Effect of body size on *Manduca sexta* midgut gene expression

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Abstract

The scaling exponent for metabolic rate in *Manduca sexta* is greater than expected based on isometric surface area scaling. We examined whether midgut genes that code for proteins involved in nutrient absorption and digestion are differentially expressed in fifth versus fourth instar larvae. RNA was isolated from middle and posterior midgut and reverse transcribed to cDNA. Real-time PCR was used to quantify expression of two genes, aminopeptidase N (msAPN3) and potassium amino acid cotransporter (KAAT), by the relative quantification method using 18s ribosomal RNA as an internal control. In posterior midgut, KAAT expression was 1.6-2.6 fold higher while APN was 1.4 fold lower in fifth compared to fourth instar larvae. KAAT was more highly expressed in middle compared to posterior midgut, whereas APN was more abundant in posterior midgut. No significant variation of KAAT expression was found between days within the 5th instar. In summary, midgut region and instar affect KAAT and APN expression.

Introduction

For isometric growth, surface area increases as the square of linear dimensions while volume scales as their cube. If metabolic rate is proportional to surface area, the ratio of surface area to volume predicts a scaling exponent of ⅔ (Rubner 1883). However, a scaling exponent of ¾ emerges from comparisons of metabolic rate across species (Kleiber 1932). What biological factors cause an organism's metabolism to scale at this faster rate?

Manduca sexta grows 10,000-fold (1mg egg to a fully developed 5th instar of more than 10g) in about 18 days and therefore makes a good model for studying scaling (Goodman *et al.* 1985). The midgut of *M.sexta* is responsible for the digestion and absorption of nutrients and is regulated by the distribution and density of digestive proteins and transporters on the gut epithelium.

We studied two midgut proteins, aminopeptidase N (msAPN3) and K+-amino acid cotransporter (KAAT), which work in series to digest and uptake amino acids (Wang *et al. 2005;* Castagna *et al.* 1998*)*. Aminopeptidase N degrades the amino terminus of peptides, cleaving them into amino acids. KAAT drives the secondary active transport of amino acids into epithelial cells by coupling transport to an inward K+ electrochemical gradient.

Our hypothesis was that midgut densities of KAAT and APN increase as body size increases to account for the higher ¾ metabolic scaling exponent. Since KAAT and APN work in series, we expected similar expression trends for the genes encoding these proteins.

Methods

Manduca sexta (Carolina Biological) were raised on an artificial wheat germ diet at 27º C with a 16L:8D photoperiod. Animals were weighed daily and new wheat germ was added every 2 days. Midgut tissues were isolated from 4th and 5th instar animals on the subsequent day following a molt. Midgut tissue was separated into anterior, middle and posterior sections and frozen at -80ºC. Total RNA was isolated using the RNA-stat 60 reagent (Tel-Test), and quantified with nanospectroscopy. Genomic DNA was removed from the total RNA samples using the TURBO DNA-free kit (Ambion). One µg of DNA-free total RNA was reverse transcribed to cDNA with random hexamers using the Taqman Reverse Transcription kit (Applied Biosystems). Primers were designed on Primer Express software (Applied Biosystems), and were synthesized (Operon) and optimized. Quantitative real-time PCR reactions (SYBR Green, Applied Biosystems) were performed in triplicate on a 96-well microtiter plate using the relative quantification ΔΔCt method on an ABI prism 7500 sequence detection system (Figure 1).

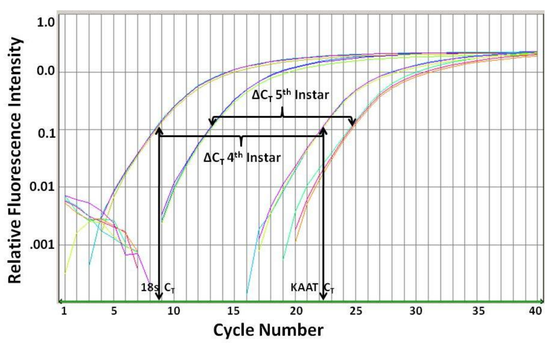

Figure 1: Fluorescence traces from Real Time-PCR amplification of KAAT in 4th and 5th instar in posterior midgut. Triplicate reactions were run for each primer/cDNA combination. Cycle-Threshold (C_T) values represent the cycle number at which the relative fluorescence of a sample crosses threshold and becomes detectable above background fluorescence. The ΔC_T is calculated by subtracting the mean C_T value of the endogenous control (18s) from the mean C_T value target gene (KAAT). The $\Delta\Delta C_T$ is the difference between 4th and 5th ΔC_T values. The RQ (fold change) = $2^{(-\Delta\Delta CT)}$

Results

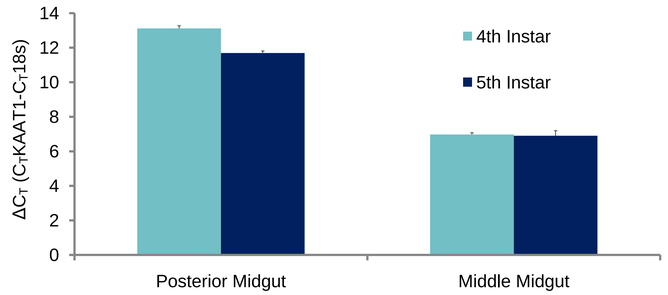

Figure 2: Comparison of KAAT expression between 4th and 5th instars using KAAT1 primers in posterior and middle midgut. The 5th instar posterior midgut had 2.68-fold higher expression than 4th instar. In contrast, the 5th instar middle midgut only had 1.05-fold higher expression compared to 4th instar (Two-way ANOVA, p_{instar} =0.005, p_{tissue}= 0.000, $p_{tissue*instar}$=0.010). Body weights (g) were taken at time of dissection and were as follows (4^{th}_{PM}=0.42g± 0.03, n=3; 5^{th}_{PM}=6.8± 1.1, n=9; 4^{th}_{MM}=0.46± 0.1, n=5; 5^{th}_{MM}=4.7±1.1, n=5)

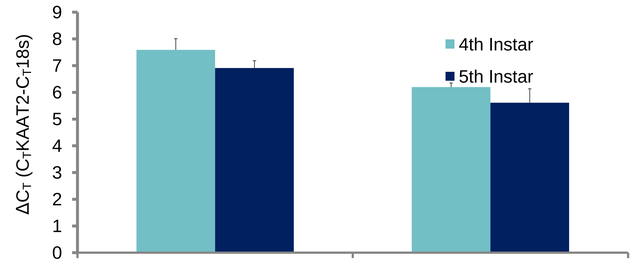

Figure 3: The relationship of KAAT expression between 4th and 5th instars using KAAT2 primers in posterior and middle midgut. The 5th instar posterior midgut had 1.6-fold higher expression than 4th instar. Similarly, the 5th instar middle midgut had 1.5-fold higher expression compared to 4th instar (Two-way ANOVA, p_{instar} = 0.118, p_{tissue}= 0.003, $p_{tissue*instar}$= 0.901). Body weights (g) were taken at time of dissection and were as follows (4^{th}_{PM}=0.48g± 0.08, n=5; 5^{th}_{PM}=3.98± 1.04, n=4; 4^{th}_{MM}=0.48± 0.08, n=5; 5^{th}_{MM}=4.69±1.07, n=5)

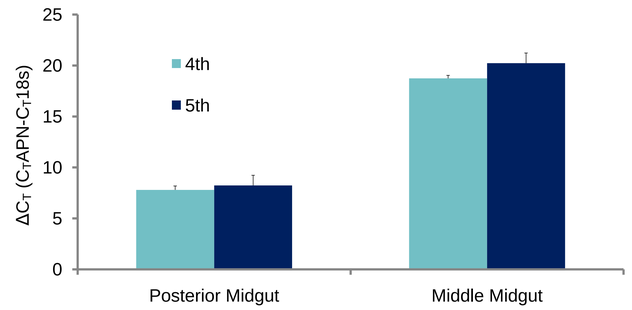

Figure 4: Comparison of APN expression between 4th and 5th instars in posterior and middle midgut. The 5th instar posterior midgut had 1.35-fold lower expression than 4th instar. In comparison, the 5th instar middle midgut had 2.81-fold lower expression compared to 4th instar (Two-way ANOVA, p_{instar} =0.002, p_{tissue}= 0.000, $p_{tissue*instar}$=0.066). Body weights (g) were taken at time of dissection and were as follows (4^{th}_{PM}=0.42g± 0.03, n=3; 5^{th}_{PM}=6.8± 1.1, n=9; 4^{th}_{MM}=0.48± 0.1, n=5; 5^{th}_{MM}=4.7±1.1, n=5)

Results

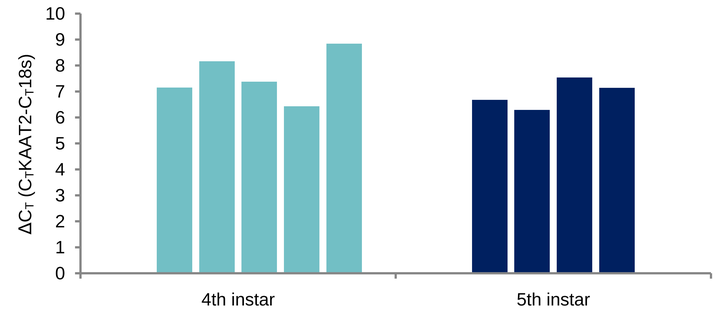

Figure 5: The distribution of ΔC_T values for a RT-PCR comparison between 4th and 5th instar expression of KAAT in posterior midgut. The average ΔC_T of 5th instars had 1.6-fold higher expression compared to 4th instar ($4th\Delta C_T$= 7.6±0.42, n=5; $5th\Delta C_T$=6.9±0.27,n=4).

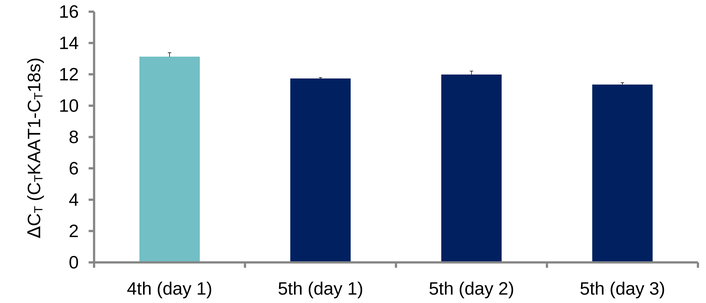

Figure 6: Variation of KAAT expression within 5th instar posterior midgut. Day 1 animals were dissected the day after molting and day 2 and 3 animals were dissected on subsequent days. Body weights (g) were taken at time of dissection and were as follows ($4th_{day1}$=0.42±0.26, $5th_{day1}$=3.0±0.06, $5th_{day2}$=7.0±0.22, $5th_{day3}$=10.4±0.12).

Summary

- The KAAT gene was expressed at higher levels in 5th instar compared to 4th instar animals in the posterior and middle midgut of *Manduca sexta (*Figure 2 and 3*)*. In contrast, APN was more highly expressed in 4th instar animals in both posterior and middle midgut (Figure 4).
- There were higher levels of KAAT expression in the middle midgut tissue compared to posterior midgut tissue (Figure 2 and 3). The expression of APN was reversed, with higher levels of APN in posterior compared to middle midgut (Figure 4).
- Expression of KAAT and APN varies among individuals (Figure 5).
- There was no significant variation in KAAT expression over different days within 5th instar (Figure 6).

References

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Conclusions

The hypothesis that APN and KAAT have similar expression trends is not supported by our data. KAAT follows the prediction that midgut gene expression increases as body size increases, while APN does not. One possibility for this result is that APN is regulated at the translational/protein level. Also, there are several other isoforms of APN which may be differentially expressed based on instar.

Acknowledgements

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