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LETTERS TO THE EDITOR

Resistance to tetrodotoxin in the isolated ileum of the rat

Su & Bevan (1970) reported the paradoxical finding that nicotine-induced contraction of the isolated pulmonary artery, was resistant to concentrations of tetrodotoxin (TTX) which inhibited neuronally-induced contractile responses to transmural electrical stimulation. They attributed the responses to nicotine or to transmural stimulation to release of noradrenaline. I now report another isolated smooth muscle preparation, the rat ileum, in which nicotine-induced contractions were not wholly blocked with TTX.

Strips of ileum from Male Charles River rats, 350–450 g, were suspended in muscle baths containing Tyrode solution at 36° and aerated with carbon dioxide in oxygen, under 1 g tension (Goldenberg, 1969). Responses to nicotine salicylate and to acetylcholine (chloride) were recorded isometrically. TTX (Calbiochem) or other antagonists were left in contact with the tissue for 10 min before adding agonist. All concentrations of drugs refer to final concentrations of their salts.

Transmural electrical stimulation was with one platinum electrode within the ileum lumen and the other surrounding the tissue. Stimuli were at 80V, 0·2 ms duration, at 3, 10 and 30 Hz. A pair of such stimuli 2 min apart, each of 5 s, was delivered at each frequency, and the mean of the two contractions recorded. An antagonist drug was left in contact with the ileum for 10 min before transmural stimulation was begun.

Nicotine, 1×10^{-5} g/ml, and acetylcholine, 5×10^{-8} g/ml, gave nearly equivalent biphasic contactile responses: a primary spike-like contraction followed by a slower progressive one (Fig. 1a). Procaine, 3×10^{-6} g/ml, elicited an 81 and a 64% inhibition of the primary and secondary contractile response to nicotine, in 10 experiments. The primary and secondary contractions in response to acetylcholine were slightly but significantly (paired *t*-test) inhibited, 14 and 7% respectively, by procaine. TTX, 1×10^{-7} g/ml, elicited a slight (21%) but significant reduction of the primary nicotine- induced contraction in 8 experiments (Fig. 1b). At 1×10^{-6} g/ml, TTX failed

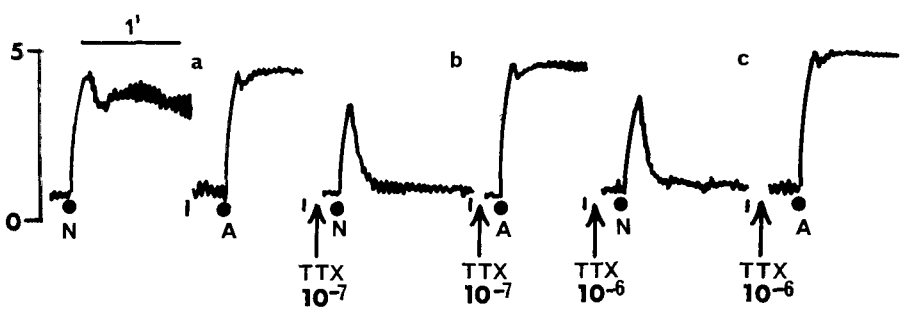

FIG. 1. Effect of nicotine (N) and acetylcholine (A) on the isolated rat ileum. (a) Control biphasic contractile responses to 1×10^{-5} g/ml of N and to 5×10^{-8} of A. (b) Responses to N and to A after 1×10^{-7} g/ml of tetrodotoxin (TTX). (c) Responses to N and to A after 1×10^{-6} g/ml of TTX. Note the resistance of the nicotine-induced primary spike-like contraction to complete blockade while the secondary contractile response was abolished. Contractile responses to A were not affected by TTX. Dot indicates when agonist was added to the bath; short vertical line represents washout of agonist or agonist-antagonist. Time mark, 1 min.

to cause any greater inhibition of the primary nicotine contraction than that observed at the lower concentration (Fig. 1c). TTX, 1×10^{-7} and 1×10^{-6} g/ml, did not inhibit the biphasic contractile response to acetylcholine in 6 strips (Fig. 1b and c).

Transmurally-induced contractions of the rat ileum were much, but not completely, diminished by hexamethonium, 1×10^{-4} g/ml. On the other hand, TTX, 1×10^{-7} g/ml, abolished contractions at all frequencies of such stimulation, in 6 experiments.

Nicotine is a ganglionic stimulant, but there have been many experiments which show the drug to act at presynaptic nerve endings where neurotransmitter substances are released (Trendelenburg, 1965: Chiou & Long, 1969; Bhagat, 1970). In the rat ileum, nicotine, at a maximal concentration, may stimulate the intrinsic nerve supply in at least two different sites. At ganglia, nicotine excites postsynaptic chemoreceptor sites and elicits excitatory postsynaptic potentials; nicotinic receptors on postganglionic nerve endings, on the other hand, are directly activated by nicotine to release acetylcholine, resulting in the primary spike-like contraction. Procaine nearly abolished the contraction and may act at both sites. TTX caused slight but not dose-related antagonism of the spike-like contraction, this inhibitory effect apparently taking place at neural sites, such that postsynaptic potentials were blocked (an effect, that is, similar to the complete blockade of transmurally-induced contractions of the rat ileum by TTX). That portion of the spike-like contraction which was resistant to TTX blockade may have been evoked by an "explosive" liberation of acetylcholine resulting from activation by nicotine of receptors in the neural endings. Nicotine stimulation, that is, unlike transmural stimulation, involves presynaptic receptor activation in the nerve endings, with sudden release of acetylcholine for a postsynaptic contractile effect. Katz & Miledi (1969) reported that the TTX-resistant portion of tetraethylammonium activity of the giant synapse in the squid stellate ganglion was localized in the terminal parts of the presynaptic axons.

The "explosive" effect, as described by Löffelholz (1970) for the release of noradrenaline from adrenergic terminal fibres by nicotinic drugs in the rabbit isolated heart, may be responsible for the nicotine-induced TTX-resistant contraction of the pulmonary artery reported by Su & Bevan (1970).

The secondary contractile response to nicotine was sensitive to TTX blockade. In this situation, nicotine activity may be ascribed to ganglionic excitation (either directly or indirectly through acetylcholine released preganglionically), with subsequent propagation of nerve impulses in the intrinsic nerve plexuses. Thus TTX, which interferes with conduction of nerve impulses, inhibits the secondary contractile response to nicotine just as TTX abolished the responses to transmural stimulation.

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