THE IMPORTANCE OF REACTIVITY IN ANIMALS IN THE SUMMATION OF TOXIC STIMULI

REPORT 1. THE INFLUENCE OF ALLERGIC REACTIVITY ON THE COURSE OF BEHRING'S PHENOMENON

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In our previous investigations we discovered some features of the phenomenon known as Behring's phenomenon. We found that success in the reproduction of this phenomenon is directly dependent on the size of the dose of toxin on each occasion (the strength of the stimulus) and inversely dependent on the length of the intervals between injections; it also depends on the modes of injection of the sublethal doses of toxin and on the functional state of the central nervous system (the course of the toxicosis is aggravated by caffeine and alleviated by bromide). It was also shown that the repeated injection in turn of toxins having the same action, yet immunologically different (for example, botulinus types A and B) may produce Behring's phenomenon just as easily as the injection of the same toxin each time.

All these rules are similar to the rules governing the course of nervous processes. In particular, they are analogous to the phenomena of the summation of stimuli. In contrast to the phenomena of the summation of stimuli observed, for example, during stimulation with a galvanic current, Behring's phenomenon develops when long intervals are present between the injections. It is evident that a certain period of time is required for the realization of the effect of each stimulus, and when this time is up the next injection of toxin strengthens the developing pathological effect. If this interval of time is not present, the next injection merely increases the dose of toxin. No effect also arises if the toxin does not cause protracted pathological phenomena. In this case, not even by a more frequent injection of the toxin can Behring's phenomenon be produced. This is observed, for example, when staphyloccal and perfringens toxins are used.

The difference which we have described between Behring's phenomenon and the phenomena of summation of stimuli from the galvanic current, etc., justifies the introduction of the concepts of "toxic stimulation" and the "summation of toxic stimuli."

These distinctive features of the summation of toxic stimuli make it imperative to study the importance of the reactivity of the animal organism. We accordingly undertook a systematic investigation, part of which is described in the present communication.

EXPERIMENTAL METHOD

In our studies of the changes in the reactivity of animals during the repeated injection of fractional doses of diphtheria toxin, we found that the animals became sensitized to the toxin, and the injection of diphtheria toxoid as a reacting preparation into the blood stream caused a typical anaphylactic reaction. The question naturally arose, how does this allergic transformation affect the course of Behring's phenomenon. It did not seem possible to solve this problem by means of toxin itself, for in addition to increased sensitivity to antigenic action, the animals also showed the development of antitoxic immunity, the effect of which would be difficult to exclude in the case of depression of the summation of the toxic stimuli in the allergic state. This problem was accordingly studied with the aid of the sensitization of animals with horse serum.

Since several days are required for the development of the toxicosis after injection of sublethal doses of a toxin, we had to choose an allergic reaction which would run a more or less prolonged course, for example, the Arthus phenomenon. Only under these circumstances could an obvious effect be expected. Nevertheless, the study of the effect of acute anaphylactic shock on the course of Behring's phenomenon was also envisaged.

The principal observations were made on 110 guinea pigs. Sensitization was induced by the subcutaneous injection of 0.05 ml of horse serum. Before each experiment, a proportion of the animals which took no subsequent part therein were tested for their degree of sensitivity by means of an intracardiac and subcutaneous injection of the same preparation. Naturally, only those guinea pigs which were adequately sensitized were included in the experiments.

In the first series of experiments (46 guinea pigs), for a period of 5 days, 10 sensitized guinea pigs received injections of 0.02 MLD of diphtheria toxin together with 0.2 ml of horse serum. The toxin and serum were injected at different places. The guinea pigs (10) of the second group received injections of the same toxin and serum in the form of a mixture. The guinea pigs (10) of the third group received their first injection of toxin immediately after an acute anaphylactic reaction had been induced in them by the intracardiac injection of 0.05 ml of horse serum, amounting to roughly half a minimal lethal dose of serum (preliminary tests showed that the injection of 0.1 ml of serum caused the death of 2 of 3 sensitized guinea pigs). In control experiments, 8 sensitized and 8 unsensitized guinea pigs were injected with toxin alone in the same dose. In this series of experiments only 5 injections were given, in order to determine the influence of the allergic reaction on the local changes caused by the toxin in the process of summation of the toxic action (this dose of toxin, when injected subcutaneously, itself caused no local changes: these appeared only as a result of 3 or 4 injections).

EXPERIMENTAL RESULTS

The group of sensitized guinea pigs receiving separate injections of toxin and serum showed no local phenomena at the site of injection of the diphtheria toxin in 9 animals and the development of infiltration in one animal.

The injection of mixed toxin and serum led to the development of areas of infiltration in all 10 guinea pigs, but these were indistinguishable from the areas of infiltration developing in the guinea pigs receiving serum without toxin. In other words, we were dealing with the development of the Arthus phenomenon, which was not prevented by the presence of small doses of diphtheria toxin.

Acute anaphylactic shock did not prevent the development of infiltration as a result of the injections of diphtheria toxin in 8 guinea pigs; in one guinea pig no infiltration was present. One guinea pig died, although probably from causes not connected with the experiment. In all the control guinea pigs, the injections of diphtheria toxin led to the development of infiltration.

The second series of experiments was conducted on 37 guinea pigs in accordance with the same scheme. In contrast to the first series, the number of injections was increased to 11. Furthermore, in this series of experiments, in a third group of animals the reacting dose was injected intraperitoneally and not into the heart. Otherwise (arrangement of the groups of animals, dosage of toxin and serum, etc.), this series of experiments was identical with the preceding series.

In the first group of guinea pigs (6), despite the large number of injections of toxin, none of the animals died and no local manifestations were observed in 5 guinea pigs. Only one animal developed necrosis at the site of injection of the toxin.

In the second group, all 8 guinea pigs developed infiltration, and this was evidently connected with injection of the serum. It is important to note that none of the animals in this group, as in the preceding group, died.

Six of the seven guinea pigs in the third group showed no local changes. One animal developed infiltration, and none of them died.

In the group of the sensitized control animals, four of the six guinea pigs died and one developed infiltration. Seven of the ten animals in the group of the unsensitized controls died, and three of the remainder developed infiltration.

The results of the two series of experiments suggested that both the local and the general toxic effects of diphtheria toxin are diminished in the presence of a focus of hyperergic inflammation. The development of Behring's phenomenon was accordingly depressed.

In order to discover the strength of this action of allergic inflammation on the toxic properties of the toxin, a further series of experiments was conducted on 28 guinea pigs; diphtheria toxin was injected intradermally into 14 sensitized and 14 unsensitized guinea pigs once only. Seven of the sensitized guinea pigs, additionally, received a subcutaneous injection of 0.5 ml of horse serum into the opposite flank (one injection only). Instead of serum, seven

of the unsensitized animals received an injection of 0.2 ml of turpentine. Preliminary tests showed that an intradermal injection of 0.02 MLD causes extensive necrosis, and injection of 0.01 and 0.005 MLD causes slight necrosis. These doses were used in the experiments.

Infiltration only was observed in the seven sensitized guinea pigs in which the Arthus phenomenon was reproduced. In some cases these areas of infiltration were covered with a thin, superficial scab, the origin of which was difficult to determine for they resembled excoriations. The lesion was probably superficial necrosis. Its severity was far less than in the seven unsensitized animals receiving corresponding doses of toxin. By the degree of the reaction, the latter were indistinguishable from the sensitized guinea pigs (7) receiving the same doses of toxin, but in which the Arthus phenomenon was not reproduced.

Rather different results were obtained after the injection of toxin into the guinea pigs receiving injections of turpentine into the opposite flank. In this group (2 guinea pigs), extensive necroses were observed not only after injection of 0.02 MLD of toxin (as in the controls); a dose of 0.01 MLD caused the same degree of necrosis in two of three guinea pigs as did a dose of 0.02 MLD in the control animals. This result was difficult to explain, although we are justified in concluding that the inflammatory focus caused by turpentine did not have the same effect as was obtained with allergic inflammation. This problem obviously requires special study. In particular, the effect of different phases of the inflammatory reaction caused by turpentine must be examined.

These experiments thus showed that the development of Behring's phenomenon, caused by the repeated injection of diphtheria toxin, is depressed if the Arthus phenomenon is reproduced in the animals at the same time. The depression of Behring's phenomenon is evidently associated with a decrease in the local and general toxic action of diphtheria toxin as a result of the presence of a focus of allergic inflammation. No decrease in the local toxic action of the toxin is observed if an inflammatory focus is produced at the same time with turpentine.

Sensitization of guinea pigs with horse serum does not in itself change the course of Behring's phenomenon: the toxicosis follows the same course in the sensitized animals as in the controls. Acute nonlethal anaphylactic shock in guinea pigs does not change the local toxic action of diphtheria toxin in the initial phase of Behring's phenomenon.

SUMMARY

Daily administration of $^1/_{50}$ MLD of diphtheria toxin for a period of 10-15 days to guinea pigs caused their death with a characteristic picture of diphtheria intoxication, although they received the total of less than 1 MLD of the toxin (Behring's phenomenon). The effect of allergic reactivity on Behring's phenomenon was revealed in this work.

As established, neither the rate of development nor the severity of intoxication changed in horse serum-sensitized guinea pigs. A nonfatal anaphylactic shock, induced prior to the toxin injection, did not reduce the local toxic effect of diphtheria toxin (infiltrates, necroses) developing during the first phase of Behring's phenomenon. Reproduction of Arthus' phenomenon in the sensitized guinea pigs has not inhibited the development of Behring's phenomenon. In this case, both the local and the general toxic effect of diphtheria toxin was seen to decrease. Terpentine-induced inflammatory focus did not diminish the local toxic action of the toxin.