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Short communication

Lack of anticholinergic effect of N^G-nitro-L-arginine methylester in the small intestine

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Abstract

The nitric oxide (NO)-synthase inhibitor, N^G-nitro-L-arginine methylester (L-NAME), has been reported to have an atropine-like action. We compared the effects of L-NAME (1 mM) and atropine on isolated small intestinal preparations of the guinea-pig, rat, rabbit and mouse. Half-maximal longitudinal contractions in response to acetylcholine (50–100 nM) were not influenced by L-NAME, but were strongly suppressed by atropine (1 nM). Cholinergic 'twitch' contractions of the guinea-pig ileum were slightly enhanced by L-NAME; this effect was prevented by pretreatment with N^G-nitro-L-arginine (L-NOARG, 100 μM), another NO synthase inhibitor. 'Twitch' contractions were concentration dependently inhibited by atropine (1–100 nM). We conclude that L-NAME is free of atropine-like activity in isolated intestinal preparations.

Keywords: L-NAME (N^G-nitro-L-arginine methylester); Atropine; Ileum, guinea-pig, rat, rabbit, mouse

1. Introduction

The nitric oxide (NO)-synthase blocker, N^G-nitro-L-arginine methylester (L-NAME), is widely used in work with cardiovascular, gastrointestinal and other types of tissue; its good water solubility, better than that of many other L-arginine analogues makes it a popular experimental tool. It has, however, been reported that L-NAME may show an atropine-like effect in some tissues (Buxton et al., 1993). This is of great potential importance, especially with gastrointestinal preparations, which possess a large number of cholinergic neurons that release acetylcholine as a result of spontaneous firing, electrical field stimulation or reflex peristaltic activity. In fact, L-NAME has been used for studying possible 'nitrergic' involvement in the peristaltic reflex of the small intestine (Waterman and Costa, 1994; Barthó and Holzer, 1995; Holzer et al., 1997). If L-NAME has significant atropine-like activity, all data obtained for this drug with gastrointestinal preparations (except when atropine or a similar drug was present) need to be re-examined.

The present study aimed to investigate the possible anticholinergic action of L-NAME in small intestinal preparations from 4 species of laboratory animals. Smooth muscle muscarinic receptors were stimulated by the administration of acetylcholine (in all species) or by electrical field stimulation (guinea-pig) that activates enteric cholinergic neurons (Paton and Vizi, 1969). The effect of L-NAME was compared to that of atropine.

2. Materials and methods

2.1. Guinea-pig ileum

Guinea-pigs of either sex weighing 300–400 g were stunned and bled. Segments of pre-terminal ileum were removed and made up as preparations of approximately 2 cm length. They were placed in 5-ml organ baths containing Krebs–Henseleit solution at 37°C. Carbogen gas (5% CO_2 plus 95% O_2) bubbling was used for oxygenation. The preparations were mounted vertically on tissue holders. The load on the tissue was 5 mN. Movements were recorded isotonically through lever transducers and bridges (Hugo Sachs Elektronik, Germany) on pen recorders.

Electrical field stimulation was delivered by a high-power stimulator (ST-1, modified for our purposes; Experimetria, Budapest, Hungary). Parameters of stimulation were: 80 V, 0.1-ms impulse width, single shocks. Stimuli

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were delivered to the tissue through a pair of platinum electrodes placed at the top and bottom of the organ bath (distance, 25 mm). Field stimulation was not applied continuously; 5–10 stimuli were delivered at a frequency of 0.05 Hz at appropriate times. Exogenous acetylcholine was administered in a concentration of 50 nM (contact time, 2 min). This was repeated at 20 to 30-min intervals until stable control responses were obtained and then the effect of L-NAME was examined.

The following experimental protocols were used.

2.1.1. Studying the effect of L-NAME alone

Control field stimulation or acetylcholine was applied, then the application was repeated in the presence of 1 mM of L-NAME. With field stimulation, the effect of L-NAME was tested at min 5, 15 and 30 after its administration. With acetylcholine, the contact time for L-NAME was 20 min.

2.1.2. Studying the action of L-NAME in the presence of N^G-nitro-L-arginine (L-NOARG)

After control field stimulation or acetylcholine exposure, L-NOARG (100 μM) was administered (contact time, 20 min) and the cholinergic stimulus was repeated. The action of L-NAME (1 mM) was then tested with L-NOARG still present in the organ bath with contact times of 5 and 15 min (electrical stimulation) or 20 min (acetylcholine).

2.1.3. Examining the effect of atropine

Control field stimulation or acetylcholine was applied, then the application was repeated in the presence of atropine (for field stimulation 1, 10 and 100 nM, added cumulatively, for acetylcholine 1 and 10 nM). Contact time for each concentration was 20 min.

2.2. Rat, rabbit and mouse ileum

In some experiments, rat ileal longitudinal muscle-myenteric plexus strips, rabbit ileal longitudinal strips and mouse whole ileal segments were used. Male Wistar rats (150–250 g), male white rabbits (3000 g) and male NMRI mice (40–50 g) were killed by a blow to the head and exsanguinated. Rat ileal longitudinal muscle-myenteric plexus strips were prepared as described earlier (Barthó et al., 1992). Using a strip was necessary because this preparation (following an equilibrium period of 60–90 min) has a lower basal tone than does whole ileum. Segments of rabbit ileum were opened along the longitudinal axis and pinned flat in a Petri dish; whole-thickness, longitudinally oriented strips of approximately 25 × 3 mm were cut with scissors.

Mouse whole ileum was made up as preparations of approximately 25 mm in length. Preparations were connected to isotonic lever transducers under a load of 2 mN (rat and mouse ileum) or 5 mN (rabbit ileum). These preparations were used to determine the influence of L-NAME (1 mM) or atropine (1 nM) on the contractile effect of acetylcholine (100 nM). The contact time for both L-NAME and atropine was 20 min.

2.3. Drugs

Drugs used were acetylcholine chloride (Miochol, Iolab Pharmaceuticals), atropine sulphate (Merck), L-NAME and L-NOARG (Sigma, Budapest, Hungary). Stock solutions were prepared as follows. L-NAME (100 mM), L-NOARG (10 mM), acetylcholine and atropine (1 mM each) were all dissolved in isotonic saline. The NO synthase blockers were applied directly from these solutions whereas acetylcholine and atropine were diluted in saline. The solvents had no effect on the preparations. L-NAME was freshly prepared each day (see Buxton et al., 1993).

2.4. Expression of data; statistics

Contractions were expressed as percentage of the maximal longitudinal spasm evoked by acetylcholine (10 μM) at the beginning of the experiment. Means ± S.E.M. are given throughout. The following non-parametric tests were used for statistical comparisons; for comparing several related samples we applied the Quade test (see Theodorsson-Norheim, 1987) and for two related samples, Wilcoxon's signed rank test. A probability of $P < 0.05$ or less was accepted as significant.

3. Results

Control experiments showed that contractions in response to field stimulation (guinea-pig ileum) or acetyl-

Table 1
Electrical field stimulation

Control	L-NAME			*n*
	(min 5)	(min 15)	(min 30)	
43.9 ± 3.6	50.9 ± 3.8*	52 ± 3.6*	52.4 ± 3.4*	5
Control	L-NOARG	L-NOARG + L-NAME		
		(min 5)	(min 15)	
40.8 ± 5.0	45.5 ± 5.9	46.1 ± 5.8	45.2 ± 5.7	7
Control	Atropine			
	1 nM	10 nM	100 nM	
53.1 ± 4.1	22.3 ± 2.5[a]	7.2 ± 1.1[b]	0[c]	10

Contractions of the guinea-pig whole ileum in response to single pulses of electrical field stimulation before and in the presence of L-NAME (1 mM), L-NOARG (100 μM) plus L-NAME (1 mM) or atropine (concentrations as indicated). Contact time for atropine and L-NOARG was 20 min; that for L-NAME was 20 min or as indicated in brackets. Contractions are expressed as percentage of the maximal longitudinal spasm. Means ± S.E.M. are given. Asterisks denote statistically significant differences; [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ (Quade test for several related samples) and *n* the number of experiments.

Table 2
Acetylcholine

Preparation	Control	L-NAME	*n*
Guinea-pig ileum	62.2 ± 5.5	66.1 ± 4.9	5
Rat LM-MP strip	48.2 ± 6.7	47.6 ± 5.3	6
Mouse ileum	60.9 ± 10.6	50.3 ± 13.3	5
Rabbit ileum	55.5 ± 2.8	62.6 ± 3.0	5

Lack of effect of L-NAME (1 mM, contact time 20 min) on contractions (expressed as percentage of the maximal longitudinal spasm) of the guinea-pig and mouse ileum, the rabbit ileal longitudinal strip and the rat ileal longitudinal muscle-myenteric plexus (LM-MP) strip to acetylcholine (50 nM in the guinea-pig ileum and 100 nM for all other preparations). Means ± S.E.M. are given. No significant changes were noted (Wilcoxon's test).

choline (all preparations) remained stable throughout the observation period ($n = 4–5$).

In the guinea-pig intestine, the contractile effect of electrical field stimulation was not inhibited by L-NAME (1 mM). Instead, a slight but significant enhancement of the electrically induced contractions was found at all times of observation (min 5, 15 and 30) (Table 1). In the presence of L-NOARG (100 μM) L-NAME (1 mM) failed to have any influence on the contractile effect of field stimulation. L-NOARG itself tended to enhance the response but this did not reach statistical significance. Atropine caused a concentration-dependent inhibition of the electrically induced contraction; 1 nM of the drug induced an approximately 60% inhibition, while that with 10 and 100 nM of atropine was approximately 85 and 100%, respectively (Table 1).

Half-maximal contractions of the guinea-pig, rat, rabbit and mouse ileal preparations challenged with acetylcholine were uninfluenced in the presence of L-NAME (1 mM) (Table 2). In the guinea-pig, L-NOARG (100 μM) and a combination of L-NOARG (100 μM) and L-NAME (1 mM) was also ineffective against acetylcholine (contractions reached 65.2 ± 8.7 and 66.1 ± 7.3% of maximum, respectively, control responses being 63.0 ± 9.1%; $n = 5$).

Atropine (1 nM) strongly suppressed the acetylcholine-evoked half-maximal ileum contractions in all species studied. Reduction of the acetylcholine-induced contraction was 95.4 ± 2.4% in the rat ileal strip, 94.0 ± 3.0% in the rabbit ileal strip, 88.7 ± 3.6% in the mouse ileum, and 71.9 ± 5.6% in the guinea-pig ileum ($n = 5$); in the latter preparation, 10 nM of atropine caused a 95.4 ± 2.7% inhibition ($n = 5$ for each group).

4. Discussion

These data, obtained with small intestine of 4 species of laboratory animals, fail to show any anti-cholinergic effect of L-NAME, in a concentration as high as 1 mM. The enhancement of the cholinergic 'twitch' response of the guinea-pig ileum by L-NAME is consistent with a reduction of stimulation-induced acetylcholine release by endogenous NO released from enteric neurons (Kilbinger and Wolf, 1994). Pretreatment with another NO synthase inhibitor, L-NOARG, prevented the enhancement of the 'twitch' response by L-NAME but failed to unmask any atropine-like action of this compound. It is known that atropine blocks the 'twitch' response; in our experiments, as little as 1 nM of atropine inhibited by half the effect of field stimulation.

Buxton et al. (1993) reported that L-NAME displayed antimuscarinic activity in a range of tests, including acetylcholine-mediated contractions of the rabbit colon smooth muscle, where an IC_{50} value of approximately 10 μM was found. Binding studies yielded partial confirmation of an affinity of L-NAME to muscarinic receptors (Hellmlich and Gyermek, 1997), whereas functional tests with the heart and bladder failed to demonstrate an atropine-like effect (Cheng et al., 1994; Hellmlich and Gyermek, 1997). Koss (1997) also concluded that L-NAME does not inhibit non-endothelial responses due to cholinergic nerve stimulation or nerve activity, and L-NAME failed to displace labelled scopolamine from spinal cord membranes (Feldman et al., 1996). On the other hand, in vivo experiments on the microcirculation of rat diaphragm support a possible antimuscarinic effect of L-NAME (Chang et al., 1997). Although the tests used in these various studies differed greatly from each other, it is difficult to find an explanation for the discrepant findings. Our primary goal was to assess the possible interference of L-NAME with muscarinic receptors in the intestinal tract.

In conclusion, L-NAME (at least up to 1 mM) can probably be safely used with intestinal preparations, in vitro.

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