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Red back spider venom and inhibitory transmission

The venom of the black widow spider, *Latrodectus mactans tredecimguttatus*, has been shown to cause release of transmitter from the aminergic systems of insects (d'Ajello, Magni & Bettini, 1971; Majori, Bettini & Casaglia, 1972; Cull-Candy, Neal & Usherwood, 1973; Griffiths & Smyth, 1973), from the crustacean neuromuscular system (Grasso & Paggi, 1967; Kawai, Mauro & Grundfest, 1972), and from the amphibian neuromuscular system (Clark, Hurlbut & Mauro, 1972). Similar effects of the venom have also been demonstrated in mammalian tissue supplied by adrenergic nerves (Frontali, Granata & Parisi, 1972) and cholinergic nerves (Longenecker, Hurlbut & others, 1970; Paggi & Rossi, 1971; Okamoto, Longenecker & others, 1971). It was previously reported that the venom of the Australian red back spider, *Latrodectus mactans hasselti*, causes the depletion of vesicles within the adrenergic nerve terminals of the mouse vas deferens, and also produces an increased frequency of spontaneous excitatory junction potentials in this tissue (Einhorn & Hamilton, 1973). As the action of the venom thus seems to be independent of the nature of the transmitter, it was of interest to study the effects of red back spider venom on the purinergic nervous system (Burnstock, 1972) of the guinea-pig ileum.

Segments of ileum isolated from adult guinea-pigs of either sex, were cut along the mesenteric border, and mounted with the serosal surface uppermost, in a 15 ml organ bath. Intracellular recordings were obtained with KCl-filled glass capillary microelectrodes of 60–100 Mohm resistance. The composition of the perfusion medium and details of circuitry have been described previously (Hashimoto & Holman, 1967). Transmural silver wire electrodes were used for electrical stimulation (pulses of 0·5 ms from a Grass S4 stimulator). 2×10^{-7} gml^{-1} atropine sulphate was added to the perfusion medium to prevent excitation due to stimulation of cholinergic neurons. Experiments were conducted at 25° to minimize spontaneous muscle movement. After each experiment the tissues were fixed in either glutaraldehyde in cacodylate buffer or glutaraldehyde in 110 mM magnesium solution, and examined with the electron microscope.

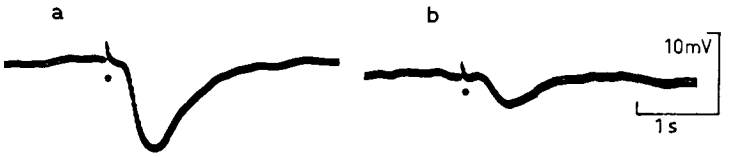

FIG. 1. Intracellular recordings from circular muscle cells of guinea-pig ileum, showing response to electrical stimulation. Dots indicate stimulus artifacts. (a) Control IJP. (b) Recording from same preparation 15 min after exposure to venom.

Intracellular recordings from the unstimulated tissue showed two types of muscle cell. The upper layer, composed of longitudinal muscle cells, had a resting membrane potential of 45 $\pm$ 8 mV, and the circular muscle cells below had a resting membrane potential of 58 $\pm$ 5 mV. Upon electrical stimulation with pulses of maximal intensity no response was observed in the longitudinal muscle cells. When recording from the circular muscle layer, a transmural stimulus evoked an inhibitory junction potential (IJP), that is a transient hyperpolarization of 12–16 mV of the muscle membrane potential (Fig. 1a).

The venom glands of two female red back spiders were removed and ground up in 1 ml of the perfusion medium. Flow through the organ bath was stopped, and the venom was added directly to the solution. In control experiments stopping the flow of the perfusion fluid for a similar length of time did not alter the resting membrane potential nor the response to electrical stimulation. Majori & others (1972) have shown that the crude gland extract, as used in this study, has a similar toxicity to gland lumen venom or purified venom obtained from a direct bite.

In 6 of 7 experiments the amplitude of the IJP was increased within 5 min of adding the venom. Subsequently the amplitudes of successive IJPs decreased until at 20 min after the addition of venom the IJPs were reduced to 20% of their control value (Fig. 1b). In the seventh experiment, recordings were first made 5 min after the venom was added and depression of IJP amplitude was observed. An experiment conducted at 37° resulted in complete abolition of IJPs within 15 min. In another experiment, the venom from 4 spiders was added to the bath and IJPs were again abolished. Return of flow of the perfusion medium and hence washout of the venom

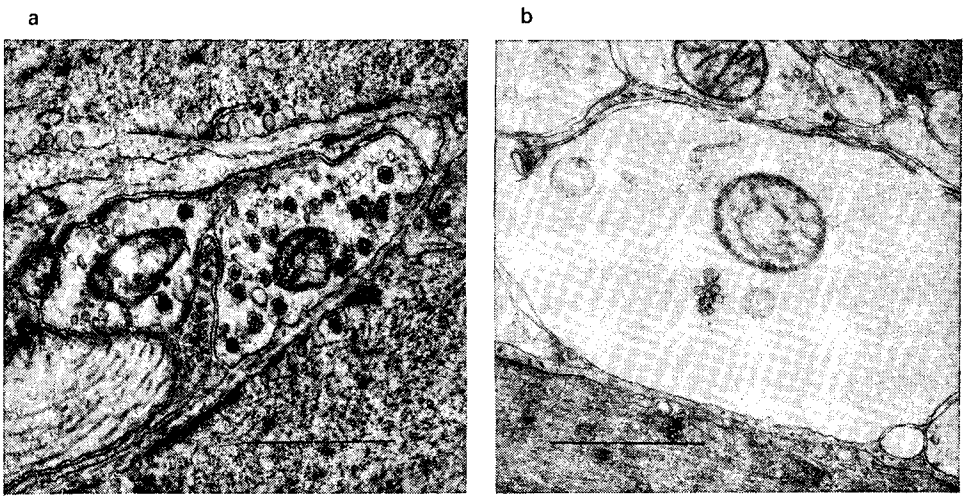

FIG. 2. Ultrastructure of nerve bundles in the circular muscle layer of guinea-pig ileum. (a Nerve bundle in control tissue. One nerve profile contains heterogeneous granular vesicles and another agranular vesicles (bar = 1 μm). (b) Nerve bundle from venom treated tissue, showing an empty dilated nerve profile (bar = 1 μm).

did not produce recovery of IJPs. Occasionally, small spontaneous hyperpolarizations of the resting membrane potential of brief duration compared with IJPs were observed in the presence of the venom. These may have been spontaneous inhibitory junction potentials, but were too infrequent to study in detail.

Studies with the electron microscope of tissues not treated with venom showed that the circular muscle layer was innervated by bundles of axons. These bundles were found in three regions: the myenteric plexus, bundles coursing through the circular muscle (sometimes in association with capillaries) and in a plexus separated from the submucosa by a thin layer of muscle cells. Each region contained at least three types of nerve varicosity. The first type had small agranular vesicles and some larger granular vesicles. The second contained many small granular vesicles, some small agranular vesicles and some large granular vesicles. The third type of varicosity consisted of large granular vesicles of varying electron density—the heterogeneous granulated vesicle described by Gabella (1972) and Baumgarten, Holstein & Owman (1970) and small agranular vesicles (Fig. 2a). The varicosities may respectively be the cholinergic, adrenergic and purinergic endings as described by Burnstock (1972).

In venom-treated tissues, few typical nerve varicosities could be found in Auerbach's plexus or the circular muscle layer. Large dilated electronlucent varicosities containing few vesicles were common (Fig. 2b). Nerve varicosities in the plexus above the submucosa seemed to be unaffected by the venom. This may be due to inaccessibility to the venom, as found in the rat and mouse vas deferens (Einhorn & Hamilton, 1973). The venom did not appear to preferentially destroy or spare a particular vesicle type.

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