LETTERS TO THE EDITOR

Resistance to tetrodotoxin in toad sympathetic nerves

During an investigation of the innervation of the gut of the toad (*Bufo marinus*), it was found that the excitatory responses of the isolated small intestine to splanchnic nerve stimulation were resistant to blockade by a wide variety of autonomic blocking drugs, including the local anaesthetic drugs procaine and cinchocaine. To check whether this response was due to a direct spread of the stimulating current to the muscle, the drug tetrodotoxin was applied. Tetrodotoxin is known to prevent the specific sodium conductance change during the action potential in nerve fibres (Narahashi, Moore & Scott, 1964; Nakamura, Nakajima & Grundfest, 1965; Kao, 1966; Takata, Moore & others, 1966), and on the other hand it has only minimal effects on action potentials in vertebrate smooth muscles (Bülbring & Tomita, 1966; Kuriyama, Osa & Toida, 1966). Because of these properties it has been possible to use tetrodotoxin to give an effective "denervation" of vertebrate smooth muscle preparations (Gershon, 1966, 1967; Bülbring & Tomita, 1966; Bell, 1968), a concentration of 5×10^{-7} g/ml sufficing to do this. However, in the present experiments it was found that tetrodotoxin in doses of up to 5×10^{-6} g/ml failed to cause any reduction of the contraction of the toad intestine caused by splanchnic nerve stimulation (Fig. 1). On the other hand, the response of the intestine was abolished by cutting the splanchnic nerves between the stimulating electrodes and the muscle.

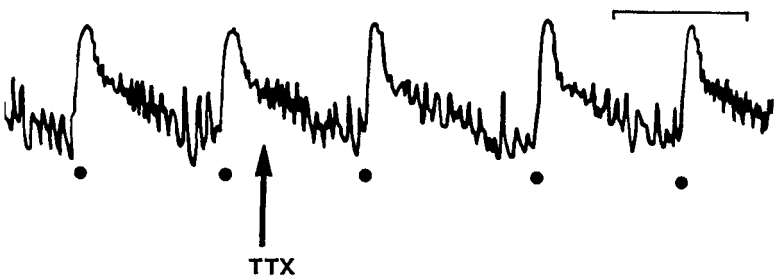

FIG. 1. Responses of isolated toad duodenum to splanchnic nerve stimulation. Tetrodotoxin (10^{-6} g/ml), added to the bath at the arrow, does not affect the responses. Stimuli (10/s, 1 ms for 30 s) at black dots. Time marker = 5 min.

These results strongly suggest that the fibres of the toad splanchnic nerve are resistant to tetrodotoxin. To investigate this further, conventional extracellular recordings were made of action potentials from the isolated splanchnic nerve, using silver wire electrodes; simultaneously, sciatic nerve action potentials were recorded in the same bath. The stimulus was adjusted to elicit an action potential peak propagating at 35 m/s (A-fibres) in the sciatic nerve and at 5·5 m/s (B-fibres) in the splanchnic nerve (Fig. 2A). Splanchnic B-fibre action potentials were studied because Bishop & O'Leary (1938) suggested that the B-fibres mediate excitatory responses of the intestine in the bullfrog. The application of tetrodotoxin (10^{-7} g/ml) rapidly abolished the A-fibre spike of the sciatic nerve, but the B-fibre spike of the splanchnic was not affected by tetrodotoxin, even when the concentration was raised to 5×10^{-6} g/ml (Fig. 2B). Thus conduction in the splanchnic B-fibres was found to be at least relatively resistant to tetrodotoxin.

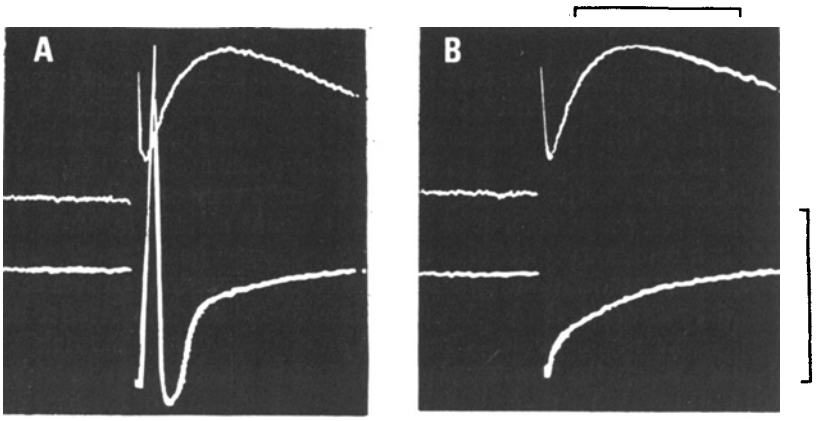

FIG. 2. Extracellular action potentials recorded from toad sciatic and splanchnic nerves. Normal action potentials shown in (A). In (B), 15 min after the addition of tetrodotoxin (5×10^{-6} g/ml), the splanchnic B-fibre action potential (upper trace) is unaffected, but the sciatic A-fibre action potential (lower trace) is abolished. Vertical calibration = 5 mV, horizontal calibration = ms.

The relative resistance of the splanchnic B-fibres to tetrodotoxin may indicate that calcium ions are carrying at least part of the inward current of the action potential. Koketsu & Nishi (1968) have reported that calcium action potentials can be sustained by frog sympathetic ganglion cells, and that these action potentials persist in the presence of tetrodotoxin. This would be consistent with the lack of effect of procaine on the intestinal responses to splanchnic nerve stimulation reported here, since this drug does not act on the specific calcium conductance mechanism of the action potential in barnacle giant muscle fibres (Hagiwara & Nakajima, 1966). But whatever the reason for the resistance, it is clear that tetrodotoxin cannot be used blindly to achieve chemical "denervation" of vertebrate smooth muscle. It must first be shown that the drug does in fact prevent the initiation of action potentials in the nerves supplying the organ under study.

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