The effect of staphylococcus α-toxin on the release of acetylcholine in the coaxially stimulated isolated ileum preparation of the guinea-pig

SIR,—Feldberg & Kellaway (1938) were the first to describe the action of staphylococcal toxin on the guinea-pig isolated ileum preparation. Since then its action has been extensively studied (e.g., Brown, Pritchard & Quilliam, 1959; Rašková & Vaněček, 1964; Brown & Quilliam, 1965). The most obvious features of the effect of the toxin on smooth muscles *in vitro* are a slowly developing contracture with subsequent diminution or absence of responses to stimulant drugs and failure to respond to a further dose of the toxin. However, the mechanism of action remains far from clear. The slow contracture it produces may be a direct effect on the smooth muscle cells or due to liberation of a contracting substance from the ileum. Botulinus toxin depresses transmitter release from myenteric plexuses (Harry, 1962) and there remains the possibility that staphylococcus toxin behaves similarly. We have examined this question.

The guinea-pig isolated ileum was stimulated coaxially according to Paton (1955). Pieces of ileum (4–5 cm), taken at least 10 cm proximal to the ileo-caecal junction, were placed in a 5 ml bath containing modified Krebs solution (Eccles, 1952) at 37° and gassed with a mixture of oxygen 95% and carbon dioxide 5%. The distal end of the ileum was tied to a short polyethylene tube allowing the gut lumen to be washed. One platinum electrode (anode) was placed in the lumen of the preparation and the other was placed in contact with the bath fluid opposite the anode and outside the lumen. The ileum was stimulated transmurally with supramaximal rectangular pulses of 400 μsec duration at a rate of 6/min. Stimulation at a frequency of 30/sec was usually required for 3 to 10 sec to produce tetanic responses. The sensitivity to acetylcholine and histamine was also tested. Contractions were recorded by an isotonic lever writing on smoked paper. Acetylcholine output was measured in the presence of eserine salicylate 5×10^{-6} g/ml in the bath. The sequence and duration of acetylcholine collections during the periods without and with the electrical stimulation is shown in Table 1. Acetylcholine activity of samples was assayed immediately on another piece of ileum in the presence of eserine salicylate 12·5 μg/litre, and morphine hydrochloride, 6 mg/litre. This ileum preparation was allowed to equilibrate with the eserinized solutions for 45 min to completely inactivate cholinesterases (Paton, 1957). That the activity was due to acetylcholine was checked. It was stable in acid but destroyed by boiling in alkaline solution, all the biological effects were antagonized by atropine (10^{-8} g/ml).

A filtrate of *Staphylococcus pyogenes* strain Wood–46 was used. Preparation of the toxin was as described by Johanovský (1956). The haemolytic potency of the toxin, expressed in haemolytic units (H.U.) assessed according to Brown & others (1959), was 5·1 H.U./mg of toxin and the toxin had an intravenous LD50 for albino mice of 4·9 H.U. (3·7–6·4 H.U.)/20 g body weight. The toxicity was lost either after heating to 90° or by gassing with oxygen 95% and carbon dioxide 5% for 10 min. After each of those two procedures, inactivated toxin or the culture media were not spasmogenic on the coaxially stimulated guinea-pig ileum.

In our experiments 13 H.U./ml or more produced a slowly developing contracture, the extent of which was dose-dependent. The contracture was followed by a gradual diminution and finally abolition of the electrical and drug-induced responses. The contracture response had a latency of from 30 to 60 sec and washing out of toxin after a contact time of 10 sec did not impair the contracture. Fig. 1A shows a contracture after 20 H.U./ml of α-toxin.

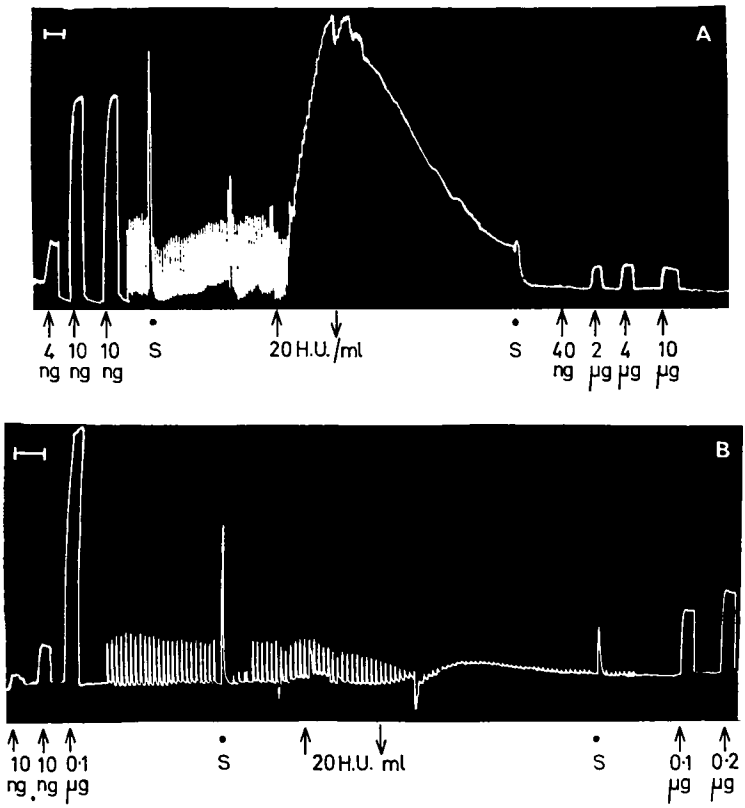

FIG. 1. Coaxially stimulated isolated guinea-pig ileum preparation. Stimulation by rectangular pulses 400 µsec duration and intensity of current to elicit maximal response. A. Experiment in which the toxin is followed by a contracture. B. An experiment without contracture. ↓↑ period of contact with 20 HU/ml of staphylococcal α-toxin. Figures are doses of acetylcholine. S. 3 sec stimulation with frequency 30 shocks/sec.

TABLE 1. ACETYLCHOLINE OUTPUT FROM THE GUINEA-PIG ISOLATED ILEUM PREPARATIONS EXPRESSED IN NG/MIN/ML OF BATH FLUID AND STATED AS ACETYLCHOLINE HYDROCHLORIDE

Expt No.	Acetylcholine output before and after the 3 min contact with 20 H.U. of α-staphylotoxin								
	Before			After					
	Spont.	Stim.	Spont.	Spont.	Stim.	Spont.	Stim.	Spont.	
1	1·3	1·7	2·6	—	1·7	1·4	1·8	1·5	During stim. periods supramaximal pulses frequency of 1/10 sec were used. Duration of collection periods 20 min.
2	1·2	1·5	1·4	—	1·1	0·5	1·2	—	
3	0·9	2·5	1·1	—	2·2	0·9	2·8	1·0	
4	1·7	2·6	2·5	1·9	2·1	1·1	1·6	1·5	
5	0·5	0·6	0·4	0·3	0·4	0·4	—	—	
6	0·3	0·3	0·2	0·2	0·3	0·3	—	—	
7	0·6	1·5	1·0	0·65	1·0	0·6	1·2	0·7	During stim. periods supramaximal pulses frequency of 2/sec were used. Duration of collection periods 10 min.
8	1·75	2·4	1·6	—	1·7	1·0	1·5	1·2	
9	2·0	4·1	3·6	2·4	3·6	3·4	4·6	2·6	
10	0·4	1·6	—	—	1·1	0·4	1·2	—	
11	0·7	1·9	0·8	—	1·7	0·8	3·2	—	

This response was found in two thirds of our experiments. In the other third, however, desensitization occurred without appearance of the contracture. In all experiments the responses to acetylcholine and electrical stimulation were eventually abolished.

The output of acetylcholine was measured during rest and during stimulation at frequencies of 1/10 sec and 2/sec. Results are in Table 1. There was no change in the output of acetylcholine before or after the addition of the toxin. Even when the frequency of stimulation was raised to 10/sec there was no fall in the acetylcholine output after the toxin at 20 H.U./ml.

Thus, no evidence for changed nervous activity was found and our results accord with those of Brown & others (1959, 1965), Brown & Quilliam (1965) and Thal & Egner (1961) that the toxin has a direct action on smooth muscle and its effect is probably not mediated through nervous plexuses. We have found that toxin does not alter the acetylcholine output.

Since the nerve plexuses are the main source of the acetylcholine output (Paton, 1957, 1963; Harry, 1962; Johnson, 1963), the action of the toxin appears to be entirely postsynaptic and on the smooth muscle itself. The irreversible action of the toxin after a short period of contact argues either for a rapid enzyme-like action with immediate damage of the smooth muscle membrane, or for a fixation of the toxin with the development of gradual changes. A direct action of the toxin upon the contractile substance does not accord with the findings of Gulda & Seč (1966) that the toxin had no effect on actomyosin.

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