Pharmacological Effects of Neurotransmitters on Earthworm Gut Contraction

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Introduction

- Earthworm gut motility can be modulated by neurotransmitters, making the earthworm a useful model to study the pharmacological effects of different neurotransmitters
- The crop-gizzard of the earthworm gut is especially sensitive to acetylcholine (ACh), an excitatory neurotransmitter important to muscle contraction
- The present study examined the relative influences of ACh, epinephrine, and serotonin (5-HT) on earthworm gut motility (i.e., frequency and amplitude of spontaneous contraction)

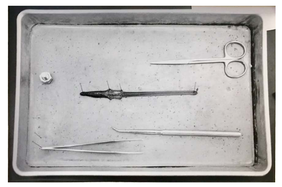
Hypotheses:

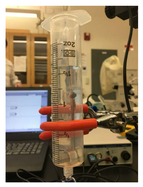
- When exposed to ACh and epinephrine in equal concentrations, the crop-gizzard will show increased contractions because it is more sensitive to ACh
- When exposed to ACh and 5-HT in equal concentrations, the crop-gizzard will show baseline-like contractions because the excitation and inhibition will cancel each other out

Method

Earthworm dissection:

- The earthworm was anesthetized in 100 ml 15% ethanol for 5-10 minutes
- The worm was placed in the dissection tray dorsal side up
- An incision was made anterior to posterior along the clitellum
- The body wall was pinned back to expose the crop-gizzard
- The crop-gizzard and a section of gut were removed





Organ bath mounting:

- Suture was tied around both ends of the tissue with a nut and bolt secured to the posterior end of the tissue and a force transducer connected to the anterior end
- The micropositioner of the force transducer was used to create tension in order to measure changes in force (i.e., contractions)

Procedure:

- Control recording, 5 minutes in earthworm saline
- Experimental recording, 10 minutes in 10^{-4} M ACh and 10^{-4} M epinephrine
- Emptied the chamber and performed a saline rinse
- Experimental recording, 10 minutes in 10^{-4} M ACh and 10^{-4} M 5-HT

Analysis:

- Contractile beats per minute was converted to contraction frequency
- Analysis of variance (ANOVA) was run to compare the difference in relative amplitude between groups

Results

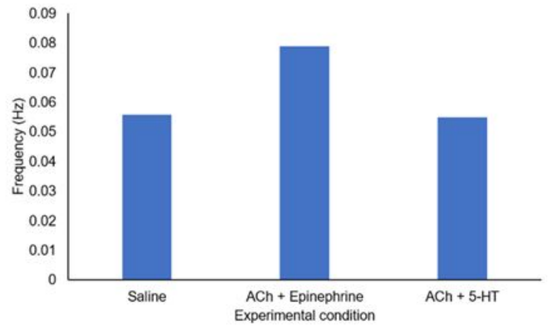


Figure 1. Frequency in Hz according to experimental condition

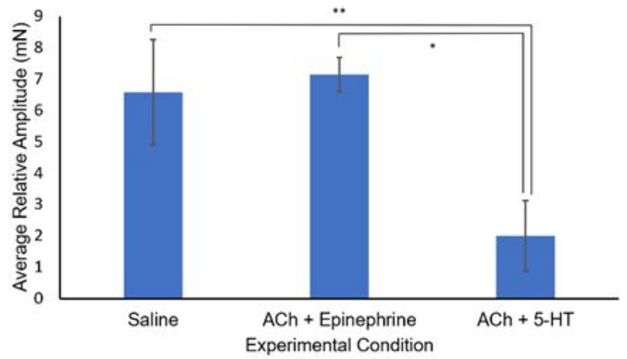


Figure 2. The average relative amplitude according to experimental condition (*p=0.0009; **p=0.0003, t-test: two-sample assuming unequal variance)

- The crop-gizzard contracted differentially according to condition
  - **Saline**: Trace data with a duration of Δt = 2.09 seconds, including 8 visible contractions and a frequency of 0.0558 Hz
  - **ACh + epinephrine**: Trace data with a duration of Δt = 1.48 seconds, including 8 visible contractions and a frequency of 0.0788 Hz
  - **ACh + 5-HT**: Trace data with a duration of Δt = 0.91 seconds, including 4 visible contractions and a frequency of 0.0549 Hz

- Post-hocs could not be run for frequency analysis with $n = 1$

Discussion

- The present study employed the crop-gizzard of the earthworm gut to examine the effects of neurotransmitters on gut motility (i.e., the frequency and amplitude of contraction). The experiments explored 1) how the crop-gizzard responds to equal concentrations of ACh and epinephrine and 2) how the crop-gizzard responds to equal concentrations of ACh and 5-HT. The results provided preliminary support for both hypotheses.

- The frequency value for the ACh and epinephrine condition was larger than those of the other two conditions, suggesting that the excitatory effect of ACh was greater than the inhibitory effect of epinephrine. Although this difference is not confirmed as statistically significant, the observable difference aligns with the first hypothesis. This is supported by Wu (1939), who found that the crop-gizzard is especially sensitive to ACh compared to epinephrine.

- The ACh and 5-HT condition had a decreased contraction force compared to the other two groups as seen in a change in mN (amplitude), suggesting that 5-HT has a strong inhibitory effect on gut motility even when combined with the excitatory neurotransmitter ACh. This finding supports the second hypothesis tha 5-HT would cancel out the excitatory effects of ACh.

- Serotonin plays an important evolutionary role as it modulates processes related to central nervous system development (Turkejski, 1996). It also regulates feeding, nutrient intake, and digestion (French et al., 2014). While 5-HT excites gut contraction in some insects and in most mammals, it inhibits gut contraction in the earthworm. Williams et al. (2018) showed that 5-HT increases neuronal excitability through disinhibition and observed reduced amplitude of depolarization-dependent calcium in *C. elegans*. Indeed, spontaneous contractions in the smooth muscle of the earthworm gut results from an increase in calcium permeability.

- A major limitation was the one replication of frequency counts. Due to lack of replicates, the present study combined neurotransmitters in concentrations that would reliably elicit a response. Future research should use a larger sample size and combine epinephrine and ACh at differing concentrations to quantify the magnitude at which ACh is more influential than epinephrine.

References

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