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Detoxification rates of wild herbivorous woodrats (*Neotoma*)

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Abstract

The detoxification systems of mammalian herbivores are thought to have evolved in response to the ingestion of plant secondary compounds. Specialist herbivores consume high quantities of secondary compounds and are predicted to have faster rates of Phase 1 detoxification compared to generalist herbivores. We tested this hypothesis by comparing the performances of a specialist (*Neotoma fuscipes*) and generalist (*Neotoma lepida*) herbivore using hypnotic state assays. Herbivores foraging in nature were live trapped and injected with hexobarbital (100 mg/kg). We measured the length of time in the hypnotic state as the time in which the animal was unable to right itself twice in 30 s. The specialist metabolized hexobarbital 1.7 times faster than the generalist ($F_{1,\ 19}=9.31$, $P=0.007$) as revealed by its significantly shorter time spent in the hypnotic state (56±9 min vs. 87±8 min, respectively). The results are consistent with the hypothesis that specialists have faster rates of Phase 1 detoxification. This is the first evaluation of the detoxification capability of mammalian herbivores foraging under natural conditions. Hypnotic state assays have broad potential applications to the study of vertebrate-plant interactions.

Keywords: Detoxification; Herbivores; Hexobarbital; Hypnotic state assay; *Neotoma*; Sleep time; Woodrat

1. Introduction

The detoxification systems of mammalian herbivores are thought to have evolved in response to the concentration and diversity of plant secondary compounds (PSCs) in their diet (Freeland and Janzen, 1974). Specialist herbivores tend to consume diets rich in PSCs and are predicted to possess detoxification systems that can metabolize large concentrations of chemically similar toxins while simultaneously minimizing energetic costs (Freeland and Janzen, 1974; Lamb et al., 2004). In particular, specialists are predicted to have high capacities of Phase 1 detoxification enzymes such as the cytochrome P450s (Boyle et al., 2000, 2001; Lamb et al., 2004). In contrast, generalist herbivores consume small amounts of various PSCs and are expected to utilize Phase 2 conjugation enzymes to process the diversity of ingested compounds (Boyle et al., 2000, 2001; Lamb et al., 2004). Phase 2 detoxification enzymes are less substrate specific than Phase 1 enzymes but require energetically costly conjugates such as glucose derivatives (Klaassen, 1996).

This long-standing detoxification hypothesis has been difficult to address for a number of reasons. First, although a number of *in vitro* techniques (e.g., microsomal assays) are commonly used in pharmacological studies of laboratory rats, such techniques have only recently been applied to mammalian herbivores (Pass et al., 2001, 2002). A limitation of many *in vitro* pharmacological approaches is that as endpoint studies, they can only be conducted on species for which adequate numbers can be harvested (8–10 individuals per treatment). Thus, they are not appropriate for endangered or protected species. Second, application of *in vitro* approaches is typically conducted on captive populations of herbivores to permit control of dietary contents and measurement of intake. However, such studies necessitate the use of artificial diets, which can introduce artifacts (Price et al., 1980, 2004; Appel, 1993).

We used a hypnotic state assay to test the detoxification hypothesis in free-ranging mammalian herbivores. A benefit of these assays is that they are relatively non-invasive, requiring only intraperitoneal injections of anesthesia from which the animals recover quickly (within a few hours). Hypnotic state assays are commonly used in nutraceutical or pharmacological studies to screen for possible drug interactions (Price et al., 2004; Kim and Shin, 2005; Nyarko et al., 2005; Oliveira et al.,

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2005). The basic premise of the hypnotic state assay is that an animal is given a hypnotic agent (e.g., hexobarbital, zoxazolamine) and the length of time the animal is in a hypnotic state is measured (Kim et al., 1993; Sasaki, 1994). The length of time in the hypnotic state is inversely proportional to the animal's ability to metabolize the hypnotic agent. We selected hexobarbital as the hypnotic agent for this study as an indicator of Phase 1 detoxification.

The mammalian herbivores used in this study were a sympatric specialist and generalist. *Neotoma fuscipes* is a specialist on oak foliage (e.g., *Quercus agrifolia*). In parts of California, the specialist occurs sympatrically with another woodrat, *Neotoma lepida*, which has greater diet diversity. The diet of the *N. lepida* in this part of its range is dominated by *Opuntia occidentalis* cactus and *Salvia apiana* (Atsatt and Ingram, 1983). We tested the hypothesis that the specialist has greater rates of Phase 1 detoxification than the generalist by injecting woodrats with hexobarbital and measuring the length of time in the hypnotic state. If the specialist has faster rates of Phase 1 detoxification, we predicted it should metabolize hexobarbital faster resulting in shorter hypnotic state times compared to the generalist woodrat.

2. Methods

Woodrats *N. fuscipes* and *N. lepida* were trapped with Sherman live traps (30×9×8 cm) at Caspers Wilderness Park, San Juan Capistrano, CA in mid June 2005. The habitat consisted of oak forest edges abutting areas of cactus (*Opuntia*) flats. Traps were set at woodrat houses ("middens") and were opened at dusk and collected the following morning at sunrise. Woodrats were identified to species using morphological characters (male genitalia) and weighed to the nearest 0.1 g with a portable balance (Mettler ED601). We restricted our study to adult male woodrats (>130 g) because females could have been pregnant or nursing at the time of capture, and such reproductive states could affect the results. We captured 8 male *N. fuscipes* and 12 male *N. lepida* for use in the study.

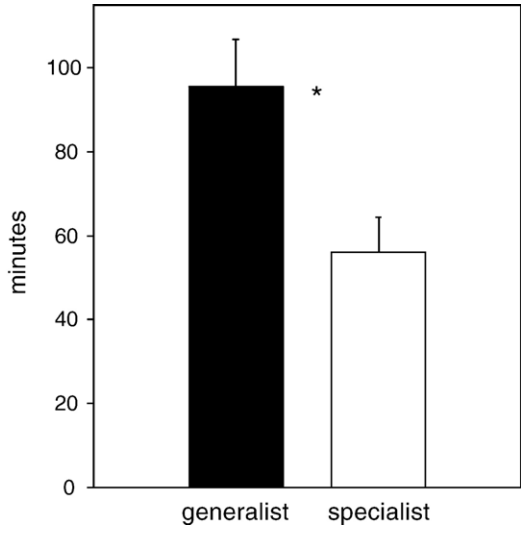

Fig. 1. Length of time in the hypnotic state for a generalist woodrat, *N. lepida* (solid bar) and a specialist woodrat, *N. fuscipes* (open bar). Asterisk indicates a significant difference ($P=0.007$) between the two species.

Male woodrats (were given an intraperitoneal injection of 100 mg/kg of hexobarbital. Hexobarbital was dissolved in 0.9% saline 0.25 N NaOH (50 mg/mL). Injection volumes ranged from 0.23 to 0.38 mL. After injection, woodrats were placed in a standard laboratory rat cage (32×23×19 cm). We measured "sleep time" beginning at the time woodrats lost the ability to right themselves twice within 30 s after being placed on their backs to the time when they could right themselves twice within 30 s after being placed on their backs (Koizumi et al., 2002). Animals were handled according to IACUC protocol 04-0212.

Sleep times were not normally distributed and were $\log(x+1)$ transformed for analysis. We compared sleep times between the specialist and generalist using a one-way ANOVA with species as the independent factor. We investigated the possible relationship between body size and sleep time independently for each species with linear regression. All analyses were conducted in JMP 4.0.4.

3. Results

Male *N. fuscipes* ($x=176\pm3.4$ g) were 5.9% larger than the male *N. lepida* ($x=166\pm2.8$; $F_{1,19}=5.5$, $P=0.03$).

Sleep times differed significantly between the specialist and the generalist ($F_{1,19}=9.31$, $P=0.007$). The generalist slept 1.7 times longer than the specialist (Fig. 1). There was no relationship between sleep time and body size for either the specialist ($r^2=0.07$, $P=0.51$) or the generalist ($r^2=0.07$, $P=0.43$).

4. Discussion

We tested the hypothesis that specialist herbivores have faster rates of Phase 1 detoxification than generalists (Boyle, 2000; Boyle et al., 2001; Lamb et al., 2004). The data from this study are consistent with this hypothesis in that the specialist herbivore metabolized hexobarbital almost twice as fast as the generalist herbivore. Future studies on more specialist and generalist herbivores are needed to determine whether this hypothesis is applicable to herbivores in general. To our knowledge, these data are the first on detoxification rates of mammalian herbivores consuming natural diets under field conditions. In the subsequent paragraphs, we discuss the implications of these results with respect to dietary strategy, detoxification physiology, putative secondary compounds, and genetic basis. We also present ideas on how this assay could be applied to other studies.

The detoxification systems of mammals consist of more than 100 enzymes (Klaasen, 1996). Across mammals, much conservation exists in these enzymes, although species-specific differences are not uncommon. With respect to woodrats, the molecular details (e.g., DNA sequences, molecular structures) have not been characterized for each enzyme in the detoxification system. However, data from model systems can be used to infer the enzymes in woodrats responsible for metabolism of hexobarbital. Cytochrome P450s, specifically cytochrome 2B