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ANAPHYLACTIC REACTIONS TO ENDOTOXIN IN GUINEA-PIG TISSUES: RELATIONSHIP TO ENDOTOXIN TOXICITY

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1 A lipopolysaccharide extract of *Escherichia coli* 026:B6 cells (026:B6(B) endotoxin) was shown to be toxic to normal adult guinea-pigs.

2 The agent had no action on isolated preparations of ileum and heart taken from normal adult guinea-pigs.

3 Ileal segments from animals actively immunized against 026:B6(B) endotoxin showed dose-dependent contractions when exposed to endotoxin. Desensitization phenomena were demonstrated.

4 Reactivity of 026:B6(B) endotoxin was transferred to isolated preparations of ileum and heart from normal animals by passive transfer of immune serum.

5 Tissue responses to 026:B6(B) were associated with release of ileal spasmogen into the bath medium. Mepyramine blocked the effects of this spasmogen at bath concentrations which caused little change in ileal responses to carbachol.

6 It is concluded that *E. coli* endotoxin can elicit anaphylactic reactions, and that this process may potentiate endotoxin toxicity in sensitized animals. However, endotoxin toxicity in guinea-pigs does not appear to depend on this kind of allergic process.

Introduction

Lipopolysaccharide complexes, termed endotoxins, may be derived from the cell walls of gram-negative bacteria by boiling or by chemical methods. (Luderitz, Westphal, Staub & Nikaido, 1971). These extracts produce a diverse range of biological effects when injected into mammals, including lethal cardiovascular disorganization (shock) (Bennett & Cluff, 1957; Gilbert, 1960; Raskova & Vanecek, 1964; Hinshaw, 1971; Milner, Rudbach & Ribi, 1971). It is thought that these agents produce the shock state associated with sepsis in man.

The mechanisms of action of these endotoxins on the circulation remain unknown. Suggested mechanisms range from direct pharmacological effects on smooth muscle (Urbaschek, 1964; Zweifach, 1964; King & Cox, 1972) and interactions with autacoid mechanisms (Zweifach, 1964; Urbaschek & Versteyl, 1965; Hinshaw, 1971) to immunologically mediated allergic reactions such as anaphylaxis (Chedid & Parant, 1971).

Recent experiments with guinea-pigs have provided evidence that a range of endotoxins produce lethal effects, yet are inert on standard pharmacological preparations utilizing guinea-pig tissues including gut, ureter, vas deferens, portal vein, uterus and atria (McLean, unpublished results). These studies also established that there was no interaction with α-adrenoceptor or H_1 and H_2-histamine receptor mechanisms. Consequently it was of interest to test the possibility that anaphylactic mechanisms were involved in this species, as this could be carried out free from interactions with the other proposed mechanisms described above.

Since the classical work of Schultz (1910) and Dale (1912–1913), it has been recognized that the anaphylactic process may be studied under *in vitro* conditions (tissue anaphylaxis). Using these methods many properties of the anaphylactic process have been defined (Mongar & Schild, 1962). However, the basic properties are those described by Dale as follows. Exposure of sensitized tissues to a specific antigen results in release of pharmacologically active agents; repeat exposure elicits a smaller reaction than the initial challenge (desensitization); reactivity to the antigen may be transferred to normal tissues through transfer of serum from an immunized animal.

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In this study, the effect of a trichloracetic acid extract of *Escherichia coli* 026:B6 cells (026:B6(B) endotoxin) on isolated tissues from normal and immunized guinea-pigs was investigated. A parallel study was made of toxicity of this extract in normal adult guinea-pigs.

Methods

Endotoxic entract

A trichloracetic acid extract of *E. coli* 026:B6 cells was purchased from Difco Laboratories. Test solutions of this endotoxin were freshly prepared from stock solutions in distilled water. This extract is termed 026:B6(B) in the text.

Toxicity testing

Normal adult guinea-pigs were injected intravenously (i.v.) and into the peritoneum (i.p.) with 026:B6(B) at a dose of either 0.5 mg/kg or 2 mg/kg.

Active immunization schedules

Animals were actively immunized according to one of two schedules, termed A and B in the text.

Schedule A. A single intraperitoneal injection of 2 mg/kg of 026:B6(B) was given. Survivors were studied four weeks after inoculation. A pool of serum was prepared after bleeding five animals using conventional techniques.

Schedule B. Footpads were injected with 0.4 ml of a 1:1 mixture of complete Freund's Adjuvant and a 2.5 mg/ml solution of 026:B6(B) in distilled water. From the fourteenth day after 'priming', the animals were 'boosted' every second day by intradermal injections into the mantle region, of 20 μg 026:B6(B) in 0.9% w/v NaCl solution (saline). Four injections were given before the animals were killed. A pool of serum was made from five immunized animals.

Passive immunization

Normal animals were given an intraperitoneal injection of 4 ml/kg of immune serum 16 h before ileal segments were removed and exposed to endotoxin (see below).

Isolated ileal preparations

Ilea were removed from normal and immunized animals after stunning and bleeding. Segments were mounted in an isolated organ bath apparatus and bathed with modified Tyrode solution (Feigen, Vaughan Williams, Peterson & Nielsen, 1960) and attached to an isotonic lever writing on a smoked drum apparatus. Dose-response characteristics to histamine were determined over the range 10^{-9} to 10^{-7} M. Preparations were exposed to 026:B6(B) by cumulative addition to the bath over a test range defined by screening studies on one ileal segment from each animal. Screening doses ranged from 0.1 to 500 μg/ml. When a reaction range was defined, responses were quantitated in terms of histamine equivalents (Feigen *et al.*, 1960) in two additional segments from each animal. The mean was calculated. All ileal experiments were carried out with 2 μg/ml of semicarbazide in the bath medium.

Isolated cardiac preparations

Guinea-pig heats were removed, dissected, and mounted in an isolated apparatus as described previously (Feigen *et al.*, 1960) under 1 g tension. Records of cardiac contractions were obtained with a tension transducer. Preparations were perfused with Chenoweth's solution (37°C) under 70 cmH_2O pressure. The perfusate was collected, flow-rate recorded and the fluid bioassayed for ileal spasmogen.

Hearts were exposed to 026:B6(B) endotoxin at a concentration of 47 μg/ml in the perfusate. After initial challenge, 3 ml of Schedule B immune serum was recycled four times through the perfusion system, then the exposure to endotoxin was repeated.

Bioassay techniques

Segments were exposed to standard bath concentrations of histamine to determine dose-response characteristics. All responses to other agents were expressed in terms of histamine equivalents according to the methods of Feigen *et al.* (1960).

Drugs

The following drugs were used: histamine acid phosphate, mepyramine maleate, carbamyl chloride and semicarbazide.

Results

Toxicity testing

There were no changes detected in the condition of 6 animals injected with 0.5 mg/kg 026:B6(B) by either of the two routes of administration. However, 13/13 animals injected with 2 mg/kg of this endotoxin showed increased respiratory rate and decreased spontaneous movement at one hour. Four hours after injection the animals appeared drowsy and did not show startle responses. Deaths (4/13) occurred

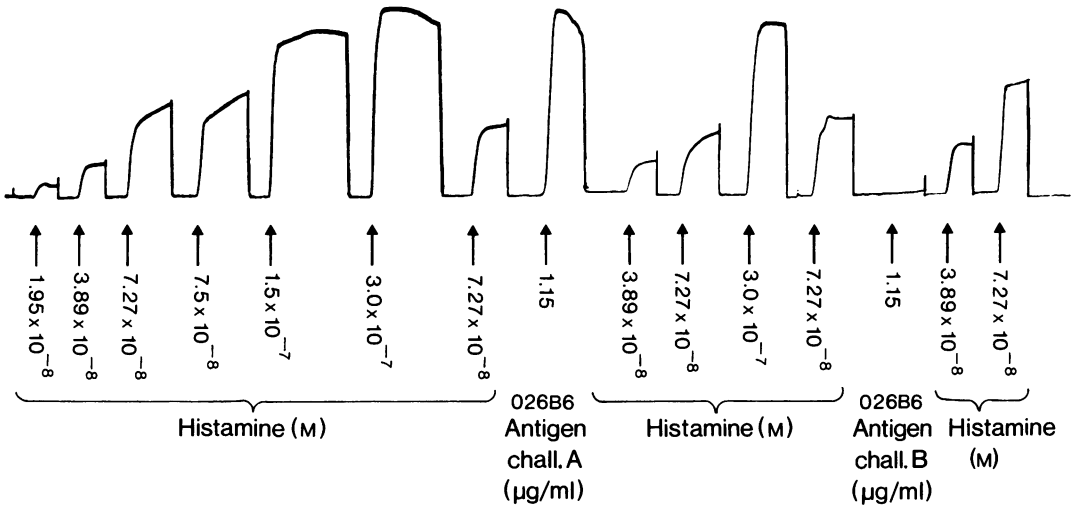

Figure 1 Records of ileal contractions (isotonic lever). The ileum was removed from an animal actively immunized according to Schedule A against an endotoxic extract of *E. coli* 026:B6 cells (See text). Doses of endotoxin and histamine were applied as shown.

between 8 and 36 h after injection. Before death, animals were lying prone with their eyes closed, breathing rapidly and shallowly and they were unresponsive to touch. There was no difference in the pattern of response to intraperitoneal injection when compared to intravenous injection.

Responses of isolated ilea to endotoxin

Segments of ileum from 10 normal animals showed no response to the addition of 026:B6(B) endotoxin to the bath in concentrations up to 250 μg/ml.

In contrast all ileal segments ($n=10$) from 5 animals immunized according to Schedule A showed contractile responses when 026:B6(B) was added to the bath over a test range defined by screening studies (see Methods section). The result of one experiment is illustrated in Figure 1. In the experiment shown, the initial exposure to endotoxin caused a contracture which terminated on washout of the bath. Repeat application of the same concentration of endotoxin caused a diminished response. The maximal responses to endotoxin in these experiments were $1.3 \pm 1.5 \times 10^{-8}$M histamine equivalents with a range of 9×10^{-9} to 3.8×10^{-8} M histamine equivalents. Similarly endotoxin elicited contractile responses in ilea obtained from 6 animals immunized according to Schedule B. The responses were dose-dependent, showing increasing responses up to an optimal concentration. The maximal responses obtained were $6.4 \times 10^{-8} \pm 1.09 \times 10^{-7}$ M histamine equivalents with a range of 0 to 2.75×10^{-7} M histamine equivalents.

Influence of passive transfer of immune serum on reactivity of ilea

Three normal guinea-pigs were injected intraperitoneally with Schedule A immune serum 16 h before study of ileal segments. Reactivity to endotoxin was demonstrated in all ileal segments studied ($n=7$). The properties of the reactions included dependence of the reaction on endotoxin concentration and desensitization as observed with actively immunized tissues.

A similar attempt was made to demonstrate passive transfer of reactivity to ileum together with transfer of Schedule B serum using five normal animals. Contractions were elicited in ilea from two animals; however, these responses could not be quantitated meaningfully as they were near to threshold for detection by this technique.

Influence of passive transfer of immune serum on reactivity of isolated hearts

The hearts were exposed to 026:B6(B) at a concentration of 47 μg/ml in the perfusate. Initial challenge caused no change in the rate, rhythm, or strength of contractions of the isolated hearts, and no change in the rate of perfusate outflow. Challenge with the endotoxin was repeated after *in vitro* exposure to Schedule B immune serum (see Methods section). The results obtained with one heart are illustrated in Figure 2. The exposure caused disorganization of cardiac rhythm with atrioventricular dissociation

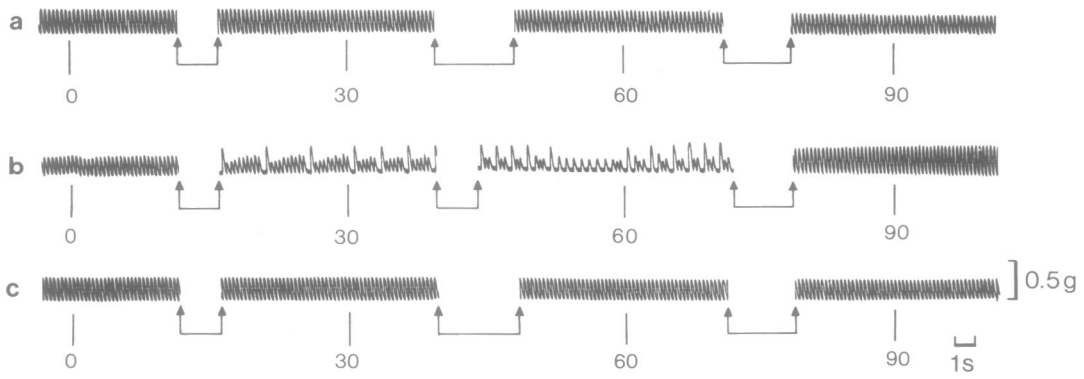

Figure 2 Records of spontaneous contractions of an isolated preparation of guinea-pig heart recorded with a strain gauge tension transducer and polygraph recorder. Panels (a) to (c) show the effects of exposure to 47 μg/ml of 026:B6 (B) endotoxin under different conditions. (a) Shows representative activity under control conditions at 0 time, 30, 60 and 90 s after the beginning of exposure to endotoxin; (b) shows the responses after passive transfer of immune serum (see text). In (c) the effects of a third application of endotoxin are shown. The antigen concentration was maintained in the perfusate for 60 s before return to toxin-free Chenoweth's solution. The symbols ↑——↑ represent discontinuity in the traces occasioned by the paper speeds used (see time calibration).

(Figure 2). The perfusion rate fell from 5.2 ml/min to 1.6 ml/min and bioassay of the perfusate showed release of ileal spasmogen at a peak rate of 61.44×10^{-8} M histamine equivalents/minute. Further challenge with endotoxin failed to produce any change in mechanical activity or perfusion rate, and there was no evidence of release of ileal spasmogen. Similar findings were recorded in experiments on two other normal hearts. The maximal release rates of ileal spasmogen in these experiments were 18 and 37.12×10^{-8}M histamine equivalents/minute.

Assay of bath fluid for reaction products

After challenge of tissues with endotoxin, bathing media were collected, heated to 100°C for 30 min and then assayed on ilea from normal animals.

There was no evidence of release of ileal spasmogen by endotoxin from cardiac or ileal preparations from normal animals. In contrast, bath fluid taken after challenge of sensitized tissues with endotoxin caused ileal contractions which were blocked by mepyramine in parallel with blockade of effects of equivalent doses of histamine. Responses to carbamyl chloride (carbachol) were little affected by the blocking doses of mepyramine, suggesting that the ileal spasmogen released was histamine.

Discussion

The endotoxin used in this study was toxic to normal adult guinea-pigs, yet it did not elicit anaphylactic reactions in ileal or cardiac preparations from normal adult guinea-pigs. From these observations it may be concluded that endotoxin toxicity is not necessarily dependent on anaphylactic reactivity as has been suggested since the earliest study of the two phenomena (Sanarelli, 1924).

The studies on tissues from actively immunized animals showed dose-dependent responses of ilea to exposure to the test endotoxin. Further challenge with the same dose of antigen produced a greatly diminished response. This phenomenon is termed desensitization, and is one of the characteristic features of the anaphylactic process, rather than being specific to any one antigen (Mongar & Schild, 1962). The tissue responses were paralleled by the release of histamine-like ileal spasmogens into the bath medium. Ileal and cardiac reactivity to endotoxin was shown to be transferred with transfer of immune serum. These observations suggest that endotoxins may elicit anaphylactic reactions in immunized animals, raising the possibility that this process might potentiate toxicity in such animals.

The reason for the lack of anaphylactic reactivity to 026:B6(B) in tissues of normal adult guinea-pigs remains unclear but may reflect a general lack of natural exposure to *E. coli* previously reported in these animals (Smith & Crabb, 1961; Smith, 1965) and confirmed for the colony used in these experiments (Boquest & McLean, unpublished observations).

Serological studies have provided evidence that most animals are naturally immunized against endotoxins. These findings possibly reflect the colonization of the bowel of most species by gram-

negative organisms, including *E. coli* (Smith & Crabb, 1961). The demonstration that 026:B6(B) endotoxin may elicit anaphylactic reactions in immunized animals might have broad implications for the pathogenesis of endotoxin toxicity in most mammals.

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