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LETTERS TO THE EDITOR

Transmitter release by red back spider venom

The venom of the Australian red back spider (*Latrodectus mactans hasseltii*) has been found to cause the disappearance of noradrenaline fluorescence and granular vesicles in the rat vas deferens (Hamilton & Robinson, unpublished findings). The venom of the black widow spider (*Latrodectus mactans tredecimguttatus*) has been shown to eliminate noradrenaline fluorescence in the rat iris (Frontali, 1972). This venom greatly increases the frequency of miniature end-plate potentials at cholinergic junctions and depletes the nerve terminals of their vesicles (Longenecker, Hurlbut & others, 1970; Clark, Mauro & others, 1970). The theory that the small granular vesicles characteristic of noradrenergic terminals are involved in the release of transmitter has received considerable support from biochemical experiments (Smith, 1971). Physiological evidence for the packaged release of noradrenaline implicit in this hypothesis has been more difficult to obtain, owing to the syncytial nature of smooth muscle and the structure of its innervation (Bennett, 1972). However, intracellular recordings from smooth muscles where axon varicosities make close contact with the smooth muscle membrane (e.g. vas deferens) have shown that spontaneous excitatory junction potentials (EJPs) occur, which are not blocked by tetrodotoxin. It has been argued that these potentials are analogous to the miniature end-plate potentials recorded at cholinergic junctions (Holman, 1970). It was decided therefore to see whether the loss of vesicles from noradrenergic terminals was associated with an increase in the frequency of spontaneous EJPs. The mouse vas deferens was chosen for these experiments since it is most likely that this muscle receives a pure noradrenergic motor innervation (Farnebo & Malmfors, 1971).

The vasa deferentia were removed from adult male mice, and placed in a 25 ml organ bath maintained at 35°. Intracellular recordings were obtained with KCl-filled glass capillary microelectrodes of 60–100 Mohm resistance. The composition of the perfusion medium and details of recording circuitry have been described previously (Hashimoto & Holman, 1967). Transmural silver wire electrodes were used for electrical stimulation (pulses of 0·15 ms from a Grass S4 stimulator). Tissue movement was observed through a binocular microscope. After each experiment, the tissues were fixed in gluteraldehyde and examined with the electron microscope.

Intracellular recordings from unstimulated muscles showed resting membrane potentials between 65–80 mV in the normal perfusion medium. Small spontaneous EJPs (Fig. 1A) were observed in the absence of any visible contractions of the tissues Electrical stimulation at a frequency of 0·66 Hz gave EJPs of increasing amplitude as the output of the stimulator was increased from 40 to 120 V (dial setting). Visible contractions were usually observed when the strength of the stimulus exceeded 90 V.

The venom glands of two female red back spiders were removed and ground in 1 ml of the perfusion medium. Flow through the organ bath was stopped and the venom was added directly to the bath; the final concentration of the venom was 0·16 glands ml^{-1}. Cessation of the flow through the bath did not alter the resting membrane potential, the frequency of spontaneous EJPs, the amplitude of EJPs, or contractile response to electrical stimulation over an observation period of 30 min.

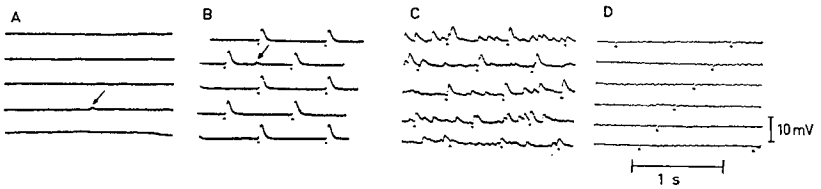

FIG. 1. Effect of red back spider venom on the frequency of spontaneous EJPs in mouse vas deferens. Arrows indicate spontaneous EJPs. Dots indicate electrical stimuli. (a) Intracellular recording from unstimulated tissue. (b) Recording from electrically stimulated tissue. (c) Recording from electrically stimulated tissue during onset of venom action. (d) Recording from electrically stimulated tissue 10 min after exposure to venom.

Within 5 min of adding the venom to the unstimulated preparation the frequency of spontaneous EJPs was greatly increased and the tissue contracted vigorously. The frequency and the amplitude of the spontaneous EJPs coon decreased; after 10 min none could be recorded from surface cells. Spontaneous EJPs could be observed in deeper cells at this time, but visible contractions of the tissue had ceased.

In other experiments the tissue was stimulated repetitively at 0·66 Hz with pulse strengths below that necessary to elicit visible contractions (Fig. 1B). Addition of the venom to the bath produced an increase in the frequency of spontaneous EJPs, associated with the onset of visible contractions (Fig. 1C). Within 5 min of addition of the venom EJPs were much reduced in amplitude. After 7 min neither spontaneous nor evoked EJPs were recorded from surface cells (Fig. 1D). If the strength of electrical stimulation was increased beyond that previously necessary to elicit a contraction in the control muscles no movement was now seen.

Return to the control perfusion medium after cessation of spontaneous EJPs did not cause a return of the response to electrical stimulation. Depolarization of smooth muscle cells was not observed even after 30 min exposure to the venom. Venom that had been denatured by boiling did not affect the frequency of spontaneous

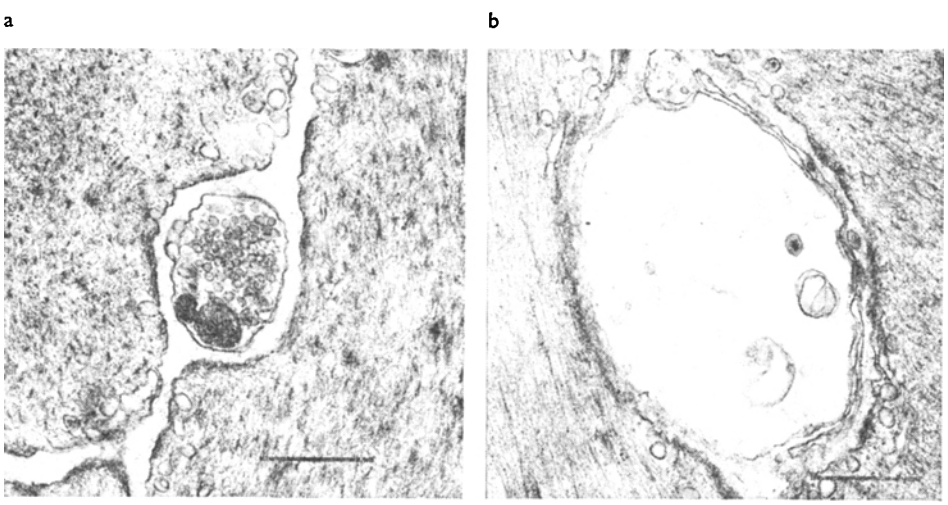

FIG. 2. Effect of red back spider venom on the ultrastructure of nerve terminals in the mouse vas deferens. (a) Section of control tissue showing nerve terminal containing numerous small granular vesicles, large granular vesicles, and mitochondria (bar = 0·5 μm). (b) Section of venom treated tissue showing swollen, electrontranslucent structure containing a large granular vesicle and mitochondrial remnants (bar = 0·5 μm).

EJPs, or the response to electrical stimulation. Tetrodotoxin (10^{-6} g ml^{-1}) did not prevent the discharge of spontaneous EJPs caused by the toxin.

When the control tissues were examined with the electron microscope, numerous nerve terminals packed with small granular vesicles were seen (Fig. 2a). In the venom-treated tissues no nerve terminals of usual appearance were found in surface cell layers. Electrontranslucent structures were seen in positions normally occupied by the nerves. These structures contained only large granular vesicles and remnants of mitochondria (Fig. 2b). In intermediate cell layers there were similar structures, containing some small vesicles, and in deeper layers, normal terminals were seen. These changes within the terminals were similar to those found in rat vas deferens (Hamilton & Robinson, unpublished). These observations suggest that the venom diffuses slowly into the tissue and this would explain why there were spontaneous EJPs in deep cells when these have ceased in surface cells. After the tissue had been treated with denatured venom all nerve terminals examined were of normal appearance. In all experiments the smooth muscle cells throughout the tissue were normal.

This study, together with a recent report that the black widow venom caused the dissolution of vesicles and a discharge of miniture end-plate potentials at a glutamatergic synapse in insects (Cull-Candy, Neal & Usherwood, 1973), indicates that the action of the toxin does not depend on the nature of the transmitter. The observation that the loss of small granular vesicles from noradrenergic synapses in the vas deferens was associated with a massive discharge of spontaneous EJPs provides additional evidence that these vesicles may be associated with the quantal release of noradrenaline.

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