NEUROTOXINS OF MICROORGANISMS OF THE ENTERIC GROUP

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Some biological properties and characteristics of the antigenic structure of neurotoxins obtained from Shigella sonnei, Salmonella typhi, and Escherichia coli are described.

It has long been known that microorganisms of the enteric group can produce toxic soluble substances, one distinguishing feature of which is their neurotropic action [1, 2, 7-9, 10]. The neurotoxin of Shigella shigae has been studied most intensively. However, neither the antigenic structure nor the chemical composition of this toxin has yet been finally established. Even less is known about the neurotoxins of other members of the Enterobacteriaceae.

The object of the present investigation was to study the antigenic structure of neurotoxins of microorganisms of the enteric group. At the same time, some of the biological properties of the neurotoxins, distinguishing them from the corresponding endotoxins, were investigated.

EXPERIMENTAL METHOD

Neurotoxins were isolated by the method of Mesrobeanu et al. [7] from 72 h autolyzates of cultures of Shigella sonnei, of serologically untyped strains of Escherichia coli, and cultures of Salmonella typhi.

Endotoxins were precipitated from supernatants of the autolyzed cultures by five volumes of 96° alcohol after removal of the neurotoxins. The toxicity of the preparations was tested in albino mice weighing 16-18 g by injection of 0.2 ml of different dilutions of the preparations into the caudal vein.

To study the action of the neurotoxins on animals, isolated segments of the rabbit intestine were used [5]. The antigenic structure of the neurotoxins was studied by Ouchterlony's agar diffusion reaction in its micromodification. Precipitating sera against neurotoxins and endotoxins were used.

EXPERIMENTAL RESULTS

When undiluted neurotoxins were injected the mice died 1-2 days later with general toxic manifestations. The development of pareses and paralyses of the limbs was observed in mice after injection of some neurotoxins obtained from recently isolated hemolytic strains of E. coli and S. typhi. Neurotoxins obtained from strains of Sh. sonnei were least toxic to the mice.

The neurotoxins and endotoxins were indistinguishable in their toxicity toward albino mice. LD_{50} for neurotoxins of E. coli, expressed as the dry weight of the preparations, varied between 1.12 and 0.16 mg, whereas LD_{50} of the corresponding endotoxins from the autolyzates was 1.29-0.2 mg.

Intracerebral injection of E. coli and S. typhi neurotoxins into mice revealed no increase in the toxicity compared with the intravenous method of administration.

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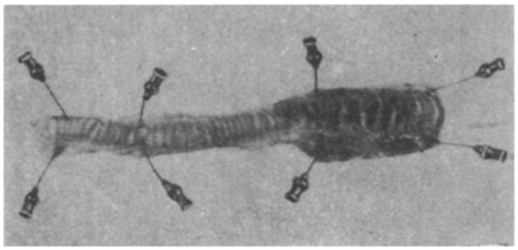

Fig. 1. Dilatation of a segment of rabbit small intestine and hemorrhages into mucous membrane of segment following injection of E. coli neurotoxin (left—control, right—experiment).

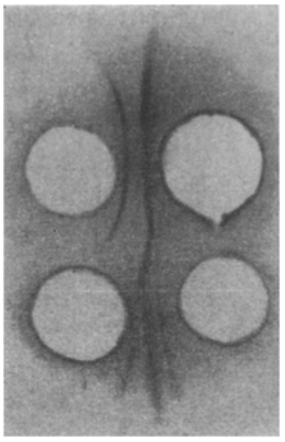

Fig. 2. Agar diffusion reaction. Upper and lower left wells; antiserum against typhoid neurotoxin; upper right well; typhoid neurotoxin (strain 5501); lower right well; endotoxin from typhoid autolyzate (strain 5501).

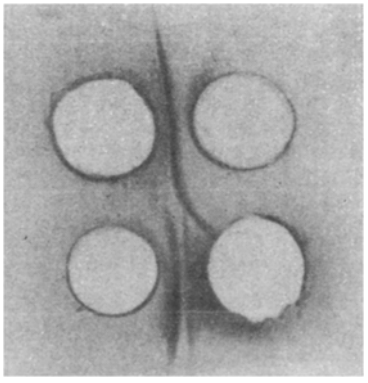

Fig. 3. Agar diffusion reaction. Upper and lower left wells; antiserum against typhoid neurotoxin; upper right well: typhoid neurotoxin; lower right well: neurotoxin of E. coli.

Injection of E. coli neurotoxins into the lumen of an isolated loop of small intestine of a fasting rabbit caused dilatation of the segment through the accumulation of a sero-hemorrhagic fluid; numerous hemorrhages appeared in the mucous membrane of the isolated intestine (Fig. 1).

The character of the changes in the isolated loop of rabbit intestine and the absence of any particularly marked toxic effect following intracerebral injection of the neurotoxins are evidence that they act indirectly rather than directly on the central nervous system. The neurotoxins evidently attack the capillaries first, as has been demonstrated in the case of the neurotoxin of Sh. shigae [4].

Unlike the endotoxins elevating the body temperature in animals, neurotoxins of microorganisms of the enteric group produced hypthermia when injected intravenously into rabbits and guinea pigs: the body temperature of the animals fell by 2-3° 3 h after injection of the neurotoxins and remained low for the next 24-48 h. Guinea pigs, which are relatively insensitive to the action of neurotoxins, are most convenient for investigation of this type.

A serological study of the neurotoxins revealed their complex antigenic structure. The neurotoxins of E. coli, S. typhi, and Sh. Shigae were similar in that they contained specific components of the corresponding endotoxins (Fig. 2), as was shown by the positive precipitation reaction between antisera of the corresponding neurotoxins and the homologous Boivin endotoxins or endotoxins obtained from autolyzates of the cultures.

These components included specific polysaccharide groups, for antisera against the neurotoxins formed up to three precipitation lines with haptens of the homologous strains. In addition, after boiling or treatment with alkali, the neurotoxins were able to sensitize untreated human or sheep's erythrocytes, further evidence that the neurotoxins contained polysaccharides.

The neurotoxins also contained antigens common to the genera Shigella, Salmonella, and Escherichia, as was shown by crossed reactions between different neurotoxins and antisera against heterologous and homologous neurotoxins (Fig. 3). These intergenetic antigens have nothing in common with the endotoxins, because antibodies against them remained in the antisera against the neurotoxins when completely absorbed with homologous endotoxins.

The intergeneric antigens were characterized by thermolability, as shown by the inability of the neurotoxins, when heated for 1 h at 80°C, to form the corresponding precipitation lines. Toxicity of the heated preparations of neurotoxins to mice was reduced. It can accordingly be concluded that the intergeneric antigens also contribute to the toxic effect of the neurotoxins, and in all probability they are protein in nature.

Neurotoxins of the thyphoid strains contained Vi and H antigens, the first was demonstrated by the hemagglutination and inhibition of hemagglutination tests; the second by the agglutination test with an H-diagnostic serum obtained from Salmonella münchen.

Some neurotoxins obtained from recently isolated hemolytic strains of E. coli contained a hemolyzin detectable by its direct action on human erythrocytes.

No antigen peculiar to the neurotoxin of any one species of microorganism was found by the agar diffusion test. The specificity of each neurotoxin was determined by the polysaccharide component, also present in the corresponding endotoxin.

The results regarding the antigenic structure of the neurotoxins thus obtained do not agree with those published by Mesrobeanu et al. [7, 8], who found no common antigens in neurotoxins from different members of the Enterobacteriaceae and concluded that the neurotoxins contain no specific polysaccharides.

In their antigenic composition, the neurotoxins studied in the present work were indistinguishable, in principle, from the neurotoxin obtained from the S-forms of Sh. shigae, to judge from the findings of El'chinova [1], who also found intergeneric antigens in the composition of this neurotoxin.

In a number of their features these last antigens differ from Kunin's common antigen [6]. Intergeneric antigens closely resemble the protein antigens described by Barber et al. [3].

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