Differential expression and activity of catechol-O-methyl transferase (COMT) in a generalist (Neotoma albigula) and juniper specialist (Neotoma stephensi) woodrat

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A B S T R A C T

Mammalian herbivores, particularly dietary specialists, must have an efficient means to metabolize the high doses of plant secondary compounds they consume. We found previously that Neotoma stephensi, a juniper specialist, upregulated catechol-O-methyl transferase (COMT) mRNA almost seven fold in response to an ecologically relevant diet (70% juniper). To further investigate the relevance of this enzyme with respect to juniper metabolism, we compared the protein expression, activity and kinetics of the two forms of COMT, soluble (S-COMT) and membrane bound (MB-COMT), in the blood, kidneys and liver of N. stephensi on its natural juniper diet to that of N. stephensi fed an experimental diet of 70% juniper as well as a non-toxic control diet under laboratory conditions. In addition, we compared these results to that of Neotoma albigula, a generalist species, in which we consume a diet of 25% juniper in the wild. The specialist consuming juniper under both field and laboratory conditions had increased S-COMT expression and activity in their livers and kidneys, and increased S-COMT activity in their blood compared to the specialist and generalist fed the control diet. The specialist showed expression and activity of S-COMT in their kidneys that was as high as or higher than that in their livers. The generalist had an elevated Vmax for MB-COMT compared to the specialist that resulted in higher activity for MB-COMT than the specialist despite lower expression of MB-COMT in the generalist’s livers and kidneys. This high activity MB-COMT may be in part responsible for differences in the behaviors of the generalist compared to the specialist. We conclude that S-COMT is important in the specialist’s ability to consume high levels of juniper.

1. Introduction

Less than 1% of mammalian herbivores are considered to be dietary specialists, where a single species of plant represents 60% or more of their diet (Freeland, 1991; Shipley et al., 2009). The detoxification limitation hypothesis proposes that dietary specialization is rare in mammalian herbivores because of limitations in their detoxification system in metabolizing large doses of one or a similar suite of plant secondary compounds (PSCs) (Freeland and Janzen, 1974; Marsh et al., 2006). The preponderance of the generalist strategy is thought to be driven by optimizing nutrient needs by incorporating multiple types of plants with different nutrient compositions (Westoby, 1974, 1978) and/or by the physiological requirement of not overloading the detoxification system by alternating the ingestion of multiple types of plants containing PSCs metabolized through different detoxification pathways (Freeland and Janzen, 1974; Torregrossa and Dearing, 2009b).

The mammalian detoxification system is separated into two phases that can work in tandem or independently (Klaassen and Watkins, 2003). Phase I or functionalization involves enzymes that oxidize or reduce their substrates to form reactive metabolites that can then be conjugated by phase II enzymes. Phase II or conjugation involves enzymes that glucuronidate, sulfate, methylate, or conjugate glutathione or other amino acids to their substrates to increase the hydrophilicity of the metabolites for excretion in urine or bile. Phase I is considered to be energetically inexpensive yet physiologically risky as the products of functionalization can often be more reactive than the substrates. Phase II is considered to be safer but more energetically demanding since it requires cofactors that need to be replenished through the diet. The loss of glucuronic acid, a conjugate, can account for 2–9% metabolizable energy intake (Mangione et al., 2004; Sorensen et al., 2005b). Since mammalian herbivores often consume diets low in nutrients that are potentially low in phase II cofactors, but high in PSCs, it has been hypothesized that dietary specialists may rely more heavily on phase I than phase II pathways for metabolism of PSCs (McLean et al., 1993; McLean and Foley, 1997; Boyle et al., 1999; McLean et al., 2001; Dearing et al., 2005a; Sorensen et al., 2005a, 2005b, 2006). While decreasing the use of conjugation may decrease the energetic costs of PSC consumption, it may increase the likelihood of the production of reactive metabolites...
by functionalization enzymes (Ayala and Cutler, 1997; Guengerich, 2006).

The phase II enzyme catechol-O-methyl transferase (COMT; EC 2.1.1.6) may permit herbivores to strike a balance between expensive but safe conjugation and cheap but reactive functionalization. COMT conjugates a methyl group to its substrate that is relatively inexpensive compared to other conjugates e.g., glucuronic acid. COMT is a well-studied enzyme in pharmacological and toxicological research because it has a number of endogenous as well as exogenous catechol containing substrates such as catecholamine, catechol estrogens, flavonoids and tea polyphenolics (Männistö and Kaakkola, 1999; Lautala et al., 2001; Zhu, 2002; O'Leary et al., 2003). COMT also metabolizes endogenously produced catecholamines, which are used as neurotransmitters in the central nervous system (CNS) and as hormones in the peripheral circulation. Thus, COMT plays an essential role in CNS function and behavior, as well as the response of many peripheral organs to the sympathetic nervous system (Männistö and Kaakkola, 1999; Wang et al., 2001; Zhu, 2002). COMT also is an important route for the metabolism of exogenous dietary phenolics such as flavonoids and tea polyphenolics (Zhu et al., 1994; Odely et al., 1997; Bravo, 1998; Piskula and Terao, 1998; Männistö and Kaakkola, 1999; Tsuda et al., 1999; Donovan et al., 2001; Hein et al., 2002; Zhu, 2002; O'Leary et al., 2003; Talavéra et al., 2005; Crozier et al., 2009). Despite COMT's importance in behavior, homeostasis and metabolizing dietary phenolics, its role has not been investigated in wild herbivores.

We chose to examine the role of COMT in a dietary specialist, Neotoma stephensi, for several reasons. Its ability to specialize on one-seeded juniper (Juniperus monosperma) is well documented (Vaughn, 1982) as is the natural products chemistry of this species (Adams et al., 1981, 1983; Adams, 2000; Utsumi et al., 2009; Adams, 2011). Juniper is an evergreen that contains high levels of PSCs (8 1981, 1983; Adams, 2000; Utsumi et al., 2009; Adams, 2011). Juniper is the only known vertebrate to specialize on one-seeded juniper (Juniperus monosperma) in the wild (Vaughn, 1982; Dial, 1988) and also the amount of juniper consumed. We compared the protein expression and activity of COMT in captive Neotoma stephensi (Vaughn, 1982; Dial, 1988) as is the natural products chemistry of this species (Adams et al., 1981, 1983; Adams, 2000; Utsumi et al., 2009; Adams, 2011). Juniperus genus is widespread throughout the Northern Hemisphere and the berries serve as an occasional source of food for many vertebrates, but N. stephensi is the only known vertebrate to specialize on the foliage of the plant (Vaughn, 1982; Dial, 1988; Adams, 2011). To get a global picture of the regulation of the detoxification system in N. stephensi, we previously used microarray technology to compare the expression of hepatic genes of the specialist to that of a sympatric generalist, Neotoma albigula that consumes a maximum of 25% juniper in the wild (Dial, 1988; Skopec et al., 2007). We found large between species differences in gene expression (albigula versus stephensi), as well as large within species differences in gene expression of the specialist on a low juniper diet versus a diet typical of that ingested in nature (Skopec et al., 2007). Of particular interest was the change in gene expression of COMT that was up-regulated almost seven fold in the specialist when on a 70% juniper diet compared to a 25% juniper diet (Skopec et al., 2007). Such induction of enzyme transcripts is indicative of an enzyme's role in detoxification of that substance.

Previous research has not found up or down-regulation of COMT in response to drug or dietary treatments (Männistö and Kaakkola, 1999; Zhu, 2002); thus the results in N. stephensi were novel. To further investigate the role of COMT in detoxification of juniper by the specialist, we compared the protein expression and activity of COMT in captive N. stephensi fed a nontoxic control diet lacking juniper, or a 70% juniper diet to that of wild N. stephensi feeding on a natural diet of juniper. We also compared the protein expression and activity of COMT in the specialist to that of the generalist N. albigula fed a control diet.

2. Materials and methods

2.1. Woodrats

*Neotoma stephensi* (N = 22) were trapped on Woodhouse Mesa, near Wupatki National Park, 45 km northeast of Flagstaff, Arizona, USA (35°30’ N, 111°27’ W). Sufficient numbers of the generalist, *N. albigula*, were not available at the Woodhouse Mesa location during the collection of *N. stephensi*; therefore, we collected *N. albigula* (N = 7) from Castle Valley, UT (38°30’ N, 109°18’ W). We have previously used this population for comparative studies between the specialist and generalist (Dearing et al., 2000). All woodrats were transported to the University of Utah, Department of Biology's Animal Facility. Woodrats were housed in individual cages (48 × 27 × 20 cm) and had ad libitum access to food and water. Environmental conditions were 12:12-h light:dark cycle, 28 °C and 15% humidity. All experimental procedures involving woodrats were approved by the University of Utah’s Institutional Animal Care and Use Committee protocol number 0702015.

2.2. Dietary treatments

*Neotoma stephensi* were separated into three groups, wild, control and 70% juniper. The wild *N. stephensi* (N=9; 2 male, 7 female) were trapped and maintained on fresh juniper foliage supplemented with rabbit chow (Harlan Teklad formula 2031) for a maximum of 48 h before dispatch. The *N. stephensi* in the control and 70% juniper groups were maintained in captivity for more than 3 months on rabbit chow prior to the experiments. The captive groups were fed either the nontoxic control diet (N = 6; 3 male, 3 female) of ground rabbit chow or a 70% juniper diet (N = 7; 4 male, 3 female) that consisted of 70% ground juniper and 30% ground rabbit chow on a dry matter basis. 70% juniper is the minimum amount of juniper consumed by *N. stephensi* in the wild (Vaughn, 1982; Dial, 1988) and also the amount of juniper fed to *N. stephensi* in our microarray study (Skopec et al., 2007). The control diet was fed for a minimum of 5 days. The specialists in the juniper treatment were fed 5% juniper diet for 1 day, 25% juniper diet for 2 days and 70% juniper diet for 5 days to give adequate time for the induction of detoxification enzymes involved in metabolizing the PSCs in juniper (Hollenberg, 2002; Bock and Köhle, 2004; Lamb et al., 2004; Haley et al., 2007; Skopec et al., 2007). The juniper used in 70% juniper diet was *J. monosperma* collected from several trees at the trapping sites and kept frozen at −20 °C until use. The juniper was ground with dry ice in a Waring blender until it passed through a 1 mm screen and again stored at −20 °C in air-tight containers until incorporated into 70% juniper diet. The 70% juniper diet was made daily to minimize volatilization of terpenes.

The *N. albigula* (N = 7; 3 male, 4 female) were maintained in captivity for more than 3 months on rabbit chow prior to the experiment and were fed only the control diet for a minimum of 5 days. *N. albigula* cannot maintain body mass long term on juniper diets of 50% or greater in the lab (McLester et al., 2004; Dearing et al., 2005b; Sorensen et al., 2005b) and consumes around 25% juniper in the wild (Dial, 1988). In our microarray study, we saw no differences in the expression of COMT mRNA in *N. albigula* on a control compared to a 25% juniper diet (Skopec et al., 2007), therefore we included only generalists fed a control diet.

Food intakes were measured daily for all captive groups i.e., *N. stephensi* fed control or 70% juniper diets and *N. albigula* fed control diet. Body masses were measured daily for all animals. Food intake was not measured for the *N. stephensi* in the wild group; however, they were observed to be consuming the fresh juniper foliage being provided and maintained body mass from date of collection.

2.3. Tissue collection and preparation

At the end of the trials, the animals were euthanized with CO2 and blood, kidney and liver tissues were harvested. While the liver tends to have the highest levels of COMT in the body, the kidney also shows high expression and activity of COMT (Ellingson et al., 1999; Männistö and Kaakkola, 1999; Tsunoda et al., 2002; Zhu, 2002). The expression and activity of COMT in the blood may mirror that occurring
elsewhere in the body (Masuda et al., 2002). Therefore, kidney and blood were analyzed for COMT expression and activity. There are two forms of COMT, soluble and membrane bound (Männistö and Kaakkola, 1999; Zhu, 2002); all tissues were separated into cytosolic fractions and membrane (erythrocytes) or microsomal (liver and kidney) fractions. The cytosolic fractions contained the soluble form or S-COMT whereas the membrane and microsomal fractions contained the membrane bound or MB-COMT.

Blood (≤ 1 mL) was collected from euthanized animals via heart puncture and processed as in Masuda et al. (2002) to first separate erythrocytes from plasma and other cellular elements and then to separate the cytosolic fraction of the erythrocytes from the membrane fraction (Masuda et al., 2002). Due to a lack of sample volume, the membrane fraction of the blood was not analyzed.

Liver and kidneys were perfused in situ by injecting cold isotonic saline into the hepatic portal vein (for liver) and renal arteries (for kidneys). The liver and kidneys were removed and weighed. Cytosolic and microsomal fractions were prepared by differential ultracentrifugation as described for laboratory rats by Franklin and Estabrook (1971). All samples were stored at −80 °C until assayed for COMT. Protein concentrations for each fraction were determined colorimetrically via the Bio-Rad Protein assay (Bio-Rad) based on the Bradford dye-binding method.

2.4. Western blots

We chose to use western blots to measure differences in the expression of COMT protein between tissues and treatments rather than measuring mRNA levels since mRNA expression does not necessarily correlate to protein expression due to differences in translational control and protein half-life (Gygi et al., 1999). We therefore are referring to protein expression and not gene expression when using the term “expression” from this point forward. Samples were diluted to 5 μg/μL with 1 M Tris–HCl pH 7.4, placed in loading buffer (4% SDS, 20% glycerol, 0.1% bromophenol blue, 250 mM Tris–HCl pH 6.9, 0.2% 2-beta mercaptoethanol) and denatured by heating at 100 °C for 3 min. Protein (100 μg) was loaded into each well of a 4–20% Tris-glycine iGeL (ISC bioexpress) and the samples were subjected to SDS-polyacrylamide gel electrophoresis and then transferred onto polyvinylidene difluoride (PVDF) membranes (Thermo Scientific). The membranes were blocked for 1 h using 5% skim milk in Tris-buffered saline with 0.01% Tween and then incubated with the primary antibody, rabbit anti-COMT antibody (1:300 FL-271 Santa Cruz Biotechnology Inc.). The blot was visualized with peroxidase labeled goat anti-rabbit secondary antibody (1:10,000 Pierce ECL Western Blotting Substrate (Thermo Scientific). A Typhoon 8600 (Molecular Dynamics 300-2483) imaging system was used to visualize and quantify protein bands on the membranes.

2.5. Enzyme assays

Enzyme assays were used to quantify the activity of COMT using catechol as the substrate and radiolabeled SAM (adenosyl-L-methionine, S-[methyl-H], specific activity: > 50 Ci/m mole, MP Biomedicals) as the cofactor as in Zürcher and Prada (1982). We checked for linearity of the assay by varying protein concentrations (25, 50, 100, 150 μg of sample protein) and time (100 μg protein incubated for 5, 10, 20, and 30 min). A cytosolic liver fraction from one animal of each species on the control diet was run in triplicate using the following reaction mixture (0.5 mM catechol, 0.1 M sodium phosphate buffer, 1 μM DTT, 1 mM MgCl₂, 200 μM SAM 1 μCi/mL H-SAM and 0.2 μM dinitrochatehol).

To determine if α-pineine, a terpene, and the most abundant PSC in one-seeded juniper, acts as a substrate for COMT, cytosolic and microsomal liver fractions from three N. stephensi on control diet were incubated with a reaction mixture that did not contain catechol and instead contained varying concentrations of α-pineine (Sigma-Aldrich) that had been dissolved in ethanol (0.01 mM, 0.05 mM, 0.1 mM, 0.5 mM, 1 mM α-pineine).

Kinetics of S and MB-COMT in N. stephensi and N. albigula were done on cytosolic and microsomal liver fractions from 3 animals of each species on the control diet using 100 μg of protein and the above reaction mixture except that catechol concentration was varied (0.01 mM, 0.05 mM, 0.1 mM, 0.5 mM, 1 mM catechol) and the reaction was allowed to proceed for 20 min. Lineweaver–Burk double reciprocal plots were used to calculate Km and Vmax for each individual. The kinetic data revealed that MB-COMT had a significantly lower Km for catechol than S-COMT in both species, therefore all further reactions for microsomal fractions were run with a concentration of 0.05 mM catechol and the cytosolic fractions were run with a concentration of 0.5 mM catechol. All other reaction mixture reagents remained the same and the reaction was allowed to proceed for 20 min.

2.6. Statistical analysis

Body mass was analyzed using analysis of variance (ANOVA) with species and diet coded as a single factor. Dry matter intake was analyzed using analysis of covariance (ANCOVA) with species and diet as a single factor but with body mass minus organ mass as the covariate. Liver and kidney weights were also analyzed using ANCOVA with species and diet as a single factor but with body mass minus organ mass as the covariate (Christians, 1999). Because there were no significant differences in expression and activity of COMT between the wild N. stephensi and N. stephensi fed the 70% juniper diet, we combined them into a single group in which we refer to as “N. stephensi juniper” in further analyses. Expression and activity were analyzed using ANOVA with species and diet as a single factor. Differences between individual means were determined by post hoc Bonferroni adjusted pairwise comparisons. SYSTAT 10 was used for all analyses (Wilkinson and Coward, 2000). All data are expressed as mean ± SE and p ≤ 0.05 was used to establish significance.

3. Results

3.1. Body mass, dry matter intake and organ weights

There was a significant difference in body mass of woodrats (F3, 25 = 4.770, p = 0.009, Table 1), with wild N. stephensi being significantly smaller than N. stephensi on control diet (p = 0.011). There were no differences among the body masses of the captive groups (N. stephensi on control diet and 70% juniper, and N. albigula on control diet). Dry matter intake did not differ among woodrats in captivity (F3, 16 = 1.289, p = 0.303, Table 1) and body mass was not a significant covariate (F1, 16 = 1.479, p = 0.242).

There was a trend for liver mass to be different among groups (F3, 24 = 2.568, p = 0.078, Table 1) and body mass minus liver mass was a significant covariate (F1, 24 = 60.697, p < 0.01). Post hoc comparison of liver masses to body mass ratios showed that wild N. stephensi had higher liver masses to body mass ratios than N. albigula fed a control diet (p = 0.024). Kidney mass significantly

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differed among the groups (F3, 23 = 2.993, p = 0.05) and body mass minus kidney mass was a significant covariate (F1, 23 = 25.03, p < 0.01). Post hoc comparison of kidney mass to body mass ratios showed that wild *N. stephensi* had a significantly larger kidney mass per gram body mass than the *N. albigula* on control diet (p = 0.005).

### 3.2. COMT expression

The expression of COMT significantly differed among groups in all tissues tested except for blood (see Table 2 for summary of ANOVAs and Fig. 1). In general, albeit with a few exceptions, woodrats on the control diets had lower expression of MB-COMT compared to both *N. stephensi* groups (p < 0.001). The *N. stephensi* fed the control diet had similar expression of MB-COMT but significantly lower expression of S-COMT in their kidneys compared to *N. stephensi* consuming juniper (p = 0.026). In the liver, *N. albigula* had lower expression of MB-COMT than both *N. stephensi* groups (p ≤ 0.03). Expression of S-COMT in the liver of *N. albigula* was significantly lower than only the *N. stephensi* consuming juniper (p = 0.05). The *N. stephensi* fed the control diet had lower expression of both MB-COMT and S-COMT in their livers compared to the *N. stephensi* consuming juniper (p ≤ 0.05).

### 3.3. COMT activity

The COMT activity assay using catechol as a substrate and 3H-SAM was linear for both protein concentration (Fig. 2A) and time (Fig. 2B) in the *N. stephensi* and *N. albigula* cytosolic liver samples (R2 ≥ 0.96). When the selective COMT inhibitor dinitrocatechol was present in the reaction mixture, no 3H-methylated products were formed (data not shown). When *α*-pinene was added to the activity assay as the substrate rather than catechol, no 3H-methylated products were detected (data not shown).

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Neotoma stephensi (n=6)</th>
<th>70% juniper diet (n=7)</th>
<th>Wild (n=9)</th>
<th>Neotoma albigula (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (g)</td>
<td>196.8±12.4a</td>
<td>182.1±16.0ab</td>
<td>142.5±7.3b</td>
<td>176.7±7.5ab</td>
</tr>
<tr>
<td>Dry matter intake (g/day)</td>
<td>13.6±0.7</td>
<td>12.3±0.6</td>
<td>NA</td>
<td>14.2±1.1</td>
</tr>
<tr>
<td>Liver mass per g body mass (g/g)</td>
<td>0.046±0.001ab</td>
<td>0.045±0.001ab</td>
<td>0.053±0.004a</td>
<td>0.039±0.003b</td>
</tr>
<tr>
<td>Kidney mass per g body mass (g/g)</td>
<td>0.012±0.001ab</td>
<td>0.013±0.001ab</td>
<td>0.015±0.001b</td>
<td>0.011±0.001b</td>
</tr>
</tbody>
</table>

* Different letters (a, b) denote means significantly different (p ≤ 0.05) as determined by Bonferroni adjusted pairwise comparisons within the same row.

### Table 2

Summary of ANOVAs for differences in the expression of COMT in the various tissues of woodrats tested.

<table>
<thead>
<tr>
<th>Source of COMT</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood S-COMT</td>
<td>0.085</td>
<td>2,10</td>
<td>0.919</td>
</tr>
<tr>
<td>Kidney MB-COMT</td>
<td>29.70</td>
<td>2,22</td>
<td>0.000</td>
</tr>
<tr>
<td>Kidney S-COMT</td>
<td>51.31</td>
<td>2,23</td>
<td>0.000</td>
</tr>
<tr>
<td>Liver MB-COMT</td>
<td>26.54</td>
<td>2,22</td>
<td>0.000</td>
</tr>
<tr>
<td>Liver S-COMT</td>
<td>5.33</td>
<td>2,20</td>
<td>0.014</td>
</tr>
</tbody>
</table>

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Table 3
Kinetics of COMT in the livers of control woodrats.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Neotoma stephensi (n = 3)</th>
<th>Neotoma albigula (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-COMT</td>
<td>MB-COMT</td>
</tr>
<tr>
<td>Km (mM catechol)</td>
<td>0.725 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.145 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vmax (mM catechol/mg protein/min)</td>
<td>19,167 ± 3,632&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9,761 ± 2,683&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Different letters (a, b) denote means significantly different (p<0.05) as determined by Bonferroni adjusted pairwise comparisons within the same row.

The kinetics of COMT for the substrate catechol differed among the liver fractions and species in the animals fed the control diet tested (Table 3). The Km for catechol was higher in S-COMT than MB-COMT in the liver fraction in both species (F<sub>3, 8</sub> = 4.834 p = 0.033). The Vmax of MB-COMT in N. albigula was higher than N. stephensi (F<sub>3, 8</sub> = 4.07 p = 0.05). While our sample size was limited to 3 animals per species, we nonetheless found significant differences between species. Our sample error is also comparable to other studies that used sample sizes of 5–6 animals (Ellington et al., 1999; Masuda et al., 2002; Tsunoda et al., 2002; Tsunoda and Imai, 2004).

In general, N. stephensi had elevated S-COMT activity that was often connected with juniper ingestion compared to N. albigula. In contrast, N. albigula had higher activity of MB-COMT (see Table 4 for summary of ANOVAs and Fig. 3). In the blood, N. albigula and N. stephensi fed the control diet had similar activities of S-COMT while the N. stephensi consuming juniper had significantly higher S-COMT activity (p ≤ 0.005). In the kidneys, N. albigula fed the control diet had significantly higher activity of MB-COMT than both groups of N. stephensi (p ≤ 0.001) but significantly lower activity of S-COMT than both groups of N. stephensi (p ≤ 0.001). The N. stephensi fed the control diet had similar activity of MB-COMT compared to the N. stephensi consuming juniper but a lower activity of S-COMT than N. stephensi consuming juniper in their livers (p = 0.005).

4. Discussion

Metabolizing and excreting large doses of PSCs may be energetically and physiologically challenging to mammalian herbivores and likely constrains most from being dietary specialists. Phase II enzymes typically produce non-reactive compounds that can be easily excreted in the urine or feces, yet the loss of an energy rich conjugate may significantly compromise an herbivore’s energy budget (McLean et al., 1993; McLean and Foley, 1997; Boyle et al., 1999; McLean et al., 2001; Dearing et al., 2005a; Sorensen et al., 2005a, 2005b, 2006). Catechol-O-methyl transferase (COMT) is a phase II enzyme that conjugates a relatively inexpensive methyl group to the xenobiotic, thus it may be an energetically efficient yet physiologically safe pathway that the juniper specialist, N. stephensi, utilizes to metabolize the PSCs present in their diets (Skopec et al., 2007). We compared the juniper specialist, N. stephensi to N. albigula, a dietary generalist, to determine if there were differences in the expression and activity of COMT. We found a number of differences in COMT expression and activity both within and between species and discuss how these differences may influence the physiology of the two species and the implications for COMT as a novel and important pathway for the specialist to metabolize juniper PSCs.

Ingestion of juniper induced the expression and activity of COMT in a range of tissues of the specialist (Figs. 1 and 3). The specialist consuming juniper either in the wild or in captivity showed similar levels of expression and activity of COMT in all tissues tested and was therefore combined into a single group. The lack of difference between the specialist in captivity consuming 70% juniper and those recently trapped from the wild confirm that our 70% juniper diet is ecologically relevant in terms of detoxification enzyme induction. The specialists consuming juniper showed increased expression and activity of S-COMT in their kidneys and livers and increased activity in the blood compared to the specialist and generalist fed the control diet. While the generalist was not fed a diet containing juniper, the upregulation of COMT in response to juniper appears unique to the specialist. In our previous experiment, the generalist showed no change in the expression of COMT mRNA on its ecologically relevant diet of 25% juniper diet compared to a control diet (Dial, 1988; Skopec et al., 2007).

MB-COMT is likely not instrumental in the metabolism of juniper PSCs. The only difference in the expression or activity of MB-COMT in the specialist was that it had a 50% higher expression of liver MB-COMT when feeding on juniper compared to the control diet (Fig. 1). Although it was a significant increase, it was much smaller than the 140% increase in expression of liver S-COMT in the specialist consuming juniper, compared to the control diet (Fig. 1). In laboratory rats and humans, MB-COMT has higher affinity for its substrates but a much lower Vmax than S-COMT and seems to play a more important role in the metabolism of catecholamines in the nervous system than...
in the metabolism of endogenous or exogenous substrates in peripheral tissues (Männistö and Kaakkola, 1999; Huotari et al., 2002; Zhu, 2002). We found that hepatic S-COMT of the specialist had higher \(K_m\) and higher \(V_{\text{max}}\) values for catechol than MB-COMT. We therefore conclude that the almost sevenfold increase in COMT mRNA documented previously (Skopec et al., 2007) was coding for mainly S-COMT translation. Both forms of COMT are transcribed from the same gene with the longer transcript coding for the larger MB-COMT and the shorter transcript coding for S-COMT (Männistö and Kaakkola, 1999; Zhu, 2002).

The COMT enzyme in the specialist consuming juniper most likely metabolizes phenolics in juniper and not terpenes. We found no evidence of a methylated product when \(\alpha\)-pinene was substituted for catechol as the substrate in the enzyme assays. As terpenes do not contain any catechol groups for COMT to methylate, it is not surprising catechol as the substrate in the enzyme assays. As terpenes do not contain any catechol groups for COMT to methylate, it is not surprising given that in other species tested (humans, mice and rats), MB-COMT tends to have higher affinity but lower \(V_{\text{max}}\) for substrates than S-COMT (Männistö and Kaakkola, 1999; Zhu, 2002). The MB-COMT \(K_m\) for catechol in the generalist was not significantly different from that in the specialist, but the MB-COMT \(V_{\text{max}}\) for catechol in the generalist was more than double that of the specialist. The much higher \(V_{\text{max}}\) in the generalist means that it can convert catechol to the methylated product (guaiacol) at a much faster rate than the specialist.

Generally MB-COMT is more highly expressed in the central nervous system and plays a critical role in the metabolism of centrally acting catecholamines and thus behavior (Männistö and Kaakkola, 1999; Huotari et al., 2002; Zhu, 2002). High MB-COMT activity is linked to aberrant human behaviors such as attention deficit disorder, antisocial behavior and polysubstance abuse (Zhu, 2002; Langley et al., 2010). Indeed, a COMT inhibitor cures compulsive kleptomania (Grant, 2011). The high \(V_{\text{max}}\) of MB-COMT in the generalist is most likely due to kinetic differences in MB-COMT between the two species. Interestingly, MB-COMT's \(V_{\text{max}}\) for catechol in N. albiguila was similar to S-COMT's \(V_{\text{max}}\) for catechol in both the generalist and specialist. The high \(V_{\text{max}}\) for MB-COMT is unusual given that in other species tested (humans, mice and rats), MB-COMT tends to have higher affinity but lower \(V_{\text{max}}\) for substrates than S-COMT (Männistö and Kaakkola, 1999; Zhu, 2002). The MB-COMT \(K_m\) for catechol in the generalist was not significantly different from that in the specialist, but the MB-COMT \(V_{\text{max}}\) for catechol in the generalist was more than double that of the specialist. The much higher \(V_{\text{max}}\) in the generalist means that it can convert catechol to the methylated product (guaiacol) at a much faster rate than the specialist.

Dopamine decreases the reabsorption of sodium in the kidney, causing natriuresis and diuresis (Aperia et al., 1997; Aperia, 2000; Odliöld et al., 2000). Both water and sodium are likely limiting to the specialist given that it is a desert dweller (Frelend et al., 1985; Kaspari et al., 2008) and juniper contains less than 0.02% sodium. Moreover, juniper in general, and \(\alpha\)-pinene, the major terpene found in one-seeded juniper, are diuretics (Dearing et al., 2001, 2002; Kumar et al., 2010), which may put further stress on the specialist’s ability to maintain water balance in its arid environment. By increasing S-COMT expression and activity the specialist may be increasing their kidneys’ metabolism of dopamine and therefore increasing sodium and water retention.

As expected, the generalist, N. albiguila, showed lower expression and activity of S-COMT in their livers and kidneys compared to the specialist consuming juniper. The generalist also had lower MB-COMT expression in kidneys and liver than the specialist, but surprisingly, the generalist had higher hepatic and nephritic MB-COMT activity than the specialist. The difference in the results of the expression versus activity of MB-COMT in the generalist is most likely due to kinetic differences in MB-COMT between the two species. Interestingly, MB-COMT's \(V_{\text{max}}\) for catechol in N. albiguila was similar to S-COMT's \(V_{\text{max}}\) for catechol in both the generalist and specialist. The high \(V_{\text{max}}\) for MB-COMT is unusual given that in other species tested (humans, mice and rats), MB-COMT tends to have higher affinity but lower \(V_{\text{max}}\) for substrates than S-COMT (Männistö and Kaakkola, 1999; Zhu, 2002). The MB-COMT \(K_m\) for catechol in the generalist was not significantly different from that in the specialist, but the MB-COMT \(V_{\text{max}}\) for catechol in the generalist was more than double that of the specialist. The much higher \(V_{\text{max}}\) in the generalist means that it can convert catechol to the methylated product (guaiacol) at a much faster rate than the specialist.

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5. Conclusions

To our knowledge this is the first study to show tissue distribution and enzyme kinetics of COMT in a wild mammalian herbivore. The specialist, N. stephensi's, upregulation of S-COMT in the liver and also kidneys is a unique response and may be an integral part of its ability to specialize on juniper. We also found that the generalist, N. albiguila has an unusually high MB-COMT \(V_{\text{max}}\) and future studies are necessary to determine if this causes unique behaviors in the generalist.
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