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Mechanisms Controlling Venom Expulsion in the Western Diamondback Rattlesnake, *Crotalus atrox*

BRUCE A. YOUNG* AND KENNETH V. KARDONG
School of Biological Sciences, Washington State University, Pullman, Washington 99164-4236

ABSTRACT Although many studies have documented variation in the amount of venom expended during bites of venomous snakes, the mechanistic source of this variation remains uncertain. This study used experimental techniques to examine how two different features of the venom delivery system, the muscle surrounding the venom gland (the Compressor Glandulae in the rattlesnake) and the fang sheath, could influence venom flow in the western diamondback rattlesnake, *Crotalus atrox*. Differential contraction of the Compressor Glandulae explained only approximately 30% of the variation in venom flow. Lifting (compression) of the fang sheath as occurs during a normal strike produced marked increases in venom flow; these changes were closely correlated and exceed in magnitude by almost 10 × those recorded from the Compressor Glandulae alone. These results suggest that variation in these two aspects of the venom delivery system—both in terms of magnitude and temporal patterning—explain most of the observed variation in venom injection. The lack of functional or mechanical links between the Compressor Glandulae and the fang sheath, and the lack of skeletal or smooth muscle within the fang sheath, make it unlikely that variation in venom flow is under direct neural control. Instead, differential venom injection results from differences in the pressurization by the Compressor Glandulae, the gate keeping effects of the fang sheath and enclosed soft-tissue chambers, and by differences in the pressure returned by peripheral resistance of the target tissue. *J. Exp. Zool. 307A:18–27, 2007.*

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Venom expulsion is any pressurized discharge or flow of venom from the venom delivery system of the snake. Venom injection represents one special form of venom expulsion in which the venom is discharged while the fang is imbedded (typically in the tissue of the target organism). Venom injection, like other forms of venom expulsion—such as venom milking or venom spitting—is characterized by variation in both the volume and pressure of the venom, that is to say, by differential venom flow. Two contrasting hypotheses have been advanced to account for differential venom flow in snakes. One hypothesis, termed the *venom metering hypothesis*, postulates that the venom delivery system is under strong neural regulation and that snakes apportion venom so as to optimize the amount of venom injected into prey. In support, experimental trials report that small prey receive less venom than large prey (e.g., Hayes, '95). Further, this apportionment of venom is proposed to occur through conscious decision making (see Hayes et al., 2002; Hayes, 2004). The second hypothesis, termed the *pressure-balance hypothesis*, postulates that the amount of venom injected is determined largely by the mechanical interactions between a snake's venom apparatus and the prey, with little neural regulation of the venom delivery system (see Young et al., 2002, 2003). The venom metering hypothesis emphasizes active regulation before the snake makes contact with the prey, whereas the pressure-balance hypothesis emphasizes passive regulation that occurs during contact.

The differences between these two hypotheses can be mapped onto the structural features of the venom delivery system. The venom gland of all

*Correspondence to: Dr. B.A. Young, Department of Biology, Washburn University, Topeka, KS 66621.
E-mail: bruce.young@washburn.edu
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viperid, elapid, and atractaspid snakes is surrounded by skeletal muscle (see Haas, '73). The presence of striated voluntary muscle abutting, if not attaching to, the venom gland clearly establishes that muscle contraction plays a role in pressurizing the venom gland and thereby driving venom through the duct system (Rosenberg, '67; Young et al., 2000). What has not been established to date is the nature of the relationship between differential muscle contraction and differential venom injection. The distal portion of the venom delivery system consists of the fang sheath, an envelope of connective tissue and epithelium, which drapes down from the roof of the mouth to surround the fang (Young et al., 2006). Within the fang sheath are several soft-tissue chambers through which venom must flow in order to reach the fang (Young et al., 2006). The fang sheath is devoid of skeletal or smooth muscle, and thus cannot actively regulate venom flow. However, physiological studies have suggested that deformation of the fang sheath can influence venom flow, presumably, at least in part, by altering the flow of venom through the soft-tissue chambers (Young et al., 2001a).

The present study was undertaken to experimentally explore the relative contributions of these two structural features—fang sheath and the compressor muscle—to venom release in the western diamondback rattlesnake, *Crotalus atrox*. This is a way to test the relative importance of neural regulation on venom injection, since the fang sheath is not an active effector organ, and therefore not under direct control by the central nervous system. This study will examine the relationship between differential contraction of the skeletal muscle surrounding the venom gland and differential venom expulsion, as well as the impact of fang sheath position on venom expulsion. In this way, we experimentally compare the predictions of the venom metering and pressure-balance hypotheses.

MATERIALS AND METHODS

Live animals

This study utilized seven live specimens of western diamondback rattlesnake, *C. atrox*, with snout lengths (svl) ranging from 66 to 135 cm. Four of the specimens were obtained commercially (Glades Herp, Bushnell, FL) while the remaining three were wild-caught in western Texas. The snakes were maintained at 27–31°C, with a 12:12 light cycle, water ad libitum, and a diet of pre-killed rodents. To ensure a normal venom supply, the snakes were not fed within 2 weeks of any experimental procedure. Maintenance and use of these animals followed guidelines for reptiles and particularly venomous snakes, and all experimental protocols were approved by the Institutional Animal Care and Use Committee of Washington State University.

Non-pressurized fang sheath compression

Two of the *C. atrox* (svls of 87 and 95 cm) were anesthetized through exposure to Isoflurane, then intubated and maintained on a low-level flow of Isoflurane. Once the animals were fully anesthetized, the head was positioned in a clamp that held one side of the head static while allowing manipulation of the contralateral side. A micromanipulator was positioned behind the quadratopterygoid joint and used to mechanically protract the upper jaw until the fang was erected to at least 70° (measured from the ventral margin of the supralabials to the leading edge of the fang).

The fang sheath was gently lifted to expose the fang tip and a rotary disk used to truncate the fang just proximal to the exit orifice; doing this exposed the venom canal at the excised surface of the fang. A length of polyethylene (PE) tubing was slipped over the fang; the inner diameter of the PE tubing was such that a tight fit was achieved with the outer surface of the fang. The free end of the PE tubing was attached to a Statham P23AA pressure transducer (Gould, Valley View, OH), and both tubing and transducer were filled with Ringer's solution allowing no air within the channel. The pressure transducer was connected to a P511 AC/DC Amplifier (GRASS, West Warwick, RI) the output of which was converted to a digital record using a PCI-PCM12 H A/D converter (SuperLogics), recorded (at a sampling rate of 10 kHz) using WINview (SuperLogics), and analyzed with WINcalc (SuperLogics, Waltham, MA).

In experiments to test the effects of the fang sheath, the fang sheath was manually lifted—dorsal to simulate the elevation and compression that occurs normally during the strike—using either forceps or pads of foam rubber. The rate and extent of the fang sheath elevation varied. At least five sheath elevations were recorded from each side of the specimens.

Fatigue of the Compressor Glandulae

To access the Compressor Glandulae, the scalation over the venom gland was removed, exposing

the Compressor Glandulae while leaving its vascular supply and innervation intact. A clamp was applied to the lower jaw to stabilize the insertion of the Compressor Glandulae. Silk suture was used to attach the origin of the Compressor Glandulae to a force transducer (UFI model 1030). The force transducer was connected to the P511 amplifier and the data acquisition system as described above.

A micromanipulator was used to position a bipolar stimulating probe against the motor nerve to the Compressor Glandulae. The stimulating probe was connected to a S88 dual channel stimulator (GRASS), which was used to stimulate (7 V for 50 msec) the Compresssor Glandulae once every 5 sec for 5 min. Fatigue was calculated as the percentage decline in twitch force over time.

Twitch venom pressure

Five specimens of *C. atrox* (svls from 66–135 cm) were anesthetized and prepared as described above to expose the Compressor Glandulae. Bipolar EMG electrodes were constructed using 0.05 mm diameter stainless-steel wire with nylon insulation (California Fine Wire), and implanted into the Compressor Glandulae via hypodermic needles. The electrodes were attached to a custom-built EMG amplifier which was coupled to the data acquisition system.

A bipolar stimulating probe was used to stimulate the surface of the Compressor Glandulae; while each stimulus was applied individually, effort was made to standardize the manual pressure applied to the stimulator, and to stimulate the same general region (the anterolateral quadrant) of the muscle. Using the S88 stimulator (GRASS), the stimulus applied to the Compressor Glandulae was varied both in terms of voltage (5–10 V) and duration (40–120 msec). We attempted to apply 10–12 stimuli to both the right and left venom delivery system of each specimen. Stimuli were not applied to one side of two different specimens; one specimen had shed its fang and the other evidenced physical trauma to the fang sheath. During data analysis (see below) stimuli were excluded if there was evidence of cross-talk or poor signal quality. Ultimately we quantified 68 twitches, total from both sides, which were roughly evenly divided among the five specimens.

The data tracings for this experiment (Fig. 1) consisted of the synchronized marker from the stimulator, the voltage output from the EMG amplifier, and the voltage output from the pressure transducer. Using the WINcalc software (SuperLogics), the EMG voltage was rectified and the area under the curve quantified. The peak venom pressure was quantified, as was the area under the pressure curve. A curve was fit to the pooled data points for each specimen (using maximal r^2 value as the criterion for curve fit). Subsequently, the data points from all the specimens were standardized—by adjusting the mean venom pressure value for a narrow range of EMG area—to minimize intraspecific variation and the data sets combined for all specimens.

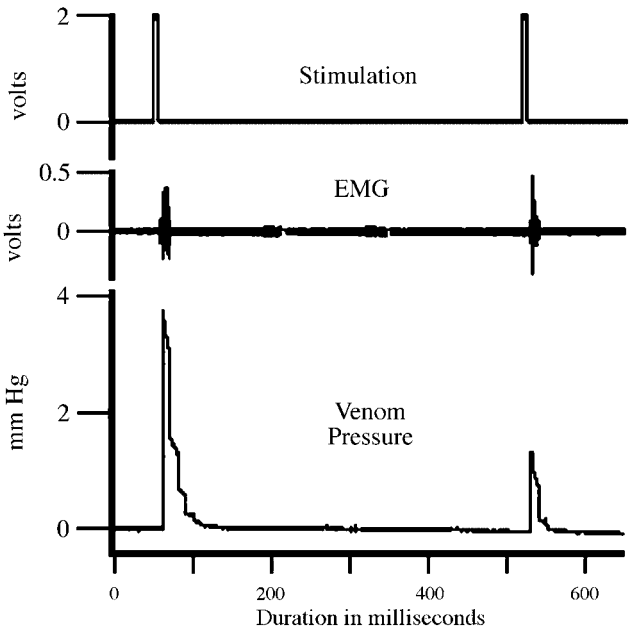

Fig. 1. Data tracing from the twitch venom pressure experiment. The EMG waves were rectified then the area under the curve calculated. The peak venom pressure was measured, as was the area under the venom pressure curve.

Pressurized sheath elevation

Following the twitch venom pressure experiments, a magnetoresistive sensor (Philips KMZ10) was positioned, using a micromanipulator, dorsolateral to the fang sheath. A train stimulus (7 V, 50 msec duration, 19.5 pps) was applied to the surface of the Compressor Glandulae; this stimulus produced nearly constant pressurization of the venom gland but did not induce a tetanic contraction of the muscle (individual pulses were still evident in the pressure tracing).

Although variable in duration, the Compressor Glandulae was always stimulated prior to the lifting of the fang sheath (Fig. 2). The fang sheath was lifted manually by sliding a small pad of foam rubber over the PE tubing and dorsally up the

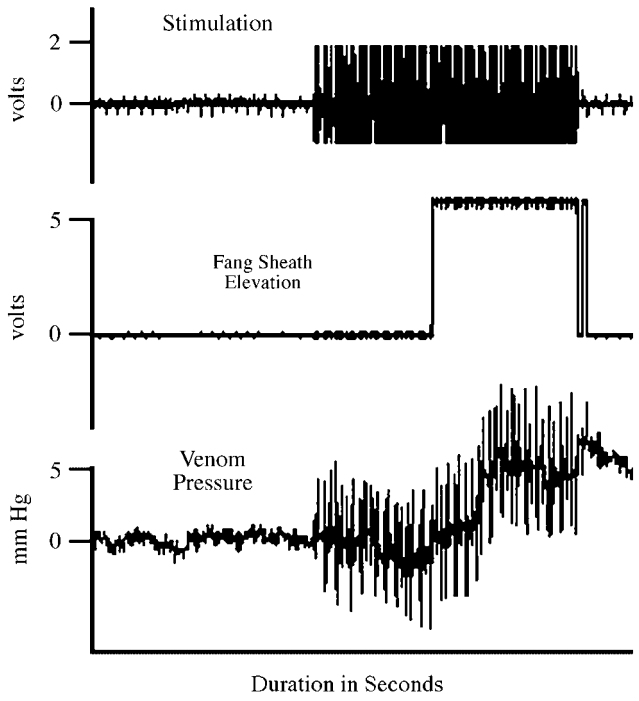

Fig. 2. Data tracing from the pressurized fang sheath elevation. In this, the first run, the gain was too high on the magnetoresistive chip, producing a supramaximal voltage output. Note that the Compressor Glandulae was active for over 500 msec prior to elevation of the fang sheath, and that during this time there was little change in venom pressure. The pulsatility within the venom pressure tracing indicates that the Compressor Glandulae was not in tetanic contraction.

fang. A 3.2 mm diameter Neodymium Iron Boron ceramic magnet (ForceField, Inc., Fort Collins, CO) was located within the foam pad in such a way that lifting the fang sheath moved the magnet linearly in relation to the magnetoresistive chip. The magnetoresistive chip was connected to an Accudata 218 bridge amplifier (Honeywell, Morristown, NJ) and then to the data acquisition system.

Only the period from the onset of magnet displacement to maximal fang sheath compression was analyzed, due mainly to the fact that the fang sheath did not rebound to its starting position immediately after mechanical lifting of the sheath was discontinued. The voltage range of the data tracings from the pressure transducer and magnetoresistive chip were balanced mathematically so that the two curves (voltage over time) could be directly compared. Subtracting one curve from the other provided useful information about the mechanics of the fang sheath. The peak venom pressure was also quantified. The compression of the fang sheath typically led to the discharge of venom, thereby altering the fluid mechanics of the system. Due to this alteration, we only performed one or two fang sheath manipulations on each side of the venom delivery system.

Pressurized fang retraction

This adjunct to the preceding experiment was performed on three of the specimens (svls of 66, 85, and 115 cm). Following the termination of the pressurized fang sheath experiment, the Compresssor Glandulae was subjected to the same stimulation regime. This time the stimulating probe was placed on the medial surface of the Compressor Glandulae. In this position, the stimulus produced contraction not only in the Compressor Glandulae but also in the adjacent musculature of the pterygoid arch. Stimulating the pterygoid arch musculature produced a retraction of the fang, and with it a displacement of the fang sheath. We had no independent marker for the displacements of either the fang or the fang sheath, but we could record the changes in venom pressure that occurred when the fang sheath was displaced without any direct physical contact.

RESULTS

Non-pressurized fang sheath elevation

Lifting of the fang sheath, in the absence of fluid pressure within the venom delivery system, resulted in retrograde venom flow. This retrograde venom flow was manifest as a decrease in venom pressure (Fig. 3). Release of the sheath allows it to drop back over the fang, and produced an increase in venom pressure that was roughly equal in magnitude to the pressure decrease recorded during compression (Fig. 3).

Fatigue of the Compressor Glandulae

The two fatigue tests yielded similar results. The force output began to decrease after approximately 13 stimuli (Fig. 4). The muscle thereafter declined to slightly less than 50% by the 35th stimulus (Fig. 4). Additional stimuli resulted in continued force decline, although at a slower rate.

Twitch venom pressure

In every specimen, the relationship between twitch EMG area and venom pressure area was best described by a power curve with an exponent of approximately 0.70. Although these curves represented the best fit to the data points, they always explained less than 40% of the variation in the data, as indicated by the r^2 values (Fig. 5). When the data from all five specimens were

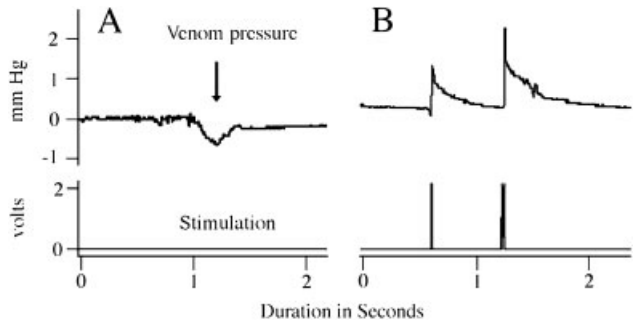

Fig. 3. Data tracing from the non-pressurized fang sheath elevation showing the retrograde venom flow (arrow) typical of those experiments (**A**) in contrast to twitch pressures (**B**) taken from the same preparation approximately 60 sec later in which the Compressor Glandulae is contracted.

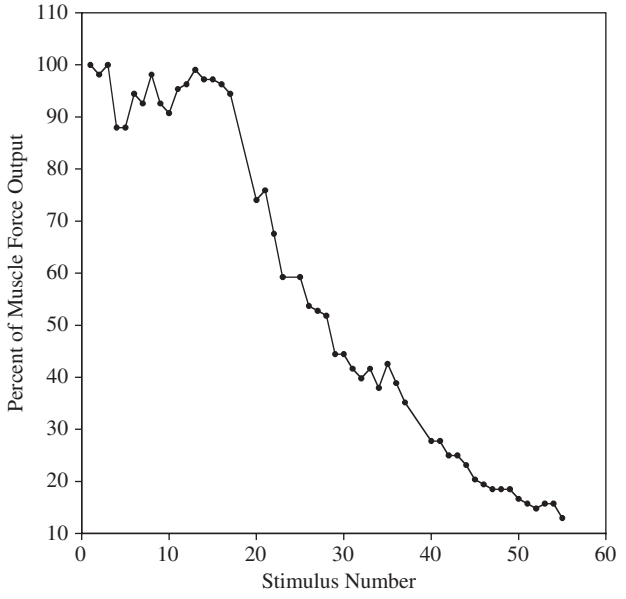

Fig. 4. Fatigue curve for the Compressor Glandulae. Note that with the exception of a short physiological ''rally'' early in the fatigue test, the muscles has a relatively constant decline in force output over the course of the 5-min trial.

combined, and the venom pressures standardized, a power curve with similar exponent still represented the best fit, although the amount of variation explained decreases to less than 25% (Fig. 6).

Pressurized venom sheath

With the venom delivery system pressurized (stimulation of the Compressor Glandulae), manual lifting of the fang sheath produced a sharp and distinctive rise in venom pressure (Fig. 7). If the range of venom output is standardized and the pressure curve subtracted from the magnetoresistive curve, two prominent deviations

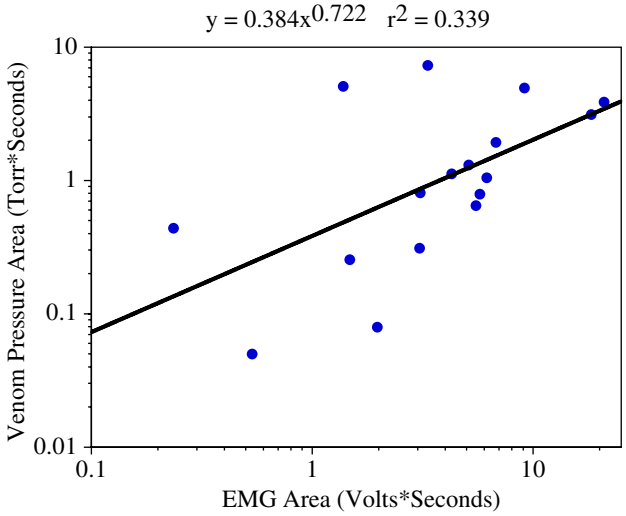

Fig. 5. Twitch venom pressure data from the 135 cm svl specimen of *Crotalus atrox*. Note the range in contractile activity of the Compressor Glandulae. The best-fit curve has the formula $y = 0.384x^{0.722}$, and an r^2 value of 0.339.

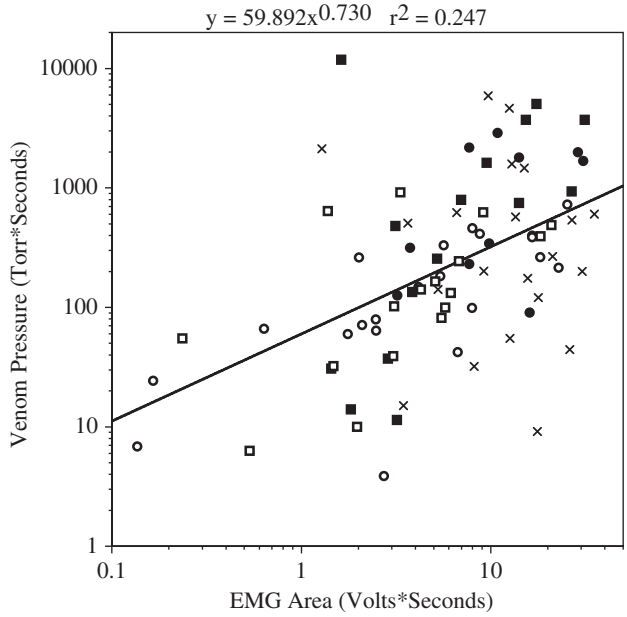

Fig. 6. Combined twitch venom pressure data set from all five specimens of *Crotalus atrox* after standardization of a mean venom pressure value. Note the marked variation in venom pressure for any given level of contractile activity. The best-fit curve has the formula $y = 59.892x^{0.73}$, and an r^2 value of 0.247.

from linearity are observed (Fig. 8). The larger deviation (Fig. 8B) is at the end of curve and is caused by the withdrawal of the magnet (and foam pad) combined with the failure of the fang sheath to naturally recoil. The smaller deviation occurs at the onset of fang sheath compression (Fig. 8A). This deviation is produced by a slight decrease in

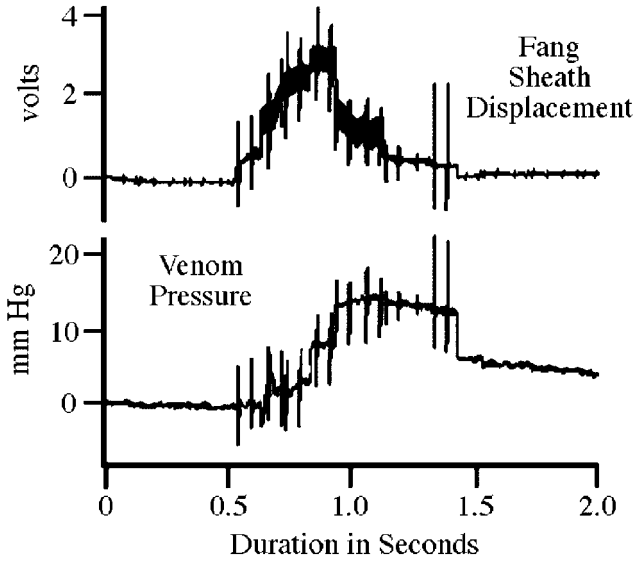

Fig. 7. Data tracing showing the elevation of the fang sheath (bottom tracing) and the associated change in venom pressure (top trace). The vertical line represents peak fang sheath elevation after which the magnet was withdrawn. Note the close relationship between the two curves during fang sheath elevation.

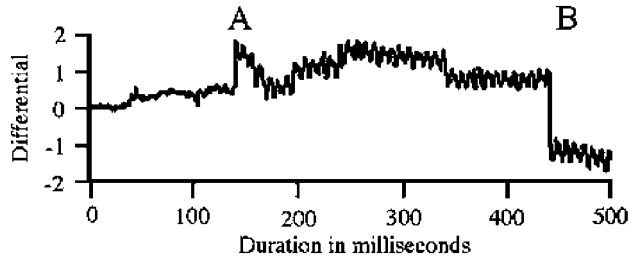

Fig. 8. Subtraction of the venom pressure curve from the magnetoresistive curve from Figure 7. Note the two prominent deviations from linearity. The first deviation (**A**) occurs at the onset of fang sheath elevation, while the latter, and larger deviation (**B**) occurs when the magnet is withdrawn but venom pressure does not rebound.

venom pressure at the onset of fang sheath elevation, and by a temporal delay between fang sheath elevation and the rise in venom pressure (Fig. 9).

In each snake, the peak venom pressures produced following the lifting of the fang sheath were approximately 10 × the mean peak pressures produced by the single twitch stimuli that were not accompanied by lifting the venom sheath (Fig. 10).

Pressurized fang retraction

Retraction of the fang, and corresponding displacement of the fang sheath produced a marked increase in venom pressure (Fig. 11). As fang retraction progressed, with stimulation constant, the venom pressure subsided but did not

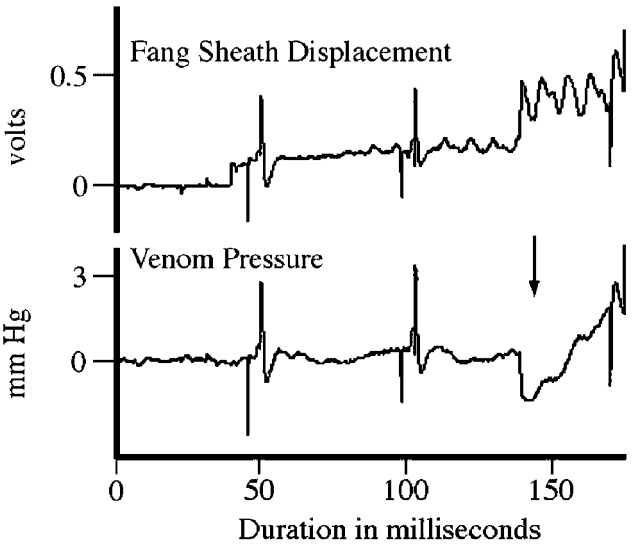

Fig. 9. Synchronized recordings of venom pressure (upper curve) and fang sheath elevation (lower curve) from the same trial episode. In this expanded section from the start of the trial the brief pulse drop in venom pressure (arrow) is evident, as is the slight temporal offset between fang sheath elevation and the rise in venom pressure. These two features combine to produce the initial deviation evident in Figure 8.

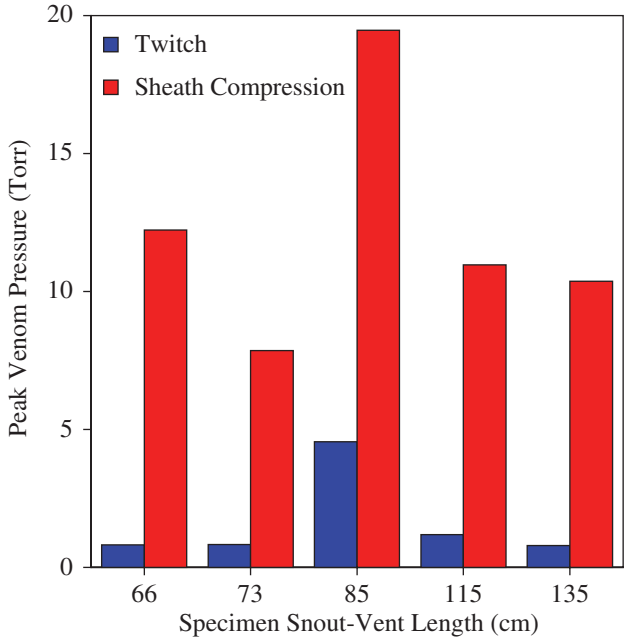

Fig. 10. Histogram of the peak pressures recorded during pressurized fang sheath elevation (solid) and the mean venom peaks produced by the muscle twitch experiments (open) for all five specimens. Note that the impact of fang sheath elevation is roughly 10 × that of the muscle compression.

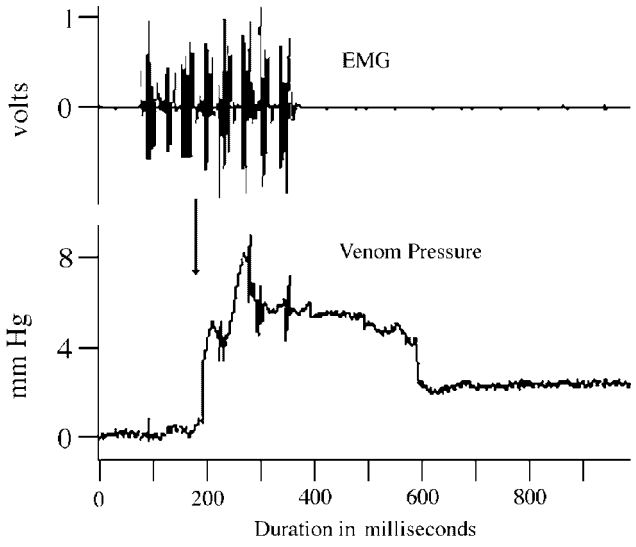

Fig. 11. Data tracing showing the change in venom pressure during pressurized fang retraction. Note that initial retraction of the fang causes an increase in venom pressure (arrow) but that with continued retraction the venom pressure decreases, even though the Compressor Glandulae is still contracting.

return to the baseline level. When the stimulation was terminated, and the fang at least partially rebounded to the protracted position, no clear pattern of change in venom pressure was observed (Fig. 11).

DISCUSSION

In both trials, the Compressor Glandulae showed rapid and sustained fatigue when exposed to repeated stimulation (Fig. 4). Although there appeared to be a brief physiological recovery in the early part of the fatigue test, the force output fell to approximately 50% of the initial value within 35 stimuli (Fig. 4). This fatigue profile is consistent with what was observed in a previous study (Young et al., 2000). The fatigue of the Compressor Glandulae informed and dictated the experimental procedures used in this study. The numbers of twitch stimuli applied were kept to a relatively low number and were temporally spread out in order to lessen the fatigue. To minimize fatigue effects, only one or two fang sheath elevations per trial were performed on each venom delivery system.

This study found only a weak relationship between differential muscle force (as induced by twitches in the Compressor Glandulae) and venom pressure. This weak relationship was present in the data obtained from each snake (Fig. 5), and in the combined data set (Fig. 6). The best fit between EMG and Pressure was a power curve with an exponent of 0.722, indicating a relative decrease in venom output with increasing muscle force. The underlying cause of this relationship could not be determined from this study.

The venom gland of *Crotalus* consists of extensive parenchyma supporting the venom secreting cells (e.g., Kochva, '78; Mackessy, '91). By using spatially localized twitch stimuli, produced by stimulating the surface of the muscle and not the motor nerve, our methodology would have preferentially expelled venom from a localized region of venom gland parenchyma. There may be a limit to how much localized venom could be extruded in this fashion, which could result in a relative decrease in venom output with muscle force. While this spatial localization might explain the exponent of the power curve, it would not account for the low level of venom pressure variation (r^2 values of under 0.25) explained by variation in muscle force.

This study used three experimental treatments to examine the effects of the fang sheath displacement. The results of each treatment revealed changes in venom flow with fang sheath displacement. Lifting of the fang sheath without pressurizing the venom gland (no muscle contraction) produced a decrease in venom pressure (Fig. 3). Lifting the fang sheath presumably altered the soft-tissue chambers within the fang sheath; this volumetric displacement moved retrograde (toward the venom gland) producing the drop in venom pressure at the tip of the fang.

When the venom gland was pressurized (Compressor Glandulae stimulated), manually lifting the fang sheath resulted in an increase in venom pressure (Fig. 7). A brief episode of negative pressure was also seen in these experiments as the fang sheath first started to compress (Figs. 8 and 9). The internal architecture of the fang sheath (Young et al., 2001a) including the internal fang membrane (a portion of the soft-tissue chambers) could physically obscure the entrance orifice of the fang. Lifting the fang sheath would then fully expose the entrance orifice to flow of the pressurized venom. This would explain both the sharp rise in venom pressure during manual lifting of the fang sheath and the rise in venom pressure during fang retraction (Fig. 11).

The difference in the directionality of the venom pressure change evident in the data tracings may reflect, at least in part, our experimental method. We connected the venom canal of the fang to a fluid-filled channel (tubing and pressure transducer). To minimize the loss of venom we kept the

exit port of the fluid transducer closed thereby creating a closed system. This meant that there was always a pressure head acting on the end of the fang tip. If the pressure within the venom gland was lower than the pressure head created by the pressure transducer, then venom flow would be retrograde, as it was during the non-pressurized fang sheath liftings. If the pressure within the venom gland exceeded the pressure head of the transducer, then changes in the soft-tissue chambers within the fang sheath would have created positive, not negative, venom flow. Although clearly the pressure head created by the transducer is artifactual, during a normal strike the fang is imbedded in the target tissue and thus encounters peripheral resistance. In fact, an early study of the kinematics of venom flow through the venom duct documented a pulse of retrograde flow at the termination of each venom injection episode (Young and Zahn, 2001).

The peak venom pressures recorded during fang sheath elevation were approximately 10 times the mean peak values obtained during the muscle twitch trials (with no fang sheath displacement) (Fig. 10). The methodology we employed ensured that this pressure difference was not the result of the muscle stimulation and/or venom gland pressurization (Fig. 2). Instead, the pressure differences are attributable to the alterations of the soft-tissue chambers within the fang sheath, and the internal architecture of the fang sheath.

Previous studies have documented a wide variation in the amount of venom injected, even during bites at the same target (e.g., Hokama, '78; Tun-Pe and Khin-Aung-Cho, '86). Other studies have shown differences in the amount of venom injected associated with differences in prey items (Hayes et al., '92; Hayes, '95). The results of this study suggest that two factors have substantial effects on the amount of venom injected (Fig. 12). The differences in the amount of venom injected could arise either through differential contraction of the Compressor Glandulae or through differential positioning of the fang sheath (thereby differentially affecting the enclosed soft-tissue chambers), or a combination of both. Although the explanatory power is low, there is a significant relationship between muscle force and venom pressure (Fig. 5). However, there is a stronger relationship between fang sheath elevation and increasing venom flow (Fig. 7). Since these two factors are both mechanically and functionally disconnected, there is no reason to conclude that a certain level of muscle activation would be associated a priori with a certain level of fang sheath displacement.

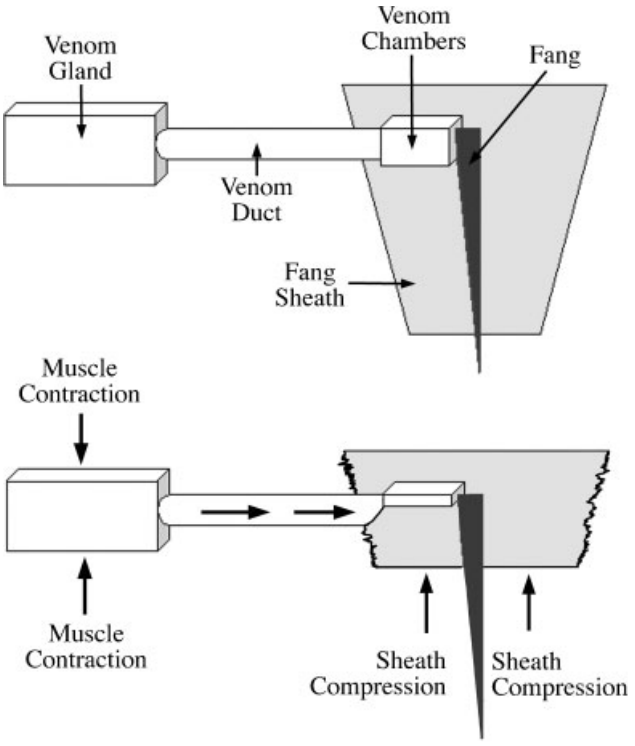

Fig. 12. Schematic of the venom delivery system. The contraction of the Compressor Glandulae would apply external force (arrows) to the venom gland which would translate into fluid pressure within the venom duct (arrows). Compression of the fang sheath caused by physical interaction with the target (arrows) would alter the volume of the soft-tissue chambers within the fang sheath. Differences in the magnitude and/or timing of these two factors would result in variation in venom expulsion.

This study concentrated on the relative magnitudes of the contraction of the Compressor Glandulae and positional changes of the fang sheath, and how these could influence venom flow. But we also recognize that the temporal pattern between these two—muscle contraction and fang sheath position—could affect venom flow. If the Compressor Glandulae contracted before fang sheath displacement, then the expelled venom could fill the soft-tissue chambers. The relative pressure within the soft-tissue chambers would reduce the effect of fang sheath displacement on venom pressure. In theory, these venom chambers could become so turgid with pressurized venom that lifting the fang sheath would affect neither the soft-tissue chambers nor venom pressure. Alternatively, lifting of the fang sheath prior to the contraction of the Compressor Glandulae would encounter minimal resistance in the venom

chambers, and thus maximal chamber deformation. A reduction in the volume of the soft-tissue chambers should maximize the volume of venom expelled. If this ''unimpeded'' deformation reached the extent that the chambers closed down, this kind of temporal imbalance could be a possible mechanism behind the phenomenon of ''dry bites'' (e.g., Silveira and Nishioka, '95). It is important to note that, as with the relative magnitude of the forces involved, there does not appear to be any mechanical or functional coupling of the Compressor Glandulae contraction and fang sheath elevation.

The results of this study suggest that venom injection in *Crotalus* is based on a two-stage mechanism, the contraction of the Compressor Glandulae and the displacement of the fang sheath. Recent experimental analyses of the functional morphology of venom ''spitting'' in cobras (Young et al., 2004) have demonstrated this same two-stage mechanism. Though different experimental analyses were applied, and the mechanics of venom expulsion are slightly different, the present study and the analysis of *Naja* (Young et al., 2004) both demonstrated that the combination of compressor stimulation and fang sheath displacement has a supra-additive affect compared to either factor alone. This commonality of basic venom mechanics, between two lineages generally taken to have independently evolved their venom delivery systems (see Jackson, 2003), suggests a high degree of functional convergence within this system.

This study was undertaken, in part, to compare the predictions of the two hypotheses for differential venom flow—venom metering and the pressure-balance hypothesis. The venom metering hypothesis emphasizes neural regulation and preparation of venom volume prior to impact with the target (Hayes et al., 2002). The results of this study suggest that neural regulation of the Compressor Glandulae is not strongly correlated with the amount of venom injected; muscle contractile force was a poor predictor of venom pressure, and the relative influence of muscle contraction on venom flow was roughly 1/10th that of the fang sheath. The lack of active regulation of the fang sheath is not surprising, as it is devoid of smooth or skeletal muscle.

It could be argued that since the snake strike is a ''voluntary'' action, the snake is regulating the forces acting on the penetrating fang (and thus, indirectly, on fang sheath position) as well as on the relative timing of fang sheath displacement and coordinated contraction of the Compressor Glandulae. However, quantitative studies of the snake strike have consistently revealed high levels of kinematic variation (e.g., Kardong and Bels, '98; Young et al., 2001b; LaDuc, 2002) and errant strikes (Kardong, '86); the strikes of most snakes, particularly the well-studied vipers, use more of a ballistic lunge than a carefully controlled positioning of the fangs. For a rattlesnake to actively regulate the timing of fang penetration and the contraction of the Compressor Glandulae, it would require that the snake could exactly determine the distance to the target, precisely control the velocity of the strike, and be able to factor in movements of the target and fang penetration angles. There is no evidence for this level of control in the snake strike.

The results of the present study are in general agreement with the predictions of the pressure-balance hypothesis (Young et al., 2002). This hypothesis posited that differential venom injection could be achieved through differences in the Compressor Glandulae, the fang sheath and enclosed soft-tissue chambers, and by differences in the peripheral resistance of the target tissue. The present study examined the first two possible sources of venom variation, which should be shared by all forms of venom expulsion. Among the different forms of venom expulsion, only venom injection incorporates peripheral resistance. Earlier laboratory (Young et al., 2003) and field (Young and O'Shea, 2004) studies have explored the influence of peripheral resistance on differential venom flow.

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LITERATURE CITED

Haas G. 1973. Muscles of the jaw and associated structures in the Rhyncocephalia and Squamata. In: Gans C, Parsons T, editors. Biology of the Reptilia, Vol. 4. New York: Academic Press. p 285–490.

Hayes W. 1995. Venom metering by juvenile prairie rattlesnakes (*Crotalus v. viridis*): effects of prey size and experience. Anim Behav 50:33–40.

Hayes W, Kaiser I, Duvall D. 1992. The mass of venom expended by prairie rattlesnakes when feeding on rodent prey. In: Campbell J, Brodie E Jr, editors. Biology of the pitvipers. Texas Selva: Tyler. p 383–388.

Hayes WK, Herbert SS, Rehling GC, Gennaro JF. 2002. Factors that influence venom expenditure by viperid and

other snakes during predatory and defensive contexts. In: Schuett GW, Hoggren M, Douglas ME, Greene HW, editors. Biology of vipers. Utah: Eagle Mountain Publishing. p 207–233.

Hayes WK. 2004. The snake venom-metering controversy: levels of analysis, assumptions, and evidence. In: Hayes WK, Beaman KR, Cardwell MD, Bush SP, editors. The Biology of Rattlesnakes. California: Loma Linda Press. In press.

Hokama Z. 1978. Study on experimental envenomation by the Habu (*Trimeresurus flavoviridis*). The Snake 10:107–113.

Jackson K. 2003. The evolution of venom-delivery systems in snakes. Zool J Linn Soc 137:337–354.

Kardong K. 1986. The rattlesnake strike: when things go amiss. Copeia 1986:816–820.

Kardong K, Bels V. 1998. Rattlesnake strike behavior: Kinematics. J Exp Biol 201:837–850.

Kochva E. 1978. Oral glands of the Reptilia. In: Gans C, Gans K, editors. Biology of the Reptilia. Vol. 10. New York: Academic Press. p 43–161.

LaDuc TJ. 2002. Does a quick offense equal a quick defense? Kinematic comparisons of predatory and defensive strikes in *Crotalus atrox*. In: Schuett GW, Hoggren M, Douglas ME, Greene HW, editors. Biology of vipers. Utah: Eagle Mountain Publishing. p 267–278.

Mackessy S. 1991. Morphology and ultrastructure of the venom glands of the northern pacific rattlesnake, *Crotalus viridis oreganus*. J Morphol 208:109–128.

Rosenberg H. 1967. Histology, histochemistry and emptying mechanism of the venom gland of some elapid snakes. J Morphol 122:133–156.

Silveira P, Nishioka S. 1995. Venomous snake bite without clinical envenoming ('dry-bites'): a neglected problem in Brazil. Trop Geol Med 47:82–85.

Tun-Pe, Khin-Aung-Cho. 1986. Amount of venom injected by Russell's viper (*Vipera russelli*). Toxicon 24:730–733.

Young BA, O'Shea M. 2004. Analyses of venom spitting in African cobras (Elapidae: Serpentes). Afr Zool 40:71–77.

Young BA, Zahn K. 2001. Venom flow in rattlesnakes: mechanics and metering. J Exp Biol 204:4345–4351.

Young BA, Zahn K, Blair M, Lalor J. 2000. Functional subdivision of the venom gland musculature and the regulation of venom expulsion in rattlesnakes. J. Morphol 246:249–259.

Young BA, Blair M, Zahn K, Marvin J. 2001a. Mechanics of venom expulsion in *Crotalus*, with special reference to the role of the fang sheath. Anat Rec 264:415–426.

Young BA, Phelan M, Jaggers J, Nejman N. 2001b. Kinematic modulation of the strike of the western diamondback rattlesnakes (*Crotalus atrox*). Hamadryad 26:316–349.

Young BA, Lee CE, Daley KM. 2002. Do snakes meter venom? Biosciences 52:1121–1126.

Young BA, Phelan M, Morain M, Ommundsen M, Kurt R. 2003. Venom injection in rattlesnakes (*Crotalus*): peripheral resistance and the pressure-balance hypothesis. Can J Zool 81:313–320.

Young BA, Daley KM, Lee CE. 2006. A contribution to the anatomy of the fang sheath and terminal venom delivery system in snakes. Zool Anz, submitted for publication.

Young BA, Dunlap K, Koenig K, Singer M. 2004. The buccal buckle: the functional morphology of venom spitting in cobras. J Exp Biol 207:3483–3494.