Final Degree Project
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Loperamide Effects in Rat Colon Motility

INTRODUCTION AND OBJECTIVES

In the gastrointestinal tract, the contraction of the muscle (longitudinal and circular segments) is regulated by neuronal **inhibitory** or **excitatory** transmission (Enteric Nervous System) or by **slow waves** from interstitial cells of Cajal (ICCs). **Loperamide** acts as **antidiarrheal** solution, but its mechanism of action is unclear.

The objective is to determine the effect of loperamide in rat colon in order to evaluate potential **mechanisms of action**.

MATERIAL AND METHODS

Table1: Summary of protocols used in the experiment. N refers to number of segments in circular (C) and longitudinal (L) layers. We use non-adrenergic, non-cholinergic (NANC) conditions and tetrodotoxin (TTX) or Krebs solution with L-NNA and MRS2500

	Pharmacology Conditions	Electric Field Stimulation	N_C	N_L
Excitatory Neurotransmission	L-NNA + MRS2500	50 Hz, 0.4 ms, 30 V, during 300 ms	7	9
Inhibitory Neurotransmission	NANC	5 Hz, 0.3 ms, 15 V during 2 min	6	8
Spontaneous Motility	TTX	---	7	10

→ 7 rat colon in organ bath.

→ Loperamide concentration ranges from **10^{-9}M to 10^{-4}M.**

❖ **Tension** (g) of contraction (spontaneous and excitatory motility) and **AUC** (inhibitory motility).

❖ **One-way ANOVA** and **non-linear regression** (loperamide pharmacodynamics).

Datawin 2001 and GraphPad Prism.

RESULTS

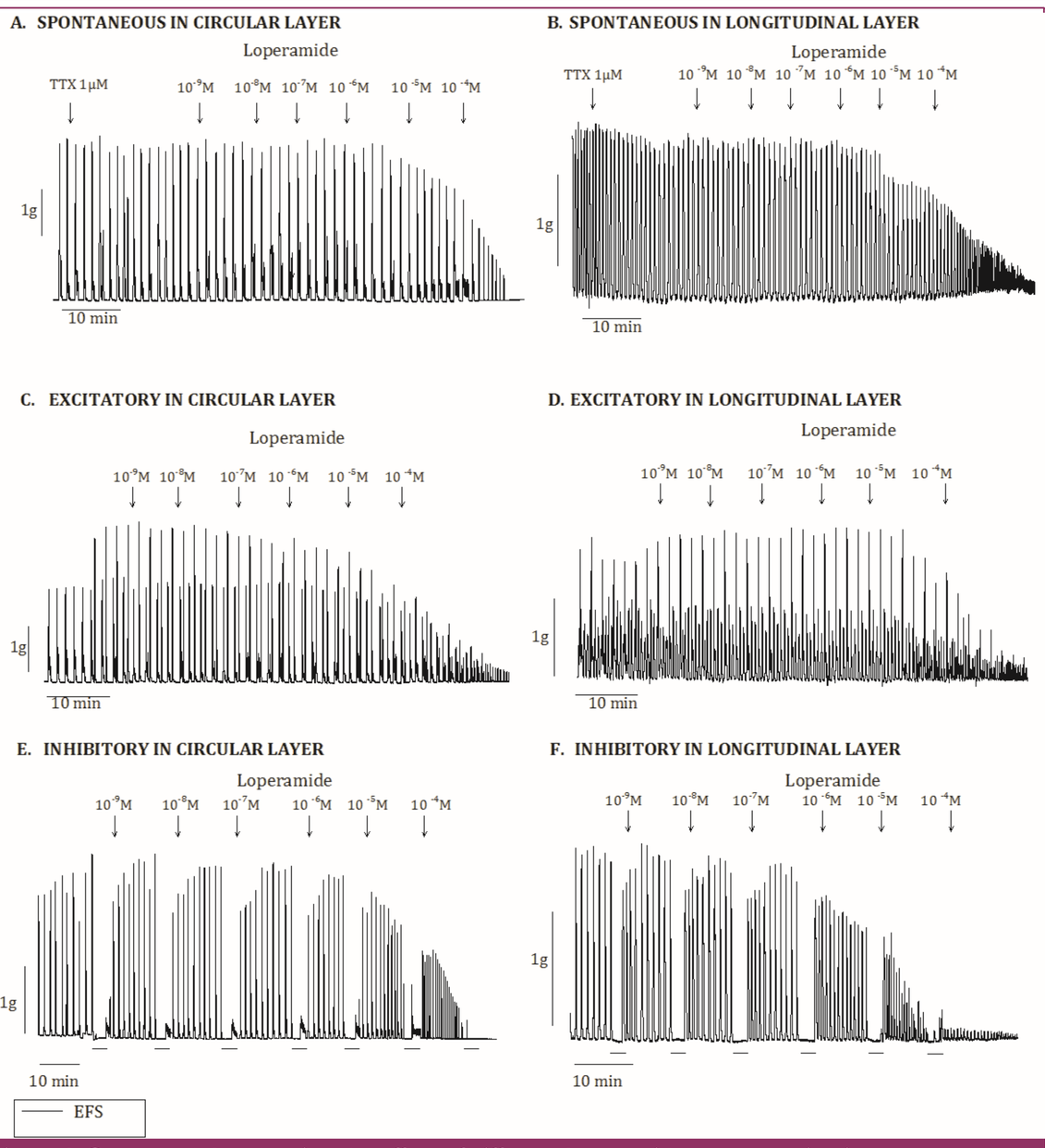

Figure 1: Organ bath log showing the effect of different loperamide concentration in spontaneous motility (A, B), neural mechanism - contraction (C, D) and relaxation (E, F) in both enteric muscle layers. In inhibitory experiments, EFS is applied every 10 minutes.

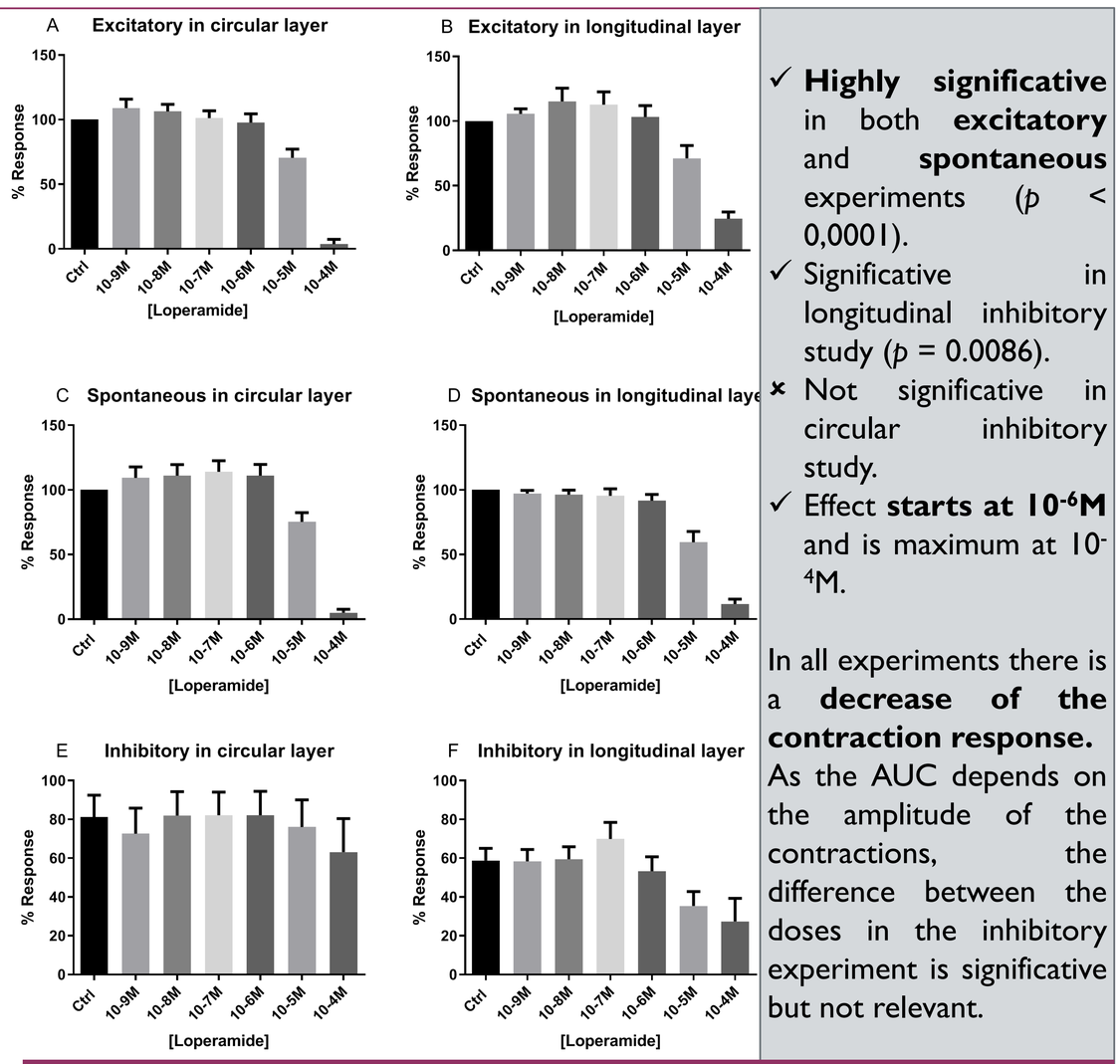

Figure 2: Graph showing the effects of loperamide on the percentage of response in different protocols and its standard mean deviation (SEM). Carried out with excitatory stimulation (A, B), TTX and no stimulation (C, D), and with inhibitory stimulation (E, F).

✓ **Highly significative** in both **excitatory** and **spontaneous** experiments ($p < 0{,}0001$).

✓ Significative in longitudinal inhibitory study ($p = 0.0086$).

✗ Not significative in circular inhibitory study.

✓ Effect **starts at 10^{-6}M** and is maximum at 10^{-4}M.

In all experiments there is a **decrease of the contraction response.** As the AUC depends on the amplitude of the contractions, the difference between the doses in the inhibitory experiment is significative but not relevant.

✓ There is a decrease of tension in all experiments

✓ Frequency of contractions seems to increase

✓ There is none or little effect until 10^{-6}M

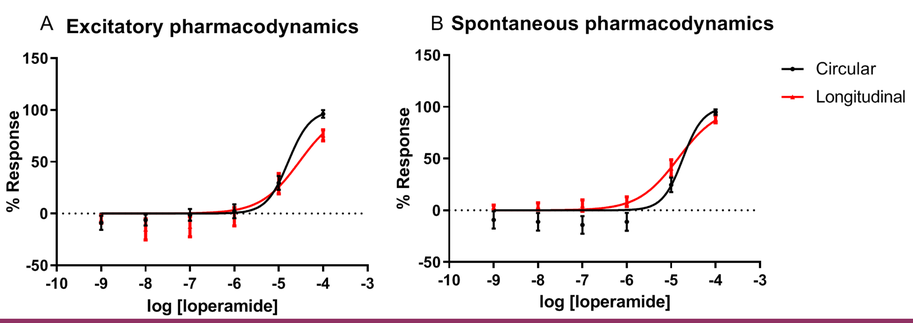

Figure 3: Dose-response pharmacodynamics with loperamide in the excitatory (A) and spontaneous (B) experiments. Much of the variation in the results would be due to the model ($R^2 > 0.60$ in all cases).

DISCUSSION

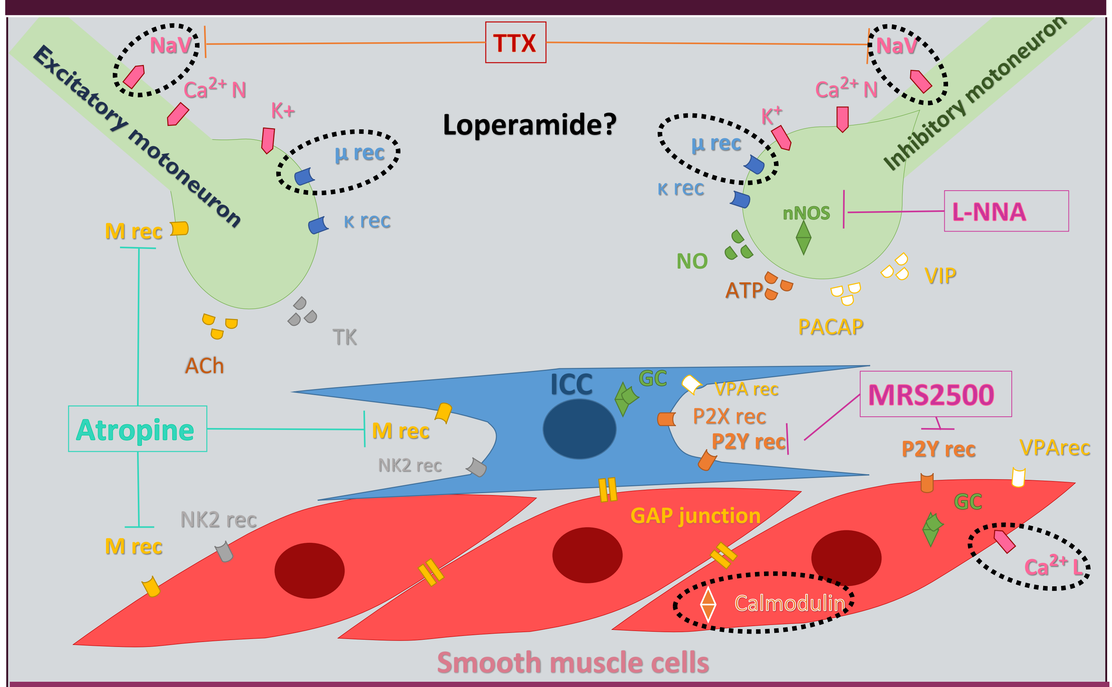

Figure 4: Scheme representing the potential routes of action of loperamide in the gastrointestinal smooth muscle. Voltage-dependent sodium channels (NaV), potassium channels (K^+), calcium channels type N ($Ca^{2+}N$) and L ($Ca^{2+}L$), opioid receptors (µ rec and κ rec), tachykinins (TK), acetylcholine (ACH), muscarinic receptors (Mrec), neurokinin receptors (NK2 rec), tetrodotoxin (TTX), guanylate cyclase (GC) nitrergic inhibitory pathway from nitric oxide synthase (NOS) that produces nitric oxide (NO), via purinergic (ATP, PACAP and VIP), which acts in ionic (P2x) and metabotropic (P2Y) receptors and vasopressin receptors (VPA rec).

CONCLUSION

➢ Loperamide causes a **decrease in tension** in the contraction of the smooth longitudinal and circular enteric muscles, both when stimulated by excitatory cholinergic motoneurons, and when it contracts spontaneously.

➢ Its effect on **inhibitory neurotransmission is less apparent** since spontaneous activity is inhibited, which makes it difficult to assess .

➢ The mechanism of action can be through **µ-type receptors**, although we do not rule out the pharmacological action on other pathways such as **L-type calcium channels**. Further studies are needed to confirm the receptors involved in the effects of loperamide at the gastrointestinal level.

❑ **µ receptors agonist**

❑ Blockage of **L-type calcium channels**

❑ Blockage of **sodium channels**

❑ Interference with **calmodulin**