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THE INFLUENCE OF CRUDE COBRA VENOM ON ELECTROLYTE LEVELS AND Na^+, K^+ ATPase ACTIVITY IN ERYTHROCYTES LIVER AND HEART CELLS

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ABSTRACT

The present study focuses on the in vitro interaction between snake venom and electrolytes and Na^+, K^+ - stimulated ATPase system in number of tissues from humans and animals.
The venom produced a change in the resting cellular sodium and potassium levels in all the tissues tested. When compared only human cancerous tissue showed apparent fall in sodium and a rise in potassium, while no significant change occurred in normal.
Me effect of snake venom (8ug/ml) on sodium pump activity, a transport enzyme, assessed in normal tissues. Human erythrocyte membrane pump was significantly was inhibited than rabbit heart and liver tissue.
With subsequent increase of dose no further change took place in these cells. A non significant and comparable stimulation in the enzyme activity was seen with rabbit heart and liver.
These findings demonstrate that human erythrocytes are more sensitive towards the venom and secondly that venom might potentiate its cellular function by modifying the sodium pump activity.

Introduction

The role of snake venom in the treatment of cancer has been subject of numerous investigators. Most of the data reported have suggested that snake venom contains components with a positive anticancer activity (Cotias *et al,* 1975) which seems to stimulate the process of tricarboxylic acid cycle (Kotiani *et al,* 1978), destroy cells during mitotic division (Dustin *et al*, 1939) or interfere with the transport mechanism of cellular membrane (Fillmore *et al.*, 1969).

Since many workers have observed a change in membrane permeability of various tissues when treated with certain poisonous substances (Fillmore *et. al*, 1969; Gerrensser, 1977) it was thought that these substances might influence the normal regulatory mechanism controlling cell growth Cole, 1973; Giosselin *et. al*, 1977).

Taking into account the abnormal electrolyte concentrations in tumor cells (Butts *et. al*, 1927; Koster, 1936), association of increased sodium levels to DNA synthesis (Cone, 1971) and heterogenecity of components of cobra venom the present study was undertaken to investigate the effect of the venom on various tissue electrolytes in order to see venom produces any change in already disturbed electrolyte levels in cancerous tissues. An attempt has also been made to asses whether the venom can alter sodium, potassium ATPase activity.

Material and Methods

Venom was collected from living Cobra snake by pressing the fange against the side of a test tube to evacuate the salivary gland content (maximally 3 drops, 0.8ug). Cobra is one of the most commonly found snake in deserts of Tharparkar, Sind, Pakistan.

Three species of animals (Rabbits, Rats, and Mice) were used in this study. The animals were sacrificed and the liver and heart were excised immediately and kept on ice. Slices were cut out in a buffer solution for electrolyte determination. The remaining tissues were homogenised in 20 vol. of a medium containing 0.25M sucrose buffered with Tris-HCL (pH 7.4). The homogenate was kept on ice and assayed the same day.

Human cancerous tissues (Liver and thyroid) were obtained from Atomic Energy Medical Centre, Liaquat Medical College Hospital, Jamshoro.

Erythrocyte membranes were prepared according to the method of Cole (1973). Blood samples were collected from six healthy adult students (both sexes) from the Department of Physiology, at University of Sind Jamshoro.

All the tissue slices, homogenates and erythrocytes were incubated with varying concentrations of snake venom for 30 minutes at 37°C in a shaking water bath.

The electrolytes (Na^+ and K^+) in serum and other tissues were detected by flame spectrophotometry (FGA 330-C). Na^+, K^+-ATPase activity was determined by measuring the amount of inorganic phosphate (Pi) released during the incubation as described by Muszbek *et. al*, (1977). All assays were run in triplicate. The enzyme activity was stopped by trichloroacetic acid (20%). Inorganic phosphate was determined according to the method of Ellengaard and Dimitrov (1973) using Spectran 20 (Bauch & Lumb) spectrophotometer.

Results for control and experimental groups were compared using Students 't' test.

Results

The effect of cobra venom (25ug/ml) on Na^+ and K^+ levels in various tissues has been studied.

Resting electrolyte values of normal and diseased liver slices and those after incubation with snake venom are shown in table-I.

During the in vitro treatment of 30 min, the venom did not produce any change in both Na^+ and K^+ in all the normal. This indicated a negligible influence of venom on these animal tissues.

In contrast, the incubation of same dose with human cancerous tissue caused a significant reduction (P.05) in Na^+ and a rise (P.05) in K^+ levels. Similar response (not shown) was also noted with thyroid when treated accordingly.

Although we failed to get more thyroid tissues to confirm, the data collected so far suggests the venom's ability to influence cell permeability in cancer state.

The influence of snake venom (25ug/ml) on tissue/membrane Na^+, K^+ - ATPase system in a variety of normal tissues is shown in table-II. The corresponding values regarding the inhibition of enzyme activity indicate that erythrocyte membranes seemed to be more sensitive to that of hepatic and cardiac tissues. The variation in response shows the discrimination made by the venom probably on the basis of cellular structural configuration.

Table-II

Tissues		Controls Mean+SEM	Treated Mean+SEM	Percent Inhibition
Human RBCs	(6)	1.49+0.16	0.668-0.21	54.4%
Rabbit Liver	(6)	5.38+1-57	5.08-1.04	5.6%
Rabbit Heart	(6)	6.83+1.43	5.38 + 1.51	21.2%

Na^+, K^+ - Stimulated ATPase activity (u moles Pi released/mg protein/30 min) of controls and treated tissues.

Fig. a b c illustrates the dose-response curve for the effect of venom on human erythrocytes (a), rabbit liver (h) and rabbit heart (c). In the erythrocytes the lower dose used (25ug/ml) produced maximal inhibition of Na^+, K^+ - ATPase. Conversely, the increasing doses did not produce any significant change in hepatic/cardiac ATPase activity. However, what would happen at doses lower than 25ug/ml we don't know at present and further investigation is required. From these observations it may be assumed that cells from different tissues differ in their susceptibility to venom.

Discussion

The results of present study demonstrate that snake venom does contain certain active components which influence the cellular membrane to alter the access of electrolytes particularly in cancer cell.

A change in membrane permeability has been proposed to be the primary cause of cell proliferation resulting in metabolic disturbances (Gosselin *et. al*, 1977) and excessive lactate production (Eligenbradt and Glossman, 1980). Other invest tigators have shown altered or abnormal levels of sodium and potassium in tumor cell (Butts *et. al*, 1927; Moravek 1932) and it was claimed that intracellular Na^+ and K^+ levels affect the process of protein synthesis (Panet and Alton, 1979; Lubin, 1980).

In the study described here we evaluated the effect of snake venom on electrolytes by comparing Na^+ and K^+ levels in normal animal liver slices and human cancer tissues (Liver and Thyroid). The data presented (Table-I) show that at resting stage the diseased tissues have significantly higher values for Na^+ and K^+ but not very different to that of normal. This might be due to species difference or the drug therapy they were kept on. Although, we did not get significant difference as one could expect, however, it may be suspected that membrane functional activity certainly gets affected when normal cell transforms into a malignant one as has been reported previously by some workers that rapid changes in cation transport are associated with rapid growth stimulation (Rozengurts and Mendoza, 1980; deLaat *et. al*, 1982).

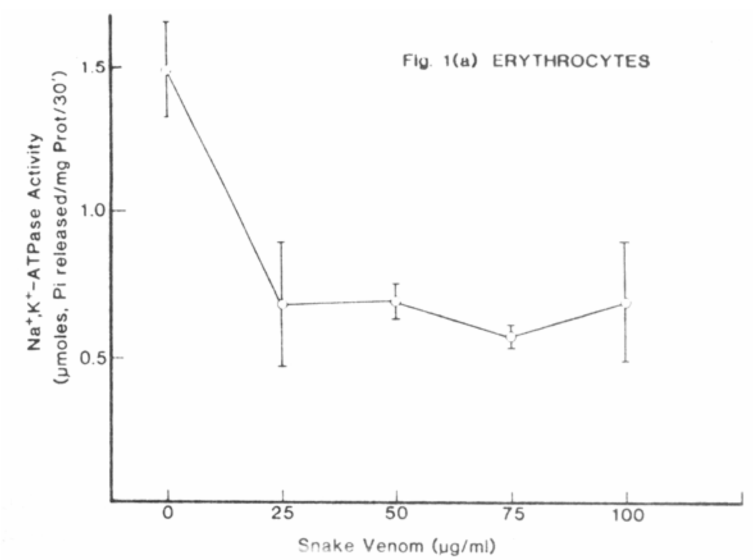
Table-I

		Sodium (mmol/L) Mean ± S.E.M.		Potassium (mmol/L) Mean ± S.E.M.	
Subject		Control	Experimental	Control	Experimental
Animals					
Rabbit	(6)	554.85±22.69	552.45±20.50	8.24±0.41	7.91±0.39
Rat	(6)	513.00±17.79	510.62±18.87	7.04±0.47	6.92±0.35
Mice	(6)	260.10±1257	253.45±13.80	4.73±0.30	4.52±034
Humans					
Patients	(6)	622.5±13.84	532.00±10.27	12.06±0.58	18.06±0.65
Normal		42.40	-	75.00	-

The effect of crude snake venom (25ug/ml) on liver tissue Na^+ and K^+ levels, obtained from various normal animals and human cancerous patients.

In order to investigate whether snake venom can modify the situation by affecting the cellular permeability towards certain ions, we incubated these tissues with 25ug/ml and measured the electrolyte levels. It may be seen from the table that a significant fall in Na and a rise in K has occurred in both the cancerous tissues while no comparable change was noted in normals. This indicated that venom might be acting through active substances on cellular surface (yet to be identified) which seems to he responsible to favour the free ionic movement in cancer and secondly, that cancer tissue seemed to be more sensitive towards venom as has been reported by Fillmore et al, (1969).

Recent investigations propose that various alkylating agents inhibit ions and amino acids fluxes across tumor cell membrane by inactivating Na^{+}, K^{+} -ATPase (Baxter *et al*, 1982; Ihlenfeldt *et. al*, 1981), or elevating CAMP levels (Halikov *et. al*, 1977). There are reports available in the literature that snake venom or its components are capable to inhibit the activity of ATPase enzyme (Mont, 1979; Zaheer and Braganca 1979; Zaki *et. al.*, 1967) and elevate CAMP levels (Halikov *et. al*, 1977) and this led us to concentrate our studies primarily on normal tissues (Human RBCs and animal liver and Heart). The results presented here (Table II) confirm that snake venom (25ug/ml) inhibits Na^{+}, K^{+} -ATPase activity in all tissues with a varied magnitude of response (maximum by RBCs and least by liver). This suggests that venom might discriminate different cell system on the basis of their structural configuration and thus strengthen the findings and view of Braganca (1978) that venom inhibits ATPase activity of those cells which are susceptibel to its destructive action. Similar results have also been reported by Zaheer *et. al*, (1979).



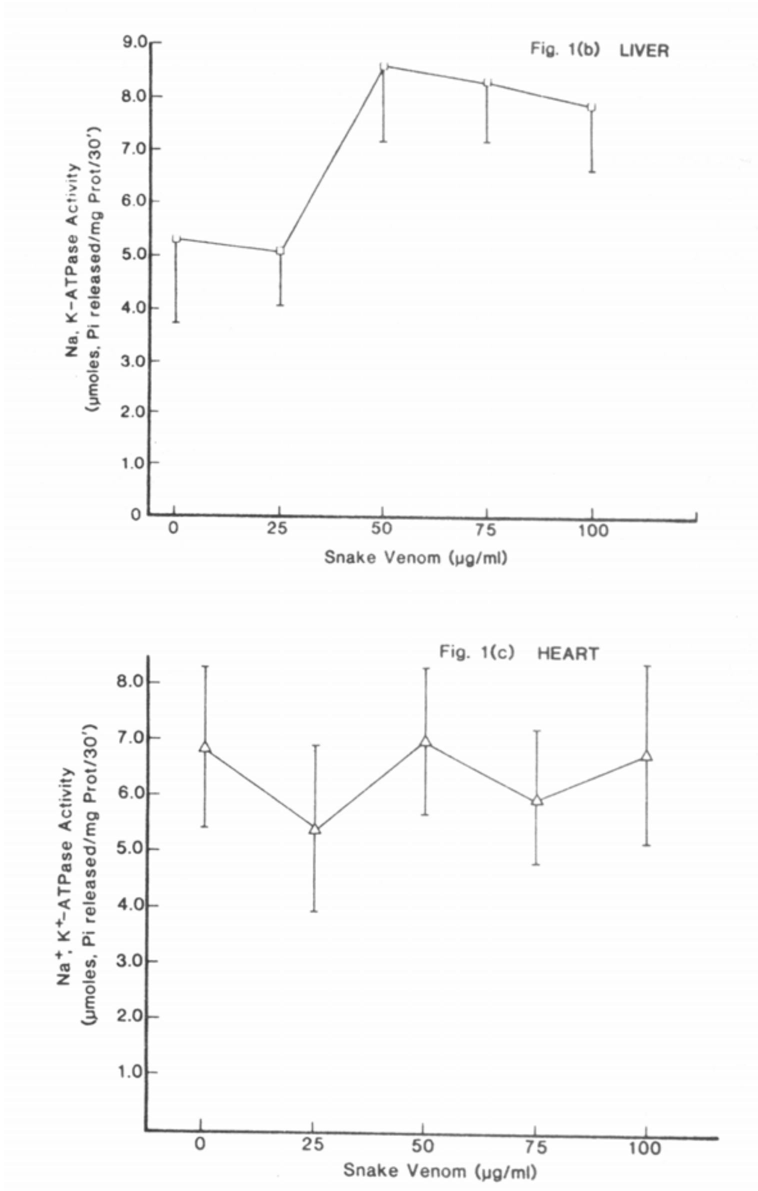


Fig 1. Showing the in vivo effect of varying doses of crude Cobra Snake Venom on cellular Sodium Potassium-ATPase activity in (a) human erythrocytes (b) rabbit liver and, (c) rabbit heart, incubated for 30 minutes.

Another interesting aspect of our studies is that with the increase of dose from 25 to 100u/ml) we revealed surprisingly a comparable elevation in the enzyme activity rather than a decrease in heart and liver tissues, whereas RBCs showed no further significant inhibitory change concomitantly (Fig. la, b, c). It is difficult to establish at present the exact mechanism involved for this elevation, however, it may be considered that the change might be due to some functional or neuroendocrinal changes taking place in order to alter membrane potential or electrochemical gradients.

Our present results can not allow us to arrive at any definite conclusions regarding the exact change in the transport enzyme activity but they conclusively show that venom in small doses cause inactivation of Na^+, K^+-ATPase. This might be one aspect through which venom interferes the cellular functions by inducing alterations in membrane activity to restrict the entry of certain substances into the cell which enhance or initiates rapid cell division. This also adds the weight to the hypothesis concerning venoms mode of action at cellular level.

It remains to be established whether these concentrations of cobra venom tested in this study show any different interaction with ATPase system of cancer cell and to workout appropriate concentration that really impairs abnormal cell growth.

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