THE MECHANISM OF INTOXICATION IN DYSENTERY

COMMUNICATION 2. THE EFFECT OF DYSENTERY TOXIN AND ANATOXIN ON INTESTINAL RECEPTORS

I. P. Myagkaya

Laboratory of Receptor Physiology (Chief – Active Member AMS USSR V. N. Chernigovsky),
Laboratory of Pathologic Physiology (Chief – Professor V. S. Galkin),
the I. P. Pavlov Institute of Physiology, AS USSR (Director – Academician K. M. Bykov) and
Department of Preliminary Instruction in Internal Diseases, the 1st I. P. Pavlov Medical Institute,
Leningrad (Director – Active Member AMS USSR M. D. Tushinsky)

(Received October 4, 1956. Presented by Active Member Acad. Med. Sci. USSR M. D. Tushinsky)

The results of experiments described in the first communication [1] showed that the action of the Shiga dysentery toxin on the mucosa of a perfused section of the intestine of an animal poisoned with the same toxin produced changes in the intensity of reflexes from intestinal chemoreceptors.

It remained unclear whether these changes were caused by the action of the toxic group of the toxin or by enhanced sensitivity to its antigenic group. The present work is concerned with elucidation of these questions.

EXPERIMENTAL METHODS

A total of 26 experiments was carried out on cats; in 5 of these the animals had had a preliminary administration of anatoxin, in 15 the animals were in a state of dysentery intoxication and in 6 the animals had been immunized by dysentery toxin 3 days prior to the experiment. Anatoxin was prepared by addition of formalin to the toxin used.

In the first series of experiments the animals received anatoxin subcutaneously (24 hours prior to the experiment) in doses corresponding to 20 lethal mouse doses per 1 kg body weight. In one experiment the dose of anatoxin corresponded to 40 lethal doses of toxin per 1 kg weight.

Two groups of animals were used in the second series of experiments. The first group (6 experiments) was given toxin subcutaneously 4-6 hours prior to the experiment, the second group (9 experiments) was given the toxin subcutaneously 24 hours prior to the experiment.

The third series of experiments was performed on animals which had been immunized by five administrations of anatoxin at 5 day intervals and in amounts corresponding to 60 lethal doses of the toxin (14-17 lethal doses per 1 kg body weight). Fourteen days after the last dose of anatoxin the animals were given 30 lethal doses of toxin subcutaneously on two consecutive days (a total of 14-17 lethal doses per 1 kg body weight). A critical experiment was performed on the fourth day.

As in the previous experiments, perfusion of an intestinal loop was used in the present work. The toxin and anatoxin were introduced into the lumen of the perfused segment of the intestine.

The order of experimental procedures remained as before [1].

EXPERIMENTAL RESULTS

In all the 5 experiments of the first series introduction of anatoxin into the lumen of the intestine exerted no effect on the pressor reactions elicited by subsequent introduction of nicotine into the vessels.

In four experiments introduction of anatoxin was followed by application of toxin to the intestinal mucosa. In one of these experiments a slight increase in the reflex blood pressure reaction was noted (a rise of 2 mm of Hg). In the remaining experiments the reflexes were unchanged. Increase of arterial blood pressure was elicited in these experiments, as in the case of healthy animals, by the addition of 1 ml nicotine in dilution 1×10^{-6} (1 γ) to the perfusion fluid.

At autopsy no changes were found in the internal organs.

Figure 1 shows the kymograms of one of these experiments. The initial stimulation of the intestinal loop receptors by nicotine (4 γ) gave rise to an increase in arterial blood pressure equal to 6 mm Hg. Introduction of anatoxin (⌊ 1) into the intestinal lumen did not cause a rise in blood pressure or stimulation of respiration. The magnitude of reflexes to nicotine following introduction of anatoxin remained as before: 6-8 mm Hg (b). Reflexes of the same magnitude were obtained in the course of 52 minutes following introduction of the anatoxin.

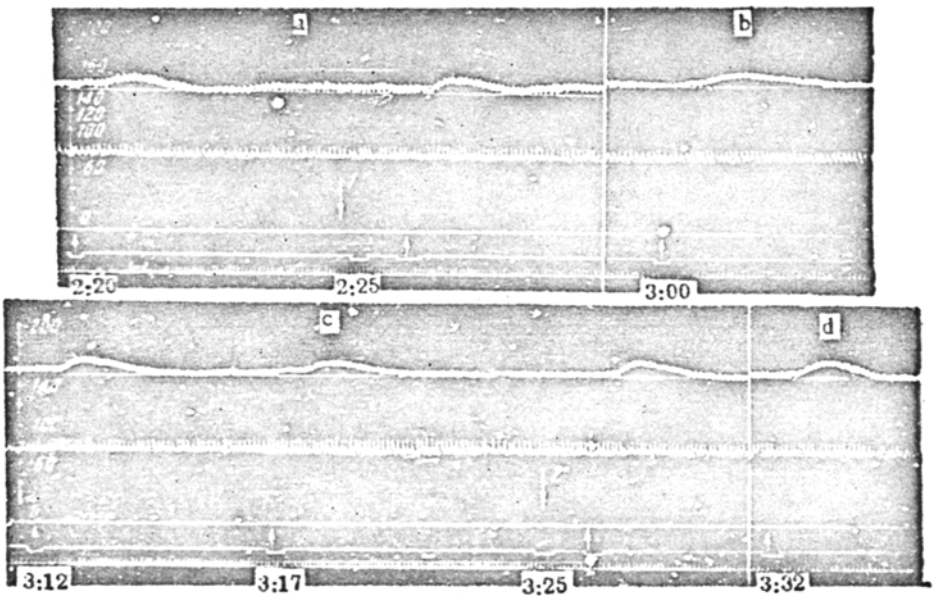

Fig. 1. Reflex reactions in a cat in experiment with preliminary administration of anatoxin in dose corresponding to 18 doses lethal for mouse per 1 kg body weight. Records from above down: arterial blood pressure, initial level of arterial blood pressure, respiration, base line of manometer, stimulus marker, time marker (2 seconds).

⌊ 1 – administration of anatoxin, ⌊ 2 – administration of toxin, ⌊ – nicotine 4 γ.

Application of toxin to the intestinal mucosa (⌊ 2) caused no change in the magnitude of reflex blood pressure and respiration reactions.

It could thus be concluded, on the basis of this series of experiments, that introduction of anatoxin as well as of toxin into the intestinal lumen of animals which had previously received anatoxin did not lead to changes in pressor reactions of arterial blood pressure in response to introduction of nicotine into the vessels.

Consequently, those changes which were obtained when toxin was given to animals which had been previously given the same toxin subcutaneously were caused by the state of dysentery intoxication and not by the action of the antigen group of the toxin.

The second series of experiments was carried out on animals in a state of dysentery intoxication. Six experiments in this series were performed on animals which received toxin 4-6 hours prior to the experiment (20-30 lethal mouse doses per 1 kg body weight). The remaining 9 animals were taken for experiment 24 hours after receiving the toxin. The dose was 15-30 lethal mouse doses per 1 kg body weight.

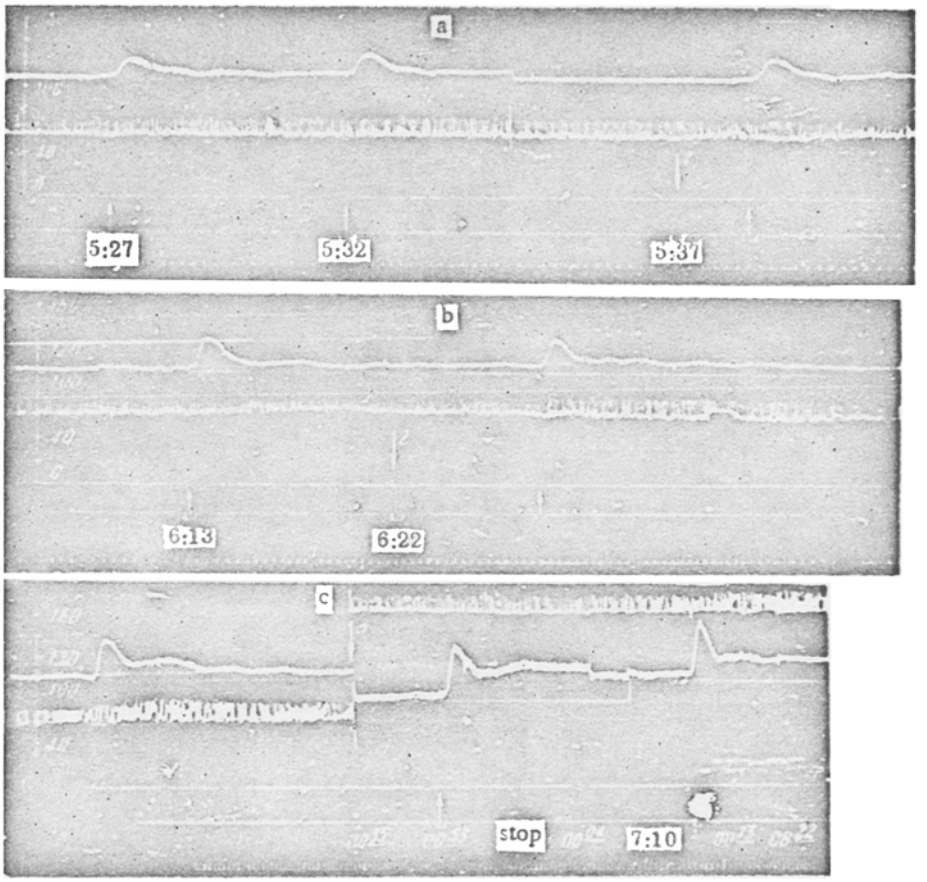

Fig. 2. Reflex reactions in a cat following preliminary administration of dysentery toxin ⌊^{3} (35 lethal mouse doses per 1 kg body weight).
Records the same as in Fig. 1. Time marker (5 seconds), ⌊ – introduction of nicotine 40 γ.

Introduction of anatoxin into the lumen of the perfused intestinal loop did not change the reflex arterial blood pressure reaction to nicotine introduced into the blood vessels of the same loop in a single one of the 6 experiments. Since the magnitude of the reflexes remained unchanged, 30-40 minutes later toxin was introduced into the intestinal lumen. In 4 experiments this led to increase of reflexes to nicotine as compared to the initial values. In two experiments there was no change in reflexes to nicotine. No changes were found in the internal organs of these animals at autopsy.

The changes described above are presented in Fig. 2. Introduction of 40 γ nicotine into the vessels of the intestinal loop was accompanied by reflex rise of arterial blood pressure by 14 mm Hg. Following application of anatoxin (⌊^{1}) to the mucosa of the perfused loop the reflexes remained unchanged over a period of 30 minutes. After 30 minutes (following introduction of anatoxin) the reflexes to nicotine rose to 24 mm Hg with an unchanged level of arterial blood pressure. It is unlikely that this rise could be connected with the action of anatoxin since usually restoration of altered reflexes was observed after 30-40 minutes. Against this background toxin was given (⌊^{2}) (120 lethal doses) 45 minutes after the introduction of anatoxin. Nicotine was given 1 minute after the toxin

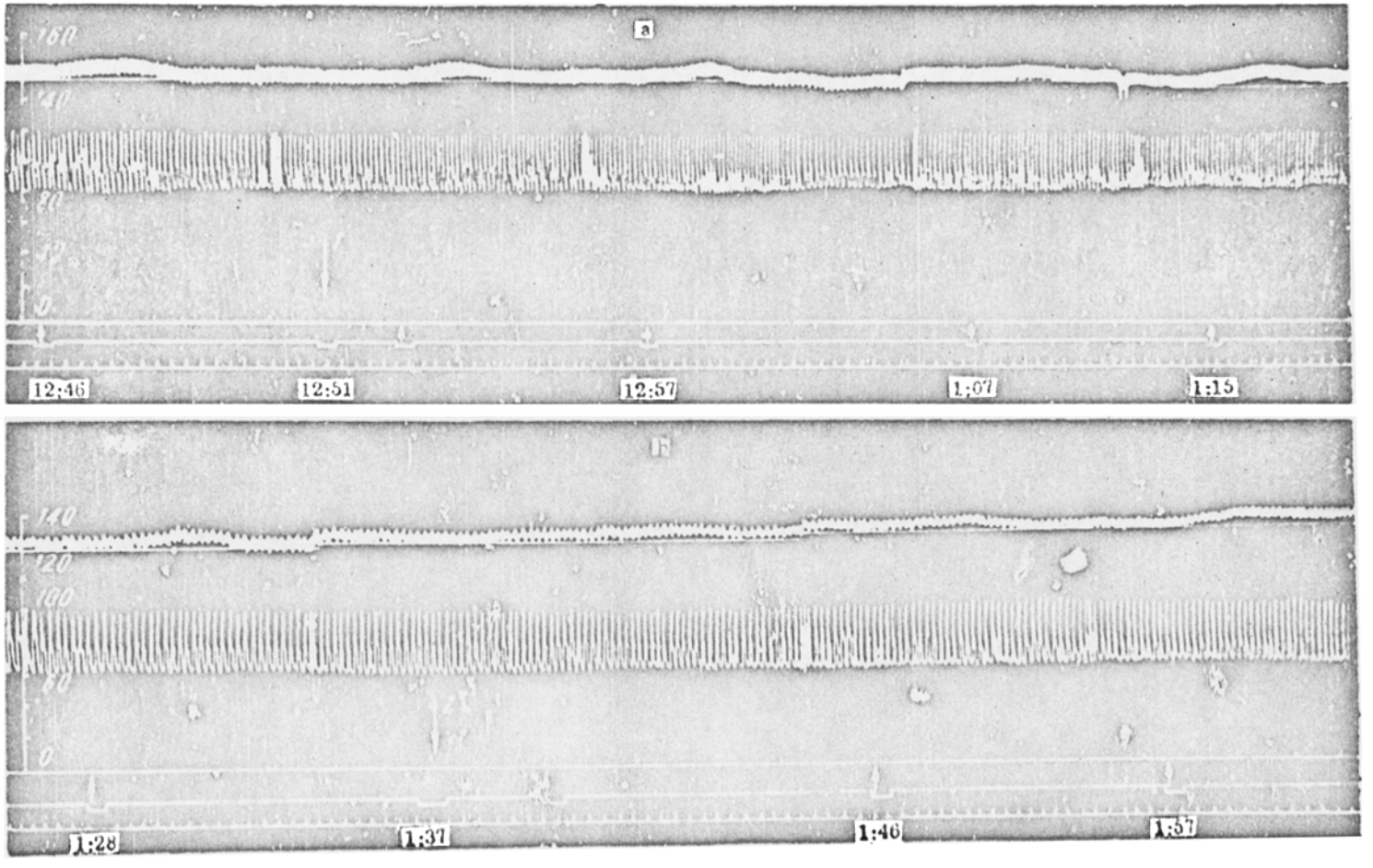

Fig. 3. Reflex reactions in a cat immunized with anatoxin (toxin given in the dose of 17 lethal mouse doses three days prior to the experiment). Records as in Fig. 1. Time marker (5 seconds), ↑ – administration of nicotine 4 γ.

and elicited a prolonged rise of blood pressure of the same magnitude. In 10 minutes the reflex to nicotine reached 32 mm Hg, exceeding by 8 mm Hg the level of the initial reflex. Deepening of narcosis ($|^1$) did not lower the magnitude of the reflexes. The experiment was completed 1 hour after administration of toxin. The reflex rise of arterial blood pressure to administration of nicotine was long lasting and reached 45 mm Hg, exceeding the level of arterial blood pressure increase prior to application of toxin to the mucosa of the intestinal loop by 21 mm.

In 9 experiments of this series, performed on the second day of intoxication, enhancement of reflex reactions to nicotine under the influence of anatoxin was observed in one case only. In the remaining 8 experiments the reflexes were unchanged.

This whole series of experiments gives grounds for concluding that the state of intoxication does not alter pressor reactions of arterial blood pressure to nicotine following introduction of anatoxin into the lumen of the intestinal loop.

It can therefore be postulated that in dysentery intoxication the sensitivity of intestinal chemoreceptors to the antigen group of the toxin remains unchanged while their sensitivity to the toxic group increases.

The third series of experiments (6 experiments) was carried out on animals in which the dysentery toxin was introduced against the background of antitoxic immunity. The animals' behavior revealed no signs of intoxication. At autopsy no changes in internal organs were found in 5 of the 6 animals. Only in one animal, which had received a very large dose of toxin (43 lethal mouse doses per 1 kg body weight), there was slight edema and hyperemia of the mucosa in the proximal part of the large intestine. In the same case application of toxin to the mucosa of the intestinal loop was accompanied by enhancement of reflexes to nicotine (10 γ and 4 γ). In the other 5 experiments introduction of toxin into the intestinal lumen did not lead to any changes of the pressor reactions of arterial blood pressure.

Figure 3 shows kymograms of an experiment in this series. Throughout the whole experiment (over one hour) the initial magnitude of the reflex reactions to 4 γ nicotine (4 mm Hg) was maintained despite two introductions of the toxin into the intestinal lumen ($|^1$, $|^2$).

In this series of experiments the threshold to nicotine stimulation was above 1γ(in 2 experiments – 4 γ, in 4 experiments – 10 γ). Such lowering of sensitivity to a nonspecific stimulant (acetylcholine) in the presence of immunity has been noted previously by G. Rashkova and co-workers [2].

In the latter experiments the repeated introduction of anatoxin could create conditions of sensitization to dysentery antigen. However, application of dysentery toxin to the intestinal mucosa in these experiments did not affect the magnitude of reflex reactions to nicotine. This can, evidently, serve as proof of lowered sensitivity of intestinal chemoreceptors to dysentery toxin in immunized animals, which is also manifested by the absence of clinical signs of the disease.

Similar lowering of sensitivity of intestinal vascular receptors to dysentery antigen was obtained by A. G. Skavronskaya [3].

Experiments on cats reported by G. Rashkova and collaborators also revealed lowering of sensitivity of receptors in intestinal vessels to the Shiga dysentery toxin in immunized animals [2].

The present investigations give grounds for stating that substantial differences exist between the action of the antigen and toxic groups of the toxin on intestinal receptors.

While in the state of intoxication the sensitivity of receptors to the dysentery toxin is apparently increased [1], this sensitivity is lowered in antitoxic immunity.

It is possible that lowering of receptor sensitivity to the toxin in immunized animals determines to some extent the fact that these animals are not susceptible to the disease under the influence of the specific pathogen.

SUMMARY

In condition of dysenteric intoxication, changes of reflexes from the chemoreceptors of intestines are due to the toxic, not the antigenic group of the toxin. In animals immunized by dysenteric anatoxin the sensitivity of intestinal receptors to dysenteric toxin is decreased.

LITERATURE CITED

[1] I. P. Myagkaya. Byull. Eksptl. Biol. i Med., 43, No. 5, 77-82, 1957.*

[2] G. Rashkova, B. Rybova, K. Rashke et al. Czechoslovak Physiology,** 2, No. 2, 203, 1953.

[3] A. G. Skavronskaya. Thesis. "Certain Problems of Pathogenesis of Flexner Bacillary Dysentery",** Moscow, 1953.

---

* Original Russian pagination. See C.B. Translation.

** In Russian.