CHANGES IN THE FUNCTIONAL PROPERTIES AND STRUCTURE OF THE PERIPHERAL NERVOUS SYSTEM OF THE SMALL INTESTINE INFECTED LOCALLY WITH *Mycobacterium tuberculosis*

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Besides causing specific lesions, tuberculosis also causes certain changes of a nonspecific character in the function and structure of the reflex arc of internal organs [1-4]. However, the functional and anatomical changes in the peripheral nerve endings have not yet been compared in a particular object.

In the present paper we describe results relating to the functional properties of the interoceptive apparatus and to the structural changes in the intramural nerve elements of an organ directly affected by specific inflammation.

EXPERIMENTAL METHOD

Experiments were conducted on cats. Under aseptic conditions and inhalation anesthesia, animals were infected locally with *Mycobacterium tuberculosis* in the wall of the small intestine, 25-30 cm in length; a laboratory strain of the Valle type was used, in a dose of 0.1 mg of culture per injection. The culture was injected into five areas of the intestine, equidistant from each other, the first injection being given very close to the ileocolic valve and the remaining four more proximally.

The animals took part in the acute experiments 3, 5, 7, 11, 16, and 21 days after inoculation (urethane anesthesia was used in these investigations). Using a technique of perfusion of the organ, isolated from its own circulation, the reflex excitability of the interoceptors of the area of small intestine infected with tuberculosis was studied. In order to provide the most favorable conditions of nutrition and activity of the isolated organ (and, consequently, experimental conditions closest to normal), during the experiments an additional healthy cat was used as a donor, supplying the experimental segment of the receipient's small intestine with blood throughout the investigation. Favorable conditions for the vital activity of the segment of intestine were ensured by providing anastomoses, made by glass cannulas and rubber tubes, between the carotid artery of the donor and the mesenteric artery of the recipient, and also between the mesenteric vein of the recipient and the jugular vein of the donor. Heparin (Richter) was used to prevent clotting of the donor's blood. From 15 to 20 min after creation of the crossed circulation, tests were made of the reflex excitability of the receptors of the affected segment of intestine. As stimulus nicotine was used in all the experiments, prepared in different dilutions immediately before the beginning of the investigation. Nicotine was applied to the receptors of the intestine by injection into the afferent cannula, introduced into the mesenteric artery of the recipient cat. The effectors were the arterial pressure and respiration; the first was recorded in the carotid artery by means of a mercury manometer, the second by means of a Marey's capsule connected to the animal's trachea. The reflex reactions from these systems were recorded on the smoked paper of an electrokymograph.

At the end of the acute experiment the animal was sacrificed and the affected area of the intestine, together with the adjacent area (with no visible morphological changes), was excised for neurohistological investigation. The material was fixed in 15% formalin solution. Serial sections were cut with a sliding or freezing microtome, and stained with picrofuchsin. The nerve structures were impregnated with silver by the Bielschowsky–Gros method.

EXPERIMENTAL RESULTS

In the earlier stages after inoculation (from the 3rd to the 7th day), an increase in the initial reflex excitability of the interoceptors of the affected organ was observed, as shown by a lowering of the thresholds of application of the stimulus. The threshold for nicotine at this period was significantly lower than in control investigations on healthy animals.

In an experiment carried out 5 days after inoculation, the threshold pressor reflex of the arterial pressure was obtained in response to injection of 1 μg of nicotine into the perfusion fluid, the reflex of the respiratory movements taking the form of an increase in the frequency and amplitude of the respiratory waves. Intensive reactions developed in response to injection of 25 and 50 μg of the stimulus. However, the high excitability of the interoceptors observed when the reflexes were tested for the first few times gradually declined in the course of the experiment. For instance, at the first test the arterial pressure reflex to injection of 50 μg of nicotine reached 24 mm, during the next two tests it was 16 mm, and later it was 8 mm. The reflex changes in respiratory movements, intensive when nicotine was injected into the perfusion fluid for the first time, also became less marked during subsequent tests. Meanwhile, in control tests of the reflexes at the same intervals of time the excitability of the interoceptors remained high.

With the naked eye the walls of the affected segment of intestine appeared edematous and cyanotic, and at the sites of injection of the culture areas of infiltration could be seen, the size of a pea, firm to the touch, and reddish-brown in color.

Microscopic investigation of the affected area revealed histological changes in the type II cells of Dogiel (shrinking and deformation). The processes of these cells also were changed: some had varicose swellings and thickenings, others vacuolation, and a third fragmentation (Fig. 1, a). The structure of the nerve cells in the part of the small intestine not affected by specific inflammation remained unchanged (Fig. 1, b). So far as the fibers in the nerve bundles are concerned, some were changed (increased argentophilia, irregularity of their edges, varicosity), while others remained morphologically unaffected.

At intervals of more than 10 days after inoculation the most characteristic finding was depression of the interoceptors which, in contrast to the experiments carried out during the first days after inoculation, now appeared at once, at the beginning of the experiment, after the first injection of nicotine. Furthermore, no differential reaction to stimuli of different strength could be observed in the animals of this group. As an example, we may cite the results of an experiment conducted on the 11th day after inoculation. It may be seen from Fig. 2 that a solution of nicotine, when injected into the perfusion fluid in a dose of 1 or 10 μg, did not produce reflex changes in either the arterial pressure or respiration. The threshold reflex affecting these particular systems appeared in response to the injection of 20 μg of stimulus. The subsequent injection of 40, 50, and even 100 μg of nicotine gave rise to reflex reactions of almost

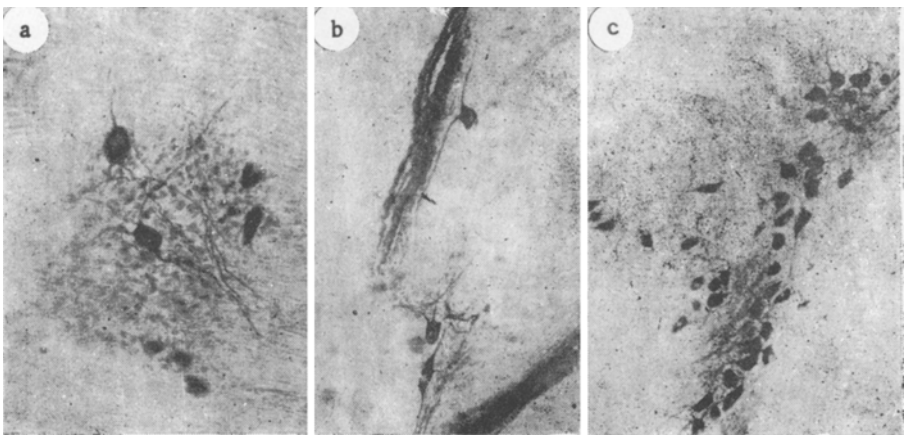

Fig. 1. Nerve cells in affected and unaffected areas of the intestine. a) Modified nerve cells in an affected area of the small intestine on the 5th day of the experiment; b) normal nerve cells in an unaffected area of the small intestine on the 11th day of the experiment; c) modified nerve cells in an unaffected area of the small intestine on the 21st day of the experiment.

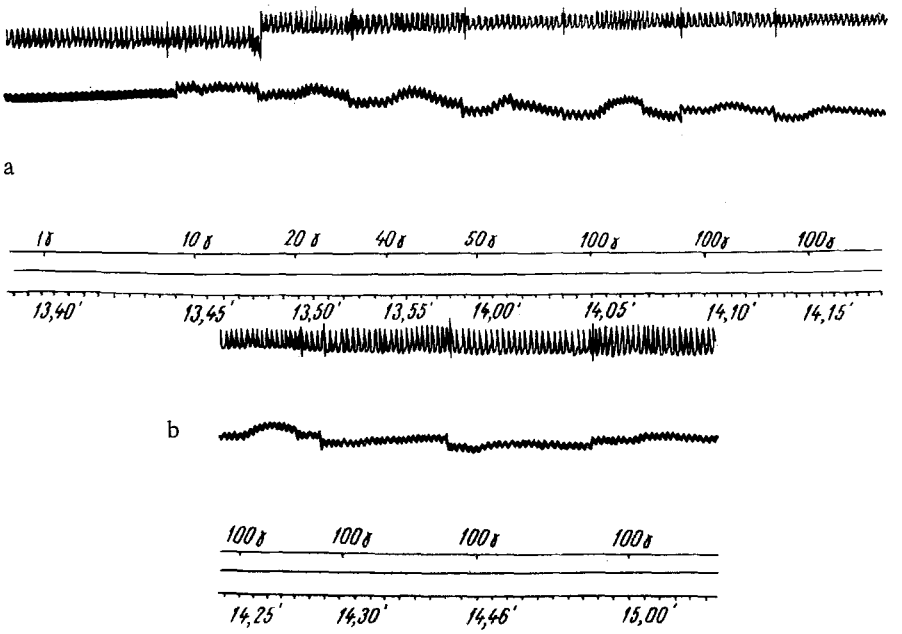

Fig. 2. Reflex changes in the arterial pressure and respiration after injection of nicotine into the vessels of the small intestine (11th day of the experiment). Significance of the curves (from above down): respiration; arterial pressure; stimulation marker; time marker (5 sec).

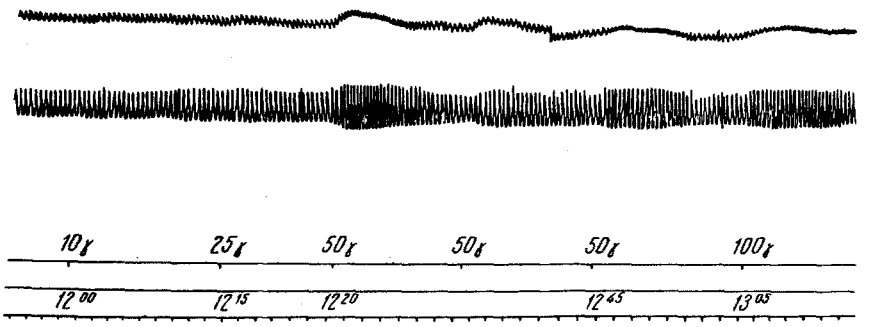

Fig. 3. Reflex changes in the arterial pressure and respiration after injection of nicotine into the vessels of the small intestine (21st day of the experiment).

equal intensity; a six-fold repetition of the injection of 100 μg of stimulus caused changes in the arterial pressure which did not exceed the threshold value. Macroscopically, the affected area was slightly bulkier than the adjacent unaffected area, and appeared hyperemic. At the sites of injection of the culture firm nodules of infiltration appeared, and destructive changes were present in the center of some of these.

Microscopic investigation of the affected areas of the intestine revealed marked pathological changes in the type II cells (shrinking, deformation, and disintegration). The processes of these cells showed degenerative changes (varicose thickenings, swellings, fragmentation). Only relatively few of the nerve cells preserved their normal structure. In the neighboring, macroscopically unchanged area of the intestine individual nerve cells showing the same degree of degenerative changes were seen.

In the last group of experiments we investigated animals infected with M. tuberculosis 20 days before making an experimental study of interoception. During this period the reflex excitability of the interoceptors in the area of the small intestine affected by specific inflammation was still more sharply depressed, and the manifestations of des-

truction of the nerve cells of the experimental organ were also more pronounced. These changes in the function of the receptor apparatus and in the structure of the nervous formations of the segment of the intestine infected with tuberculosis are illustrated by the experiment carried out on the 21st day after injection. In this case the stimulus was applied in doses of 10, 25, 50, and 100 μg. It is clear from Fig. 3 that the threshold reaction of the arterial pressure and respiration developed in response to injection of 50 μg of nicotine. At the next injection of the same dose (50 μg) of the stimulus into the perfusion fluid, the reaction of the arterial pressure was very weak, and even by injecting twice the dose of nicotine (100 μg) to act on the interoceptors its strength could not be increased.

To the naked eye the epxerimental segment of the intestine appeared thickened, adherent here and there to the mesentery, and covered with films of a whitish color. At the sites of injection of the culture nodules of inflammation the size of a large pea were seen, undergoing caseous degeneration in the center.

Microscopic examination of the experimental segment of the intestine showed that the type II cells in most cases had undergone marked destruction (shrinking, deformation of the cell body); the processes of the cells were irregular and thickened.

In addition to investigating the reflex excitability of the affected segment of the small intestine, revealing marked depression of the functional properties of its receptors, in order to make a control check of the reflex excitability we investigated an apparently unaffected area of the intestine (far from the site of injection of the culture of M. tuberculosis, with no macroscopic changes) of the same animal in the course of the experiment. In this case the excitability of the interoceptors was found to be high, with no tendency towards depression as the experiment proceeded. Most of the type II cells in the unaffected area of the intestine preserved their normal structure (the cell nucleus situated in the center, the processes slender and uniform). However, besides these morphologically normal cells, other type II cells were observed which had undergone various changes. The cells were intensively impregnated, irregular in shape, and their nucleus was situated at the periphery (Fig. 1, c).

The results of these experiments demonstrate that after local injection of a culture of M. tuberculosis into the wall of the small intestine considerable changes occur both in the functional state of the interoceptors and in the structure of the peripheral nervous formations of the affected organ. These changes are to some extent parallel. For instance, in the early period after injection with M. tuberculosis signs predominantly of irritation are observed in the intramural nervous formations: hyperpigmentation of nerve cells, varicose thickenings along the course of the axis cylinders, and so on. The initial excitability of the interoceptors of the affected area of the intestine at this period was actually slightly greater than in the controls. In these experiments, too, however, "exhaustion" of the receptors was observed, becoming more and more marked as the interval after injection grew longer.

Depression of the excitability of the interoceptors, as expressed by a considerable diminution of the reflex reactions, developing regularly in the later periods of the investigation (in our experiments on the 20th-21st day), is accompanied by the most obvious signs of destruction of the nerve cells and their fibers.

SUMMARY

An analysis of the functional properties of the interoceptive apparatus and structural changes occurring in the intramural nervous formations of the organ directly affected by specific inflammation is given. The data were obtained in experiments on animals (male cats) in which tuberculosis foci were created in a restricted area of the small intestine wall. To ascertain the functional state of interoceptors of the affected area, a method of organ perfusion with blood of a healthy animal of the same species was used. Nicotine served as a stimulus for the interoceptors. The structure of the intramural nervous formations was studied by special morphological methods. There was a reduction of interoceptor excitation in the experimental organ with the development of specific inflammation. A phenomenon of receptor "exhaustion" was noted; its degree was proportional to the duration of the period from the time of M. tuberculosis injection into the organ. The mentioned functional peculiarities were attended by marked destructive phenomena in nerve cells and fibers in the experimental area of the small intestine.

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