UNIVERSITY OF GOTHENBURG

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INVESTIGATING THE CONTROL OF GUT MOTILITY IN FISH AND HOW IT IS AFFECTED BY TEMPERATURE

Hugo Åklint

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Supervisor: Catharina Olsson, Department of biological and environmental sciences

Examiner: Elisabeth Jönsson Bergman, Department of biological and environmental sciences

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Abstract

Efficient transport, breakdown and absorption of food in the gut is crucial for animals. This is achieved through coordinated movements of smooth muscle cells inside the gut wall. It is regulated by autonomous nerves (especially the enteric nervous system) and hormones. Interstitial cells of Cajal (ICC) play a role in initiating motility and modulating control. The aim of this study was to investigate how an acute temperature increase affects the control of gut motility in rainbow trout (*Oncorhynchus mykiss*) *in vitro* by analyzing cholinergic response and ICC. Muscle contractions were recorded at different temperatures (10 °C, 15 °C and 20 °C). Cholinergic activity was mimicked by adding increasing concentrations of carbachol and the mean force developed at each concentration as well as $LogEC_{50}$, i.e. the concentration where 50% response was attained, were calculated. I found that there was an effect of temperature in different aspects of both organs. In the intestines $LogEC_{50}$ increased from -5.56±0.07 at 10 °C to -5.21±0.05 at 20 °C. But the controls also indicate a decrease in sensitivity and loss in developed force over time. ICC activity was studied by adding 50 µM benzbromarone which inhibited the activity of the stomach where only 63±9 % of the initial basal contractile force was retained, while no effect of benzbromarone was recorded for the intestine. We then examined the effects of ICC on cholinergic stimulation. The variation of results in both controls and treatments made it difficult to interpret if the decrease in response is caused by a change in temperature or due to time. Intestines at 10 °C decreased in sensitivity from $LogEC_{50}$ (-5.76±0.10 to -5.25±0.09) for treatments and controls (-5.97±0.13 to -5.42±0.09). The extent of how temperature affects ICC remains unclear but could give implications for improvements of techniques.

Svensk sammanfattning

Effektiv transport, nedbrytning och upptag av föda är kritiskt för djur. Det uppstår genom koordinerade rörelser av glatta muskler inuti tarmväggen. Magtarmkanalen regleras av autonoma nerver (särskilt enteriska nervsystemet) och hormoner. Interstitiella Cajal-celler (ICC) påverkar genom att initiera rörelser och modifiera nervsignaler. Syftet med denna studie var att undersöka hur akuta temperaturökningar påverkar kontrollen av magtarmrörelser hos regnbåge (*Oncorhynchus mykiss*) *in vitro* genom att analysera kolinerg respons och ICC-aktivitet. Muskelkontraktioner dokumenterades vid olika temperaturer (10 °C, 15 °C and 20 °C). Kolinerg aktivitet härmades genom ökande koncentrationer av acetylkolin-agonisten karbakol där medelkontraktion vid varje koncentration och $LogEC_{50}$ dvs., koncentrationen där 50% av responsen uppnåddes, beräknades. Det fanns en tydlig effekt av temperatur på båda organen men samtidigt upptäcktes drift hos respons över tid. För tarmar ökade $LogEC_{50}$ från -5.56 ± 0.07 vid 10 °C till -5.21 0.05 vid 20 °C och liknande trend uppstod för kontroller. Effekten av ICC undersöktes genom att tillsätta 50 µM benzbromaron som hämmar aktiviteten hos mage där 63±9 % av basal kontraktion återstod efter tillsättning men ingen tydlig effekt upptäcktes för tarm. Slutligen genomfördes en kombinerad undersökning hur ICC påverkar kolinerg respons vid 10 °C och 15 °C. Variationen i resultat för både kontroller och behandling medförde svårigheter att jämföra om minskningen i respons var en effekt av temperatur eller tid. Tarm vid 10 °C minskade i känslighet av karbakol från $LogEC_{50}$ (-5.76±0.10 till -5.25±0.09) för behandlingar och kontroller (-5.97±0.13 till -5.42±0.09). Huruvida temperatur påverkar ICC är fortarande oklart men kan ge insikt för utvecklandet av bättre teknik.

Introduction

The movement of food in animals is a crucial process for the survival of an individual. One of the main functions of the gastrointestinal tract is to digest and absorb nutrients. Through alternating contractions and relaxation of smooth muscle cells in the gut wall, food is transported along the digestive tract as well as mixed with gut juices for breakdown (Olsson & Holmgren, 2001).

The motility patterns of the gut are usually highly regulated by nerves, hormones and interstitial cells of Cajal (Browning & Travagli, 2014; Sanders et al, 2012; Wu et al, 2013). Additionally, there are several outer factors such as presence of food and temperature (Gräns & Olsson, 2024). With the events of global warming affecting aquatic habitats (Jutfelt et al, 2024), how temperature affects the control of gut motility of ectotherms is necessary to understand if a species can adapt to climate change.

What is gut motility?

The anatomy and functionality of the gastrointestinal in fish tract follows the general structure of other vertebrates (Wilson & Castro, 2010). The gut wall is made up several lavers (Fig 1) including two smooth muscle layers, the inner circular and the outer longitudinal.

The combined contractions and relaxations of these smooth muscles are what give rise to gut motility. There are two main motility patterns (Fig 2). Propagating motions called peristalsis that transport food along the digestive tract and non-propagating standing contractions that cause mixing of luminal contents (Gräns & Olsson, 2024). There are also movements that occur during the fasted state called migrating motor complex (MMC) that acts as a housekeeping activity to remove waste and prevent bacterial overgrowth (Kumral & Zfass, 2018; Nieuwenhuijs et al, 1998). It is crucial that these occur at the correct speed and frequency. If transport for example occurred too quickly it could lead to malnutrition (Olsson et al, 2025).

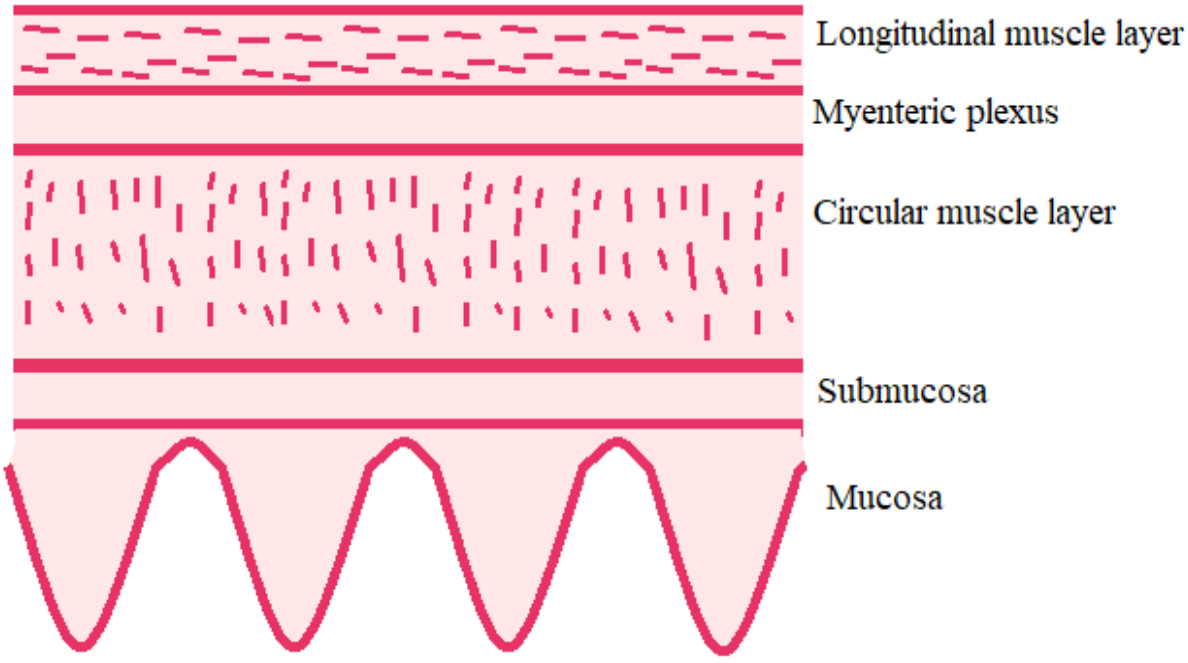

Figure 1 . Overview of the layers in the gut wall showing the longitudinal and circular muscle layers. The mucosa borders the lumen where the food is transported.

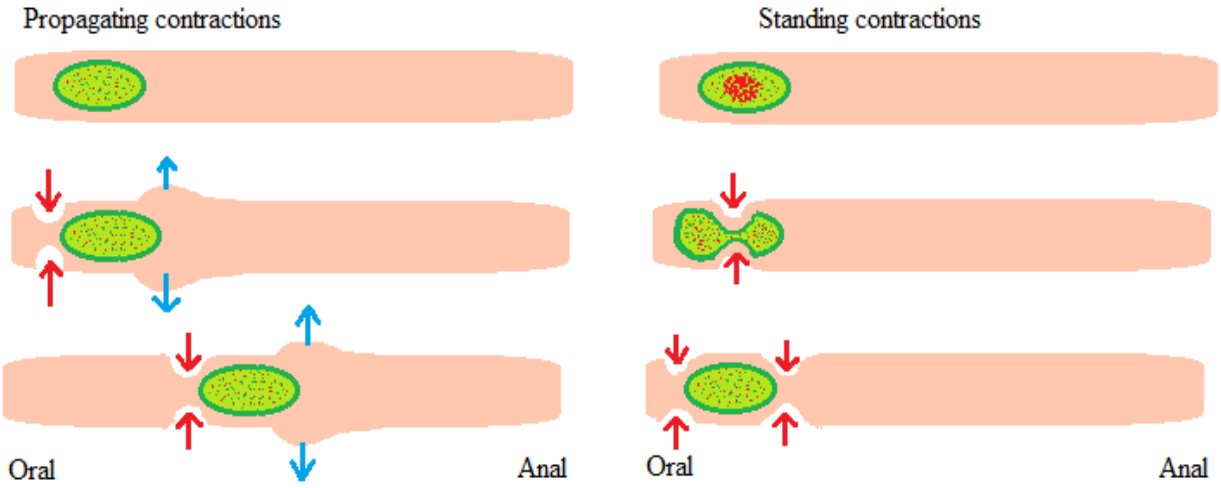

Figure 2. The main movement patterns in the gut are non-propagating standing contractions that mixes food contents, and propagating peristalsis that transports food from the mouth towards the anus.

The control of gastrointestinal movements

Contractions are caused by depolarizations that are strong enough to create action potentials in the membrane of smooth muscle cells. Additionally, there is endogenous activity in smooth muscles called slow waves that are low-amplitude depolarizations. The added stimuli from nerves at the peaks of the slow wave lead to action-potentials (Sanders et al, 2012; 2019).

The smooth muscles are regulated by excitatory and inhibitory neurons stemming from the autonomous nervous system that are located either intrinsically or extrinsically. The enteric nervous system plays the biggest role. It is the part of the autonomic nervous system situated within the gut wall and is able to act independently of neural input from the central nervous system (Spencer & Hu, 2020). It is made up of a large number of neurons (10^7 to 10^8) which are interconnected and innervate the muscles, blood vessels and secretory epithelium in the gut (Furness & Costa, 2006). The majority of nerve bodies are found in the myenteric plexus (Fig 1) situated between the longitudinal and circular muscle layers (Hansen, 2003). Extrinsic innervation from the autonomous nervous system acts directly on the enteric nerves and mainly plays a role in coordinating activity over larger distances (Olsson & Holmgren, 2011).

There are several different neurotransmitters with either excitatory effects such as acetylcholine and tachykinins, or inhibitory for example VIP and nitric oxide (Olsson, 2024a). Acetylcholine is one of the major excitatory neurotransmitters for the enteric nervous system as well as the parasympathetic nervous system (Olsson, 2024; Nilsson 2012). Acetylcholine released from the enteric nervous system acts on muscarinic receptors of smooth muscle cells (Olsson & Holmgren 2001). To study the effects of cholinergic activity, carbachol is commonly used to stimulate smooth muscle activity as it is not broken down by acetylcholinesterase (Gräns et al, 2013; Lester, 1977; Parekh & Brading, 1991).

Interstitial cells of Cajal

Interstitial cells of Cajal are another type of cells involved in regulating gut motility. They are found within the gut wall and are coupled to each other via gap-junctions to form networks along the digestive tract (Sanders, 2019). There are several types of ICC that are characterized by their form, function and location along the gut (Komuro, 2006).

The most well studied subtype, often called ICC-MY is located around the myenteric plexus in most parts of the gastrointestinal tract (Blair et al, 2014). They generate rhythmic slow waves (as mentioned earlier) that act as pacemakers. The slow-waves are generated by spontaneous opening of an ion channel called anoctamin-1 (Ano1) which is a Ca^{2+}-activated Cl^{-}-channel (Sanders et al, 2023).

ICC-IM are located in close proximity to motor neurons within the muscle layers and are thought to be involved in mediating neurotransmission (Burns et al, 1996). This is achieved by these ICC expressing receptors for both excitatory (such as acetylcholine) and inhibitory neurotransmitters. The signal is then passed onto smooth muscle cells via gap junctions (Blair et al, 2014; Iino & Horiguchi, 2006).

In addition, there are other types of ICC such as ICC-DMP which are also involved in neurotransmission and can be found in the circular muscle layer of the small intestine (Komuro et al, 1996), ICC-SMP which are found in the submucosal plexus of the stomach and ICC-SM which are found in the submucosa of the colon (Horiguchi et al, 2001; Berezin et al, 1988).

ICC have been identified in several species of fish (Andersson, 2024; Ball et al, 2012; Brijs, 2017; Uyttebroek et al, 2013). In the study by Brijs et al (2017) they used benzbromarone (a common inhibiting agent of ICC) to study the role of ICC in shorthorn sculpin (*Myoxocephalus scorpius*) and they found that blocking ICC-activity, nearly all contractile activity was stopped in the proximal intestine, suggesting that ICC may have a more encompassing role in fish than in mammals.

The effects of temperature

Climate change is an imminent issue for large number of reasons. In the case of ectotherms such as fish, their internal body temperature is determined by the surrounding environment and an increase in temperature threatens their capability to deal with heat stress and physiological functions (Jutfelt et al, 2024). Most fish tend to live in species-specific temperature ranges that are close to their thermal performance optimum (Volkoff & Rønnestad, 2020).

When it comes to temperature effects on gut motility a study showed that gut blood flow and contraction frequency increases with higher temperatures which may speed up transport of food in the gut (Gräns et al, 2013). An earlier study also suggests that increased temperatures in rainbow trouts lead to a larger basal energy cost as well as a lower cardiovascular capacity (Gräns et al, 2009). The correlation between transit speed and digestive efficiency is not a vastly studied subject and there is evidence that uptake can differ between nutrients at different temperatures (Pafilis et al, 2007). As to whether differences in transit time lead to decreased performance in overall gut function the answers are complicated. While there are some upsides for improved breakdown and uptake of some nutrients at higher temperatures, a shorter transit time might cause a mismatch between them and lead to malnutrition (Olsson et al, 2025).

Aims

The aim of this study was to examine the effects of temperature on the different control pathways of gut motility. More specifically, I aimed to answer these questions:

- How does an increase in temperature affect cholinergic response?
- How does an increase in temperature affect pacemaker ICC activity?

Furthermore, I wanted to examine to which extent cholinergic signaling is mediated through ICC and how it may be affected by increased temperature.

Methods

In vitro smooth muscle preparation

Rainbow trout (n = 26; 116 – 340 g) collected from Vänneåns Fiskeodling AB (Knäred, Sweden). They were kept at 10°C with a photoperiod of 12 hours light and 12 hours dark. Feeding occurred 1-3 times per week. Before starting any procedure, the fish was euthanized by a sharp blow to the head according to the permit number: 5.8.18-10907/2020. The cardiac stomach as well as proximal intestine were dissected and cut into longitudinally. The approximate sizes of the individual strip preparations were 10 x 2 mm.

The individual preparations (2 or 4 from each region) were placed in organ baths filled with 10 ml Ringer's solution (in mM: 140 NaCl, 2.5 KCl, 1.5 $CaCl_2$, 0.8 $MgSO_4$, 15 $NaHCO_3$, 1.0 KH_2PO_4, 5 Hepes buffer, 10 glucose, pH = 7.8) and bubbled with air. Temperature was controlled by a circulating water bath with an adjustable thermostat. The developed force was recorded by force transducers hooked onto the tissue and stored on a pc via a Powerlab 16/30 as Labchart files (Version8.1.30) provided by ADInstruments.

After being subjected to an initial force input of 10 mN, the tissue were left to stabilize for circa 45 minutes during which the tissue were washed with fresh Ringer's solution at least twice.

The effects of temperature on cholinergic response

Concentration-response curves were performed by adding increasing concentrations of carbachol (in molar 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}) to the organ baths at 10°C. The time between each addition was at least 4 minutes or until response had reached a plateau. After the response to the final concentration had been recorded, the tissue were washed three times with ringer and left to re-stabilize for ca 45 min before repeating the curve at 15°C and 20°C. Controls were run alongside the treatments where the concentration-curves were repeated without changing temperature. Tissue were run in duplicates and results were pooled if the response to concentrations varied substantially or selected as singular if response was equal. Tissue showing no response to carbachol were excluded.

The effects of ICC on endogenous activity at different temperatures

A single dose of 50 µM benzbromarone was added to the organ baths when the tissue showed stable activity for at least 20 minutes (either rhythmic contractions or stable basal tone). The activity was then recorded for circa 20 minutes. Controls were run at the same temperature without the addition of benzbromarone. Half of the preparations from each fish were run at 10°C and the others at 15°C.

The effect of cholinergic stimulation mediated by ICC at different temperatures

An initial concentration-response curve was performed at either 10°C or 15°C (same concentration and stabilization times as before). After washout and recovery, and the tissue had been stable for 20 minutes, 50 µM benzbromarone was added to the organ baths whereafter a

second concentration-response curve was performed after circa 20 minutes. Controls for this procedure were carried out by performing the two concentration-response curves without the addition of benzbromarone.

Data analysis

The recorded data were used to calculate the developed mean force using Labchart 8 and analyzed in GraphPad Prism (Version 10.4.2).

The concentration-response curves were calculated using the mean response during 180 seconds before each subsequent addition of carbachol (i.e. initial point was calculated during 180s before 10^{-8}). Using the "sigmoidal-dose response (variable slope)" package from GraphPad Prism, curve fits were applied.

Data for cholinergic response was presented as both the developed mean force in millinewtons at each concentration, and the sensitivity to carbachol with normalized response where the baseline and peak of each curve were defined as 0% and 100% respectively in order to calculate the EC_{50} (the concentration where 50% of response was attained).

Comparisons in the first experiment were made using one-way multiple comparisons ANOVA to compare the parameters between the three temperatures. In the event of a significant difference in the controls, additional unpaired t-tests were performed to compare groups within its corresponding control (i.e. 15°C compared with step 2 in control).

Comparisons in the third experiment were made using unpaired t-test between the first and second curve for each group. A categorical multiple regression was also conducted on the treatments to compare effects of temperature or any interaction between the addition of benzbromarone and temperature. In the event of a significant difference in the controls, unpaired t-tests were applied to the second curve of each corresponding temperature.

To estimate the effect of benzbromarone on basal activity, the mean force developed during 500 seconds before the addition of benzbromarone as well as when activity had been stable for 20 minutes after addition were calculated. The effect of benzbromarone was expressed as percentage of initial force.

Comparisons in the second experiment were done using categorical multiple regression by using temperature and treatment group as factors to estimate the effects of benzbromarone on the retained base line. The same thing was then repeated with all groups for each organ to investigate a possible interaction between the addition of benzbromarone and temperature.

Results

The effect of temperature on cholinergic response

Both cardiac stomach as well as proximal intestine showed concentration-dependent contractions in response to carbachol (See Fig 3-4). $LogEC_{50}$ for most preparations were between -6 and -5. While there weren't many spontaneous contractions in most preparations nor any direct effect of either carbachol or temperature on observed spontaneous contractions, increased concentrations led to an increase in tonal contractions with some spontaneous contractions occurring within the curves.

The maximal mean force developed for the stomachs decreased at increased temperatures (Fig 3A). From 10.85 ±1.12 down to 6.04 ± 0.82 ($p = 0{,}007$) and 6.07 ± 0.85 ($p = 0{,}005$) for 15°C and 20°C respectively. There was no difference between 15°C and 20°C. The controls showed no

difference between either time step which indicated that the maximum contractility didn't deteriorate over the same time period (Fig 3B). The attained $LogEC_{50}$ showed no significant difference either between the temperatures or the controls (Fig 3C, D).

The developed force for the intestines didn't vary with temperature (Fig 4A). The developed force at the highest concentration was calculated to 13.06 ± 1.35 mN at 10°C, 11.31 ± 1.40 at 15°C (p = 0.55), and 8.68 ± 1.07 at 20°C (p = 0.07).

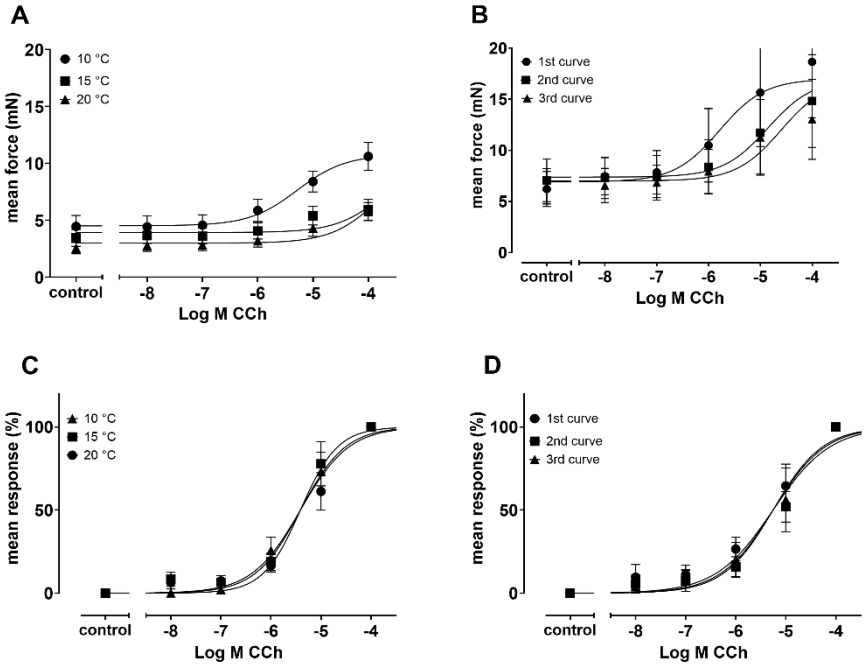

Figure 3. Carbachol-induced sigmoid concentration-response curves for longitudinal preparations of cardiac stomachs from rainbow trout (Oncorhynchus mykiss). A&C (n = 12) are treated at increased temperatures. B&D (n = 5) are repeated at 10 °C. Curves A and B show the measured developed force at each concentration. While C&D are normalized to % of maximum response. Values are mean ± SEM.

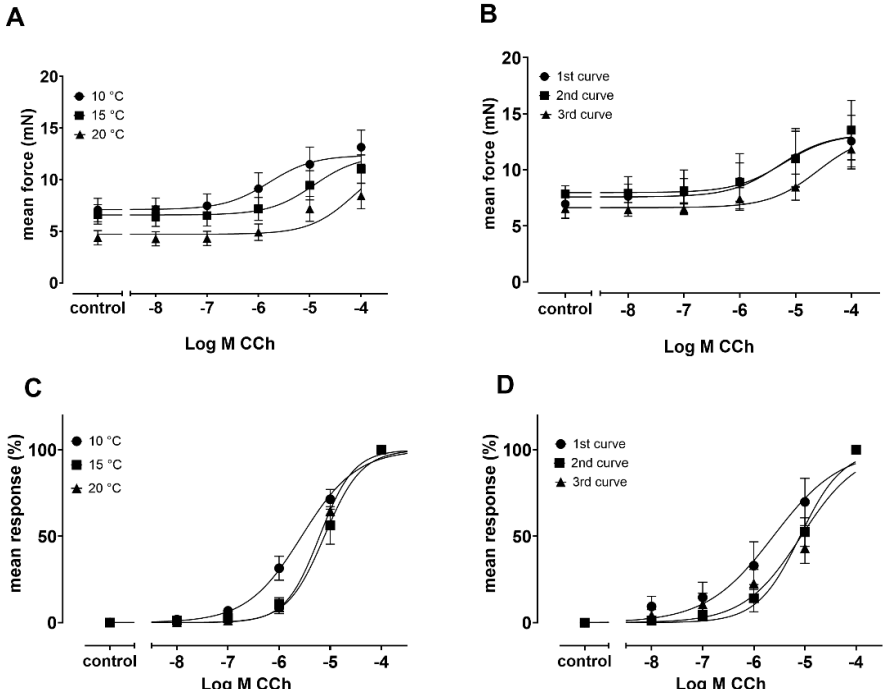

Figure 4. Carbachol-induced sigmoid concentration-response curves for longitudinal preparations of proximal intestines from rainbow trout (Oncorhynchus mykiss). A&C (n = 9) are treated at increased temperatures. B&D (n = 5) are repeated at 10 °C. Curves A&B show the developed response at each concentration while curves C&D are normalized to % of maximum response. Values are mean ± SEM.

The controls similarly didn't differ at the highest concentration varying from 12.51 ± 2.06, 14.03 ± 2.29 and 13.01 ± 1.97 (p = 0.9495 and 0.9719) for the different timesteps (Fig 4B).
The $LogEC_{50}$ values increased with higher temperatures (Fig 4C) from -5.56 ± 0.07 to -5.11 ± 0.07 and -5.21 ± 0.05, However the same thing occurred with the controls (Fig 4D). $LogEC_{50}$ increased from -5.62 ± 0.17 to -5.10 ± 0.07 and -5.07 ± 0.12 (p = <0.0001 and 0,0003) for the respective time steps. There was no difference between the temperature and its respective control step.

The effects of temperature on pacemaker ICC activity

The addition of benzbromarone yielded varied responses. In most cases the baseline and any spontaneous activity decreased while a few increasde in baseline contractions and amplitude of spontaneous contractions.

On average the control stomachs retained 87 ± 8% of baseline force at 10°C and when benzbromarone was added, the baseline was lower at 63 ± 9% (p = 0.02). The controls at 15°C had a baseline of 106 ± 10% and when benzbromarone was added (Fig 5A), it decreased to 75 ± 12% (p = 0.02). When comparing the retained baseline of the treatments at different temperatures there was no difference nor any interaction between the addition of benzbromarone and temperature. In total, 24 out of the 31 stomachs showed a gradual decrease over time.

The control intestines retained 95 ± 5 % of the original baseline at 10 °C, the addition of benzbromarone had no effect with a calculated baseline of 92 ± 7% (p = 0.78). at 15°C, the controls had a baseline of 85 ± 4%. The addition of benzbromarone (Fig 5B) led to a higher baseline at 98 ± 5% (p = 0.04). There was no effect of temperature or interaction. Like the stomachs there was a gradual decrease in 22 out of the total 31 intestines over time.

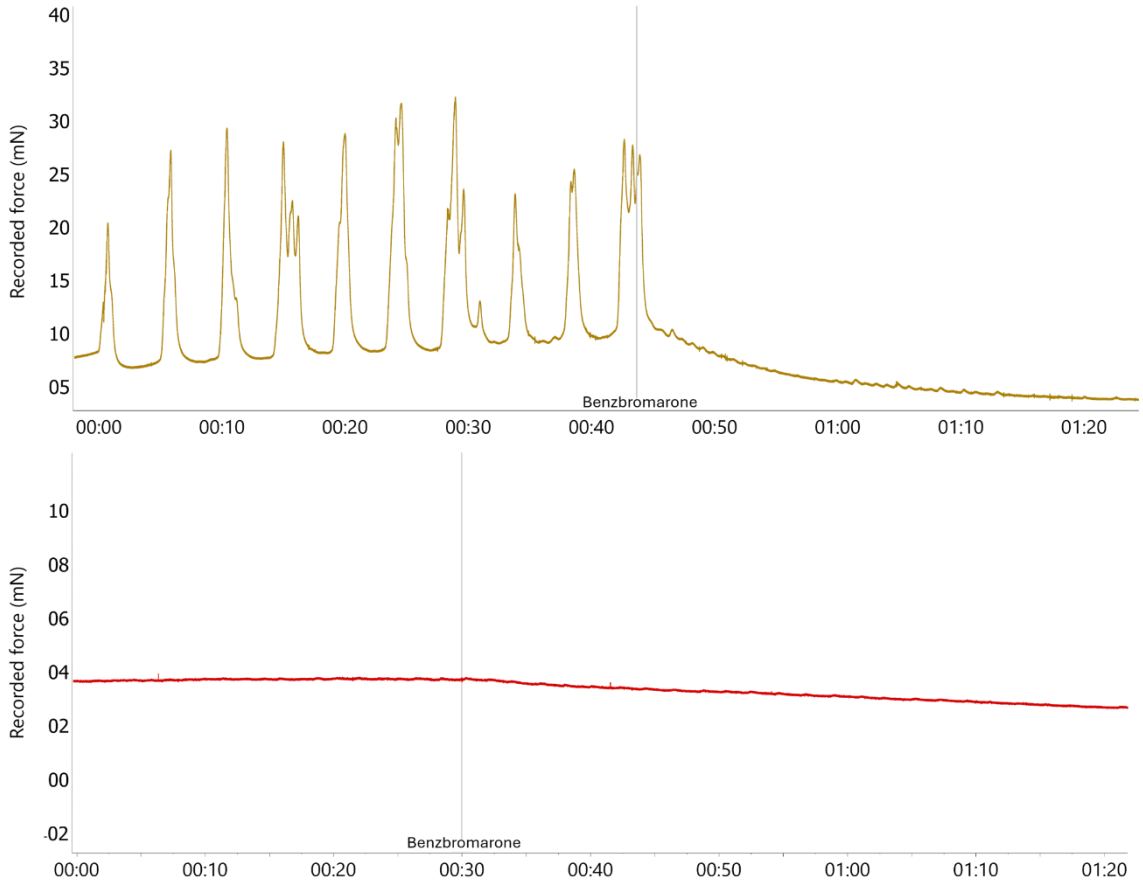

Figure 5. Original tracings of longitudinal smooth muscle from rainbow trout (Oncorhynchus mykiss) treated with benzbromarone. Figure A is a cardiac stomach at 10°C and Figure B is a proximal intestine at 15°C. Both organs show a decrease of baseline contractions in response to benzbromarone. Time starts at 00:00 when prepatations have been stabilizing for at least 45 minutes.

How is cholinergic activity mediated by ICC activity at different temperatures

Addition of benzbromarone has similar effects on baseline force as in previous experiments. However, there were also observed effects on the controls as well (Fig 6B, D and Fig 9B, D).

The maximal mean contractile response for the stomachs at 10°C (Fig 6A) decreased from 10.95 ± 1.02 to 6.49 ± 1.45 ($p = 0.008$) after the addition of benzbromarone whereas the controls decreased similarly from 15.06 ± 1.65 to 9.60 ± 2.34 ($p = 0.03$). At 15°C the max response decreased from 13.32 ± 1.23 to 6.24 ± 1.73 ($p = 0.001$) after addition of benzbromarone, and controls decreased from 13.11 ± 1.08 to 9.64 ± 1.53 ($p = 0.04$). When all stomachs were compared by multiple linear regression there was a small decrease in the stomachs that were treated with benzbromarone with an overall mean of 6.49 ± 1.60 compared to the global mean of 10,95 ± 1,128 ($p = 0.009$), but there was no difference between temperatures nor any interaction. The $LogEC_{50}$ also increased in all groups. At 10°C the stomachs increased from -6.69 ± 0.37 to -5.32 ± 0.10 ($p = 0.0002$) after the addition of benzbromarone, and controls increased from -6.03 ± 0.09 to -5.34 ± 0.05 ($p = 0.0001$). At 15°C the stomachs increased from -6.35 ± 0.11 to -5.52 ± 0.11 ($p = <0.0001$) after the addition of benzbromarone while the controls increased from -6.52 ± 0.16 to -5.99 ± 0.10 ($p = 0.01$). Comparisons between the $LogEC_{50}$ of the second curves at 15°C showed a higher $LogEC_{50}$ when benzbromarone was added than the controls ($p = 0.003$). There was no difference in LogEC50 between the second curve of control and treatment at 10°C ($p = 0.86$).

The maximal mean developed force for the intestines showed no difference when benzbromarone was added. At 10°C the measured response was 15.22 ± 2.66 and 13.47 ± 1.68 ($p = 0.61$) before and after benzbromarone, while the controls measured at 15.69 ± 2.60 and 18.47 ± 3.68 ($p = 0.48$) for the first and second curve respectively. At 15 °C the maximal mean force was calculated to 11.89 ± 1.82 and 9.39 ± 2.57 ($p = 0.35$) before and after benzbromarone was added, and the controls were calculated to 9.87 ± 1.62 and 9.16 ± 2.29 ($p = 0.77$) for the first and second curve.

The $LogEC_{50}$ for the intestines showed varied results (Fig 9). There was an increase in $LogEC_{50}$ in both the treatment and the control at 10°C. The treatment increased from -5.76 ± 0.10 to -5.25 ± 0.09 ($p = 0.0006$) when benzbromarone was added, and the controls increased from -5.97 ± 0.13 to -5.42 ± 0.09 ($p = 0.001$). At 15 °C there was an increase in the controls from. -5.54 ± 0.14 to -5.06 ± 0.16 ($p = 0.04$), while there was no difference when benzbromarone was added. $LogEC_{50}$ was calculated to -5,48 ± 0,15 and -5,40 ± 0,24 ($p = 0,77$) before and after benzbromarone. No difference was found when comparing treatments with the second curve of the controls.

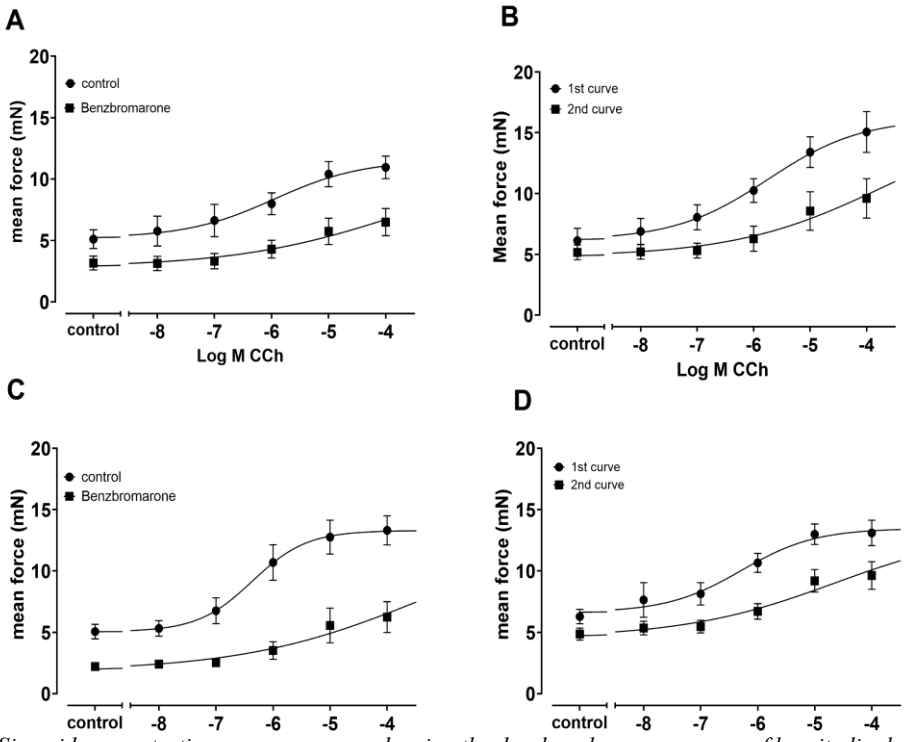

Figure 6. Sigmoid concentration-response curve showing the developed mean response of longitudinal preparation of cardiac stomachs from rainbow trout (Oncorhynchus mykiss) stimulated by benzbromarone (A&C, n=8) or repeated without benzbromarone (C, n=8 & D, n=7). A&B were treated at 10 °C while C&D were treated at 15 °C. Values are mean ± SEM. All curves indicate a significant difference from curve 1 to curve 2.

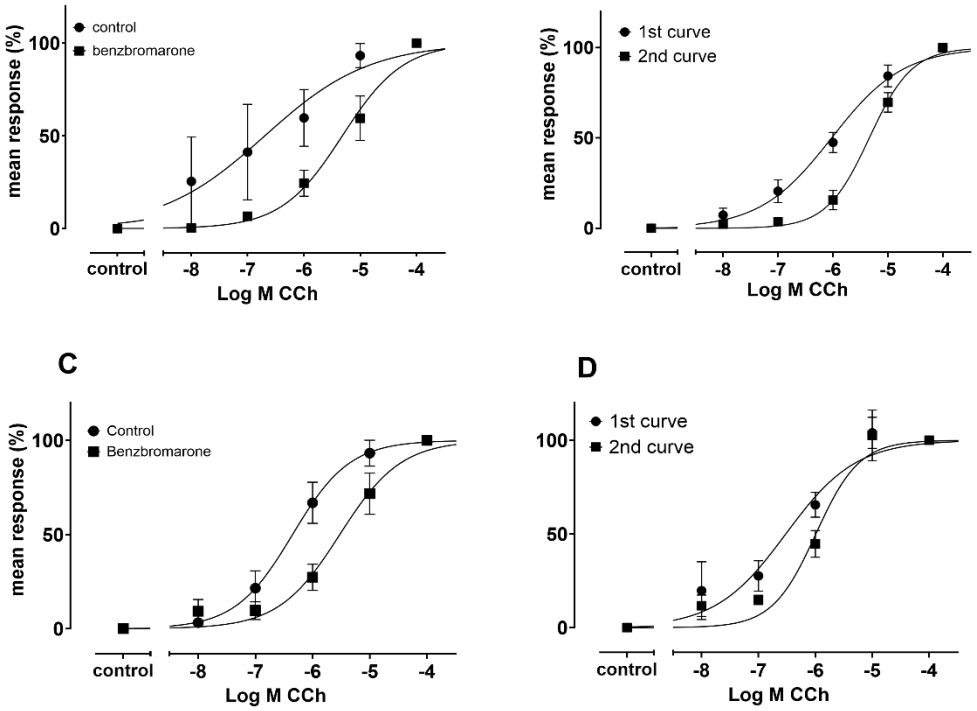

Figure 7. Sigmoid concentration-response curves of longitudinal preparations of cardiac stomachs from rainbow trout (Oncorhynchus mykiss) at either 10 °C (A&C) or 15 °C (B&D). the 1st curve in A&C (n = 8) are induced by carbachol and the 2nd curves are carbachol-induced and treated with benzbromarone. Curves B (n = 8) D (n = 7) are repeated with carbachol without the addition of benzbromarone. Values are mean ± SEM.

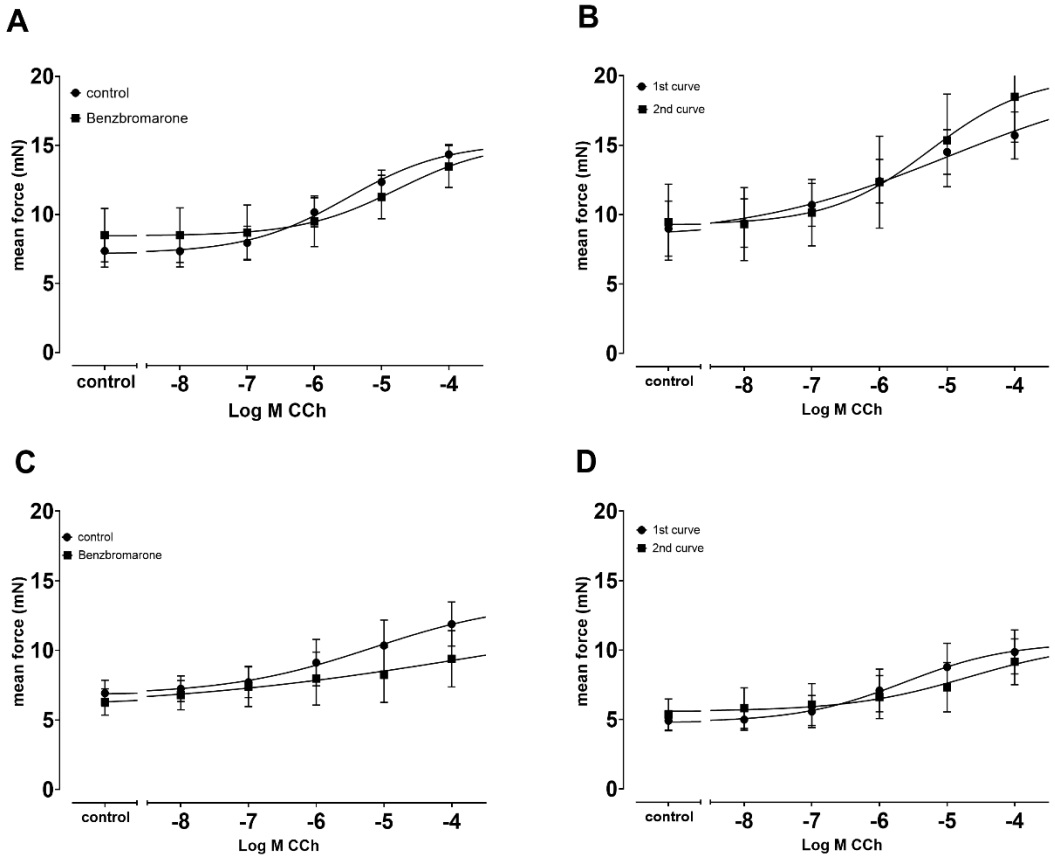

Figure 8. sigmoid concentration response curves showing the mean developed response for longitudinal preparations of proximal intestines of rainbow trout (Oncorhynchus mykiss) treated either with (A&C) or without (B&D) benzbromarone at either 10°C (A&B) or 15°C (C&D). Values are mean ± SEM.

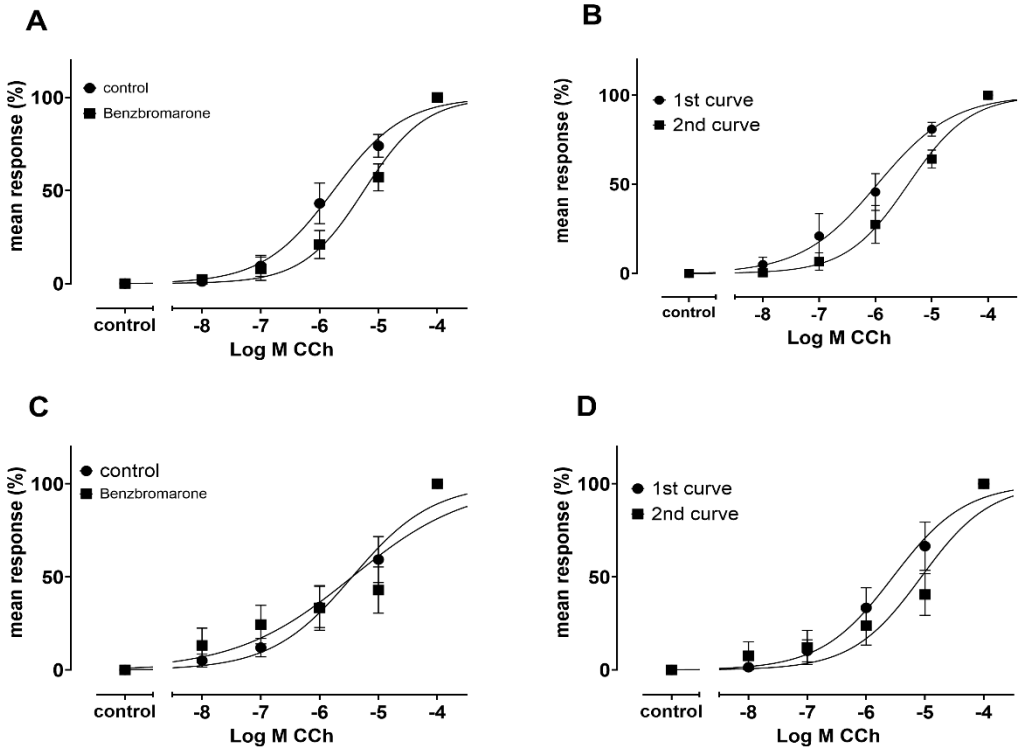

Figure 9. sigmoid concentration response curves showing the normalized responses for longitudinal preparations of proximal intestines of rainbow trout (Oncorhynchus mykiss) treated either with (A&C) or without (B&D) benzbromarone at either 10°C (A&B) or 15°C (C&D). Values are mean ± SEM.

Discussion

In this study I studied how different control pathways of gut motility react to acute temperature changes. For most of the experiments performed there was a decrease in mean developed force in response to carbachol at higher temperatures, while EC_{50} showed varied responses. What is interesting in this case is how time seems to be a consistent factor in most procedures.

Cholinergic signaling at higher temperatures

It is clear that contractile force decreases with temperature in the cardiac stomach. This is consistent with earlier studies from e.g Gräns et al (2013) where they observed a similar decrease at 14°C compared to 4°C and 9°C in shorthorn sculpin. In that study however, they also observed a decrease in sensitivity to carbachol (lowest $LogEC_{50}$ presented as -7.1 ± 0.2 at 9°C). Why this didn't occur for me might be due to errors in the experimental methods. One possible reason could be that each concentration of carbachol was added at too fast intervals so that each response didn't reach a plateu, potentially shifting most responses to the right of the curve and also affecting the EC_{50}.

It is also interesting that there was no significant decrease in developed force of the proximal intestines. It is important to note however that the net decrease in the intestines is very similar to the stomachs, so in trying to answer this I performed a multiple regression based on the absolute developed force at the different temperatures (i.e. force at 10^{-4} minus baseline force). By doing this I found that there was a significant decrease in absolute developed force. At 10°C the absolute developed force (in mN) was 7.24 ± 0.84 and decreased to 4.83 ± 1.19 ($p = 0.05$) and 4.75 ± 1.19 ($p = 0.05$) for 15°C and 20°C respectively. What this could indicate is that the baseline might not have recovered completely before a new concentration-response curve was performed and that I might have found an effect of temperature if I had waited for the baseline to stabilize more. For standardizations' sake I also tried this on the controls of the intestines, and didn't see any difference in absolute developed force. This might also explain why there are differences in the EC_{50} in both controls and treatments for the intestines. If carbachol was added too early in the second and third procedures, the preparations might not have had time to recover from the earlier stimulation and therefore respond later than the first curve.

To connect this to overall gut motility, it is difficult to assess whether a decrease in contraction force would indicate an increase or a decrease in transport speed. Higher temperatures tend to increase contraction frequency (Olsson, 2025) but we have as of today no knowledge if that is what causes decreased force and more so, how the motility patterns are affected by this. To understand how decreased force affects motility patterns, we would need to investigate further into the patterns and how they are affected when contraction is decreased.

Effect of ICC on passive gut motility at higher temperatures

The stomachs showed a response to benzbromarone with a very strong decrease in retained baseline, indicating that ICC are involved in maintaining the basal tone in the stomach. Why no effect of temperature was found is difficult to explain but considering that increased temperatures tend to increase contraction frequencies of smooth muscles (Olsson, 2025) and the fact that I only analyzed the response as retained baseline, any effects of temperature are likely to have been missed using my methods. Focusing on the direct effects on spontaneous frequency and amplitude might have been more appropriate in this case.

The presence and role of ICC in the proximal intestine has been described by Brijs et al (2017) where they observed a well distributed Ano1-network in this region that also responded to benzbromarone by abolishing most contractile activity in shorthorn sculpin. This is also further supported in rainbow trouts by an earlier master's thesis by Julien (2020) where Ano-1 positive cells were detected although the response to benzbromarone was varied. The response in the proximal intestine in this study are also varied. Adding benzbromarone led to a higher baseline than in the controls at 15°C and no difference at 10°C. One possible reason could explained by

ICC containing receptors for both inhibitory and excitatory neurotransmitters (Blair et al, 2014), meaning that in this case benzbromarone mainly blocked inhibitory pathways which potentially could lead to a higher basal tone in some preparations.

The effect of ICC on cholinergic stimulation and at higher temperatures

Making any interpretations from this experiment is very complicated considering that there were decreased responses in both the controls and treatments for the cardiac stomachs. In addition to this it also contradicts the results in experiment 1 considering curves 1 and 2 for the controls at 10°C are essentially the same setup only with a significant reduction in mean force and sensitivity (14.8 vs 9.6 mN for stomachs in experiment 1 and 3 respectively). Why this occurred might be due to carbachol curves being performed too quickly, without giving the tissue time to recover between curves.

The multiple categorical regression for the stomachs does however show that the preparations where benzbromarone was added show a slightly higher decrease than controls, so while ICC seem to have some effect on cholinergic response, I don't want to make any conclusions on the extent that they mediate the pathways when there are clearly artifacts produced by the experiment.

The lack of a response for the mean values of the intestines isn't too unexpected when compared with the results in experiment 2 which might further suggest that the ICC that were affected, primarily contain inhibitory receptors for basal activity. When looking at the significant increase in the EC_{50} at 15°C a likely explanation is the low sample size for this group (n = 4) which then might be caused by an outlier to affect the results.

The fact that there was no effect of temperature in either the cardiac stomach or proximal intestine when comparing the treatments is also quite interesting. It has been observed before that at the very least slow-wave activity is sensitive to temperature in mammalian cells (Zheng et al, 2014; Huizinga et al, 2002) so it's possible that using muscle contractions as a measurement isn't precise enough and we should instead look further into the transmission between nerves and ICC at increased temperatures.

Implications for future studies

Hopefully the results of this project shows that there are benefits of optimizing the experimental methods. If the cholinergic response was affected by procedures occurring at too small intervals, then it would be interesting to investigate the required resting time to reach maximal contraction.

The fact that the maximal response in most preparations was at M 10^{-4} in these trouts. It would be interesting to see if the response increases at higher concentrations of carbachol

The effects of ICC both on endogenous and cholinergic activity would need more samples that don't decrease in activity over time to unmask the decrease caused only by benzbromarone and investigating the effects on spontaneous frequency and amplitude could potentially reveal effects of temperature in that aspect. Increasing the temperature range to see if there is a trend in activity for the different regions of the gut could also be of interest.

Conclusions

Temperature mainly seems to affect enteric innervation of smooth muscle activity while the effects on ICC are still unclear. ICC are shown to be involved in slow-wave endogenous contractility in the cardiac stomach of rainbow trout while the proximal intestine provides a more varied answer. This study suggests that using this method provides some degeneration in activity in both sensitivity to stimulation and developed force caused by experimental methods over time which need to be accounted for to be able to predict how gut motility could be impacted in future climate scenarios.

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