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Short communication

Comparison of total protein and enzyme levels in successive regenerations of venom from individual coralsnakes

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ABSTRACT

Coralsnakes produce highly potent neurotoxic venoms, but little is known about variations in specific enzyme components within a species or from one replenishment of venom to the next within the same animal. Since published studies are often conducted using venom pools from multiple snakes, individual differences are masked and variations among individual snakes and between subsequent venom regenerations from the same snake have rarely been documented. This study involves the analysis and comparison of four successive venom collections from each of nine individual coralsnakes in order to detect these differences. Significant variation was found within the successive re-synthesis of venom components. Even greater differences were observed between the venoms from similar individual snakes. Since studies of variation in enzymatic activity would be significant only if they were above these normal variations, it is important to be aware of these differences. These results suggest the importance of understanding the variations present within and between individuals of the same species when interpreting the potential significance of differences found as the result of genetic, environmental or ecological factors.

1. Introduction

Snake venom is a complex mixture of diverse enzymes and small peptides used for predation (Thomas and Pough, 1979) and self-defense or predator deterrence (Pintor et al., 2011). These components are selectively expressed and stored in the venom glands and function to immobilize, kill, and/or digest prey (Fry et al., 2006). Venom composition and potencies vary significantly between families, from genus to genus, and between species (Koh et al., 2006). Within fifteen different coralsnake taxa, significant differences have been reported as a function of phylogeny, geographical location, season, age, gender, and diet (da Silva and Aird, 2001).

Once expended, it is important for venom stores to be replenished. Many conflicting studies can be found in the older literature concerning the effect of frequent or repeated milkings on the yield and composition of venoms from a variety of venomous organisms (Chippaux et al., 1991). Seasonal and geographic factors were shown to influence these results in several snake species, and regeneration of venom protein sometimes showed individual variation (Oron and Bdolah, 1973; Willemse et al., 1979). The dynamics of venom re-synthesis is important but poorly understood. Early studies indicate that RNA and protein levels peak at 3 and 4–8 days after venom expenditure respectively (Paine et al., 1992), but little was known about the expression dynamics of individual components. A recent study confirmed this time scale of venom RNA and protein re-synthesis and followed the relative expression of several specific enzymes and differences between individual snakes (Currier et al., 2012).

The observation that snakes exhibit considerable variation in the amount of venom injected during a predatory or defensive envenomation has led to the idea that snakes can control their venom dosage. This raises the question of how metabolically expensive it is to replenish venom stores after an expenditure. The "venom optimization hypothesis" (Wigger et al., 2002) or "venom metering" (Hayes, 2008) postulates that since venom is

Abbreviations: PLA_2, phospholipase A_2.

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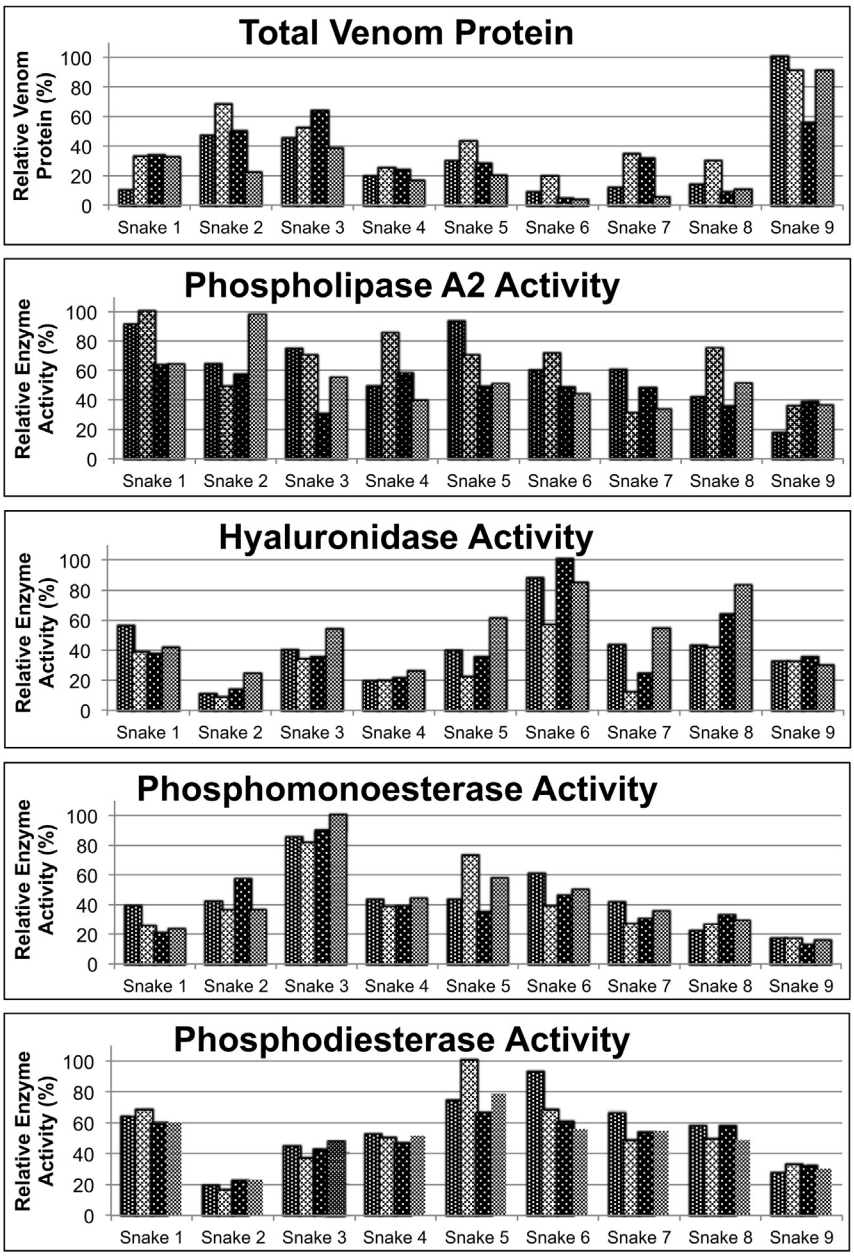

Fig. 1. Comparison of venoms from 4 successive collections from each of 9 individual coralsnakes. The total venom protein and the activity of each enzyme, calculated as activity per mg total venom protein, are scaled and expressed as a relative percentage of the highest value obtained.

metabolically expensive, it is used frugally through behavioral control. It was also shown that the cost of venom production is not trivial as demonstrated by an 11% increase in metabolic rate during the course of venom regeneration (McCue and Mason, 2006). This suggests that there is a significant metabolic load associated with the maintenance of a venom system, so venom would likely be used as economically as possible (Kuhn-Nentwig et al., 2011). In contrast, an even more recent study found that snakes that had venom extracted did not have significantly higher metabolic rates than control snakes (Smith et al., 2014). This indicates that venom may not be energetically costly to produce and the metabolic cost of venom re-synthesis may be small relative to the resting metabolic state and normal homeostatic controls. Information concerning the consistency of venom from one regeneration to the next is almost completely lacking.

Coralsnakes produce highly potent neurotoxic venoms (Tanaka et al., 2010), but little is known about variations in specific enzyme components within a species or from one replenishment of venom to the next within the same animal. Since published studies are often conducted using venom pools from multiple snakes, individual differences are masked (Chippaux et al., 1991). Therefore, variations between individual snakes and between subsequent venom samples from the same snake have rarely been documented. Any differences in levels of enzymatic activity as a result of any environmental, ecological, or genetic factors would be significant only if they were above the normal variations within successive venom regenerations in a given snake or between similar individuals.

The present study involves the analysis and comparison of four successive venom collections from each of nine individual coralsnakes in order to detect these differences. All of the snakes were captured from a similar geographical region at the same time and