THE INFLUENCE OF VARIOUS AMOUNTS OF PYROGENAL ON THE FORMATION OF SCARS OF THE SPINAL CORD

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 56, No. 9,
pp. 115-119, September, 1963
Original article submitted November 28, 1962

In recent years increasing attention has been directed towards pyrogenous substances which elicit phenomena of stress in man and animals and which influence the organism in various ways [4]. It has been repeatedly shown [1,2, 3,5] that pyrogenal slows down the formation of a scar of the spinal cord, but until now the dose required has not been worked out. For clinical and for experimental use with animals as a rule amounts are given which cause a temperature response and which bring about side effects [7,9]. It was thought useful to test the effectiveness of these substances when given therapeutically in amounts too small to induce a temperature response.

The object of the present work has been to study the action of various doses of pyrogenal on the formation of a scar of the spinal cord.

EXPERIMENTAL METHOD

The work was carried out on white female rats weighing 120-150 g. The damage to the spinal cord was inflicted under nembutal anesthesia in the region of the VIII-IX thoracic vertebrae. At the region of the laminectomy a sheet of thin plastic was placed in position with a slit corresponding to the middle diameter of the spinal cord at this level. Razor blades were introduced of the same thickness as the slit in the plastic sheet. In this way the operation protected the periosteum of the lateral and anterior vertebral canal from damage and divided only the posterior columns and the grey matter of the cord. In most of the experiments no damage was caused to the anterior columns. As a rule the rats tolerated the operation quite satisfactorily.

Altogether 23 rats were used and of these 9 received 0.65 μ g/kg pyrogenal (first group), 6 received 10 μ g/kg (second group), and 5 received 40 μ g/kg (third group). Three rats made up the fourth or control group. The preparation was injected intraperitoneally starting at the day of the operation: for the first month it was given daily, for the next $1\frac{1}{2}$ months it was given every second day. Observations were continued for $2\frac{1}{2}$ months, and the rats were then killed.

Histological study of the cord was made from serial longitudinal sections stained with hematoxylin-eosin or hematoxylin-picrofuchsin.

In comparison with other animals rats are relatively insensitive to pyrogenal though they give well-marked stress responses even as a result of being fixed to a stand or receiving injections; it was therefore not possible to establish the extent of the leucocytic and temperature changed induced in the course of experiments on normal laboratory rats. For this purpose we carried out a special set of experiments on rats of the Wistar strain, which are comparatively resistent to autonomic changes and which received 0.65, 10, and 40 μ g/kg pyrogenal; in them the rectal temperature was measured and white cell counts were made. In the control rats we made the same measurements without the injection of pyrogenal.

EXPERIMENTAL RESULTS

The results of 300 temperature measurements made on 21 rats were treated statistically and are shown in Fig. 1. In the control group and to a somewhat lesser degree with doses of 0.65 and 10 μ g/kg there was a tendency for the body temperature to fall five hours after injection of the pyrogenal. Only the maximum dose of 40 μ g/kg caused some temperature increase, and it occurred three hours after the injection. This change was statistically significant. We may suppose therefore that 40 μ g/kg for the rat is the dose which causes a small temperature reaction.

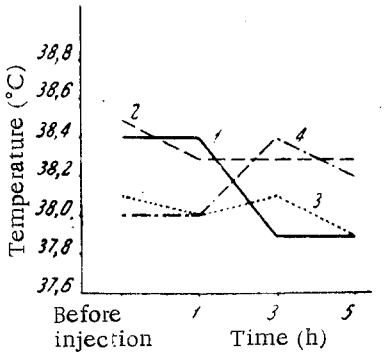

Fig. 1. Temperature reaction of rats (1) in the control group, (2) in response to 0.65 μ g/kg; (3) in response to 10 μ g/kg, (4) in response to 40 μ g/kg of pyrogenal.

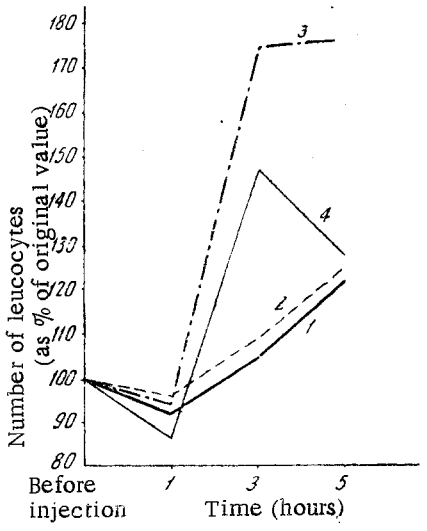

Fig. 2. Leucocytic reaction of rats under the influence of various doses of pyrogenal. Indications as in Fig. 1.

As can be seen from Fig. 2, where the curves were also taken from 300 measurements, a dose of 0.65 μ g/kg causes no appreciable change. A leucocytosis without any noticeable leukopenia occurred 2-3 h after the injection of 10 μ g/kg. A dose of 40 μ g/kg given one hour later caused a leukopenia which changed over into a leucocytosis. The difference in the curves was statistically significant.

Therefore a dose of pyrogenal of 0.65 μ g/kg in rats does not give the characteristic leucocytic reaction, while a dose of 10 μ g/kg is critical and causes a leucocytosis without any leukopenia; a dose of 40 μ g/kg causes a characteristic leukopenia followed by a leucocytosis.

To analyze the results of the histological investigations we divided them into three categories according to the structure of the scar in the spinal cord.

Coarse structure. The connective tissue scar consists of cords of fibroblasts having oval nuclei and very fine bundles of collagenous fibers arranged as a reticulum. The broad meshes of this reticulum were filled with undifferentiated cells having rounded but irregular nuclei, with granular spheres, and with a large amount of thin-walled blood vessels (Fig. 3, a).

Compact structure. In the central part of the scar there were broad bundles of collagenous fibers directed chiefly transverse to the axis of the spinal cord. The comparatively narrow spaces between them consisted of loose connective tissue and a few, mainly thick-walled blood vessels. In the peripheral portions of the scar the structure was less compact (Fig. 3, b).

Scar Structure and Pyrogenal Dose

Group of animals	Number of animals	Structure of scar in cord		
		coarse	transitional	compact
First	9	8	1	
Second	6	4	2	
Third	5	2	3	
Fourth	3			3

Transitional structure. Here the difference from the scar with the compact structure was that the bundles of collagenous fibers were thinner, the spaces with loose connective tissue were broader and contained more blood vessels, most of which are thin walled (Fig. 3, c, d). A comparative analysis showed that the structure of the scar of rats in the last group more closely resembled the loose than the compact scar.

Despite the small number of animals there was a clear relationship between the nature of the scar formed and the dose of pyrogenal given (see the table). The formation of the scar was restrained by pyrogenal given in any dose, but the dose

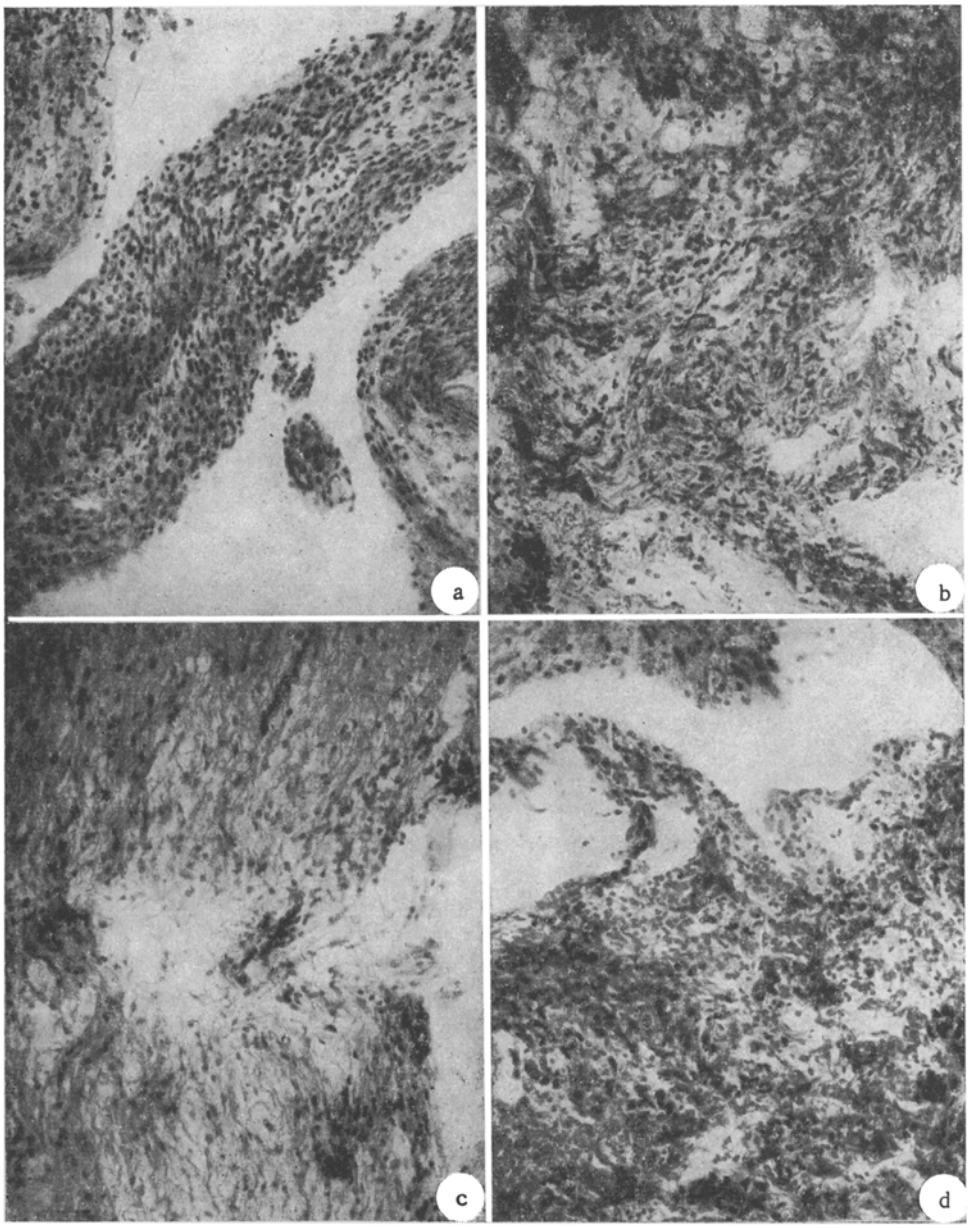

Fig. 3. Structure of the scar in the cord. a) Coarse scar; b) dense scar; c, d) transitional scar. Objective 20. Gam IV. Stain hematoxylin-picrofuchsin.

which caused no increase to temperature produced a more noticeable action than did a pyrogenic dose. The results obtained with large and moderate doses show that under the conditions of the present experiment a small dose causing no definite leucocytic change was somewhat more effective.

The inverse relationship which we have found between the density of the scar and the pyrogenal dose shows that there is no need to give pyrogenal in cases when it is required to influence scar formation in the cord; again, perhaps there is no need to give it in other cases when there is no direct indication that the temperature should be raised. Judging from the results a leucocytic reaction is not inevitable.

Several indications have been published concerning the great effectiveness of small doses of pyrogenic substances [6,7,8]. Most investigators ascribe the greater effectiveness of subfebrile doses to the absence of side effects. None of them considers the leucocytic reaction. It is known that leycocytosis caused by pyrogens develops independently of a temperature increase, but it does not occur in animals from which the adrenal cortex has been extirpated. This means that the leucocytic reaction is related to the activity of the adrenocorticotropic system. From the results of experiments

in which the influence on scar formation in the cord was most marked in the absence of any leucocytic response we may conclude that the specific influence of pyrogenal on connective tissue is not related to stimulation of the adrenocorticotropic system but is brought about by other means. Possibly, however, this influence is the result of very mild stimulation of the adrenal cortex, so that phenomena related to enhanced adrenocortical function could not be observed.

SUMMARY

To determine the action of various doses of pyrogenal on the formation of a scar caused by incomplete division of the spinal cord, three different doses of this substance were tested. The first dose was of 0.65 μ g/kg and caused no leucocytic or temperature reaction; the second was of 10 μ g/kg and induced a distinct leucocytosis, but did not affect the body temperature. The third dose of 40 μ g/kg induced leukopenia followed by a leucocytosis and a slight rise of temperature.

The results show that $2\frac{1}{2}$ months after the operation the character of the scar was related to the dose of pyrogenal given. The scar was most rarified in the group receiving the smallest dose. A scar of medium density was seen in the group in which a moderate dose was employed, and the scar was densest in the control group. In the group receiving the maximum dose most of the animals developed a scar of medium density whereas the rest showed a rarified scar.

Therefore to delay the formation of a scar in the spinal cord subfebrile pyrogenal doses proved the most effective.

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