic blockers have any effect on them. The relaxation or contraction elicited by electric stimulation on the muscle strip containing intact nerve plexus were completely blocked by tetrodotoxin(TTX)(Li and Rand 1990), which proved that those phenomena are mediated by a certain class of nerve.

There are many NANC neurotransmitters. Among them, the candidates which could play a major role in the receptive relaxation of the proximal stomach during ingestion are vasoactive intestinal polypeptide(VIP), adenosine-

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triphosphate(ATP), and nitric oxide(NO). Since 1988 when NO was suggested to be an endothelium-derived relaxing factor(Furchgott 1988), NO has been recognized to be responsible for inhibitory neural regulation in many tissues including the trachea(Kannan and Johnson 1992; Belvisi *et al.* 1991) and gastrointestinal tract (Toda *et al.* 1990; Knudsen *et al.* 1992; Hata *et al.* 1990). Much evidence that NO plays a role as an inhibitory neurotransmitter in the GI tract has been reported. Some of it is as follows; 1) Immunohistochemical studies have shown that the enzyme necessary for NO synthesis is expressed in the enteric plexus of the guinea pig(Bredt *et al.* 1991; Costa *et al.* 1991). 2) Electric field stimulation(EFS) on the muscle strip of rat stomach increases the release of cGMP(Ito *et al.* 1990). 3) Inhibitory junction potential evoked by electric field stimulation is reduced by application of NO synthase inhibitor(He and Goyal 1992; Dalziel *et al.* 1991).

In the stomach, it has been recognized that NANC inhibitory neural regulation is responsible for receptive relaxation(Li and Rand 1990; Boeckxstaens *et al.* 1991). The object of this study is to investigate whether NO plays a major role in regulating inhibitory effect through inhibitory junction potential.

MATERIALS AND METHODS

Guinea pigs of either sex, weighing about 300g, were killed by stunning and exsanguination. The whole stomach was excised and placed in a bath containing oxygenated phosphate-buffered Tyrode solution(NaCl 147 mM, KCl 4 mM, $MgCl_2 \cdot 6H_2O$ 1.05 mM, $CaCl_2 \cdot 2H_2O$ 2 mM, $NaH_2PO_4 \cdot 2H_2O$ 0.42 mM, $Na_2HPO_4 \cdot 12H_2O$ 1.81 mM, glucose 5.5 mM, pH 7.35) at room temperature. Corporal regions were obtained and cut in the longitudinal direction along the lesser curvature. After the contents of the stomach were removed, patches of the muscle coat were obtained by removing the mucosal layer in Tyrode solution. Circular muscle strips, 2 mm wide and 10 mm long, were made by dissecting along the direction of the circular muscle.

Using a vertical chamber which has a capacity of 100 ml, isometric contraction was recorded. One end of the muscle strip was tied to a hook made of glass fixed to the chamber and the other end was tied to a movable glass rod which was connected to a Havard force transducer. Parallel to the muscle strip, a pair of platinum plates were set up to make electric field stimulation(90 V, 1 ms, 32 Hz, for 1 s). The electrical and mechanical responses were recorded simultaneously using the conventional glass capillary microelectrode method in a horizontal chamber which has a capacity of 2 ml. In the chamber the strips were pinned out at one end with tiny pins on a rubber plate and was connected to the Harvard force transducer at the other free end. The microelectrode filled with 3 M KCl with a tip resistance between 40-60 MΩ was impaled from the mucosal side and electrical activity was recorded with the Device's pen recorder. Junction potentials were recorded by transmural electrical stimulation (10-60 V, pulse duration 0.3 ms, single or repetitive) by using a platinum stimulating electrode (diameter 0.5 mm)(Fig. 1).

Drugs used were as follows and purchased from Sigma Chemical Co. ; apamin(Sigma), atropine sulfate(Sigma), guanethidine sulfate (Sigma), tetrodotoxin(Sankyo), tetraethylammonium(Sigma), acetylcholine chloride(Sigma), L^G-nitro-L-arginine(Sigma), methylene blue (Sigma), L-arg(Sigma), norepinephrine(Sigma), $PGF_{2\alpha}$(Sigma).

RESULTS

Effect of electric field stimulation on the corporal circular muscle of guinea pigs

Electric field stimulation with varying frequencies or intensities produced initial contraction followed by relaxation with slow recovery. The initial contraction was sensitively inhibited by atropine, the muscarinic receptor blocker, and the following relaxation was inhibited by tetrodotoxin. Guanethidine, the blocker of adrenergic transmission, had no

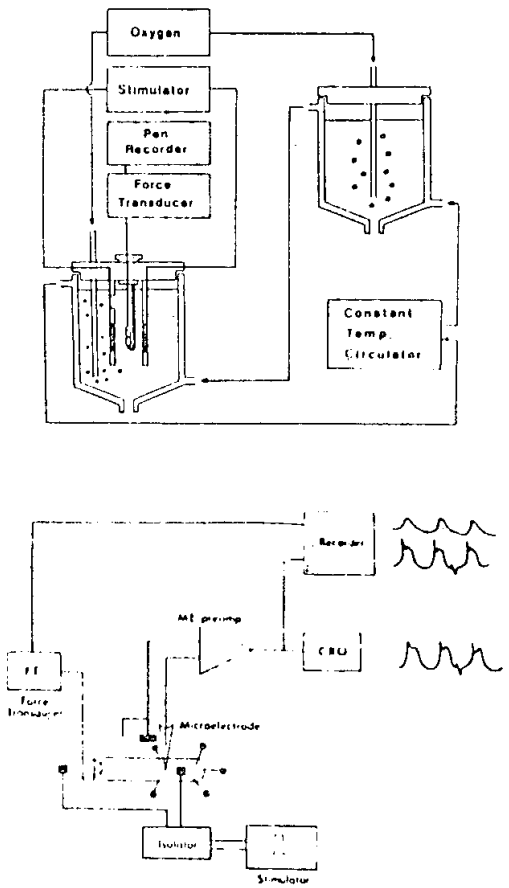

Fig. 1. a) A schematic representation of the 100 ml vertical chamber with isometric contraction recording system and transmural electrical field stimulation system. The transmural electrical stimulation was done with platinum plate electrodes that was 0.5 cm^2 in area, positioned parallel with preparation, and separated by 1.5 cm. b) A schematic representation of the recording system for isometric contraction and the electrical activity. The isometric contractions were recorded through a tension transducer from the smooth muscle preparation. And the microelectrode puncture technique for intracellular recording of the electrical activities was employed in this experiment. CRO: Cathode Ray Oscilloscope ME: Microelectrode

effect on EFS-induced relaxation(Fig. 2). These results suggested that the first initial contraction was mediated by cholinergic nerves and the following relaxation was mediated by nonadrenergic noncholinergic nerves.

Effect of L-NNA on the EFS-induced relaxation

In NANC state, $PGF_{2}\alpha$ was applied to increase basal tone. The following relaxation was inhibited by the application of L^{G}-nitro-L-arginine(L-NNA) which is known as an inhibitor of nitric oxide synthase. The inhibitory effect of L-NNA on the EFS-induced relaxation was antagonized by L-arg which is known as a substrate of nitric oxide synthase(Fig. 3). These results suggest that nonadrenergic noncholinergic relaxation is mediated by NO.

Effect of L-NNA on the cholinergic contraction induced by EFS

To reconfirm the possibility that the EFS-induced relaxation is developed by NO, a similar experiment was done in non NANC state. The application of L-NNA potentiated the EFS-induced cholinergic contraction which was selectively inhibited by application of L-arg, which suggests that this phenomenon is related to NO mediated system. Such potentiation was limited to EFS-induced contraction only, whereas the contraction induced by externally applied acetylcholine was not potentiated (Fig. 4).

Effects of apamin and L-NNA on inhibitory junction potential(IJP) and mechanical contraction

To investigate the possibility that EFS-induced relaxation was elicited by hyperpolarization, the effect of L-NNA on IJP was tested. Apamin, small conductance potassium channel blocker, blocked the IJP, while L-NNA did not (Fig. 5). In NANC state(Fig. 6), the EFS-induced relaxation was not affected by apamin. The spontaneous contractions recorded in the presence of atropine, guanethidine and L-NNA were changed in shape by the EFS. Apamin influenced these contractions evoked by EFS in

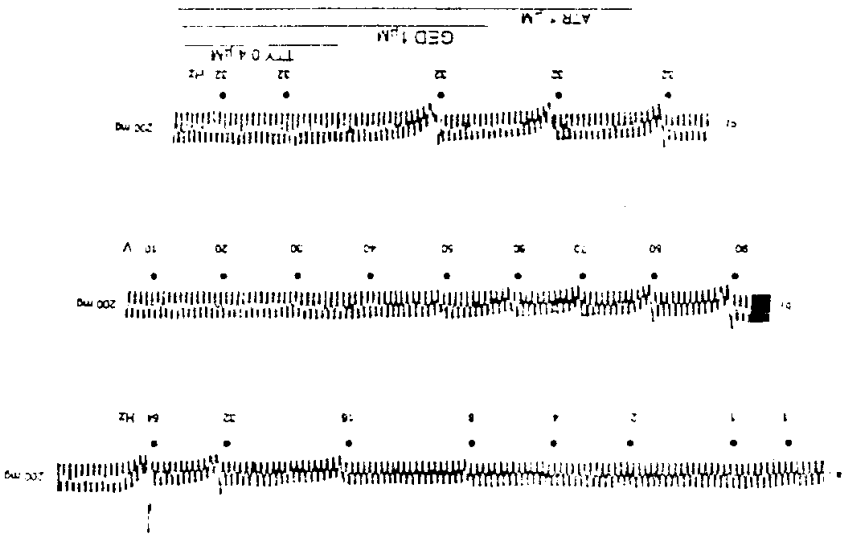

Fig. 2. The effect of electric field stimulation(EFS) on the spontaneous contraction of gastric circular muscle of the guinea pig. a) With varying frequencies(Hz) at constant time, voltage, and pulse duration(●, 1 s, 90 V, 1 ms). b) With varying voltages(V) at constant time, frequency, pulse duration(●, 1 s, 32 Hz, 1 ms). c) EFS(●, 90 V, 1 ms, 1 s) at different states(ATR: atropine, GED: guanethidine, TTX: tetrodotoxin)

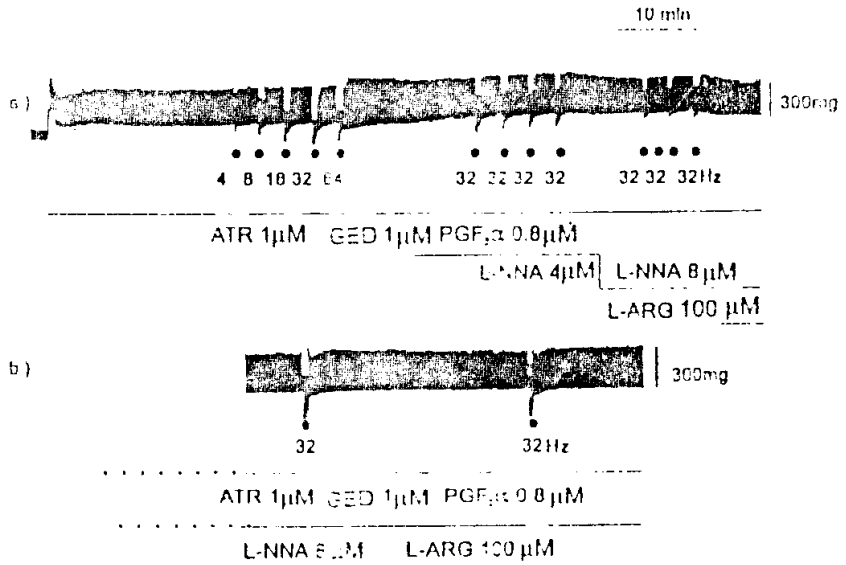

Fig. 3. The effect of L-NNA and L-arg on the isometric contraction. a) In the presence of atropine (ATR), and guanethidine(GED),$PGF_{2}\alpha$ increased both basal tone and amplitude of phasic contraction. When EFS(●, 90 V, 1 ms, for 1 s) was applied, it showed frequency-dependent relaxation reaching maximal relaxation at 32 Hz. After the treatment with L-NNA, the maximal relaxation reduced as time elapsed. b) The reduced relaxation by L-NNA restored its maximal relaxation at 32 Hz about an hour after the administration of L-arg.

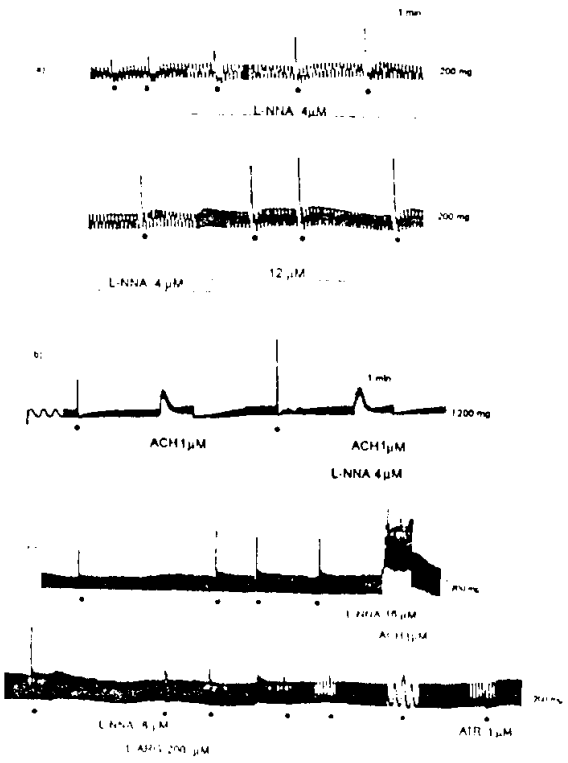

Fig. 4. The potentiation effect of L-NNA on cholinergic contraction. a)EFS-induced cholinergic contraction was potentiated in the presence of L-NNA while showing a saturation phenomenon. b) Both EFS (●, 90 V, 1 ms, 32 Hz for 1 s) and exogeneously applied acetylcholine (ACH) produced contractions. However, only the EFS-induced contraction was potentiated in the presence of L-NNA. c) The EFS-induced contraction potentiated by the pretreatment with L-NNA was reduced after incubation of L-arg for about an hour. After washing out, EFS(●, 90 V, 1 ms, 32 Hz for 1 s) also produced contraction which was sensitive to atropine(ATR).

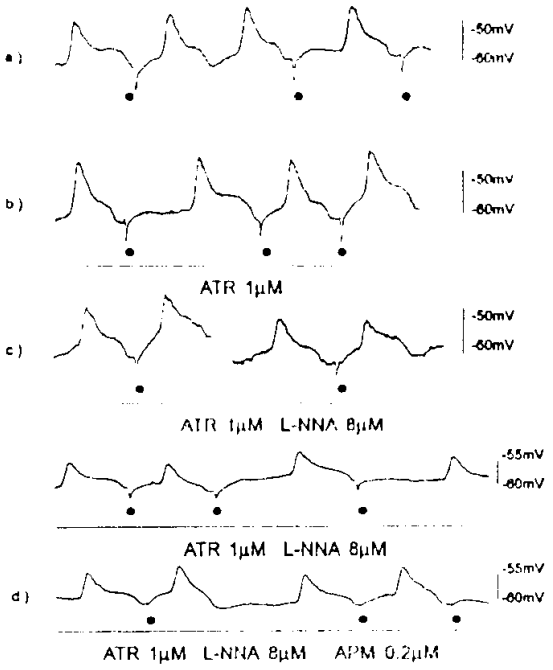

Fig. 5. The effects of L-NNA, atropine, and apamin on the inhibitory junction potential. Inhibitory junction potential (a) produced by single pulse(●, 10 V, 0. 3 ms) was not blocked by atropine(ATR) or L-NNA as shown in (b) and (c). But it was almost abolished by apamin(APM) in (d).

NANC state and L-NNA(Fig. 7). These results suggest that NO plays a major role in the EFS-induced relaxation, the mechanism of which is not through IJP.

Effect of sodium nitroprusside(SNP) on mechanical contraction and slow wave

To investigate the effect of NO on mechanical contraction and slow wave, SNP which is known to release NO was used. While contraction and membrane potential was recorded simultaneously using the conventional microelectrode technique, SNP was applied. SNP reduced the amplitudes of both mechanical contraction and slow wave(Fig. 8). Mechanical contractions were almost completely suppressed, while slow waves were reduced in amplitude and slope and resting membrane potential was not changed.

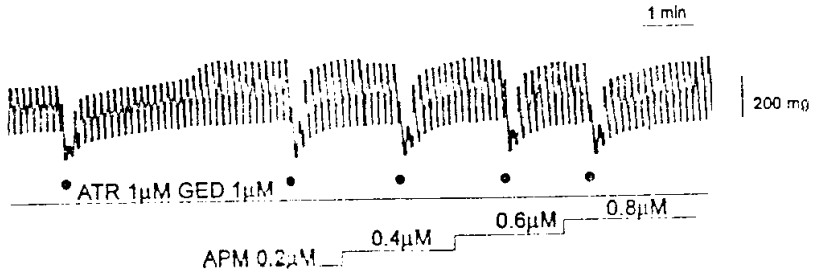

Fig. 6. In the presence of atropine and guanethidine(ATR: atropine, GED: guanethidine), apamin had no effect on EFS(●, 90 V, 8 Hz for 5 s)-induced relaxation.

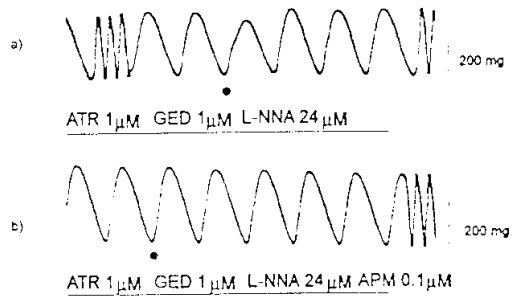

Fig. 7. The effect of apamin on the L-NNA treated muscle strip. a) EFS(●, 90 V, 0.6 ms, 32 Hz for 1 s) produced a contraction a little different in shape. b) Apamin(APM) changed it.

DISCUSSION

In this study, the relationship between EFS-induced relaxation and the NO-mediated system was investigated using L-NNA(LG-nitro-L-arginine). Recent trends in approving involvement of NO in some responces are such that 1) NO synthase inhibitor can inhibit those responses and L-arg can reverse it. 2) Methylene blue, known as soluble guanylate cyclase inhibitor, can inhibit the responses. 3) Oxyhemoglobin, known as a scavenger of oxygen free radicals, inhibits the responses. 4)

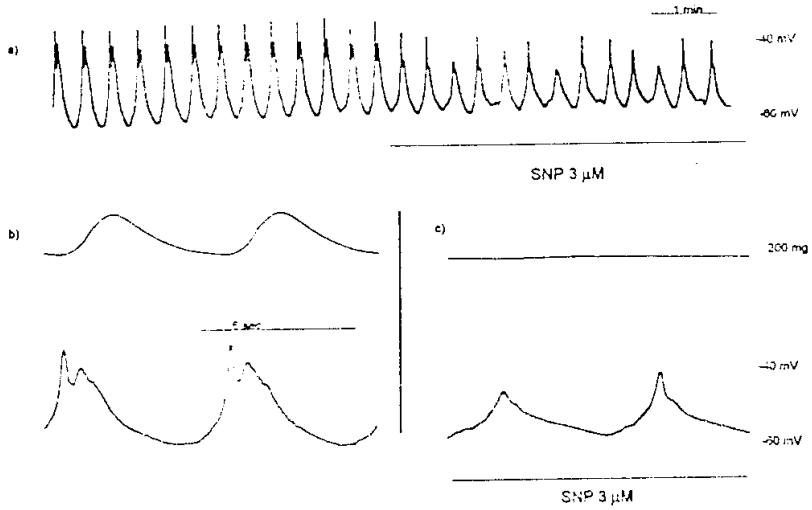

Fig. 8. The effect of sodium nitroprusside(SNP) on the mechanical contraction and slow wave. Mechanical contraction and slow wave were recorded simultaneously with slow paper speed(a) or with rapid speed (b) and (c) in the absence (b) or presence (c) of SNP. a) SNP decreased the amplitude of slow wave. b) Upper trace showed the isometric contraction and lower trace showed slow wave in the absence of SNP. c) SNP abolished phasic contraction in upper trace and suppressed upstroke phase of slow wave shown in lower trace in b).

responses(Sanders and Ward 1992). Only NO synthase inhibitor was used in this study.

NO is known to bind heme moiety of soluble guanylate cyclase. The binding of NO is known to activate soluble guanylate cyclase. The increased cGMP is known to relax smooth muscle(Rapoport *et al.* 1989). It is suggested that NO relaxes the smooth muscle by the following mechanisms; 1) Increased cGMP might directly regulate ionic channels(Fesenco *et al.* 1985). 2) cGMP might reduce intracellular [Ca^{2+}] to relaxation(Ramagopal *et al.* 1989). 3) cGMP might decrease the Ca^{2+} sensitivity of smooth muscle to non effective excitation-contraction coupling(Ozaki *et al.* 1992; Nishimura *et al.* 1989). 4) The hyperpolarization resulting from the summation of EFS-induced IJP might relax smooth muscle(Thornbury *et al.* 1991). From the results obtained in this study, IJP could be ruled out as a cause of EFS-induced relaxation at least in the guinea pig stomach. The others could not be investigated in this system. But the result which showed that SNP influenced mechanical contraction more than electrical activity suggested that Ozaki's hypothesis might explain our data.

The fact that the application of L-NNA potentiated EFS-induced cholinergic contraction might correspond with the experiments done in canine cerebral arteries and mesenteric arteries(Toda *et al.* 1990). Those phenomena might be explained in view of a functional antagonism between NO and cholinergic nerves. The other possibility that NO might inhibit the acetylcholine release in resting state must be ruled out.

The other neurotransmitters such as VIP, a frequently mentioned candidate of inhibitory neurotransmitter(Crist *et al.* 1992), might be released by EFS. But considering the fact that L-NNA almost blocked relaxation, the contribution of VIP may be minimal.

From the above results, it is suggested that NO might be a major neurotransmitter of EFS-induced relaxation in the guinea pig stomach.

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