

Full Paper

The Role of Nitric Oxide in the Electrical Field Stimulation-Induced Contractions of Sphincter of Oddi and Gallbladder Strips in Guinea PigsHulya Gultekin¹, S. Remzi Erdem², Selda Emre-Aydingoz³, and Meral Tuncer^{4,*}¹Department of Pharmacology, Faculty of Medicine, Erciyes University, Kayseri 38039, Turkey²Department of Pharmacology, Faculty of Medicine, Baskent University, Ankara 06530, Turkey³1703 Bluejay Cove, St. Louis, MO 63144, USA⁴Department of Pharmacology, Faculty of Medicine, Hacettepe University, Ankara 06100, Turkey

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Abstract. The aim of this study was to investigate the modulatory role of nitric oxide (NO) in the electrical field stimulation (EFS)-induced contractions of isolated sphincter of Oddi (SO) and gallbladder strips from guinea pigs. EFS was used to activate the intrinsic nerves in SO and gallbladder strips. EFS produced frequency-dependent biphasic contractile responses in the SO strips. A smaller contraction, “on response”, occurred during EFS, which was followed by a bigger contraction, “off response”. Both responses were completely and irreversibly abolished by tetrodotoxin (TTX) (10^{-6} M). Atropine (10^{-6} M) inhibited the “on response”, but not the “off response”. EFS produced frequency-dependent monophasic contractile responses in gallbladder strips, which were completely and irreversibly abolished by TTX (10^{-6} M) and atropine (10^{-6} M). A nitric oxide synthase (NOS) inhibitor, *N*^G-nitro-L-arginine (10^{-4} M and 3×10^{-4} M, in SO and gallbladder strips, respectively), significantly increased all EFS-induced contractions of SO and gallbladder strips. L-Arginine, but not D-arginine reversed the effect induced by the NOS inhibitor, at all frequencies, in both strips. These results suggested that NO released from nitrergic nerve endings might play a regulatory role in the cholinergic neurotransmission of guinea pig SO and gallbladder strips. The “off response” in the SO preparations might be a rebound increase that was modulated by the nonadrenergic, noncholinergic inhibitory mediator NO.

Keywords: sphincter of Oddi, gallbladder, nitric oxide, electrical field stimulation, *N*^G-nitro-L-arginine

Introduction

There are several reports on the response of gallbladder muscle strips and sphincter of Oddi (SO) preparations to electrical field stimulation (EFS). Gallbladder motility is modulated by intrinsic cholinergic neurons and by the gastrointestinal hormone cholecystokinin that acts in part through the local release of acetylcholine in the gallbladder (1, 2). Control of SO motility involves a complex interaction between extrinsic and intrinsic nerves and gastrointestinal hormones (3). Nonadrenergic, noncholinergic (NANC) nerve fibers are the main source

of neurally induced SO relaxation in several animal species including the guinea pig (4). Noradrenergic innervation has only a minor role (5).

Nitric oxide (NO) has been reported to be a putative inhibitory neurotransmitter of NANC events (6). Involvement of NO in neural relaxation of the SO has been proposed (7). Furthermore, it has been reported that NO may be a mediator of the “off response” and of NANC responses in gallbladder smooth muscle (8). Low frequency electrical stimulation (8 Hz with 0.1-ms pulses for 5 s using 10 – 130 V) of intramural nerves of gut smooth muscle produces a contraction during the stimulation and a contraction after a latency from the end of the stimulus. The contraction that occurs during the stimulation is known as the “on response” and the contraction that follows the turning off of the stimulus is

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called the "off response" (8). The off response coincides with a depolarization of the muscle following an EFS-induced hyperpolarization of that muscle (9). On the other hand, it has been reported that a prompt "off" contraction occurred immediately after termination of EFS, in SO preparations (7). A number of investigators have suggested that the contraction that occurs during the "off response" represents the physiological equivalent of peristalsis (10, 11). Alternatively, some authors consider an "on response" observed *in vivo* and *in vitro*, and thought to be cholinergic in nature, as the intrinsic mechanism of the peristalsis (9). The latency gradient of peristalsis in the smooth muscle of the esophagus might be due a changing balance between the cholinergic and NANC influence (12).

The SO and gallbladder are anatomically closely related organs. Therefore, it would be of great importance to investigate the on and off contractions induced by EFS in these two tissues obtained from the same animal. Thus, our goals were to investigate the EFS-induced contractions of gallbladder and SO strips and whether NO plays a role in the regulation of these responses.

Materials and Methods

Animals

All animals used in this study were obtained from the Laboratory Animal Breeding House of Hacettepe University and kept in cages at room temperature in the Pharmacology Department for 48 h before the experiments. During the first 36 h, animals had unlimited access to food. After an overnight fast, guinea pigs of either sex (300–350 g) were studied in a randomized manner.

Preparations

Animals were anesthetized by urethane (1.5 g/kg, *i.p.*). An abdominal incision was made and the gallbladder was removed by cutting the cystic duct. The extrahepatic biliary tree, duodenum, and stomach were exposed. Then the choledochus with the choledochoduodenal junction, a block of duodenal tissue to a distance of approximately 1.5 cm from the papilla, and a piece of gastric antrum were removed *in toto*, and the SO, as a tube-like preparation, was obtained by the method described previously (13). It was then opened longitudinally. The circular muscle strips obtained from this preparation and gallbladder longitudinal muscle strips (6–8 mm long \times 2–3 mm diameter) were mounted between hooks from the cut edges in 30-ml organ baths filled with Krebs-Henseleit solution. The composition of the Krebs-Henseleit solution is as

follows: 118.2 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, and 11.1 mM glucose. The solution was maintained at 37°C and bubbled with a gas mixture of 95% O₂ and 5% CO₂.

An initial resting tension of 1 g was applied to the tissues, which were allowed to equilibrate for 120 min prior to the addition of test agents and application of EFS. Isometric contractions were displayed on a Grass polygraph (Model 7B) by means of a force-displacement transducer (FT03). After the response to carbachol (10⁻⁵ M, to test the viability of the tissues) was obtained, EFS was applied to the preparations. EFS was delivered by a Grass S48 stimulator through the isolation unit (SIU 5). Two platinum wire electrodes were placed parallel at each side of the strips with a distance of 1 mm to the preparation and were connected to the output of the isolation unit. Impulses were sent with a duration of 0.5 and 1 ms to the gallbladder and SO, respectively. Supramaximal voltage was applied to both preparations. Impulse frequency varied from 4 to 20 Hz and from 1 to 30 Hz, for SO and gallbladder, respectively.

Protocol

When stable responses were obtained (less than 5% variation between three successive stimulations), guanethidine, atropine, tetrodotoxin (TTX), or *N*^G-nitro-L-arginine (L-NOARG) were added 20–30 min prior to the reapplication of EFS in order to test whether responses to stimulation were changed or not. The preparations were pretreated with L-arginine or D-arginine for 10 min to test the role of NO in these responses when NO synthase (NOS) inhibitor was used. Drug effects were expressed as a percentage change in the amplitude of the maximum response to field stimulation.

Drugs

Atropine sulphate (Merck, Darmstadt, Germany), guanethidine (Ciba-Geigy, Basel, Switzerland), D-arginine hydrochloride, L-arginine hydrochloride, carbachol, *N*^G-nitro-L-arginine (L-NOARG), and tetrodotoxin (TTX; Sigma, St. Louis, MO, USA). All drugs were dissolved in distilled water.

Data analysis

Results were expressed as the mean \pm S.E.M. of the number (n) of experiments. Differences between group means were evaluated by Student's paired *t*-test. Statistical significance was defined as *P* < 0.05.

Results

EFS (4–20 Hz, supramaximal voltage, 1-ms pulse

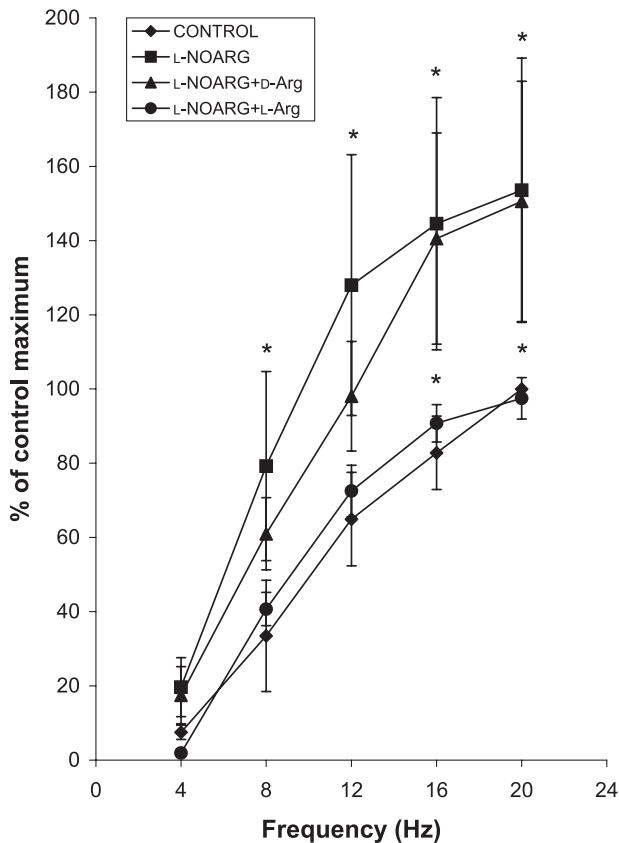


Fig. 1. The effects of N^G -nitro-L-arginine (10^{-4} M) alone (square) and together with L-arginine (5×10^{-3} M, circle) or with D-arginine (5×10^{-3} M, triangle) on EFS-induced frequency-dependent contractions (Control, diamond) in the guinea-pig sphincter of Oddi strips (n = 6). * $P < 0.05$ vs control.

width, 10-s train duration) produced frequency-dependent responses in the SO strips (Fig. 1).

In these preparations, the stimulation induced a rapid contraction “on response” which was followed by a bigger contraction “off response” that occurred shortly after EFS ended (Fig. 2).

Both responses were completely and irreversibly abolished by TTX (10^{-6} M). The “On response” was inhibited by atropine (10^{-6} M, Fig. 3), whereas the “off response” was not. Guanethidine (10^{-6} M) had no effect on both contractions in SO strips.

L-NOARG (10^{-4} M) significantly increased EFS-induced both contractions of SO (Fig. 1). The increase in the contractions induced by NOS inhibitor was reversed by L-arginine (5×10^{-3} M), but not by D-arginine (5×10^{-3} M) at all frequencies in SO strips (Fig. 1).

In gallbladder strips, EFS (1–30 Hz, 100 V, 0.5-ms pulse width, 30-s train duration) caused frequency-dependent monophasic contractile responses (on response) (Fig. 4). Atropine (10^{-6} M) and TTX (10^{-6} M) completely and irreversibly inhibited these contractions.

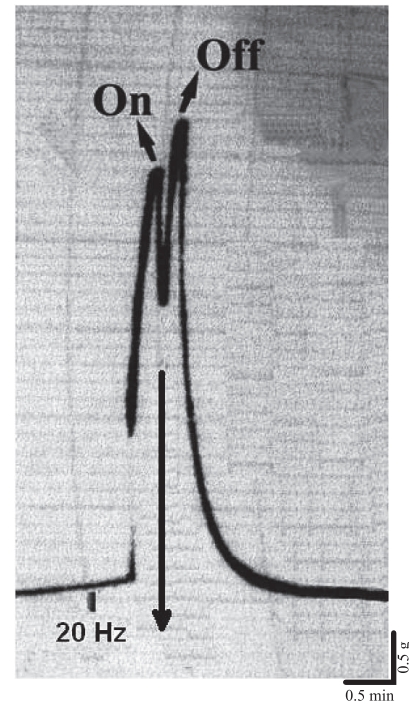


Fig. 2. Representative tracing of EFS (20 Hz)-induced “on” and “off” contractions of the guinea-pig sphincter of Oddi strips. The arrow shows the end of the stimulus given.

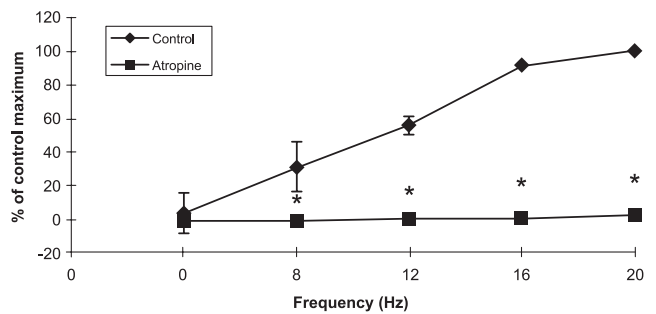


Fig. 3. The effect of atropine (10^{-6} M, square) on the EFS-induced contractile “on” responses (Control, diamond) in the guinea-pig sphincter of Oddi strips (n = 5). * $P < 0.05$ vs control.

Guanethidine (10^{-6} M) did not change EFS-induced contractions of gallbladder.

The NOS inhibitor (3×10^{-4} M) significantly increased the contractions induced by EFS. The increase in the contractions was reversed by L-arginine (5×10^{-3} M), but not by D-arginine (5×10^{-3} M) in gallbladder strips (Figs. 4 and 5).

Discussion

Several studies have investigated the nonadrenergic, noncholinergic inhibitory innervation in SO and gallbladder smooth muscle. McKirdy et al. demonstrated

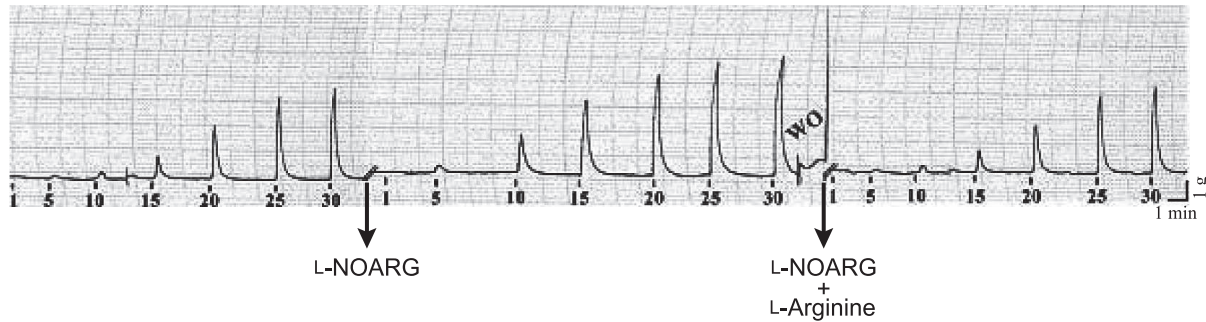


Fig. 4. Representative tracing of EFS (1–30 Hz)-induced contractions in the guinea-pig gallbladder strips before and after the administration of N^G -nitro-L-arginine alone (3×10^{-4} M) and together with L-arginine (5×10^{-3} M), respectively. WO = Washout.

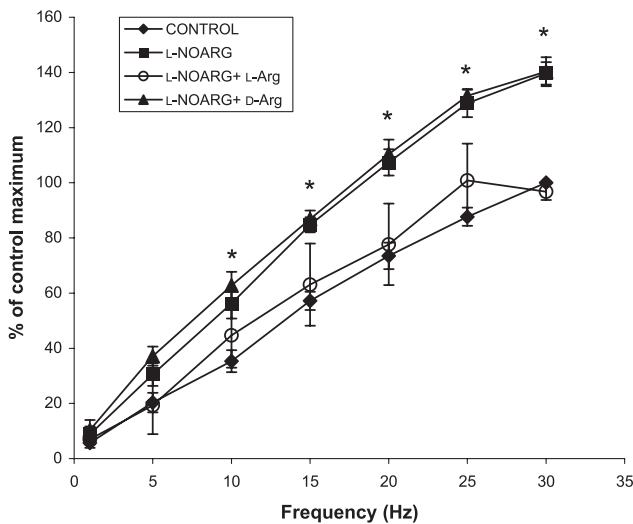


Fig. 5. The effects of N^G -nitro-L-arginine (10^{-4} M) alone (square) and together with L-arginine (5×10^{-3} M, circle) or with D-arginine (5×10^{-3} M, triangle) on EFS-induced frequency-dependent contractions (Control, diamond) in the guinea-pig gallbladder strips ($n = 6$). * $P < 0.05$ vs control.

the relaxation to EFS in precontracted human gallbladder strips (14). The difference in our study was that we did not precontract the SO and gallbladder strips. Thus, EFS did not cause a relaxation response. Instead, we obtained EFS-induced contractions in both tissues and we evaluated the augmentation induced by an NOS inhibitor. Additionally, we used the substrate for NO synthesis in order to determine the role of NO in the responses.

Our study demonstrates that the “off response” (contraction occurred after EFS is stopped) is present in SO preparations, confirming the finding reported by Pauletzky et al. (7), but not in gallbladder strips, in contrary to the finding reported by Cullen et al. (8) This inconsistency found in gallbladder strips may result from the different animal species used in the experiments. Cullen et al. used opossum as an experimental

animal (8). It is well known that the responses to the same stimuli or the same agonist applied to the same tissues obtained from different animal species may be different. TTX abolished the contractions obtained during the stimulation (“on response”) and “off response” in SO and “on response” in gallbladder of guinea pig. Acting by inhibiting Na^+ conductance in nerve fibers, TTX is the standard agent used for discrimination between neurogenic and myogenic responses, and in the prevailing concentration of TTX, it has no action on smooth muscle excitability (15). Consistent with the literature, our results with TTX indicate that all responses to EFS are neurogenic in nature.

The stimulatory effect of EFS which yielded basic responses has been described elsewhere, namely, the “on” and “off” response (16). The nature of the “off response” has been debated: it was suggested that the “off” contraction seen in smooth muscle strips from the opossum oesophageal body was the result of a rebound effect occurring after hyperpolarization of the smooth muscle membrane by inhibitory nerves as this contraction was not blocked by atropine or any other blocker (12, 16). Likewise, in our study, the “off response” observed in SO strips was not abolished by either atropine or an adrenergic blocker. However, L-NOARG significantly increased the responses that were reversed by L-arginine. In the light of our experiments, it may be suggested that a rebound effect contributes to the “off response” which is regulated by NO in SO strips of guinea pig. NO stimulates soluble guanylate cyclase enzyme in the smooth muscle cells and leads to the formation of 3',5'-cyclic-guanosine monophosphate (cGMP) that facilitates the dephosphorylation of myosin light chains, preventing the interaction of myosin with actin. Thus, NO may relax smooth muscle structures or inhibit the contractions mainly through this mechanism.

Although atropine did not inhibit the “off response”

of SO, it inhibited the frequency-dependent contractile “on responses” of SO and gallbladder strips of guinea pig. EFS-induced contractions were found to be mediated primarily by postganglionic cholinergic neurons in guinea pig gallbladder similar to that of SO (17). Because the blockade of excitatory muscarinic receptors by atropine at the same time was associated by a decrease in contractile amplitude in “on responses” in both tissues, indicating the presence of excitatory muscarinic receptors, we consider the one-nerve hypothesis to be valid in these responses (12).

The contractions obtained during the stimulation (on responses) of both tissues were augmented by the NOS inhibitor, and pretreatment of the tissues with the substrate for NO synthesis L-arginine prevented this augmentation. Our findings suggested that NO might be a modulator of these NANC responses in SO and gallbladder smooth muscles of guinea pig. Blockade of SO inhibitory nerves after administration of N^G -nitro-L-arginine methyl ester (L-NAME) has also been observed in a study of the isolated guinea pig SO and in vivo in prairie dogs (7, 18). However, pre-incubation of the rabbit tissues with L-arginine has not reversed this blockade (19). On the other hand, inhibitory motor innervation of the gallbladder musculature by intrinsic neurons containing NO has been reported in Australian Brush-tailed possum (20). We obtained definite evidence that L-arginine is the substrate for the NO synthesis, but D-arginine, the enantiomer used as the inactive control compound in our current study, is not.

We used guanethidine in our study to achieve a presynaptic adrenergic blockade. Since no change with guanethidine was observed in the responses in both the SO and gallbladder preparations, an adrenergic modulation for the contractile responses induced by EFS could be excluded in these two tissues. This finding is in accordance with the results obtained by other investigators for human gallbladder muscle (14).

Taken all together, it may be suggested that a rebound effect contributes to the “off response” in SO strips and NO may be a modulator for this “off response” in addition to being a modulator for the NANC responses in both SO and gallbladder smooth muscles of guinea pig.

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