A Presynaptic Effect of Whole Venom of *Dendroaspis jamesoni* on the Hemidiaphragm of Rat

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Summary. Dendroaspis jamesoni venom in a dose of 12.5 µg/ml restricts the uptake of Ca^+ leading to inhibition of release of Ach from the motor nerve terminals of the hemidiaphragms of rats.

Presynaptically active components are known to be present in *Bungarus multicintus* and *Notechis scutatus scutatus*[2-4]. In a recent paper it was shown that the effect of whole venom of *Dendroaspis jamesoni* on skeletal nerve-muscle preparations produced an irreversible depolarization type of postsynaptic block[5]. The authors, however, have not excluded the possibility of a multiple mode of action of the snake venom. In our paper we have shown that the venom acts on presynaptic nerve terminals by preventing the release of acetylcholine (Ach).

Materials and methods. Dessicated venom was obtained from Mr. J. H. Leakey, Baringo Snake Farm, P.O. Box 1141, Nakuru, Kenya. The phrenic nerve-diaphragm preparation obtained from male albino rats was set up in a 40 ml organ bath[6]. The Kreb's solution in the bath was maintained at a temperature of 37 °C. Experiments were also carried out at 20 °C. The phrenic nerve was stimulated by electrical pulses of 50 µsec duration at a frequency of 0.5 Hz and voltage 10 V. Control experiments did not show any reduction of response over a period of 3 h.

The changes in the Ca^+ concentration in the bath fluid during electrical stimulation were determined by an atomic absorption spectrophotometer (Pye Unicam SP 90B, series 2) with a wavelenth of 422.7 nm, maximum current 7 mA and slit width 0.1 mm. The gaseous mixture used was air: acetylene in a ratio of 5: 1.6 (v/v). A standard curve was drawn for each experiment with a Ca^+ concentration varying from 20 ppm to 100 ppm in steps of 10 ppm; the standard solutions of Ca^+ were made in Kreb's solution. It was found that the physiological Kreb's solution used for the experiments contained Ca^+ of the strength of 100 ppm.

The hemidiaphragms were immersed in physiological Kreb's solution and allowed to equilibrate. No electrical stimulation of nerve was carried out, and at the end of the equilibratory period of 30 min, 10 ml of the Kreb's solution was pipetted out (reading I). The tissue was stimulated for 3 min and 10 ml of bath fluid pipetted out during stimulation (reading II). Snake venom (12.5 µg/ml) was added to the bath and 10 ml of the bath fluid was pipetted during stimulation at intervals of 0, 5, 10, 15, 20, 25, 32 min (immediately after block) and 180 min (reading III). Each time the 10 ml of bath fluid was replaced by an equivalent quantity of Kreb's solution from the reservoir. The difference between reading I and II gave the amount of Ca^+ uptake before addition of the venom and difference between reading I and III gave the amount of Ca^+ uptake after addition of the venom.

In another series of experiments, the effect of snake venom on post-tetanic potentiation[7] and the inhibition of release of Ach[8] was determined. The biological indicator for assay of Ach was the hypotensive response in cats. It

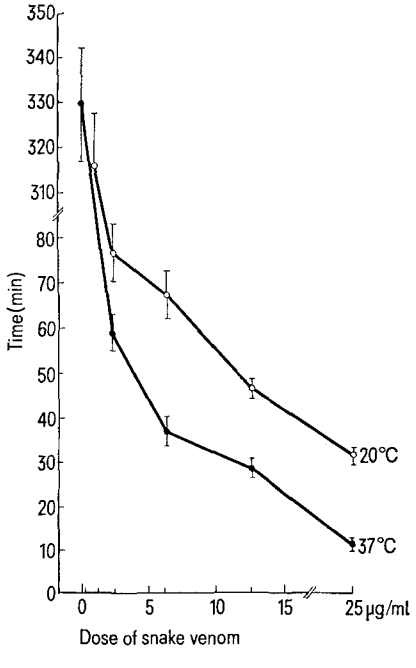

Fig. 1. Effect of snake venom on the time for complete block of hemidiaphragms of rats. The Kreb's solution was maintained at 20 °C and 37 °C. Snake venom (µg/ml) in doses of 0.25, 1.25, 2.5, 6.25, 12.5 and 25 was added to the bath fluid and the time for complete block determined. Ordinate: dose of snake venom (µg/ml). Abscissa: time in min. The values are the mean of 10 hemidiaphragms ± SE.

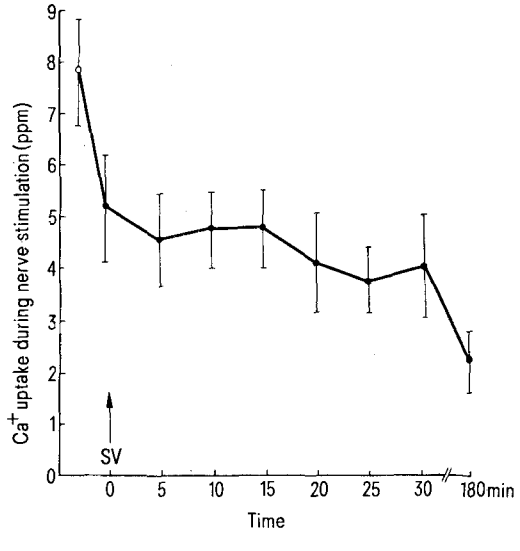

Fig. 2. Effect of snake venom (SV: 12.5 µg/ml) on the Ca^+ uptake during phrenic nerve stimulations in hemidiaphragms of rats. Ordinate: Ca^+ uptake during nerve stimulation in ppm. Abscissa: time in min. The values are the mean of 10 readings ± SE. ○, control readings; ●, readings after addition of SV.

was found that the rat blood pressure gave quite an amount of depressor artifacts.

Results and discussion. From Figure 1, it is evident that reduction of temperature from 37 °C to 20 °C increased the time for complete block of the neuromuscular junction at various doses of the snake venom (0.25, 1.25, 2.5, 6.25, 12.5, and 25 µg/ml). This is in agreement with the work of Patel and Excell[5], who have suggested involvement of at least two different types of mechanisms.

Post-tetanic potentiation was found to be depressed from the control values (considered as 100%) to 17.21% ± 7.84 (Mean ± SE; $p < 0.01$) and 2.07% ± 2.07 ($p < 0.01$) at 12 and 17 min respectively. The frequency of electrical pulses for post-tetanic potentiation being 20 Hz and repeated at intervals of 5 min after addition of 12.5 µg/ml snake venom to the bath. There was no post-tetanic potentiation at end of 22 min. Post-tetanic potentiation has been found to be a phenomenon concerned with presynaptic nerve terminals specifically dependent on the movement of Ca^+ and associated with an intracellular accumulation of Ca^+ during tetanus[9,10].

In Figure 2, the uptake of Ca^+ is reduced to less than half at the end of 32 min ($p < 0.01$) and the uptake of Ca^+ falls to about $^1/_4$th the control values after 180 min ($p < 0.001$). Ca^+ is essential for the release of Ach from presynaptic nerve terminals[11]. It was found that, in the present series of experiments, the Ach released from nerve terminals fell from the control values 78.10 ± 7.90 ng of the base to 26.28 ± 3.73 ng ($p < 0.001$) immediately after the complete block. The values fell to 15.43 ± 1.57 ng ($p < 0.001$) 180 min after the block.

From the present investigation it can be concluded that *D. jamesoni* venom has a multiple mode of action and one of the possible ways the venom acts is by affecting the release of Ach from presynaptic nerve terminals by restricting the uptake of Ca^+ necessary for the release of the neurotransmitter.

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The Influence of Salt Intake on Glomerular Count in Compensatory Kidney Hypertrophy in Rats of Different Ages

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Summary. Drinking saline instead of water elevated the glomerular count in hypertrophied kidneys of rats uninephrectomized as adults. No changes occurred in glomerular concentration in kideny tissue indicating a more marked increase of other kidney structures. This procedure was ineffective in immature animals.

The glomerular count was found to increase during postnatal kidney growth up to the age of 100 days[1,2], and even more so in kidneys undergoing compensatory hypertrophy induced by uninephrectomy before the 50th day of age[2]. At this period of development, a higher sensitivity of rats has been reported to hypertensogenic action of chronically increased salt intake[3]. The kidney plays a critical role in the pathogenesis of hypertension from the point of view of maintaining the water and salt balance[4] as evidenced, among other things, by the exaggerated hypertensive response to chronic excess salt ingestion in unilaterally nephrectomized rats[5]. Because of this, the influence of chronically increased salt intake on the number and 'concentration' of glomeruli was investigated in the normal and compensatory growing kidneys.

Methods. Male Wistar strain rats, maintained on a balanced pellet diet containing 1% NaCl and tap water ad libitum, were unilaterally nephrectomized at ages 18 and 80 days. 1 week after surgery, during which the young animals were left in the nest with the mother, an aliquot of these groups was exposed to a high salt intake (1% saline as the only drinking fluid) and the rest were left on the original dietary regime ('normal' salt intake). Intact animals of the same ages and maintained either on high or normal salt intake served as controls. At ages 55 and 117 days the animals were killed, the kidneys weighed and glomerular count determined by the method of Damadian et al.[6], as modified by Bonvalet et al.[2]. In order to compare the kidney weights in young and adult animals with different body weights at the end of the experiment, organ weight was expressed as: (mg organ wt./g body wt.^{2,3}) × 0.1, since in Wistar rats this expression does not change in the body weight range 30–680 g[5]. It corresponds basically to the formula used

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