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Effect of cholinergic agonists on muscular tonus of the lizard small intestine and esophagus

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ABSTRACT

The underlying mechanisms of acetylcholine-induced intestinal relaxation in the lizard *Liolaemus tenuis tenuis* are still unknown. By using a classical model of intestinal recording of isometric contraction and relaxation in conjunction with specific pharmacological tools, this article studies the possible influence of EDRF/NO and nicotinic ganglionar receptors on the Ach-induced relaxation in an effort to elucidate the probable mechanisms involved in ACh effect.

It was observed that the relaxation of the lizard intestine elicited by ACh (10^{-7} - 4 x 10^{-4} M) was not affected by hexametonium (5 x 10^{-4} M) or tetrodotoxin (10^{-6} M). Nicotine (10^{-7} to 10^{-4} M) induced relaxation was significantly antagonized by hexametonium; however, it was not influenced by tetrodotoxin.

These results allow us to discard a neuronal pathway in cholinergic-induced relaxation, suggesting a more direct cholinergic effect on the smooth muscle, perhaps mediated by an unknown substance released by some specialized tissue.

N-nitro-L-arginine, used to block NO-synthase and NO production, induced no changes in ACh-induced relaxation. Methylene blue, a soluble guanylate cyclase inhibitor, induced no changes in ACh-induced relaxation. These results allow us to discard a probable role of EDRF/nitric oxide in the ACh-induced relaxation of lizard small intestine, providing evidence that this mechanism could be different from that reported in other species.

Key terms: lizard *Liolaemus tenuis tenuis*, small intestine, acethylcholine, ganglionic nicotinic receptors, nitric oxide, relaxation

INTRODUCTION

We previously reported the inhibitory effect of acetylcholine (ACh) on muscular tonus of the small intestine of the lizards *Lioalemus gravenhorsti and Liolaemus tenuis tenuis*. The results obtained suggested that this inhibitory effect was not mediated by α or ß-adrenoceptors (Wacyk *et al.*, 1984, 1989).

In the canine isolated ileocolonic junction, ACh induces relaxation, and this effect is blocked by inhibitors of nitric oxide (NO) biosynthesis (Boeckxstaens *et al.*, 1990). NO has been reported to be involved in nonadrenergic-noncholinergic (NANC) relaxation in different portions of the digestive tract (Tottrup *et al.*, 1991; Stark *et al.*, 1993; Baker *et al.*, 1993; Boeckxstaens *et al.*, 1993; Lefebvre *et al.*, 1995). It has been further proposed that either NO or a related substance accounting for the biological activity of the vascular endothelium-derived relaxing factor (Palmer *et al.*, 1987, Salas, 1998) should be the inhibitory NANC neurotransmitter in the canine ileocolonic junction (Boeckxstaens *et al.*, 1990).

The purpose of the present study was to investigate whether the ACh-evoked relaxation of lizard intestinal muscle is mediated by ganglionic nicotinic receptors and

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NO. Our second aim was to compare the responses of the small intestine to nicotine (Nico) with those of another segment of the lizard digestive tract. The esophagus was chosen. The muscular coat of this portion of the digestive tube of the lizard *Liolaemus tenuis tenuis* contains only smooth muscle (Wacyk *et al.*, 1980).

MATERIAL AND METHODS

Lizards *Liolaemus tenuis tenuis* of either sex, weighing between 2.5 and 6 g and captured in the central zone of Chile, were used in these experiments. The animals were decapitated and the esophagus and small intestine quickly removed under Tyrode solution gassed with a mixture of 95% O_2 and 5% CO_2. This solution was used in all experiments, and the temperature was maintained at 21 ± 1°C. The esophagus (6-12 mm in length) and segments of the mid-section of the small intestine (8-14 mm in length) were placed in 25 ml organ baths under a resting tension of 500 mg.

After the preparations underwent a minimum 30-minute equilibrium period, ACh and Nico were cumulatively applied in increasing concentrations. In other experiments the cholinergic agonists were added approximately 20 minutes after pretreating the esophagus with hexamethonium (Hex) and tetrodotoxin (TTX) and the small intestine with Hex, TTX, N-nitro-L-arginine, and methylene blue. The changes of isometric muscle tension were recorded with a FT03 force transducer connected to a polygraph (Grass 79 D) and expressed in grams per 100 mg of wet tissue. Results are given as mean ± SEM. Statistical analysis was performed using ANOVA. Differences with a P-value of 0.05 or less were considered significant.

The following drugs were used: acetylcholine chloride, (-)-nicotine, hexamethonium chloride, tetrodotoxin, N-nitro-L-arginine, and methylene blue (Sigma Chemical Co.). Solutions were freshly prepared in Tyrode (pH 7.4) at appropriate concentrations before use.

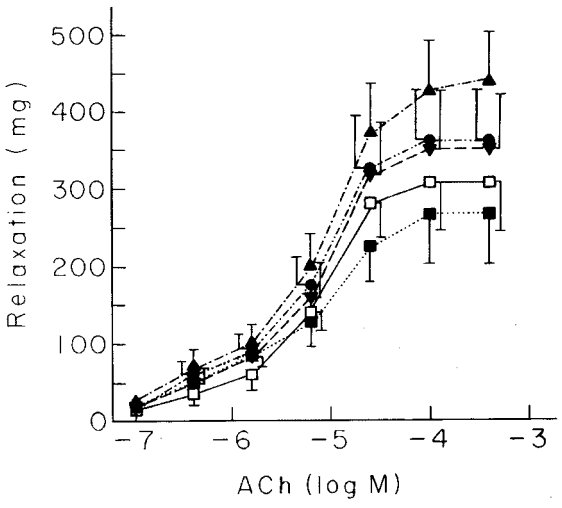

Figure 1

RESULTS

ACh (10^{-7} - 4 x 10^{-4} M) elicited a concentration-dependent relaxation of the intestinal muscle that was unaffected by Hex (5 x 10^{-4} M). In the presence of 10^{-4} M N-nitro-L-arginine and 10^{-5} M methylene blue, no significant changes were detected with respect to the action of ACh on the muscular tonus. TTX (10^{-6} M), previously added, did not antagonize the inhibitory effect of ACh on the small intestine (Fig 1). ANOVA analysis allowed us to estimate a P-value of 0.4566, showing that the variability between group means was not significantly different than the residual variability within the groups. The obtained data provide no evidence that the group means differ from one another.

To compare the effects of the different drugs used, maximal ACh-induced relaxation values, as shown in Table 1, were analyzed by means of one-way ANOVA. There were no significant differences among the experimental groups. Nicotine added to the small intestine in cumulative concentrations from 10^{-7} to 10^{-4} M, evoked a sigmoidal curve of relaxation. The intestinal muscle response was antagonized by 5 x 10^{-4} M Hex ($P < 0.05$ at 10^{-4} M Nico); however, it was not influenced by 10-6 M TTX (Fig 2).

TABLE I

Effects on maximal relaxation induced by acetylcholine

	MAXIMAL RELAXATION (mg/100mg)*	N	SIGNIFICANCE ∞
CONTROL	299.0 ± 58.1	10	---
HEXAMETHONIUM	260.7 ± 54.5	8	ns
L-NNA	359.1 ± 64.7	8	ns
METHYLENE BLUE	354.5 ± 65.4	7	ns
TTX	438.2 ± 78.1	8	ns

* Significance with respect to the control relaxation (one-way ANOVA).
ns = non significant.
∞ mg of tension/100 mg of wet tissue.

On the other hand, the esophagus smooth muscle exhibited a concentration-dependent tension development after the cumulative addition of nicotine. Figure 3 shows that the contraction curve obtained with (10^{-7} - 10^{-4} M) Nico was significantly ($P < 0.05$) inhibited by the addition of Hex (5×10^{-4} M) and TTX (10^{-6} M).

DISCUSSION

We have reported elsewhere that the inhibitory effect of ACh on muscular tonus of the small intestine of the lizards *Liolaemus gravenhorsti* and *Liolaemus tenuis tenuis* was not mediated α by or ß-adrenoceptors (Wacyk *et al.*, 1984, 1989) and the site of ACh action remained unknown.

It has been shown in dogs that ACh-induced relaxation of the ileocolonic junction is blocked by inhibitors of NO biosynthesis (Boeckxstaens *et al.*, 1990). Furthermore, NO has been reported to be involved in NANC relaxation in different portions of the digestive tract (Tottrup *et al.*, 1991; Stark *et al.*, 1993; Baker *et al.*, 1993; Boeckxstaens *et al.*, 1993; Lefebvre *et al.*, 1995).

The results obtained in this work show that in the lizard, the relaxant effect of ACh is not blocked by N-nitro-L-arginine, a potent inhibitor of NO-synthase (Ishii *et al.*, 1990), nor by methylene blue, a well known inhibitor of EDRF/NO effects (Mar-

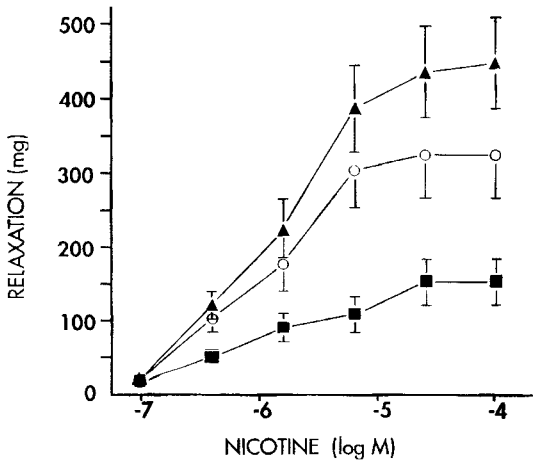

Figure 2

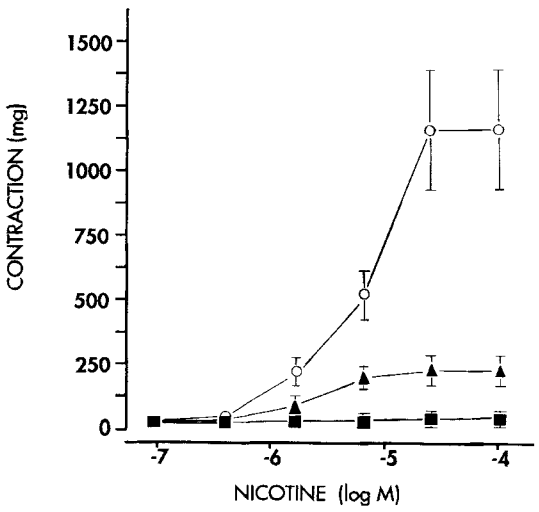

Figure 3

tin *et al.*, 1985). This suggests that the decrease of the muscular tonus induced by this cholinergic agonist is not mediated by NO. The inhibitory effects of Nico and ACh were not antagonized by TTX, and Hex failed to antagonize the relaxant action of ACh. These results suggest that under the experimental conditions described above, these two cholinergic agonists are able to cause intestinal muscle relaxation through a non-neuronal pathway. Moreover, the possibility that ACh-induced relaxation could be mediated by ganglionic nicotine receptors is discarded.

It appears reasonable to assume that Nico and ACh act on non-neuronal tissue. The finding that some secretory and glandular cells express muscarinic and nicotinic receptors supports this possibility (Goyal, 1989; Kuijpers *et al.*, 1994). It is also known that ACh causes an increase in gastric secretion (Mihm and Wetzel, 1987). Unlike the small intestine, the Nico-induced contraction of the esophagus was blocked by Hex and TTX, suggesting that this response was mediated by intramural neurons.

It is suggested that there is a cholinergic-induced mechanism in the lizard small intestine that is able to decrease the muscular tonus through a non-neuronal pathway, ruling out nitric oxide mediation. We do not discard the possibility that ACh could be acting directly or releasing a still unknown mediator with hyperpolarizing consequences. Electrophysiological studies are being accomplished in order to elucidate this point.

SUMMARY

1. The responses to acetylcholine and nicotine of the small intestine and to nicotine of the esophagus of the lizard *Liolaemus tenuis tenuis* were investigated *in vitro*.

2. Both cholinergic agonists caused relaxation of the intestinal muscle. The effect of acetylcholine was not antagonized by hexamethonium, tetrodotoxin, N-nitro-L-arginine nor by methylene blue. The effect of nicotine was blocked by hexamethonium, though not by tetrodotoxin.

3. The results suggest that the cholinergic agonists should activate non-neuronal tissue inducing the release of an unidentified mediator, distinct from nitric oxide, which causes a decrement of the muscular tonus.

4. Nicotine increased the tonus of esophageal smooth muscle. This effect was blocked by hexamethonium and tetrodotoxin, suggesting that it was mediated by nerve cells.

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