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Evidence that inhibitory neurotransmission differs between the proximal and distal segments of guinea-pig taenia caeci

Catalina Caballero Alomar *, Carmen Santos, Pedro Puig-Parellada

Unit of Pharmacology, Faculty of Medicine, University of Barcelona, Casanova 143, 08036 Barcelona, Spain

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Abstract

The effect of atropine (1 μM) and N^G-nitro-L-arginine (L-NOARG, 10 μM) on electrical field stimulation induced relaxation in proximal and distal segments of guinea-pig taenia caeci in the presence of guanethidine (4 μM) was studied. The frequency-dependent relaxations were lower in proximal than in distal segments both in the presence and in the absence of atropine. The effect of L-NOARG (an inhibitor of nitric oxide (NO) synthase) on relaxation in the presence of atropine depended on the frequency of electrical stimulation and the segment used; the effect of L-NOARG was greater in proximal segments than in distal segments. In the absence of atropine, the inhibitory effect of L-NOARG was the same in both segments at all frequencies tested. This study demonstrates differences between the opposite extremes of guinea-pig taenia caeci in relaxations induced by electrical stimulation. Our data also show a role of NO that is dependent on the integrity of cholinergic transmission.

Keywords: Nitric oxide (NO); NANC (non-adrenergic–non-cholinergic) nerve; Cholinergic nerve; taenia caeci, guinea-pig

1. Introduction

Non-adrenergic–non-cholinergic (NANC) inhibitory innervation was first demonstrated by Burnstock et al. (1966) in guinea-pig taenia caeci. The nature of the NANC inhibitory transmitter(s) has been the subject of much debate. Mediators implicated in the NANC inhibitory transmission in guinea-pig taenia caeci are adenosine 5′triphosphate (ATP) (Satchell, 1981), vasoactive intestinal polypeptide (VIP) (Grider et al., 1985) and pituitary adenylyl cyclase activating peptide (PACAP) (McConalogue et al., 1995). Other studies indicate a possible role for nitric oxide (NO) in NANC transmission. Evidence that NO is a transmitter is provided by immunohistochemical techniques (Furness et al., 1992), and inhibitors of its biosynthesis caused a partial decrease in NANC relaxations in precontracted guinea-pig taenia caeci in response to electrical stimulation (Gustafsson et al., 1990; Piotroswski et al., 1993). However, Rand and Li (1990) found that the inhibitors of NO synthase did not modify NANC responses to electrical stimulation, and Bridgewater et al. (1995) did not obtain any effect of N^G-nitro-L-arginine (L-NOARG) in electrophysiological studies. On the other hand, Knudsen and Tottrup (1992) and Ward et al. (1996) obtained an effect of NO-inhibitors in the absence of atropine, thus they suggested that the inhibitory effects of L-NOARG depended upon the integrity of cholinergic neurotransmission. Contradictory evidence concerning the effect of inhibitors has also been presented by Williams and Parsons (1995), and Selemidis et al. (1997) suggested that NO is released during NANC nerve stimulation, but does not affect NANC relaxations unless the effects of another apamin-sensitive nerve-derived hyperpolarizing factor is blocked.

Some reports have indicated that neurotransmission of the enteric inhibitory neurons varies from one region of the gut to another (Suthamnatpong et al., 1993; Konturek and Konturek, 1995). The possibility that the involvement of NO may vary along the longitudinal smooth muscle of taenia caeci has not been studied.

The purpose of this study was to determine the role of NO in inhibitory neurotransmission in the proximal and distal segments of guinea-pig taenia caeci in relaxations induced by electrical stimulation by looking at the effect of the NO-synthesis inhibitor L-NOARG.

* Corresponding author. Tel.: +34-93-402-45-26; Fax: +34-93-403-58-81; E-mail: catalina@medicina.ub.es

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2. Materials and methods

2.1. Preparations of segments of guinea-pig taenia caeci

Male albino guinea-pigs weighing 300–350 g were fasted, with free access to water, for 24 h before they were killed by a blow to the head followed by exsanguination. The caecum was exposed via midline incision. Silk ligatures were tied to each end of dorsal and ventral taeniae: the end near the colon was defined as the distal part and the opposite end as proximal. The taeniae were dissected out and placed in Krebs solution (composition in mM: NaCl 119.78, KCl 4.7, $MgSO_4 7H_2O$ 1.17, KH_2PO_4 1.17, $NaHCO_3$ 24.9, glucose 11.1, $CaCl_2 2H_2O$ 2.56). Guanethidine (4 μM) was routinely added to the Krebs solution in order to prevent adrenergic relaxations. When atropine (1 μM) was used, it was also present throughout the experiment. Four segments were cut from each taenia: proximal, proximal medium, distal medium and distal, and mounted in thermostatically controlled (37 ± 0.5°C) organ baths containing Krebs solution. The pH of the organ bath fluid was kept constant at 7.4 by bubbling the Krebs solution with 95% O_2 and 5% CO_2. A resting tension of 2 g was applied to each strip and the responses were recorded isometrically. During an equilibration period of 60–120 min, the preparations gradually relaxed until a continuous baseline tension (of no more than 0.5 g) and showed spontaneous contractions, the amplitude of which was expressed in grams. We also measured the time interval in minutes between the peak amplitude of contractions. Throughout the experiment, the resting tone, the amplitude and the frequency between contractions were constant. Strips with no spontaneous contractions were excluded from further study.

2.2. Electrical field stimulation

The segments that exhibited spontaneous contractions were stimulated between parallel platinum electrodes (0.3–20.0 Hz; 40 V, 1 ms) and the stimulus was applied at the peak of spontaneous contraction for 5 s, instead of inducing a constant level of tone with a contracted substance. Electrical field stimulation produced a transient relaxation followed by frequency-dependent contraction after the stimulation was stopped (Fig. 1). The relaxation is expressed as a percentage of the amplitude of each spontaneous contraction. When L-NOARG was assayed, only one frequency was tested in each tissue set-up. Before the addition of L-NOARG (control) the relaxation responses remained constant. The control and L-NOARG results were expressed as an average of the electrical relaxations for 20 min. The inhibitory effect of L-NOARG was calculated as a percentage inhibition compared with control. When L-arginine was used it was added simultaneously with L-NOARG. Drugs (L-NOARG 10 μM, tetrodotoxin 1 μM and L-arginine 1 mM) were added as single concentration.

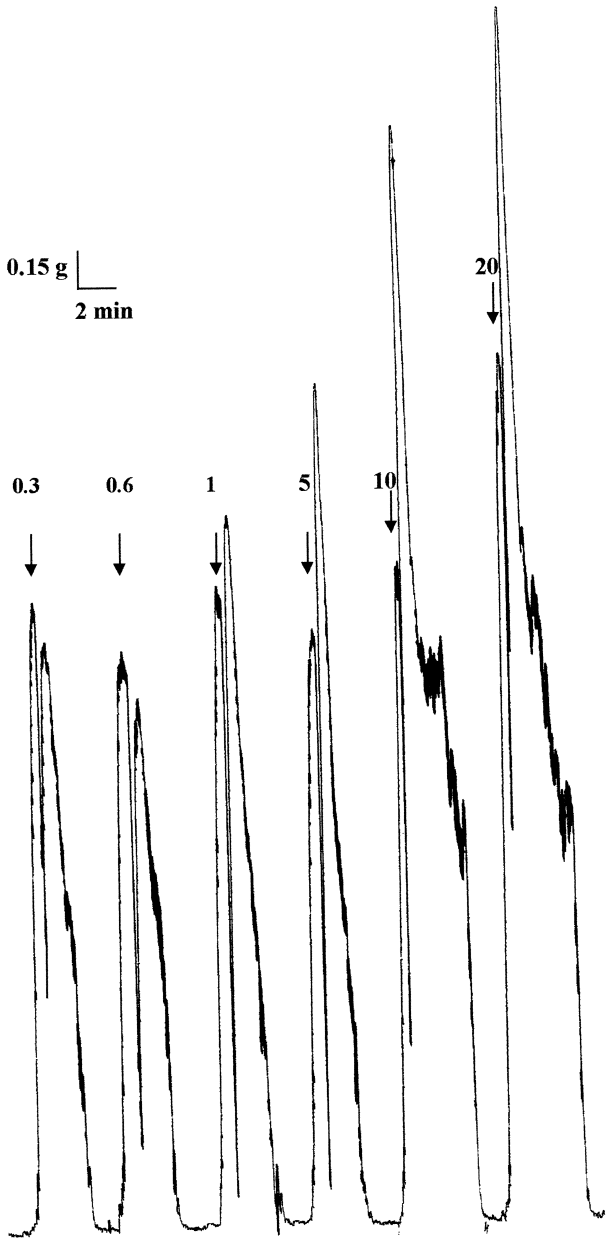

Fig. 1. NANC relaxation responses to electrical field stimulation (0.3–20.0 Hz, 1 ms, 40 V) applied for 5 s at the peak of the spontaneous contractions on distal segment of guinea-pig taenia caeci.

2.3. Drugs

The following drugs were used: atropine sulfate, guanethidine sulfate, L-NOARG, L-arginine hydrochloride, tetrodotoxin; all were from Sigma-Aldrich Química (Madrid, Spain).

Table 1
Effect of atropine (1 μM) on relaxation induced by electrical field stimulation

Frequency (Hz)	Proximal relaxation	Distal relaxation
In the presence of atropine (1 μM)		
0.3	39 ± 4 (17)	70 ± 3[c] (19)
0.6	49 ± 5 (17)	84 ± 3[c] (21)
1	86 ± 8 (3)	98 ± 2 (3)
2	87 ± 8 (7)	97 ± 2 (5)
In the absence of atropine		
0.3	21 ± 4[e] (15)	48 ± 4[c,f] (11)
0.6	34 ± 4[d] (5)	62 ± 13[a] (4)
1	55 ± 8[d] (4)	85 ± 8[a] (4)
2	50 ± 8[e] (13)	77 ± 4[b,f] (15)

Electrical relaxation (%) in proximal and distal segments of guinea-pig taenia caeci in the presence and in the absence of atropine (1 μM). Electrical field stimulation: 0.3–2 Hz, 40 V, 1 ms was applied at the top of the spontaneous contractions. The relaxation is expressed as a percentage of the amplitude of each spontaneous contraction. Data are given as mean ± S.E.M. The number of experiments is shown in parentheses. Differences between proximal and distal segments ([a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$). Differences in the presence and in the absence of atropine ([d]$P < 0.05$, [e]$P < 0.01$, [f]$P < 0.001$).

2.4. Statistical analysis

Results were analysed statistically by Student's paired or unpaired *t*-test (two-tail). N = number of animals, n = number of experiments. Data are given as mean ± S.E.M.

3. Results

In 89% of segments, spontaneous contractile activity was observed with an amplitude of 4 ± 0.3 g ($N = 26$) and a time interval between contractions of 5 ± 0.4 min ($N = 25$); no statistical differences between segments were observed.

The amplitude of the spontaneous contractions were not affected by atropine (control: 3.4 ± 0.3 g; atropine 1 μM: 3.3 ± 0.3 g; $N = 7$), L-NOARG (control: 3.7 ± 0.2 g; L-NOARG 10 μM: 3.8 ± 0.2 g; $N = 4$) and tetrodotoxin (control: 4.4 ± 0.4 g; tetrodotoxin 1 μM: 4.7 ± 0.5 g; $N = 3$). The tone was also not affected.

3.1. Responses of proximal and distal segments of taenia caeci to electrical field stimulation

The electrical relaxation responses of the taenia caeci segments were in increasing order from proximal, proximal medium, distal medium to distal (data not shown). Because the difference was significant only between proximal and distal segments, we decided to carry on the study with these two segments.

Electrical field stimulation in the presence of atropine resulted in a frequency-dependent relaxation from 0.3 to 1 Hz. Significant differences ($P \leq 0.001$) between proximal

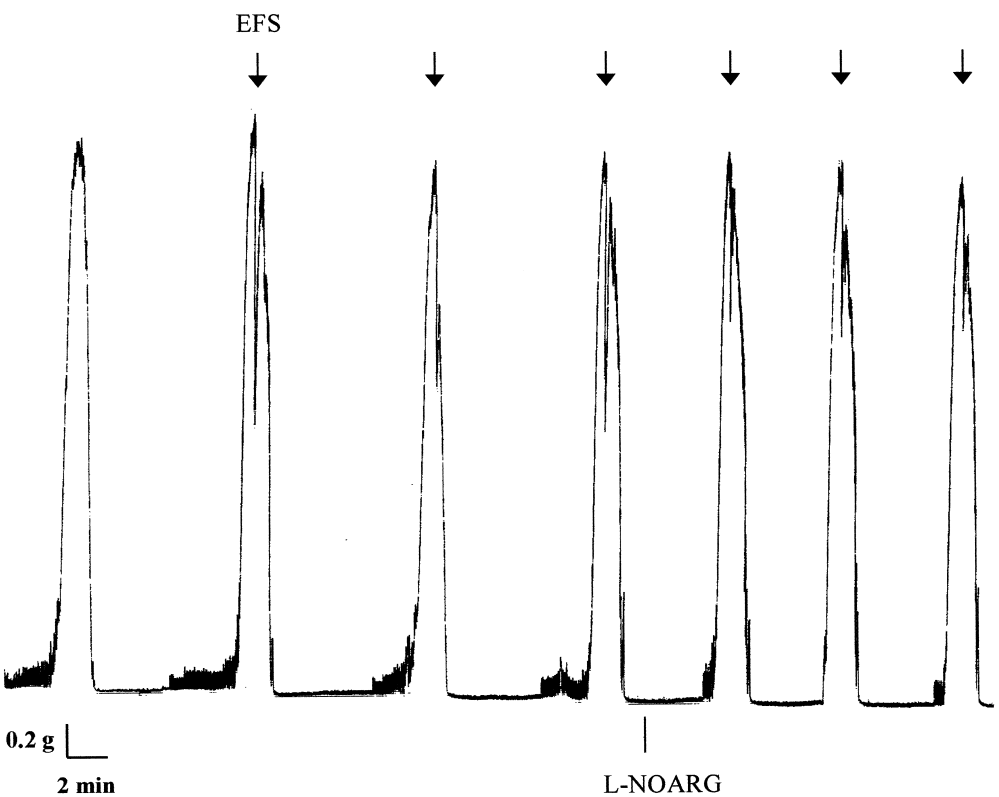

Fig. 2. Inhibitory effect of L-NOARG 10 μM on NANC relaxation induced by electrical field stimulation on proximal segment of guinea-pig taenia caeci. Electrical field stimulation (EFS): 0.3 Hz, 40 V, 1 ms were applied for 5 s at the peak of the spontaneous contractions.

Table 2
Effect of L-NOARG (10 μM) on relaxation induced by electrical field stimulation

Frequency (Hz)	Proximal			Distal		
	Control	L-NOARG	Inhibition (%)	Control	L-NOARG	Inhibition (%)
In the presence of atropine (1 μM)						
0.3	39 ± 3	27 ± 2[f] (7)	29 ± 1	68 ± 5	57 ± 5[e] (6)	16 ± 3[b]
0.6	50 ± 7	42 ± 7[d] (8)	17 ± 5	81 ± 8	73 ± 7[d] (8)	9 ± 3
2	90 ± 4	86 ± 4 (7)	5 ± 2	96 ± 2	93 ± 4 (4)	4 ± 3
In the absence of atropine						
0.3	32 ± 3	24 ± 2[d] (6)	24 ± 5	49 ± 4	37 ± 4[f] (7)	27 ± 5
2	42 ± 13	36 ± 13[d] (7)	18 ± 6	74 ± 5	58 ± 8[f] (11)	24 ± 5

Inhibitory effect of L-NOARG 10 μM on electrical relaxation of proximal and distal segments of guinea-pig taenia caeci in the presence and in the absence of atropine (1 μM). Electrical field stimulation: 0.3–2 Hz, 40 V, 1 ms was applied at the top of the spontaneous contractions. The relaxation of control and L-NOARG treated tissues is expressed as a percentage of the amplitude of each spontaneous contraction. Data are given as mean ± S.E.M. The number of experiments is shown in parentheses. Significance of difference by unpaired Student's *t*-test ([b]$P < 0.01$) between proximal and distal L-NOARG inhibition. Significance of difference by paired Student's *t*-test ([d]$P < 0.05$, [e]$P < 0.01$, [f]$P < 0.001$) from the corresponding control value.

and distal segments were observed at 0.3 and 0.6 Hz. Maximum relaxation was reached at 1 Hz (Table 1).

In the absence of atropine, the relaxation responses were lower than in the presence of atropine at all frequencies tested in both proximal and distal segments. We found statistical significant differences between proximal and distal segments at all frequencies tested (Table 1).

The relaxations induced by electrical field stimulation were abolished by tetrodotoxin (1 μM).

3.2. Effects of L-NOARG on relaxations induced by electrical field stimulation of proximal and distal segments of taenia caeci

To determine whether NO production was different in proximal and distal segments, L-NOARG was added at 10 μM to both segments. In the presence of atropine, L-NOARG caused a partial inhibition of relaxation in both proximal and distal segments, this inhibition decreased when the frequency increased. The maximum inhibition was 29% and 16% at 0.3 Hz in proximal (Fig. 2) and distal segments, respectively ($P \leq 0.01$) (Table 2). L-Arginine (1 mM) completely blocked the inhibitory effect of L-NOARG at all frequencies tested (data not shown).

Since the maximum inhibition produced by L-NOARG was obtained at 0.3 Hz, we studied the effect of L-NOARG in the absence of atropine at this frequency (we excluded those segments in which relaxation was less than 20%) and at 2 Hz. The inhibitory effect of L-NOARG was similar in proximal and distal segments at these frequencies (Table 2).

4. Discussion

Electrical field or transmural stimulation in precontracted preparations is commonly used as a convenient method to evoke relaxations. However, it is not easy to analyse data obtained by this method because the use of a contractile substance (such as prostaglandin, carbachol or histamine) could interfere with the results (Gibson et al., 1994). Since taenia caeci exhibited spontaneous rhythmic activity, we decided not to use a contractile substance and apply the electrical field stimulation at low frequencies at the peak of the spontaneous contraction.

In these conditions, our results show clear differences between proximal and distal segments of the taenia caeci in relaxation induced by electrical stimulation. The relaxation was frequency-dependent and progressively greater from the proximal to the distal segment. This could be of physiological importance in the propagation of intestinal contents.

The effect of atropine on the electrical relaxation is consistent with the findings of Knudsen and Tottrup (1992), Ward et al. (1996) and Selemidis et al. (1997), who reported an increase in electrical relaxations in the presence of atropine in guinea-pig taenia caeci. The effect of atropine suggests that the muscarinic action of acetylcholine has a contractile effect per se, as proposed by Bennet (1966) and Campbell (1966). Electrical field stimulation is known to excite both inhibitory and excitatory nerves and the responses to electrical stimulation could be conceived as the sum of actions of the released mediators on the smooth muscle. The differences observed in electrical relaxation between proximal and distal segments may be due to the different contribution of excitatory and inhibitory transmission.

The inhibitory effect of L-NOARG on NANC relaxations decreased in both segments as the frequency of electrical field stimulation increased; the effect in proximal segments was approximately double that in distal segments. At 2 Hz, the inhibitory effect of L-NOARG was very slight. Thus, the effect of NO-synthase inhibitors on NANC relaxation depends on the frequency of electrical stimulation and the segment used. These data could explain why Rand and Li (1990) and Bridgewater et al.

(1995) did not find any effect of L-NOARG. The effect of L-NOARG in the absence of atropine was the same in both segments and the inhibition did not change with the frequency; which may explain why Knudsen and Tottrup (1992) and Ward et al. (1996) conclude that L-NOARG reduced the neurogenic relaxation of precontracted taenia caeci only in the absence of atropine.

The fact that the inhibitory effect of L-NOARG on NANC relaxation decreased when the frequency increased, suggests that NO was co-released with another frequency-dependent substance that masked or inhibited the NO. In the presence of acetylcholine (absence of atropine), the effect of L-NOARG was the same in both segments at all frequencies tested, which could imply that the muscarinic effect of acetylcholine has an inhibitory effect on the unidentified frequency-dependent co-released substance referred above. The presence of this unidentified substance is consistent with the work of Ward et al. (1996), who proposed another excitatory substance with a muscarinic regulation such as tachykinin(s). When blocked by atropine, this excitatory substance masks the postjunctional effects of NO. Alternatively, our results are also compatible with the report of Selemidis et al. (1997), who proposed that the NO is co-released with another apamin sensitive transmitter from NANC inhibitory nerves, which could cause relaxation of guinea-pig taenia caeci as well as inhibition of the release of NO.

This study demonstrates differences in relaxations induced by electrical stimulation between the opposite ends of guinea-pig taenia caeci and also that NO contributes to these relaxations, depending on the integrity of cholinergic transmission.

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