A POSSIBLE ROLE OF NITRIC OXIDE IN THE RELAXATIONS INDUCED BY ELECTRICAL FIELD STIMULATION OF ISOLATED FROG OESOPHAGEAL STRIPS

KANSU BÜYÜKAFSAR*
FIRUZ BAYSAL*
SERPIL ÖNDER*
ATILLA DIKMEN*
ERGIN SINGIRIK*

SUMMARY: Electrical field stimulation (EFS) caused voltage dependent relaxations in the isolated frog upper oesophageal circular segments precontracted with carbachol (CCh). The influence of N^G-nitro L-arginine (L-NOARG) on these relaxations was investigated. 10^{-6} M, 10^{-5} M, $5x10^{-5}$ M and 10^{-4} M concentrations of this substance inhibited them in a dose dependent manner. $4x10^{-4}$ M L-arginine (L-ARG) prevented the inhibitory action of $5x10^{-5}$ M and 10^{-4} M L-NOARG while $4x10^{-4}$ M D-arginine (D-ARG) did not protect against the effect of L-NOARG. Neither L-ARG nor D-ARG had any significant effects on the electrically-induced relaxations. The results suggested that nitric oxide (NO) is involved in the relaxations induced by EFS possibly mediated by non adrenergic non cholinergic (NANC) nerves.

Key Words : Frog oesophageal strips, nitric oxide, electrical field stimulation.

INTRODUCTION

In our laboratory (1), we determined that EFS results in voltage dependent relaxations in the circular strips prepared from frog upper oesophageal segments. They were inhibited by ouabain, suggesting a role for sodium pump. A similar inhibition was also observed after reduction of external sodium concentration (2). However, the treatment of tissue with various substances such as indomethacin, acetyl-salicylic acid, diclophenac sodium, SC-19220 and 2,4-dinitrophenol did not affect the relaxation (3).

Recent studies (4) showed the existence of an extensive and complex NANC innervation in the various parts of gastrointestinal tract including oesophagus. Several substances such as VIP and NO have been proposed as NANC inhibitory transmitter. As oesophageal tissue, whole oesophagus and lower oesophageal sphincter of opossum (5), rat oesophageal tunica muscularis (6) isolated guinea-pig lower oesophageal sphincter (7) and isolated dog lower oesophageal sphincter (8) were studied and experimental results suggested an essential role for NO in relaxations elicited by EFS and some substances.

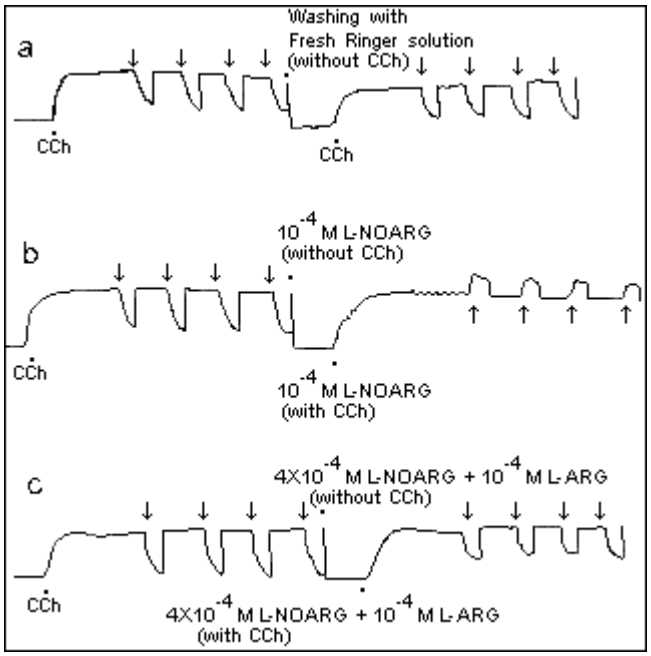
Findings have raised the question about the contribution of NO in relaxant responses of frog oesophagus to EFS. Thus, we made some experimental attempts to solve this problem.

MATERIAL AND METHODS

Circular muscle strips prepared from the oesophagus of pitted frog (fresh water frog, weighing 20-25 g) were used in these experiments. The method was described earlier (1). Briefly, the oesophagus was removed and cut open by a longitudinal incision. The upper segment was then incised circu-

*From Department of Pharmacology, Çukurova University, Faculty of Medicine, Balcali, Adana.

Figure 1: Isotonic tension recordings showing (a) Control, (b) the effects of 10^{-4} M L-NOARG and (c) the interactions between 10^{-4} M L-NOARG and $4x10^{-4}$ M L-ARG on the relaxations of the upper circular strips of frog oesophagus induced by EFS (3Hz, 2ms, 25V). Strips were contracted with $2.76x10^{-7}$ carbachol (CCh).



larly to obtain a strip (approximately 1 cm long and 0.3 cm wide). Strips were mounted between two platinum electrodes embedded in plexiglass in an organ bath filled with Ringer solution. The composition of Ringer solution was NaCl 111.2, KCl 1.87, $CaCl_2$ 1.08, $NaHCO_3$ 2.38, $NaH_2PO_4.2H_2O$ 0.8, Glucose 11.1 mM. Tension on the strips was 0.5 g. The bath medium was bubbled with oxygen and maintained at room temperature (about 20°C). The preparation was allowed to equilibrate for 60 minutes. During this time, tissue was washed with fresh Ringer solution every 20 minutes. Changes in the muscle length were recorded by means of an isotonic lever (X8-10 magnification) on a smoked drum. After equilibrium period basal tension was recorded for three minutes then the tissue was treated with fresh Ringer solution containing $2.76x10^{-7}$M CCh. This resulted in an active tone that reached a stable level in about ten minutes. Thereafter rectangular wave pulses (3Hz, 2ms, 25V) by a student stimulator (Harvard) were applied for 2 minutes to the tissue at 20 minutes intervals. In preliminary experiments a separate group of strips (n=11) were used to investigate the effect of various frequencies (1, 2, 4, 8 Hz, 2ms, 25V) and thereafter 3Hz was chosen for the remaining experiments. Following each stimulation the strips were washed with fresh Ringer with CCh. Totally four stimuli (first series) were so applied to the tissue. After the last stimulation, carbachol was washed out of the organ bath and the strips were allowed to rest 1 hour before the tone was raised again. We applied four further stimuli (second series) again in a similar manner and thus retested the sensitivity of tissue to EFS.

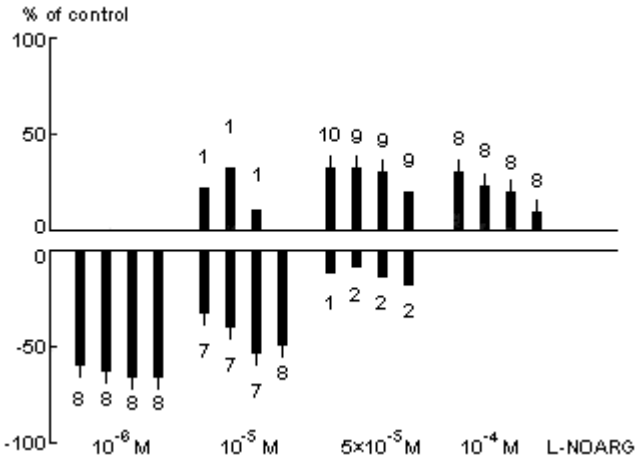
The influences of L NOARG (10^{-6} M, 10^{-5} M, $5x10^{-5}$ M and 10^{-4} M), L-ARG ($4x10^{-4}$ M) and D-ARG ($4x10^{-4}$ M) were investigated on the electrically induced relaxations. Drugs

Table 1: Relaxant responses induced by EFS (2ms,25V) with various frequencies (1,2,4,8Hz). The same frequency was applied two times to a strip in the below indicated order.

	1	2	4	8	1	2	4	8Hz
n=11	*17.63±2.56	45.00 ± 4.23	78.09 ± 7.38	90.21 ± 9.28	19.98 ± 2.57	40.30 ± 3.64	76.08 ± 7.00	83.43 ± 8.36

* mean values (± SE) expressed as % peak reduction of active tone yielded by CCh.

Figure 2: Effects of various concentrations (10^{-6} M, 10^{-5} M, $5x10^{-5}$ M, 10^{-4} M) of L-NOARG on the relaxation of the upper circular strips of frog oesophagus induced by EFS (3Hz, 2ms, 25V). Results were expressed as percentage of control in column graphics. 10^{-4} L-NOARG completely reversed the relaxant responses. Numbers of evaluated strips are situated below and above the columns.



were added to the bath medium 60 minutes prior to the second series of electrical stimulations. Each concentration of drugs was studied on a separate group.

Finally, we examined the effects of L-ARG and D-ARG on L-NOARG action. $5x10^{-5}$ M and 10^{-4} M concentrations of L-NOARG were tested in these experiments. The amino acids (L-ARG and D-ARG) were applied at the concentration $4x10^{-4}$ M. L-NOARG plus one of this amino acids were added to the bath medium in the same manner as described above. Each concentration of L-NOARG plus amino acid was studied by using a separate group.

Drugs were dissolved by adding them to Ringer solution before experiments. The relaxations were calculated as % peak reduction of CCh-induced tone. The percentage of relaxation of second series in regard to mean value obtained from four mean values of the first series of total strips (including control groups) evaluated in this study were also determined and expressed as % control. Their mean values (±SE) were then established. All data were analyzed by using Student's t test for paired and unpaired observations. P values of less than 0.05 were considered to be significant. The drugs were purchased from Sigma.

Table 2: Effects of L-ARG ve D-ARG (both $4x10^{-4}$ M) on EFS (3Hz, 2ms, 25V) induced relaxations (expressed as % peak reduction of active tone yielded by CCh) of upper circular strips of frog esophagus.

	1st*	2nd	3th	4th	5th	6th	7th	8th
Control n=14	53.90 ± 4.91	61.05 ± 4.62	63.16 ± 5.17	67.56 ± 5.32	58.81 ± 4.11	60.37 ± 4.00	62.87 ± 4.66	65.59±5.00
L = ANG n=9	62.00±7.85**	67.03±7.26**	70.79±8.24**	72.84±8.59**	74.00±10.0**	76.70±9.13**	81.3±19.00**	82.20±9.32**
D= ARG n=8	45.00 ± 3.44	47.42 ± 4.06	52.05 ± 5.70	56.81 ± 5.65	47.48 ± 5.35	47.48 ± 5.35	51.19 ± 6.60	56.75 ± 4.32

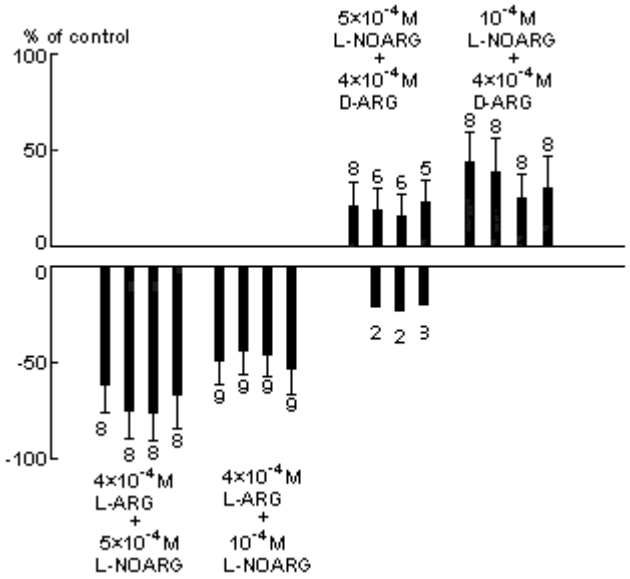
* shows the order of EFS applications.

** Comparison of mean value of first mean values with that of second four mean vlaues resulted in p value (< 0.40) of greater than 0.05.

¡ indicates the addition of aminoacid to the bathing medium.

n = Number of evaluated strips.

Figure 3: Column graphics showing the effects of $4x10^{-4}$ M L-ARG and $4x10^{-4}$ M D-ARG on the inhibitions of the EFS (3Hz, 3ms, 25V) induced relaxations observed after $5x10^{-4}$ M and 10^{-4} M L-NOARG applications in the upper circular strips of frog oesophagous. Numbers of evaluated strips are situated below and above the columns.



RESULTS

When electrically stimulated, the upper circular strips of the frog oesophagus responded with relaxation which was frequency dependent (Table 1). In experiments in which 3Hz was used a partial increase was observed after the first stimulus and this tended to disappear in second series of electrical stimulations (Table 2, Control). Relaxations were fast in onset. After the cessation of electrical stimulation and washing with fresh Ringer, the tissue returned to its original level (Figure 1) L-NOARG (10^{-6}, 10^{-5}, $5x10^{-5}$, 10^{-4} M) caused concentration related inhibitions on electrically induced relaxations (Figure 1b, Figure 2). They were completely reversed by 10^{-4} M L-NOARG concentration. Contractions observed after reversal were small but observable. The presence of $4x10^{-4}$ M L-ARG prevented the inhibitory effect of $5x10^{-5}$ M and 10^{-4} M L-NOARG on the relaxations (Figure 1c, Figure 3). In contrast, in the presence of $4x10^{-4}$ M D-ARG L-NOARG still inhibited the responses to EFS (Figure 3). Neither L-ARG nor D-ARG (both $4x10^{-4}$ M) caused any significant change in the relaxations (Table 2).

DISCUSSION

In the present work, inhibition of electrically-induced relaxation by a potent inhibitor of NO biosynthesis, L-NOARG (9,10) in a concentration dependent manner and prevention of L-NOARG action by the precursor L-ARG but not by D-ARG strongly suggest that NO plays an important role in the relaxation elicited by EFS of the upper circular strips of frog oesophagus precontracted with CCh. The similarities between electrically-induced relaxation of frog oesophagus and NO relaxations of other tissues (4) also support our notion.

On the other hand, the fact that the relaxations are frequency dependent may be explained by a direct action of electrical stimulation on the neuronal structure rather than on the other cell population such as epithelial cells. An additional supporting evidence which seems to be reasonable because of the similarities between the effect of Na^+ free medium and that of tetrodotoxin (11) is the experimental finding that the external sodium depletion inhibits the relaxant responses to EFS (5).

In conclusion, we obtained evidence suggesting that transient relaxations induced by EFS are mediated

by NO or NO releasing substance. The source of NO in our preparation may be NANC nerve as was shown in other smooth muscle preparations (4).

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Correspondence:
Firuz Baysal
Department of Pharmacology,
Çukurova University,
Medical Faculty,
Balcali, Adana, TÜRKIYE.