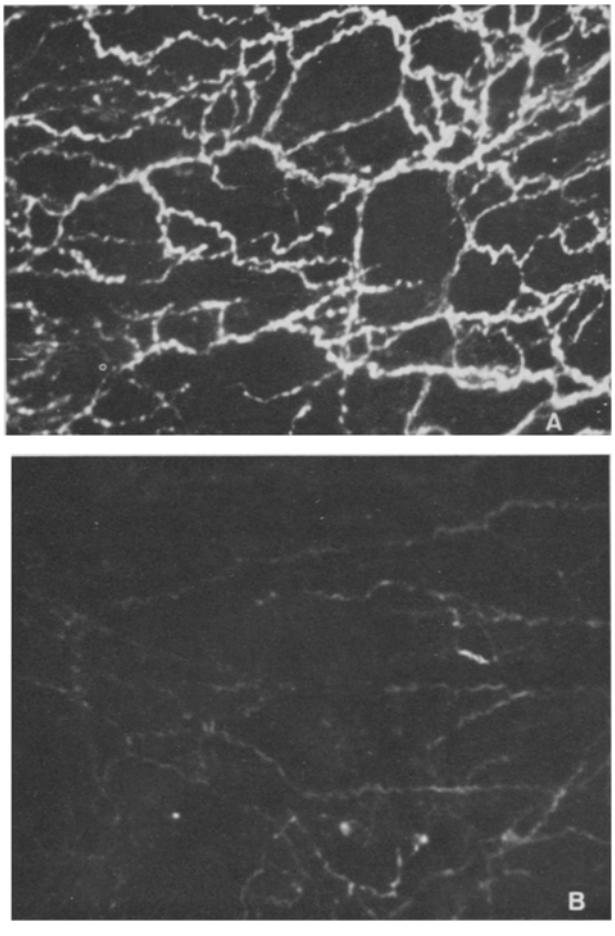
Catecholamine-Depleting Effect of Brazilian Scorpion (*Tityus serrulatus*) Venom on Adrenergic Nerves of the Rat Atrioventricular Valves

Physio-pharmacological studies have shown that scorpion venom is a potent autonomic stimulant, and the nerve endings of both sympathetic and parasympathetic autonomic nervous systems are the possible sites of the action of the venom[1–7]. The mechanism of this activity, however, remains unclear. It might act directly on the target, as many hormonal and vasoactive substances are known to do, or indirectly through the release of neurotransmitter from the postganglionic nerve endings.

The presence of noradrenaline and other amines in the adrenergic nerves can be demonstrated by means of the highly specific and sensitive fluorescence histochemical method of FALCK and HILLARP, see[8–13]. The semiquantitation of catecholamines by subjective estimation of the catecholamine content by eye has proved fairly trustworthy[14].

To our knowledge the effect of scorpion venom on the catecholamine content in the adrenergic nerves has not been investigated. Such a study is reported in this paper, which presents histochemical evidence of depletion of catecholamines in the sympathetic nerve endings of rat atrioventricular valves following administration of scorpion venom.



A) Atrioventricular valve of a control rat. Whole-mounted stretch preparation. ×250. B) Atrioventricular valve of a rat injected with scorpion venom (0.5 mg/kg of body wt., 60 min before death). Very weakly fluorescent nerve endings and barely seen varicosities. Whole-mounted stretch preparation. ×250.

Materials and methods. Adult albino rats (Wistar, 150–200 g) were used for the present study. They were divided into 4 groups of 8 animals each. The 1st group (control) was treated with an i.v. injection of normal saline, in a volume of 1 ml/kg, 60 min prior to sacrifice. The 2nd, 3rd and 4th groups were injected with a single i.v. dose of crude venom of the scorpion *Tityus serrulatus*[15] dissolved in saline, 0.5 mg/kg of body wt., respectively 30, 60, and 120 min prior to sacrifice. It was known from previous experiments[16] that this dose, when administered i.v., gave strong autonomic responses and did not usually kill the rat before the 2nd h.

Tissues from both control and experimental groups were processed in exactly the same fashion. All animals were killed by exsanguination under light ether anesthesia. The hearts were quickly removed, and as soon as the ventricular chambers had been opened, the atrioventricular valves were dissected with the help of a stereomicroscope. To obtain consistent and duplicable results, the heart valves were excised 10 to 15 min after sacrifice.

In accordance with FALCK-HILLARP's method, the excised valves were rinsed in cold calcium-free Tyrode solution for 2 min, stretched and mounted whole on glass slides and allowed to dry in the air at room temperature for 15 min. In our laboratory, the relative humidity of the air was maintained at approximately 50%, and this proved satisfactory for drying the valves and did not produce any serious changes in the morhpology of the adrenergic nerves as revealed by the fluorescence method. This agrees with other observations[8, 11]. The tissue was then treated with formaldehyde gas of optimal humidity (70%) generated from paraformaldehyde at 80 °C for 1 h, mounted with non-fluorescent immersion oil and examined with a Zeiss fluorescence microscope.

Results. Dense networks of adrenergic fibres could be demonstrated in the atrioventricular valves of control hearts. The fibres run in all directions throughout the entire extent of the valves, crossing one another, in a wavy course, and seem to penetrate deep into the valves. The appearance of the adrenergic nerves in control valves were very similar and can be compared to those described by other investigators[12, 17, 18].

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In Figure A) typical anastomosing fluorescent nerve fibres with their intensely fluorescent varicosities can be seen in a control atrioventricular valve.

A marked decrease in the specific fluorescence of the valvular nerve plexuses could be demonstrated following the i.v. administration of scorpion venom to the rats. The degree of reduction was dependent on the interval between administration and death. A general reduction was seen within 30 min, and after 60–120 min there were almost no nerves clearly visible in about 70% of cases. Only a few weakly fluorescent fibres remained visible. The decrease in fluorescence was located mainly to the varicosities.

Figure B) shows very weakly fluorescent nerve fibres and barely visible varicosities in an atrioventricular valve from a rat which had been injected with scorpion venom 60 min before death.

Discussion. The results of the present investigation clearly demonstrate that administration of Brazilian scorpion venom (*Tityus serrulatus*) to adult rats causes a depletion of catecholamines in the sympathetic nerves from the atrioventricular valves. Some of the symptoms of scorpionism in vertebrates are ascribed to a sympathomimetic overactivity. Elevation of plasma adrenaline content and of catecholamines and their metabolites in the urine in patients after scorpion sting has been reported[19,20]. More recently it was shown that scorpion venom produces an increase in blood pressure accompanied by a massive discharge of catecholamines into the blood[21]. The cardiac stimulation which follows administration of scorpion venom has been shown to be a peripheral phenomenon, probable through the release of tissue catecholamines[5]. These and other manifestations of scorpion envenomation, such as myocardial lesions[22], pulmonary edema[16], tachycardia[5], piloerection[23], and mydriasis[23], could be due to the release of noradrenaline and other amines from the adrenergic nerve endings. Scorpion venoms markedly influence excitable membranes, altering the permeability to various ions with resulting electrophysiological and physicochemical changes[24,25]. Probably scorpion venom releases catecholamines by activating the fundamental physiological mechanism.

Further experiments, to investigate the neurotransmitter-depleting action of Brazilian scorpion venom on sympathetic nerve endings, are in progress.

Zusammenfassung. Mittels Fluoreszenztechnik wurde ein mikrochemischer Nachweis der Freisetzung von Katecholamin an adrenergischen Strukturen in den Arterioventrikularklappen des Rattenherzens nach Verabreichung von Skorpiongift (*Tityus serrulatus*) erbracht.

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The Effect of Applied Tension on the Length of Striped Muscle

The effect of applied tension on skeletal muscle has considerable relevance to orthopaedic surgery. Forceful stretching of contracted muscles forms an important part of treatment in many patients with musculo-skeletal deformities. In addition, limb traction is frequently used to maintain the position of a fractured bone after reduction, or to overcome painful muscle spasm.

Although many workers have described the relationship between tension and length in isolated muscle fibres, or in actively contracting muscles, much less has been written on the effects of applied tension on individual muscles. In view of the importance of this aspect of muscle physiology in orthopaedic practice, a study of the effect of applied tension on the length of intact skeletal muscles was undertaken.

Materials and methods. 30 male Wistar albino rats weighing 285–400 g were used to assess the effect on muscle length of increasing, followed by decreasing tensions. The animals were divided into 3 groups of 10. Each group was used to study 1 of 3 selected muscles, namely the tibialis anterior, the tibialis posterior and the peronei taken as a single entity. The tendons of the 4 rat peroneal muscles together provide a tendinous cord above the ankle joint, just large enough to be handled.

An apparatus was constructed which allowed known tensions to be applied to the living muscles and permitted their length to be measured. It consisted of a wooden base, at one end of which a heavy wooden block was screwed down. The anaesthetised rat was laid upon this block and was prevented from sliding off it during application of tension by two small posts. The rat's tail lay between the posts and its hind limbs on either side.

A length of No. 36 gauge stainless steel wire was tied firmly to the distal part of the tendon of the muscle under investigation. The other end of the wire was fixed to the cantilever incorporated in a Pye TS1 strain gauge. This was rigidly mounted on a movable brass block whose movement was controlled by turning a finely threaded screw. The strain gauge and movable brass block were fixed to the wooden base of the apparatus opposite the anaesthetized rat (Figure 1). By moving the strain gauge in the longitudinal axis of the rat's hind limb, the tension in the muscle under investigation was varied as required.

The tension applied to a muscle was measured electrically by incorporating the strain gauge as one of the resistances of a Wheatstone Bridge. By applying tension to the strain gauge its resistance was altered. A galvanometer connected across the two ends of the bridge indicated any alterations in the balance of the circuit. The galvanometer was calibrated before each experiment by attaching known weights to the strain gauge. The range of tensions used varied from 0–200 g.

Measurements of muscle length under different tensions were made with a travelling microscope placed between the rat and the strain gauge (Figure 1). The rats were anaesthetized by an i.p. injection of 6–12 mg Nembutal,