J. King Saud Univ., Vol. 7, *Science* (1), pp. 63–69 (A.H. 1415/1995).

Effects of the Venoms of *Walterinnesia aegyptia* and *Echis coloratus* on Solute Levels in the Plasma of Albino Rats

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(Received 23 October 1993; Accepted for publication 27 March 1994)

Abstract. The effect of LD_{50} injections of *Walterinnesia aegyptia* and *Echis coloratus* venoms on plasma solute levels in rats have been investigated. *W. aegyptia* venom caused significant increases in plasma sodium and urea level and osmolality, together with a significant decrease in plasma potassium level. Five days of venom injection caused similar rise in plasma sodium and urea levels. However, *E. coloratus* venom resulted in a significant reduction in plasma sodium level together with a significant elevation in plasma urea concentrations and osmolality after 120 min. Five days of venom injection resulted in similar changes. Plasma potassium and chloride levels did not change significantly following *E. coloratus* venom injection after any time interval. The effects of the two snake venoms have been discussed in relation to their respective effects on the reabsorption of solutes by rat kidneys.

Introduction

The significant role of the solutes in body fluid balance is well known [1, pp. 1-28]. However, little is known of the effects of snake venoms on solute concentrations either in man or nonhuman primates following snakes bite. Reid [2], observed significant hyperkalemia in three patients fatally bitten by hydropheid snakes but a fourth fatal case was accompanied by a normal plasma potassium concentration. A sublethal dose of *W. aegyptia* venom caused an initial rise in blood sodium and decrease in blood potassium concentration in dogs, but the opposite occurred with the lethal dose of the snake venom [3]. Whereas, in the case of some Egyptian and African snakes, the sublethal dose of the venom has produced an eleveation in potassium and a decrease in sodium concentration in blood, but the lethal dose has resulted in the opposite in the blood of rabbits [4]. However, *Creastes creastes* venom has significantly decreased both serum sodium and potassium levels in anaesthetized rabbits [5].

The present study was designed to determine the effects of a sublethal dose of the venoms of the Saudi Arabia snakes, *W. aegyptia* and *E. coloratus*, on the plasma solutes of male albino rats over a period of time.

Materials and Methods

Experimental animals

Male albino Wistar rats weighing 200-250 g maintained on a normal chow diet were used. Food was withheld for 12 h prior to each experiment, but water was provided *ad lib*. The rats were divided into the following groups:

Group I

control group, the animals were injected i.p. with physiological saline (0.2 ml) and were killed one hour later.

Group II

The animals received a single LD_{50} dose of *W. aegyptia* venom (0.2 mg/kg) and were subdivided into four subgroups; a, b, c, and d of 6-8 rats each that were killed at 30, 60, 90 and 120 min postinjection respectively.

Group III

animals of this group received a single LD_{50} dose of *E. coloratus* venom (1.1 mg/kg) i.p. and were subdivided into two subgroups; a and b of 6-8 rats each that were killed 60 and 120 min postinjection, respectively.

Group IV

rats of this group were divided into two subgroups of 6-8 rats each was injected daily with a LD_{50} i.p. dose of snake venom for five consecutive days.

Venom

Venoms were milked from adult *W. aegyptia* and *E. coloratus* snakes as previously described [6] and LD_{50} were determined from two different dose mortality curves set up for each venom.

Blood analysis

All experimental animals were killed by decaptiation and blood was collected into centrifuge tubes coated with EDTA and centrifuged at 600 g for 15 min. Plasma was divided into several aliquots for solutes anlayses in the same day, or stored at –20°C for later use.

Plasma samples were analysed for sodium and potassium using a flame photometer (NOVA 1, Massachusetts), for chloride using a chloride meter (PECL M_3, Jenway, England). for osmolality using an advanced osmometer (Micro-Osmometer, Model 3MO, Massachusetts) and for urea using kits from Ames, USA.

Statistical analysis

The data are presented as means ± standard errors (S.E.) of means. Statistical analysis was performed using a student's 't' test for unpaired samples.

Results

Effect of *W. aegyptia* venom on plasma solute levels

Plasma sodium level of animals envenomated with LD_{50} dose of *W. aegyptia* venom significantly increased ($P < 0.001$) only after 60 and 90 min time intervals, with no significant changes at any other time intervals. While plasma potassium level decreased over almost all time intervals, the decrease was only significant ($P < 0.001$) after 90 min of venom injection (Table 1). Moreover, there was a significant rise ($P < 0.05$) in plasma urea level in animals of group IV together with significant increase ($P < 0.05$) in plasma osmolality 60 and 90 min postenvenomation which become even more significant ($P < 0.001$) following five days of venom treatment. Small, non-significant variations occurred in plasma chloride concentration over all time intervals following envenomation (Table 1).

Table 1. Effects of *Walterinnesia aegyptia* venom on plasma solute levels in albino rats

Solute	Group I	Group II				Group IV
	Control Mean ± SE	a (30 min) interval Mean ± SE	b (60 min) interval Mean ± SE	c (90 min) interval Mean ± SE	d (120 min) interval Mean ± SE	a (5-day) interval Mean ± SE
Sodium (μ mol. ml^{-1})	143.6 ± 0.4 n = 8	142.9 ± 0.66 n = 8	145.5 ± 0.43** n = 7	146.6 ± 0.92** n = 6	143.4 ± 0.83 n = 6	145.1 ± 1.21** n = 6
Potassium (μ mol.ml^{-1})	6.62 ± 0.08 n = 8	6.62 ± 0.18 n = 8	6.45 ± 0.23 n = 6	5.27 + 0.13** n = 6	5.85 ± 0.56 n = 6	5.99 + 0. n = 6
Chloride (μmol.ml^{-1})	94.4 ± 0.29 n = 8	96.00 ± 1.99 n = 7	96.88 ± 1.67 n = 8	97.33 ± 1.38 n = 6	96.67 ± 1.23 n = 6	91.5 ± 1.43 n = 6
Urea (μ mol. ml^{-1}) n = 8	6.44 ± 0.29 n = 8	6.43 ± 0.30 n = 6	6.27 ± 0.48 n = 6	7.64 ± 0.56 n = 7	6.05 ± 0.41	8.01 ± 0.60
Osmolality (Osmole. g^{-1} H_2O)	291.3 ± 1.5 n = 8	287.4 ± 1.8 n = 8	298.0 + 2.8* n = 8	299.0 ± 2.8* n = 6	292.8 + 1.7 n = 6	306.7 ± 1.7** n = 6

Group II: a, rats examined 30 min after venom injection; b. rats examined 60 min after venom injection; c, rats examined 90 min after venom injection;
d, rats examined 120 min after venom injection.

* = significant

** = highly significant

Effect of *E. coloratus* venom on plasma solute levels

Plasma sodium levels of rats envenomed with a LD_{50} dose of *E. coloratus* venom decreased significantly after 120 min ($P < 0.05$) and over five days ($P < 0.01$) of venom treatment. While plasma urea concentration was elevated significantly ($P < 0.001$) after 120 min and over five days of venom treatment, neither plasma potassium nor chloride level changed significantly over all time intervals tested (Table 2). Moreover, an elevation in plasma osmolality was also observed in all injected rats, but it reached a significant value ($P < 0.001$) only after 120 min of venom injection (Table 2).

Table 2. Effects of *Echis coloratus* venom on plasma solute levels in albino rats

Solute	Group I	Group III		Group IV
	Control Mean ± SE	a (60 min) interval Mean ± SE	b (120 min) interval Mean ± SE	b (5-day) interval Mean ± SE
Sodium (μ mol. ml^{-1})	143.6 ± 0.42 n = 8	143.8 ± 0.45 n = 8	140.7 ± 1.14* n = 7	136.9 ± 1.99** n = 6
Potassium (μ mol.ml^{-1})	6.62 ± 0.08 n = 8	6.87 ± 0.07 n = 7	6.89 ± 0.34 n = 6	6.94 ± 0.29 n = 6
Chloride (μ mol.ml^{-1})	94.4 + 1.02 n = 8	97.63 ± 1.64 n = 8	92.63 ± 1.99 n = 7	94.17 ± 2.32 n – 6
Urea (μ mol. ml^{-1})	6.44 ± 0.29 n = 8	7.98 ± 0.77 n = 7	10.61 ± 0.38** n = 8	9.41 ± 0.93** n = 6
Osmolality (Osmole.g^{-1} H_2O)	291.3 ± 1.5 n = 8	293.9 ± 3.3 n = 7	298.4 ± 1.2** n = 8	298.2 ± 3.8* n = 6

Group III: a, rats examined 60 min after venom injection; b. rats examined 120 min after venom injection
Group IV: b, rats injected with venom for five days.
* = significant
** = highly significant

Discussion

The present study extended that of Mohamed [3] in dogs, and provide more details on the effects of a sublethal dose of *W. aegyptia* venom on plasma solute concentrations. The significant rise in sodium and decrease in potassium levels after 60 and 90 min of venom injection could be due to a direct effect of venom on reabsorption of the two solutes by the kidney, possibly due to a moderate congestion of the kidney or to cortical subcapsular haemorrhages similar to those inflicted by the

venom on the kidneys of mice [7]. This might also be due to an acute renal failure and glomerular damage as have been reported following viper bites [8; 9, pp. 997-1012], or possibly through direct stimulation of the adrenal cortex which leads to secretion of aldosterone. Such a mechanism has been suggested to explain similar data obtained after the injection of venoms of some Egyptian and African snakes into rabbits [4]. It is also likely that part of the venom effect is due to the release of catecholamines which are known to influence sodium reabsorption via stimulating renin secretion [10, pp. 119-131].

The increase in plasma osmolality observed after 60 and 90 min and over five days of venom injection might reflect an increase in sodium and urea concentrations during those periods. While the rise in plasma urea levels observed over five days of venom injection might suggest a direct effect of the venom on the ability of the kidney to concentrate urea which correlates with data previously reported on the elevation of plasma proteins following the venom injection [11]. The absence of significant changes in the levels of plasma solutes after 120 min of venom administration could probably be due to the adaptive mechanisms of the rats to overcome the effects of the venom as in stress conditions. However, such adaptation did not seem to last for long, as the changes in the levels of plasma solutes were the dominant features in rats injected for five days with the venom. On the other hand, the effects of *E. coloratus* venom on plasma sodium and potassium levels followed a different pattern. Though the effect on plasma sodium levels observed during the present study are different from those observed in rabbits treated with *Cerastes vipera* venom [4], yet they are in agreement with those observed in rabbits injected with either *Cerastes cerastes* venom [5], or with scorpion venom [12-14]. Various mechanisms have been suggested to explain the decrease in plasma sodium and the increase in plasma potassium levels following envenomation and these include a reduction of aldosterone secretion, which is secondary to the inhibition of the adrenal cortex activity [4]. Meanwhile, the rise in plasma urea level observed with *E coloratus* venom in the present study is in agreement with that observed in rabbits injected with scorpion venom [14], and could probably be due to the impairment of renal function [7; 14]. Such a rise might explain the increased plasma osmolality observed in the present study after 120 min of venom injection. However, the plasma chloride level did not vary significantly with the venom of both snakes used in the present study. This might reflect the stability of chloride reabsorption by the kidneys of rat, since sodium and chloride ions do not always move together in the nephron [15, pp. 136].

The variations in the effects of the venom of both snakes used in the present study, especially on plasma sodium and potassium levels might emphasize the importance of variability in the venom of snakes of different species.

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دراسة تأثيرات سُم الثعبان الصلّ وحيّة السجّاد الشرقي على تركيز الأملاح في بلازما الجرذان

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(استلم في ١٤١٤/٥/٨هـ؛ قبل للنشر في ١٤١٤/١٠/١٥هـ)

ملخص البحث. لقد تمّ في هذا البحث دراسة تأثيرات الجرعة نصف المميتة من سُم الثعبان الصلّ وحيّة السجّاد الشرقي المحضّر من ثعابين جمعت من المملكة العربية السعودية، على تركيزات الأملاح في الجرذان بعد حقنها في أوقات مختلفة. ولقد أظهرت النتائج أن سُم الصلّ قد أحدث زيادات معنوية لتركيز الصوديوم واليوريا والأسموليّة في البلازما ونقصًا معنويًا لتركيز البوتاسيوم وذلك بعد الحقن بأوقات متفاوته. كذلك أظهرت الدراسة عدم حدوث أي تأثير لسُم الصلّ بعد الحقن بنصف ساعة فقط على أي من الأملاح التي تمت دراستها. أما سُم حيّة السجّاد الشرقي فقد سبب نقصًا معنويًا لتركيز الصوديوم وزيادة معنوية لتركيز اليوريا والأسموليّة وذلك بعد ساعتين وخمسة أيام من حقن السُم. كذلك أظهرت الدراسة عدم تأثير تركيز كل من أيونات البوتاسيوم والكلوريد بعد الحقن في الأوقات المختلفة وكذلك لم يتأثر تركيز الأملاح التي درست بعد الساعة الأولى من حقن سُم حيّة السجّاد الشرقي.

إن تأثيرات كل من سُم الصلّ وحيّة السجّاد الشرقي على تركيزات الأملاح المختلفة في البلازما ربما يكون سببه تأثير هذا السُم بطريقة مباشرة أو غير مباشرة على وظيفة الكُلى الفسيولوجية لامتصاص تلك الأملاح في الجرذان.